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Pediatric Gastrointestinal Disease

Pathophysiology • Diagnosis • Management

CHAPTER 1

MALDIGESTION AND MALABSORPTION

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Malabsorption syndromes are characterized by the association of chronic diarrhea, abdominal distention, and failure to thrive. During the period between 1955 and 1970, the widespread use of intestinal biopsy, the emergence of basic concepts such as lipolysis at an interface, miscellar solubilization, Na^+-coupled solute transport, and brush border as a digestive-absorptive organelle permitted the breakdown of clinical malabsorption into many distinct congenital and acquired conditions affecting one or several of the different steps in hydrolysis or transport of nutrients. Thus, the term malabsorption syndrome now also designates such different situations as those characterized by exocrine pancreatic insufficiency (eg, cystic fibrosis), intestinal villous atrophy (eg, celiac disease), specific hydrolysis (eg, congenital lipase or sucrase deficiencies), or transport (eg, glucose-galactose malabsorption) defects. To understand how the clinician should interpret chronic diarrhea, the main symptom of these disease states, and how to orient the approach to the precise defect involved, it is necessary to recall the physiology and pathophysiology of digestion and absorption.

PATHOPHYSIOLOGY OF DIGESTION AND ABSORPTION

Carbohydrates

Physiology. Carbohydrates in food comprise mainly starch (50–60% of total energy supplied by carbohydrates), sucrose (30–40%), and lactose (from 0–20% in adults, 40–50% in infants). Only starch molecules (amylose and amylopectin), which are glucose polymers of high molecular weight (MW), require preliminary intraluminal digestion by salivary and (predominantly) pancreatic amylases. These structurally related endoamylases only split α_{1-4} bonds at some distance from the ends of the glycosidic chains and from the branching (α_{1-6}) positions. They release mainly maltose, maltotriose, and residues of a higher degree of polymerization (branched-α-limit dextrins if the substrate is amylopectin) but no glucose.[1] Intraluminal α-amylase activity is 10 times that required for digesting the amount of starch ingested daily.[2]

The final hydrolysis of di- and oligosaccharides occurs at the brush border of enterocytes where act three main glycoproteins of high MW (greater than 200 kD), the disaccharidases: two α-glycosidases: sucrase-isomaltase, which accounts for 75 to 80% of the hydrolysis of maltose in the intestinal mucosa, the total hydrolysis of sucrose, and the nearly total hydrolysis of isomaltose (α_{1-6}), and glucoamylase, an exoamylase that is responsible for 20 to 25% of total mucosal maltase activity and releases glucose from glucose polymers of four or more residues, and one β-galactosidase, lactase-phlorhizin hydrolase, which accounts for over 95% of lactase activity in the intestinal mucosa and for the hydrolysis of glycosyl ceramides, complex glycolipids that are important constituents of milk globule membranes.[3] Sucrase-isomaltase and lactase activities are highest in the proximal intestine, whereas glucoamylase activity is highest in the ileum.[4] The ingested disaccharides, which physiologically are not absorbed as such, are thus ultimately broken down into their constituent monosaccharides: glucose, galactose, and fructose.

Entry into the enterocytes through the brush border membrane occurs via carrier molecules. Entry of glucose and of galactose, occurring through the same carrier, sodium glucose linked transporter (SGLT)1, is linked to the entry of Na^+ along its electrochemical gradient; the latter blocks glucose exit from the cell and eventually provides the energy necessary for its accumulation in the cell against a concentration gradient.[5] The electrochemical Na^+ gradient is maintained by Na^+, K^+-adenosine triphosphatase located in the basolateral membrane of the enterocyte. Thus, glucose and galactose absorptions are indirectly active. Mutations of the gene coding for SGTL1 are the cause of congenital glucose-galactose malabsorption.[6]

Entry of fructose occurs through another specific carrier (glucose transporters [GLUTs]). It is not Na^+ dependent.[7] Fructose is more metabolized in the enterocyte than glucose; exit from the cell of both monosaccharides occurs via a facilitated transport system (a carrier) similar to the one present in red blood cell membranes (GLUT2).[8]

Final hydrolysis and absorption of carbohydrates are closely integrated in the brush border so that when sucrose is perfused into the jejunum, no or low amounts of glucose diffuse back into the intestinal lumen. Perfusion studies in adults, as in children, have shown that the limiting factor in the overall process of disaccharide absorption is absorption of glucose and fructose in the case of sucrose and of maltose but lactase activity in the case of lactose. This relationship is not modified in cases of mucosal atrophy.[9]

Pathophysiology. Pancreatic amylase insufficiency occurs normally in newborns, whose amylase activity remains extremely low during the first weeks of life. Yet substantial amounts of starch (greater than 40 $g/m^2/d$) can be given to 1-month-old infants before fermentation, the sign of carbohydrate malabsorption, occurs.[10] Similarly, in cases of exocrine pancreatic insufficiency (cystic fibrosis or Shwachman syndrome), symptoms related to amylase insufficiency are modest. In the stools, volatile fatty acids are notably increased, but lactic acid is low or absent, and pH is above 5.5. This is probably due to the fact that starch is a poorer substrate for colonic bacteria than are oligosaccharides.[11]

Indeed, congenital or acquired defects of intestinal digestion and absorption of oligosaccharides lead to a major digestive symptom: fermentative diarrhea. It is characterized by watery stools whose volume is roughly proportional to the amount of ingested carbohydrates. Stool volume may thus be extremely variable from one day to another. The stools have an acidic smell, resembling that of rotten apples or vinegar. They have an acidic pH (5.5–4.0) and usually contain unabsorbed reducing sugars or undigested disaccharides. The child may be thirsty and presents some degree of abdominal distention—the more impressive, the younger the child.[12]

Diarrhea and abdominal distention (with gas-fluid levels on plain abdominal radiographs) are secondary to a cascade of events. Maldigested di- or trisaccharides or malabsorbed monosaccharides are small, osmotically active molecules that drive water inside the lumen of the gut in direct proportion to their amount. The increased volume of chyme leads, in turn, to an increased peristaltic activity and a reduced transit time, decreasing the chance of digestion and/or of absorption.[13] In the cecum, the unabsorbed small carbohydrate molecules are readily fermented by colonic bacteria. The latter produce CO_2, H_2, and mainly acetic but also butyric and propionic acids. If the availability of carbohydrates further increases, lactic acid, a strong acid with a low pK_a, is produced and the pH decreases below 5.5 (a stool pH less than 5.5 indicates lactic acid in the stools[12]). This low pH disturbs Na^+ absorption by the colonic mucosa, which tends to further increase stool volume, as does the presence of the osmotically active molecules of volatile fatty acids in the lumen of the colon.

The same mechanism applies in all cases of carbohydrate malabsorption: in isolated congenital defects of intestinal hydrolysis (congenital sucrase-isomaltase, lactase, or trehalase deficiencies, or late-onset lactase deficiency) or of absorption (congenital glucose-galactose malabsorption) or as a consequence of mucosal atrophy (mainly in cow's milk protein intolerance and celiac disease). Excluding the malabsorbed carbohydrate(s) from the diet stops diarrhea in a few hours; it is again triggered in a similar short period of time if the malabsorbed oligosaccharide is reintroduced in the diet. Dextrins or starch, however, may lead to diarrhea only after several (2–6) days in sucrase-isomaltase deficiency, probably because of the complementary glucoamylase activity.[12]

Proteins

Physiology. Digestion of proteins starts in the lumen of the stomach, where gastric acid denatures them and activates pepsinogens I and II into the corresponding pepsins. The latter are inactive at a pH of less than 5 and have a broad specificity, splitting peptide bonds mostly involving phenylalanine, tyrosine, and leucine.[14,15] In view of the buffering capacity of food, it is unlikely that gastric secretion plays a major role in protein digestion. In contrast, the efficiency of pancreatic proteolysis is demonstrated by the fact that as soon as 15 minutes after a test meal, about half of the amino acids in the lumen are free or in the form of small peptides.[16] After activation by enterokinase, a glycoprotein of high MW synthesized by and anchored in the brush border membrane of enterocytes in the proximal small intestine,[17] trypsinogen is converted into trypsin, which, in turn, activates the other zymogens into active proteases. The endopeptidases—trypsin, chymotrypsin, and elastase—are serine proteases of similar MW (25–28 kD) but with different and strictly defined specificities. Trypsin splits only bonds involving at the amino end basic amino acids (lysine and arginine); chymotrypsin splits those involving aromatic amino acids (phenylalanine, tyrosine, tryptophan), and elastase splits those involving uncharged small amino acids (such as alanine, glycine, and serine), which are left at the carboxy end of the newly formed peptide. They are released by exopeptidases; by carboxypeptidase A, which releases from a peptide its last amino acid when it is aromatic, neutral, or acid; and by carboxypeptidase B, when the last amino acid is basic.[1,18]

In contrast to carbohydrates, peptides enter enterocytes either after preliminary digestion by brush border peptidases into amino acids, or as peptides, in the case of di- or tripeptides, which are then split inside the cell by cytoplasmic peptidases.[19] In humans, the following brush border peptidases are now well defined: several aminopeptidases (oligoaminopeptidase or neutral aminopeptidase, the main brush border peptidase, acid aminopeptidase, dipeptidyl-peptidase IV, an ileal *N*-acetylated α-linked acidic dipeptidase with dipeptidase and dipeptidyl peptidase IV activity[20]), two carboxypeptidases (carboxypeptidase P and angiotensin-converting enzyme), two endopeptidases (including para-aminobenzoic acid [PABA] peptidase), and

γ-glutamyl transpeptidase. These enzymes are glycoproteins of MW somewhat lower than that of disaccharidases and altogether are able to hydrolyze all peptide bonds, except those involving a proline at the carboxyl side.[3] Their highest activities are in the ileum.[4,21] The released amino acids are absorbed through the following systems: neutral amino acids enter the enterocytes mainly through the B°, Na^+-dependent system, whose defect is probably responsible for Hartnup disease[22]; they can also use at least two other systems shared with basic amino acids: $B^{+,0}$ Na^+ dependent and $b^{+,0}$ Na^+ dependent, defective in type I cystinuria.[23,24] Proline and hydroxyproline mainly use the Na^+, Cl^--dependent imino carrier and, in a lesser proportion (~ 30%), the B° carrier to enter the enterocyte. Bicarboxylic acids enter through a specific Na^+-dependent, electroneutral X^-_{AG} system whose defect is responsible for dicarboxylic aminoaciduria.[23,24]

Di- and tripeptides can also cross the brush border membrane as such via a (main) peptide transport system that has been shown to have a broad specificity.[25] This carrier protein is able to transport dibasic and diacid peptides as well as di- and tripeptides (but no tetrapeptides).[15] Transport of peptide is coupled to a proton rather than to a Na^+ gradient.[26,27] Once in the absorbing cell, di- and tripeptides are split into amino acids by soluble peptidases of which glycine-leucine dipeptidase, a prolidase hydrolyzing X-Pro bonds, and a tripeptidase are known.

At the basolateral membrane, neutral amino acids leave the enterocyte using the Na^+-dependent L system; basic amino acids use Na^+-dependent y^+ and y^{+L} systems, which also accept neutral amino acids. Mutations in the gene coding for a subunit of y^{+L}, y^{+LAT} are the cause of lysinuric protein intolerance.[28] Ubiquitious Na^+-dependent A and ASC systems, of high specificity and low capacity, are probably more involved in the metabolism of the enterocytes than in protein absorption. Recently, a peptide transporter has been characterized in basolateral membranes of Caco-2 cells.[29]

Small peptides are the form in which amino acids are, in general, the more readily absorbed. Even when a di- or tripeptide is susceptible to rapid hydrolysis by brush border peptidases, an important proportion of it (30–50% depending on its concentration) is directly absorbed as such.[19,30] Thus, peptides represent the main physiologic route of entry of amino acids in the enterocytes.

Pathophysiology. In adults, nitrogen absorption is not affected by gastrectomy, which indicates that gastric acid and pepsins do not play a critical role in protein digestion. In contrast, selective absence of pancreatic protease activities—in congenital enterokinase deficiency, for example—leads to dramatic situations. Although stools are only moderately overabundant and foul smelling, fecal losses of nitrogen are massive, with the consequence of early failure to thrive. The latter is more probable and severe because the protein content of milk is low (as in human milk). Hypoproteinemia develops, leading to edema. Replacing the standard formula by a formula containing a protein hydrolysate is usually enough to normalize the stools, to decrease the fecal loss of nitrogen, and for the infant to start catching up its growth retardation.[31]

The consequences of pancreatic exocrine insufficiency on protein digestion are similar to those of selective pancreatic proteolytic deficiency. They are associated with significant losses of energy in the stools owing to the defect in starch and, mostly, in fat digestion with a massive steatorrhea. The stools are often whitish, greasy, soft but not liquid, and extremely foul smelling. Malnutrition and growth failure follow when food intakes are not sufficient to compensate for the fecal losses.

No specific congenital defect of peptide digestion or absorption by the intestinal mucosa is known, although assays of the main peptidase activities have been systematically included in the workup of diarrheal states in several pediatric gastroenterology teams for several years now. This is not surprising considering that peptides may enter enterocytes by two routes of roughly similar physiologic importance: when one is blocked, the other one can still be used. Indeed, such defects may exist but go undiagnosed. Similarly, specific absorption defects involving neutral (Hartnup disease), basic (cystinuria), or imino (prolinuria) acids are well known. Yet they have been recognized not because of digestive symptoms, which do not exist in these conditions, but because of associated specific aminoaciduria, reflecting defective tubular reabsorption of the homologous amino acids by the kidney. The absence of diarrhea in these diseases is clearly due to the fact that the specifically malabsorbed amino acids can cross the brush border membrane as peptides.[19,25] Indeed, the only known disease affecting amino acid absorption in which diarrhea is a real problem, often associated with severe malnutrition, is protein intolerance with lysinuria (or lysinuric protein intolerance). In this condition, the congenital defect does not affect the entry of arginine, lysine, and ornithine into the absorptive cell but rather their exit out of the cell through the basolateral membrane. Whatever the way of entry into enterocytes, these basic amino acids cannot get out, and symptoms occur.[32] Diarrhea is usually liquid, being probably mainly osmotic in its mechanism.

Nonspecific inflammatory alterations of the intestinal mucosa, such as those seen in celiac disease, do not lead to symptoms that can easily be assigned to protein or peptide maldigestion or absorption. A certain degree of creatorrhea exists in children with mucosal atrophy, yet it is usually mild and, because of the complex origin—both endogenous and exogenous—and the fate of protein in the human gut—absorbed or used by colonic bacteria—it is difficult to relate it directly to malabsorption. Similarly, it is difficult to decide whether the fecal losses of nitrogen observed in cases of intestinal malabsorption are the only explanation of the hypoalbuminemia that may be seen in severe celiac disease when anorexia (and, consequently, decreased protein intake) and protein-losing enteropathy are also symptoms of the disease.

Fat

Physiology. Unlike carbohydrates and proteins, fats are insoluble in water; although they diffuse through the lipid phases of the brush border and basolateral membranes of

enterocytes, they have to be "wrapped" in outwardly hydrophilic, inwardly lipophilic particles—bile salt micelles in the gut lumen, chylomicrons in the absorbing cell and circulation—to reach their site of metabolic use.[33]

Digestion of fat starts in the stomach, acted on by a lipase that has been shown recently to originate exclusively from the gastric fundus in humans,[34] whereas it is produced by the serous glands of Ebner at the base of the tongue in the rat (hence the often used term lingual lipase). In humans, this lipase has an acidic optimum pH (4.5–5.5); at the pH of the stomach, it hydrolyzes medium-chain triglycerides at the same rate as long-chain triglycerides, it preferentially splits the outer ester bonds of triglycerides, and it is not appreciably dependent on bile salts. It acts as a "starter" of pancreatic lipolysis by favoring emulsification of lipid droplets by the free fatty acids (FFAs) it releases. It plays a particularly important role in neonates whose pancreatic lipase activity is low.[35]

In the duodenum, pancreatic lipase acts only at the oil-water interface, adsorbed to the lipid droplets. Bile salts both increase the interface by emulsifying the ingested lipid droplets, thus favoring lipase activity, and, on the contrary, by forming a film between oil and lipase, inhibit its action. Colipase restores lipase activity by anchoring lipase to the interface and by keeping its active site open, giving it access to its substrate.[36] In the presence of bile salts, pancreatic lipase has an optimum pH of 8. It has an absolute specificity for the outer ester bonds (positions 1 and 3) of the glyceride molecule and releases FFAs and 2-monoglycerides.[36] Other lipolytic enzymes are secreted by the pancreas: (1) carboxylesterhydrolase, whose structure resembles that of human milk, bile salt–dependent lipase, and which acts on soluble substrates; in the presence of bile salts, it becomes active toward cholesterol esters and esters of vitamins A and E, whose absorption is thus dependent on normal bile salt secretion[37]; and (2) phospholipase A_2, which releases lysophosphoglycerides and fatty acids from phosphoglycerides, major membrane constituents.

From the onset of lipolysis, FFAs and 2-monoglycerides are solubilized in bile salt micelles, forming bigger "mixed micelles," which, in their turn, can solubilize more hydrophobic lipids such as diglycerides, un-ionized FFAs, cholesterol esters, and lipid-soluble vitamins whose absorption is therefore improved when ingested with other fats.

Primary bile acids (cholic and chenodeoxycholic acids) are synthesized in the liver from cholesterol; they are glyco- and tauroconjugated, excreted, and transformed by colonic bacteria into secondary acids (deoxycholic and lithocholic acids). Because of their lower pK_a, conjugated bile acids allow a much better micellar solubilization of the products of lipolysis.[38] Bile acids, whose pool amounts to 1 to 2.5 g, are efficiently reabsorbed in the distal ileum by a Na^+-dependent, carrier-mediated process that is responsible for the reabsorption (and recirculation) of 95% of the bile salts secreted in bile.[39]

Micelles diffuse from the gut lumen through the unstirred water layers lining the luminal surface of brush borders, where the products of lipolysis are liberated. They diffuse across the apical cell membrane. At low concentrations, there is evidence that FFAs can enter enterocytes by a fatty acid binding membrane protein with high affinity for saturated or unsaturated long-chain fatty acids[40,41]; entry of cholesterol is also mediated by brush border membrane lipid exchange proteins.[42] In enterocytes, FFAs of 12 carbon atoms or more bind to small carrier proteins, the fatty acid–binding proteins (I, ileal, the majority, and L, liver).[43] In the smooth endoplasmic reticulum, long-chain fatty acids are activated in acyl coenzyme A before entering the monoglyceride pathway responsible for at least 70% of postprandial triglyceride resynthesis (whereas the role of glycerophosphate pathway in triglyceride resynthesis increases between meals and during fasting).[33] Microsomal triglyceride transfer protein transfers resynthesized triglyceride in the rough endoplasmic reticulum where, together with phospholipids and cholesterol, triglyceride is joined by newly synthesized apolipoproteins, A-I, A-IV, and B-48. The absence of one of the microsomal transfer protein subunits, but not of B-48,[44] has recently been shown to be responsible for abetalipoproteinemia.[45] Chylomicron formation is then completed in the Golgi apparatus. Chylomicron-containing Golgi vesicles are released from the Golgi apparatus. After fusion of these vesicles with the basolateral membrane, chylomicrons are excreted by exocytosis into the intercellular spaces, from which they reach the lymphatics and, by the thoracic duct, the systemic circulation.[46]

Pathophysiology. Because of their hydrophobicity, digestion and absorption of dietary fats are dependent on many auxiliary molecules other than enzymes and carriers: bile salts, colipase, fatty acid–binding proteins, and apolipoproteins. It is thus remarkable that, given this complexity, more than 95% of ingested fat should be ultimately absorbed in children over the age of 1 year.

However, these auxiliary mechanisms are also sites for potential disturbances; indeed, causes of fat malabsorption and steatorrhea are much more numerous than those of carbohydrate and protein malabsorption. Fat malabsorption may result from lipase and/or colipase deficiency(ies); abnormal bile salt synthesis, excretion, deconjugation, and reabsorption; impaired triglyceride resynthesis; chylomicron formation and/or excretion; or obstruction of intestinal lymphatics.

Isolated fat malabsorption is extremely rare. In the case of congenital lipase deficiency, for example, stools are greasy but not liquid or even soft: nonabsorbed fat constitutes an oil phase distinct from, or surrounding, otherwise nearly normal stools. Steatorrhea is massive, with the fat absorption coefficient being less than 50%. Such a steatorrhea is painless and may have no other consequence for the patient than greasy soiling and a strong appetite to compensate for the loss of energy in the stools.[47] Colipase deficiency leads to less severe steatorrhea, and biliary atresia, although leading to no other digestive symptom, presents usually with its own signs, such as jaundice and hepatomegaly.[48]

In most cases, fat malabsorption is part of a more general pathologic condition. It may result from exocrine pancreatic insufficiency: severely reduced lipase and colipase secretions result in abnormally great quantities of neutral triglyceride reaching the colon, where the bacterial flora hydrolyzes part of them, releasing FFAs and glycerol. Steatorrhea is usually

severe, with fat absorption being reduced to 70 to 40% of ingested fat. Because undigested starch is not so osmotically active, stools are soft or pasty, not liquid, like mastic.

Absorption of fat and particularly of fat-soluble vitamins is severely impaired in congenital disorders of bile salt synthesis or secretion (Byler disease) with massive steatorrhea and, occasionally, prominent rickets.[49] However, bile salt metabolism abnormalities other than reduced or absent secretion may disturb fat absorption. Bile salts may be deconjugated by certain bacterial species in cases of bacterial overgrowth. Nonconjugated bile salts are less ionized than conjugated ones at the pH of the gut lumen; thus, they have a lower ability to form micelles than the latter. Significant steatorrhea may follow. It is usually associated with other symptoms of bacterial overgrowth in a stagnant loop: increased degradation of protein and particularly of vitamin B_{12} binding proteins, fermentation of carbohydrates with production of H_2 released in breath and of volatile fatty acids resulting in diarrhea, hypoproteinemia, loss of weight, and megaloblastic anemia.[50] Bile salts also may not be absorbed because of a congenital defect[51] or ileal disease (Crohn disease) or because of ileal resection with two consequences: on the one hand, abnormally high amounts of nonabsorbed bile salts reach the colon, where they inhibit Na^+ and water absorption; on the other hand, depletion of the bile salt pool progressively leads to poor micellar solubilization and steatorrhea. In this situation, however, the direct effect of bile salts on colonic mucosa is probably more responsible for the abnormal loose or watery stools than is the increased fecal loss of fat.

Intestinal mucosal abnormalities never affect only fat absorption, even when, as in abnormal chylomicron formation and/or excretion, fat transport alone is disturbed by the disease. Most frequently, fat malabsorption is secondary to nonspecific intestinal mucosal atrophy, as observed in celiac disease or cow's milk protein intolerance. In these conditions, malabsorption of fat, as of other nutrients, results from both a decreased absorptive surface and a disturbed enterocyte metabolism. In fact, abnormal accumulation of lipid droplets occurs in active celiac disease by mechanisms that are not completely elucidated but may involve impaired apolipoprotein synthesis. It is not clear whether lipids in the stools originate directly from ingested fat or indirectly from desquamated fat-filled enterocytes. In any case, fat lost in the stools is mainly FFA partly hydroxylated by the colonic flora, which accounts for at least 1 g daily of obligatory loss of fat in the stools.[52] Interestingly, steatorrhea is usually far less severe in intestinal mucosal disorders than in exocrine pancreatic insufficiency. This may be due to the fact that most intestinal pathologic conditions, such as celiac disease, affect only the proximal small intestine or to the existence of "accessory" pathways of fat absorption, as suggested by the paradoxic absorption of 50 to 70% of ingested fats in situations in which no absorption is expected, as in congenitally impaired formation or excretion of chylomicrons.[53] The moderate steatorrhea observed in conditions with subtotal villous atrophy is usually not sufficient to make the stools grossly greasy; in fact, stool features in these situations result more from the degree of associated carbohydrate fermentation than from the degree of steatorrhea.

Even when chylomicron formation and/or excretion is blocked, as in abetalipoproteinemia or Andersen disease, with enterocytes filled with fat droplets, malabsorption is not restricted to fat. Balance studies in these conditions show fecal excretion of fat, nitrogen, and volatile fatty acids that is similar to that observed in celiac disease.[53] Stools have the same aspect. Yet a fat-free diet is sufficient to normalize the fecal excretions of nitrogen and volatile fatty acids. This indicates that nitrogen and carbohydrate malabsorption is induced by the accumulation of fat observed in enterocytes in these conditions.

Similarly, in intestinal lymphangiectasia, reflux of absorbed fat into the intestinal lumen because of blocked lymph flow is never isolated, and steatorrhea, which is usually moderate, is associated with signs of enteric loss of the other constituents of intestinal lymph: albumin, immunoglobulins (Igs), and T lymphocytes. However, fats lost in the lumen are excreted in the stools, whereas proteins are easily digested and reabsorbed, and balance studies usually fail to find a significant increase in fecal nitrogen. Diarrhea is often moderate in this condition, in which low serum albumin levels and edema are major symptoms.[54]

NUTRITIONAL CONSEQUENCES OF MALABSORPTION

Malabsorption may have no or minor nutritional consequences (eg, lipase deficiency) or, at the opposite extreme, may lead to severe malnutrition and eventually death (eg, celiac disease). Furthermore, the same disease may be life-threatening in infancy and well tolerated in adolescence (eg, celiac disease or congenital sucrase-isomaltase deficiency). Finally, malnutrition may become the consequence of a stable state of malabsorption under the influence of exogenous factors (eg, bronchopulmonary infection in cystic fibrosis). Thus, a given nutritional disturbance is not strictly linked to a given malabsorption syndrome, and it seems appropriate in dealing with nutritional consequences of malabsorption to describe first the different consequences that malabsorption syndromes may have on the nutritional status of the child and then to analyze the general mechanisms by which malnutrition occurs. Nutritional disturbances involving micronutrients will not be considered here.

States of Malnutrition

The most obvious nutritional consequences of malabsorption syndromes concern the growth of the child. Malabsorption always first reduces weight gain before it slows down growth rate. The clinical situation may deteriorate, with disappearance of subcutaneous fat, muscle wasting, and the appearance of the skin being too large for the child. Growth stops. Life may be threatened. Such a severe evolution was described in toddlers with historic celiac disease. Nutritional consequences are not so dramatic in older children with celiac disease, for example; a certain equilibrium may be reached, the child having a weight roughly adapted to his height, yet growth is retarded, and the child

may progressively become a dwarf. In adolescents, puberty may be delayed.[55]

In most severe and/or rapidly evolving malabsorption syndromes, not only is growth affected but also other general functions; malabsorption of vitamin K, whatever its mechanism (mucosal atrophy, fat malabsorption, bacterial overgrowth), may result in decreased synthesis of blood clotting factors and disturbed hemostasis, with hematomas and easy bleeding; long-term Ca^{2+} and vitamin D malabsorption, as occurs primarily in celiac disease, usually leads to osteoporosis, with hypocalcemia, hypocalciuria, and, in severe cases, "spontaneous" bone fractures, and, rarely, to rickets.[55] Bone mineral density, now easily assessed by dual-energy x-ray absorptiometry, is, indeed, decreased at diagnosis of celiac disease[56] and, to a lesser extent, in the silent form of the disease.[57] Severe protein-losing enteropathy, often associated with malabsorption syndromes (celiac disease, bacterial overgrowth, Crohn disease, intestinal lymphangiectasia), may be an additional factor responsible for hypoalbuminemia and edema and for hypogammaglobulinemia.[54]

Other biologic anomalies, also present in children with overt malnutrition, may be used in others to detect malnutrition when it is not clinically certain. They include hyposideremia, microcytic anemia with low reticulocyte counts secondary to iron malabsorption, low serum folate levels usually without hematologic consequences in case of mucosal atrophy, low serum vitamin A levels (whose ophthalmologic consequences are nonetheless rare), and low vitamin E levels, which may lead, after years of evolution, to loss of proprioception and tendon reflexes and to ataxia, in cases of fat malabsorption; vitamin E deficiency is most severe in biliary atresia and in abetalipoproteinemia.[58]

Mechanisms of Development of Malnutrition

Our knowledge concerning the mechanisms resulting in malnutrition is limited because of the complexity of a phenomenon with intricate causes, always studied when already present, with often only crude parameters (weight and height).

Although malabsorption per se is the most obvious mechanism leading to malnutrition, it is probably less often responsible for failure to thrive than decreased food intake. This has been clearly demonstrated in the case of Crohn disease,[59] but it may be also true for active celiac disease, in which anorexia is a major symptom; more generally, decreased food intake may be a main factor of malnutrition in all situations in which fermentation is a major consequence of malabsorption (congenital disaccharidase deficiencies or disaccharidase deficiencies secondary to villous atrophy) leading to abdominal distention, discomfort, and poor appetite, whereas fecal losses of protein and energy are usually modest. At the opposite end of the spectrum, fecal losses of up to 40% of ingested protein and energy in cases of exocrine pancreatic insufficiency, associated with no major discomfort, do not lead to malnutrition as long as they are compensated by an increased appetite.[60] In this situation, any factor[61] decreasing food intake—bronchopulmonary infection in cystic fibrosis, for example—triggers malnutrition and retards growth.[61]

Malabsorption per se—independently of anorexia—may induce malnutrition only when fecal loss cannot be compensated by increased intake; this is the case in infants with undiagnosed exocrine pancreatic insufficiency when nutrient intakes are low, particularly proteins, the levels of which in commercial formulas tend to parallel those of human milk. Weight gain decreases, and hypoalbuminemia and edema develop. The same symptoms occur in the short-bowel syndrome when losses of nutrients in the stools are too high to be compensated by increased intakes without digestive symptoms.

Other mechanisms may induce or contribute to malnutrition: (1) chronic inflammation, which has been shown, as in Crohn disease, to increase protein degradation and thus protein turnover, with consequent increased energy needs for rest metabolism[62]; (2) protein-losing enteropathy, which may exceed the capacity of the liver to synthesize albumin and which occurs not only in intestinal lymphangiectasia but also in celiac disease and Crohn disease; and (3) bacterial overgrowth, which results in energy losses (fermented carbohydrates, malabsorbed fat) and leads to diversion of nitrogen fluxes toward bacterial growth, with the ultimate consequences of hypoalbuminemia and loss of lean body mass. In all of these situations, protein malnutrition is responsible for low plasma levels of insulin-like growth factor I (IGF-I or somatomedin C), whereas growth hormone secretion is normal.[63] Thus, decreased plasma levels of IGF-I explain, at least in part, growth retardation during malnutrition, and IGF-I levels may be used as accurate markers of nutritional sufficiency.[63]

Finally, impaired absorption appears to be much more harmful than impaired digestion because in the vast majority of cases, mucosal lesions affect not only absorption of major nutrients but also of vitamins (lipid-soluble vitamins [A, D, E, K] and folic acid, when intestinal lesions are proximal, as in celiac disease; vitamin B_{12} when they are distal, as in ileal Crohn disease), of major minerals such as iron and Ca^{2+}, and of trace minerals. These deficiencies hasten and aggravate malnutrition, whereas their absence, in states of impaired pancreatic digestion, contributes to the relatively satisfactory nutritional tolerance of the latter conditions.

PRACTICAL APPROACH TO MALABSORPTION

The main clinical expression of malabsorption is diarrhea. It is the direct consequence of malabsorption, which, in its turn, when chronic, may result in malnutrition and failure to thrive, the usual other features of malabsorption syndromes. Because the consequences of malabsorption on growth have been dealt with elsewhere, this part of the chapter focuses on chronic diarrhea, or more properly on "abnormal stools,"[64] as the main clue to the diagnosis and etiology of malabsorption.

Chronic diarrhea is usually defined as diarrhea lasting for more than 14 days. In toddlers who already control their stools, abnormal stools are not missed by the family, which often tends to exaggerate their importance. On the contrary, when diarrhea starts from birth or soon after, the abnormal features of the stools are less easily recognized, especially if the infant is breastfed and is the first child in the family.

For many years, balance studies with recording of ingested foods and collection of the stools during at least 3 days have been extremely useful in ascertaining malabsorption and showing that chronic diarrhea had different biochemical features, explaining its gross macroscopic characteristics (consistence, volume, smell, pH), according to its pathophysiologic mechanism: impaired intraluminal digestion, intestinal malabsorption, or fermentation (Table 1-1). It is with this pathophysiologic classification in mind that the cause of chronic diarrhea can best be looked for.

A careful clinical history remains the most important step in getting to the diagnosis of malabsorption. The fluidity, number, size, color, and smell of the stools should first be ascertained. Stools may be as liquid as water and mistaken for urine in infants with congenital chloride diarrhea, for example[65]; be passed noisily with flatus, be loose and bulky; or be pasty and yellowish. The number of stools may vary from 2 (bulky) to 10 or more (small and liquid). Stools may be homogeneous or, on the contrary, may contain undigested pieces of vegetables and mucus. Whether the stools are greasy or not is often difficult to ascertain; the less liquid the stools are, the easier this is to determine. Liquid stools may be odorless, or they may have an acidic smell owing to fermentation. In exocrine pancreatic insufficiency, stools have a penetrating, cheesy odor. Finally, the mode of evolution of diarrhea should be recorded: stools may be abnormal every day or periodically.[64]

The necessary time, then, should be spent in trying to correlate the occurrence of diarrhea with modifications in the diet—introduction or elimination of cow's milk proteins, wheat flour, lactose, sucrose, and vegetables, for example. Associated symptoms should be systematically looked for: anorexia (intestinal malabsorption), increased appetite (exocrine pancreatic insufficiency), thirst (when diarrhea is fluid and severe as in sugar intolerance), abdominal pain, cramps, discomfort, bloating (indicative of fermentation), vomiting (protein intolerance), asthenia, and weakness (celiac disease, Crohn disease).

Equally important is to establish whether the growth of the child is normal or not by recording carefully his growth charts from birth for height and weight. Clinical examination will appreciate the activity and psychomotor development of the child. One should look for abdominal distention, which is best observed in the standing position in profile, taking into account the physiologic distention in toddlers, and for finger clubbing. It is important to evaluate the state of nutrition by recording skinfold thickness, muscle tone and volume, paleness of the skin and conjunctiva, color and quality of hair, and dryness of the skin.

At the end of this clinical evaluation, the most frequent cause of chronic diarrhea in childhood—"nonspecific chronic diarrhea" or "toddler's diarrhea" (characterized by periods of frequent, heterogeneous [with vegetable matter], often mucus-containing, foul-smelling stools, often alternating with periods of normal stools or even constipation, and a normal state of nutrition)—has been eliminated. Frequently, the clinical history, the growth chart, and the physical examination of the child allow evoking of one of the following three main mechanisms of malabsorption (Figure 1-1).

Diarrhea owing to Impaired Intraluminal Digestion. Diarrhea owing to exocrine pancreatic insufficiency, the predominant cause of impaired intraluminal digestion, is remarkable by the macroscopic appearance of the stools: they are more frequently loose and pasty than liquid, homogeneous, often obviously greasy with undigested triglycerides oozing like oil from the stool when it is passed in a pot or floating on the surface of the water in the toilet, and pale (hence the mastic aspect of the stools), with an offensive cheese smell. The volume of the stools is rather constant from one day to another. These features are well explained by the large amounts of fat, nitrogen, and volatile fatty acids they contain (see Table 1-1).[66] Fecal elastase 1 appeared recently to be a relatively simple yet sensitive and specific test of exocrine pancreatic function.[67,68]

Apart from acquired surgical conditions (short-bowel syndrome, stagnant loop), such a massive fecal loss of the three classes of nutrients can be explained only by exocrine pancreatic insufficiency, whose most frequent cause in children is, by far, cystic fibrosis.The sweat test

TABLE 1–1 RESULTS OF BALANCE STUDIES IN CHILDREN WITH CHRONIC DIARRHEA, ACCORDING TO PATHOPHYSIOLOGIC MECHANISMS

		STOOLS				
PATHOPHYSIOLOGIC MECHANISM	ETIOLOGY (*N*)*	VOLATILE FRESH WEIGHT (G/DAY)	NITROGEN (G/DAY)	FAT (G/DAY)	FATTY ACIDS (MMOL/DAY)	LACTIC ACID (MMOL/DAY)
Intraluminal digestion insufficiency	Cystic fibrosis	235 ± 36†	3.2 ± 0.5	24.9 ± 3.5 (42 ± 9)‡	42.3 ± 8.0	2.8 ± 5.1
	Shwachman syndrome (9)					
Intestinal malabsorption	Celiac disease < 3 yr (28)	118 ± 75	0.88 ± 0.27	6.7 ± 2.0 (75 ± 8)	16.6 ± 11.3	4.4 ± 5.8
	> 3 yr (22)	161 ± 88	1.48 ± 0.39	7.3 ± 3.2 (85 ± 7)	24.9 ± 11.5	2.1 ± 2.8
	CMPI (11)	118 ± 68	0.64 ± 0.26	4.7 ± 2.4 (76 ± 8)	15.1 ± 8.0	9.2 ± 2.1
Fermentation	CSID (13)	413 ± 175	0.95 ± 0.79	4.6 ± 2.3 (85 ± 7)	44.6 ± 17.9	30 ± 15.3

CMPI = cow's milk protein intolerance; CSID = congenital sucrase-isomaltase deficiency.
*N = number of children studied during 6-day balances.
†Mean ± 1 SD (personal data).
‡Fat absorption coefficient (%).

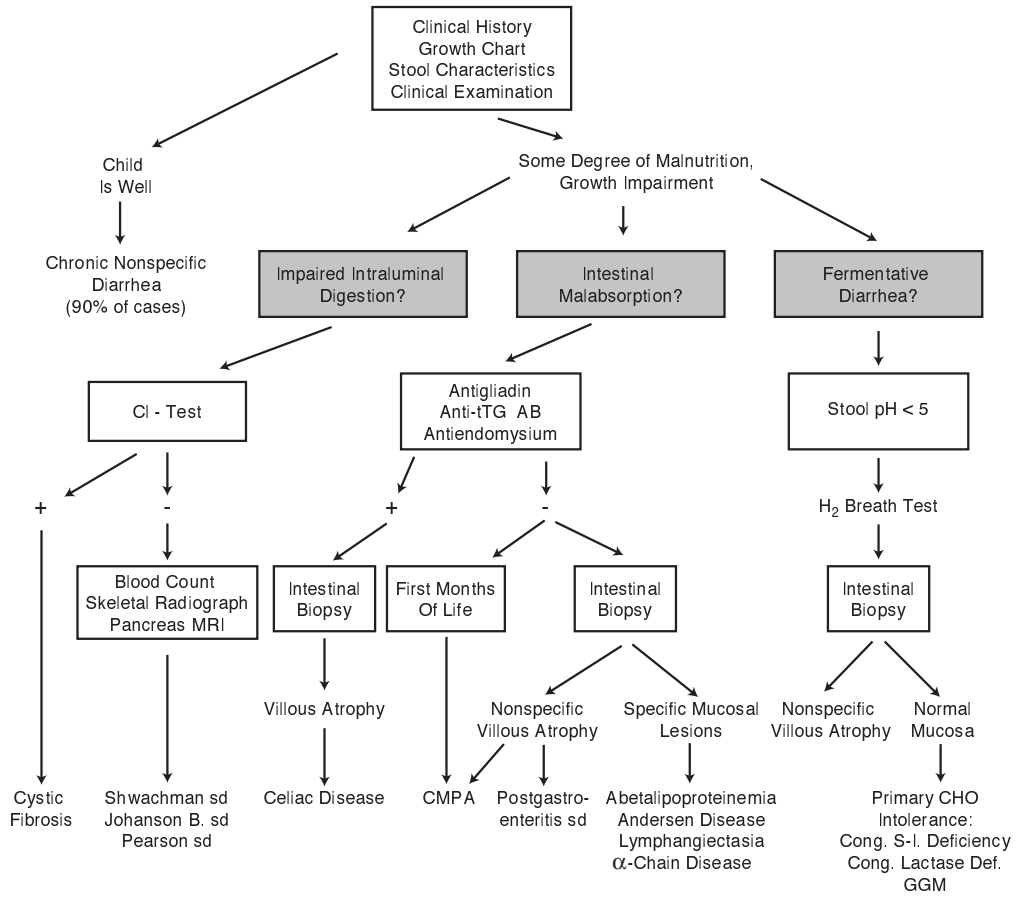

FIGURE 1-1 Diagnostic algorithm in a child with chronic diarrhea, suspect of malabsorption. CHO = carbohydrate; Cong. = congenital; CMPA = cow's milk protein allergy; Def = deficiency; GGM= glucose-galactose malabsorption; MRI = magnetic resonance imaging; sd = syndrome; S-I = sucrase-isomaltase; tTG = tissue transglutaminase.

confirms the diagnosis. If chloride concentration in sweat is normal, exocrine pancreatic insufficiency is due to hypoplasia of the pancreas. Hypoplasia may be associated with congenital lipomatous infiltration. Such is the case when exocrine pancreatic insufficiency is part of several syndromes, the most frequent of which is Shwachman syndrome. In this rather rare condition, exocrine pancreatic insufficiency is associated with chronic or cyclic neutropenia or other hematologic abnormalities, with metaphyseal chondrodysplasia, especially in ribs and hips, often with severe growth retardation.[69] Rarer is Johanson-Blizzard syndrome, in which exocrine pancreatic insufficiency is associated with morphologic abnormalities: congenital aplasia of the alae nasi, ectodermal scalp defects, and imperforate anus.[70,71] In both cases, magnetic resonance imaging may be useful in showing the typical fatty infiltration of the pancreas.[72] In Pearson syndrome, pancreatic hypoplasia is linked to fibrosis and exocrine pancreatic insufficiency is associated with refractory sideroblastic anemia and vacuolization of marrow precursors. This syndrome was recently shown to be due to mitochondrial deoxyribonucleic acid (DNA) deletions.[73] Finally, seldom in children is exocrine pancreatic insufficiency due to chronic pancreatitis. It has been described in the course of cystinosis.[74] In all cases, exocrine pancreatic insufficiency may be confirmed by direct assay of pancreatic enzyme activities in the duodenal juice (Table 1-2). Whereas the canalicular water and electrolyte secretion is impaired in cystic fibrosis, the lobular enzyme secretion is affected in cases of exocrine pancreatic insufficiency owing to lipomatosis.[75]

Impaired intraluminal digestion rarely involves only one class of nutrient. A massive isolated steatorrhea may be due to isolated congenital lipase or colipase deficiency, which can be confirmed by the direct assay of lipase activity in the duodenal juice.[47,48,76] In this situation, normal lipase activity would orient the diagnosis toward defective micellar solubilization owing to congenital absence of bile acid synthesis,[49,77] abnormal biliary excretion (congenital biliary atresia),[78] interrupted bile acid enterohepatic circulation because of bacterial overgrowth,[50] ileal resection, Crohn disease, or congenital bile acid malabsorption.[51] Bile acid assay in blood or the duodenal juice, or, less easily, in stools, may lead to one of these diagnostic possibilities. Isolated pancreatic proteolytic insufficiency has also been described; congenital trypsinogen deficiency has been reported, yet it is less well established than congenital enterokinase deficiency suspected in a malnourished infant, failing to thrive, with abnormal stools from birth.[31] Diagnosis is suspected when exogenous enterokinase restores a previously severely reduced or absent proteolytic activity in the duodenal juice;

TABLE 1–2 DIARRHEA OWING TO IMPAIRED INTRALUMINAL DIGESTION

PATHOPHYSIOLOGY	DIAGNOSIS	
	SUSPECTED	EVIDENCE FOR PROBABLE OR CERTAIN DIAGNOSIS
Impaired digestion affecting all nutrients	Cystic fibrosis	Sweat test positive
	Pancreatic hypoplasia with lipomatosis (Shwachman syndrome)	Neutropenia
	Metaphyseal chondrodysplasia	
	Johanson-Blizzard syndrome	Morphologic anomalies
	Fibrosis (Pearson syndrome)	Sideroblastic anemia
	Cystinosis	Tubular acidosis
Impaired digestion affecting fat proteins		
Fat	Isolated lipase or colipase deficiency	Direct assay in duodenal juice
	Abnormal micellar solubilization	
	Impaired bile acid synthesis	Bile acid assay in blood, duodenal juice, stools
	Bile duct atresia	Cholestasis
	Interrupted enterohepatic circulation	
	Ileal resection	Clinical history
	Crohn disease	Clinical history
	Congenital malabsorption of bile acids	Bile acid assay in blood, duodenal juice, stools
	Blind loop syndrome	Clinical history, H_2, breath test
Proteins	Congenital trypsinogen deficiency	Direct assay in duodenal juice
	Congenital enterokinase deficiency	Assay in duodenal mucosa

it is confirmed by the direct measurement of enterokinase activity in the duodenal mucosa (see Table 1-2).

Diarrhea owing to Intestinal Malabsorption. Diarrhea owing to intestinal malabsorption is loose or liquid, often with an acidic smell, typical of fermentation with production of acetic acid. The stools are rarely greasy (steatorrhea is usually mild).

Such a diarrhea in a child with abdominal distention and suboptimal growth should evoke celiac disease, the most common cause of intestinal malabsorption. Whereas tests of malabsorption (xylose test) or blood markers of malnutrition (low hemoglobin, folate, or cholesterol levels) have long been used to assess the jejunal absorption function before the necessary small intestinal biopsy, serologic markers of celiac disease (antigliadin, antiendomysium, antitransglutaminase antibodies) are now by far more reliable tests to perform before prescribing a biopsy of the jejunal mucosa. In this context, antiendomysium antibodies remain the most reliable test (nearly 100% sensitivity and specificity) of celiac disease.[79] The intestinal biopsy mandatory in such clinical situations discloses a flat mucosa (total villous atrophy) ascertaining the diagnosis, which, especially in younger children, may have to be confirmed several years later by relapse of the intestinal lesions after challenge with gluten.[80]

In cases in which the serologic tests specific for celiac disease are negative, the intestinal biopsy may be necessary to unravel the other causes of intestinal malabsorption. Nonspecific inflammatory alterations leading to partial villous atrophy in an infant of less than 6 months of age are most often secondary to sensitization to food proteins, most often to cow's milk proteins and more seldom to soy, rice, or wheat proteins. Given the lack of a reliable laboratory test to confirm such a sensitization, proof of it relies mainly on the curative effect of an exclusion diet and eventually on relapse of symptoms after challenge with the suspected protein.[81] Partial villous atrophy may also occur in the postgastroenteritis syndrome or in *Giardia lamblia* infestation; motile trophozoites may then be seen on histologic sections in the mucus layer covering the mucosa. Finally, partial villous atrophy and chronic diarrhea may reveal a state of immune activation[82] or of immunodeficiency: hypogammaglobulinemia or combined immunodeficiency syndromes, some of which may be linked to the absence of expression of human leukocyte antigens.[83] Measurements of Ig levels and of specific antibodies, counts of Ig-containing cells in the intestinal mucosa, and immunohistochemical or in situ hybridization studies should be performed in these situations as studies of delayed-type hypersensitivity. Although it is probable that intestinal lesions, in these situations, are linked to bacterial overgrowth,[60] the latter is often difficult to demonstrate, either indirectly by H_2 breath measurements after a glucose load[84] or directly by bacterial counts in the intestinal juice. A combination of several of these factors—food sensitization, depressed immune status, bacterial overgrowth—probably explains the partial villous atrophy often observed in children with protracted diarrhea (Table 1-3).

In other, much rarer, cases, the intestinal biopsy reveals specific lesions. The mucosal architecture is normal, but enterocytes appear full of lipid droplets that reflect abnormal chylomicron assembly or excretion. Such a disorder may reveal abetalipoproteinemia, which will be quickly confirmed by the finding of acanthocytosis, extremely low plasma cholesterol levels, absence of low-density lipoprotein and apolipoprotein B from the plasma, loss of tendon reflexes, and, eventually, retinitis[85]; the same intestinal lesions may also be the main finding in Andersen disease (or chylomicron retention disease), the clinical features of which are similar to those of celiac disease.[86,87] In plasma, cholesterol levels are low and apolipoprotein B is present, although at a low level.

TABLE 1–3 DIARRHEA OWING TO INTESTINAL MALABSORPTION

PATHOPHYSIOLOGY	DIAGNOSIS: SUSPECTED	DIAGNOSIS: EVIDENCE FOR PROBABLE OR CERTAIN DIAGNOSIS
IIntestinal biopsy: nonspecific inflammatory lesions		
Total villous atrophy (flat mucosa)	Celiac disease	Antigliadin antibodies, relapse at gluten challenge
Partial villous atrophy*	Sensitization to food proteins: CMP, rice, soya, wheat	Relapse at challenge
	Dermatitis herpetiformis	Dermal IgA deposit
	Giardia lamblia infestation	*Giardia* on biopsy specimen
	Immunodeficiency status, among these absence of HLA expression	HLA typing
	Bacterial overgrowth	Bacterial counts in duodenal juice, H_2 test
	"Protracted diarrhea" syndrome (may be postgastroenteritis)	Clinical history
Intestinal biopsy: specific lesions		
Fat-filled enterocytes	Abetalipoproteinemia	Absence of plasma LDL, apolipoprotein B; acanthocytosis
	Andersen disease	Decreased levels of plasma LDL, apolipoprotein B
Villi distorted by ectatic lymphatics	Lymphangiectasia	Lymphopenia, hypoalbuminemia, increased α_1 PI clearance
Dense monomorphic lymphoplasmocytic infiltrate	α-Chain disease	Monoclonal abnormal IgA in plasma
Normal intestinal biopsy	Lysinuric protein intolerance	Dibasic aminoaciduria Severe osteoporosis

CMP = cow's milk protein; HLA = human leukocyte antigen; Ig = immunoglobulin; LDL = low-density lipoprotein; PI = pancreatic insufficiency.
*In severe cases, villous atrophy may be subtotal.

In other cases, lymphangiectasia distorts the shape of villi. Lymphopenia, hypoalbuminemia with eventual edema, and hypogammaglobulinemia are usually associated with a modest steatorrhea. Measurement of α_1-antiprotease clearance confirms the loss of lymph in the digestive tract.[54] Finally, the lamina propria of the intestinal mucosa may be densely infiltrated by a monomorphic lymphoplasmocytic population, composed of packed plasma cells or, later, of lymphoblasts, which disrupts crypts and widens and flattens villi, whose epithelium is barely altered. In such cases, α-chain disease should be suspected. The presence of an abnormal monoclonal IgA in plasma confirms the diagnosis (see Table 1-3).[88] In rare cases, plasma cells produce polyclonal IgA.[89]

Diarrhea owing to Fermentation. Diarrhea owing to fermentation is liquid often passed with flatus and acidic, with a pH less than 5.5 owing to the abnormal presence of lactic acid in addition to the usual volatile fatty acids. Such an acidic pH is extremely evocative of fermentation owing to carbohydrate intolerance and must lead to a systematic search for reducing substances in the stools.[12] The stool volume is variable and roughly proportional to the amount of malabsorbed carbohydrate that has been ingested (see Table 1-1).

Although overexcretion of H_2 in breath after an oral load of a suspected sugar may orient the diagnosis, the most useful investigation, here also, is an intestinal biopsy. The intestinal mucosa appears normal on histologic sections in cases of congenital (or primary) sugar intolerance. The assay of disaccharidase activities in a homogenate of the mucosa detects sucrase-isomaltase deficiency,[90,91] the most frequent of these intolerances, more often than glucoamylase deficiency[92] or congenital lactase deficiency,[93] which has been ascertained principally in Finland.[93] In nonwhite children or adolescents, late-onset lactase deficiency[94] is, on the contrary, frequently the cause of a mild lactose intolerance. Normal enzyme activities, as well as clinical trials with different sugars, lead to the possibility of congenital glucose-galactose malabsorption, which can be proven by studies in an Ussing chamber (glucose does not trigger any short-circuit current, as it should) with brush border vesicles (glucose is not taken up even in the presence of Na^+),[95] eventually using molecular genetics (Table 1-4).[6]

However, much more frequently, the intestinal mucosa looks abnormal, with more or less severe villous atrophy. Disaccharidase activities are, like peptidase activities, nonspecifically decreased as a consequence of mucosal damage. Sugar intolerance and fermentation are, then, secondary to villous atrophy, as in celiac disease.[96] In the latter condition, secondary sugar intolerance is probably the main factor responsible for the volume of stools (see Table 1-4).[97]

Diarrhea Starting in the Neonatal Period. Chronic diarrhea starting in the hours or days following birth is usually extremely severe, leading in a few weeks to life-threatening malnutrition. The features of a malabsorption syndrome are thus gathered, although in some of the conditions characterized by such a diarrhea, malabsorption may involve only one ion. The age at which these conditions are discovered—the neonatal period—the often abundant and watery character of the stools, the severity of the dehydration and of malnutrition resulting from diarrhea, and the fact that these conditions are all familial justify their grouping independently from their pathophysiology (Table 1-5). Keeping these conditions in mind

TABLE 1–4 DIARRHEA OWING TO FERMENTATION

PATHOPHYSIOLOGY	DIAGNOSIS: SUSPECTED	DIAGNOSIS: CONDITIONS OF PROBABLE OR CERTAIN DIAGNOSIS
Intestinal biopsy: normal or subnormal intestinal mucosa	CSID	Assay of saccharidases in mucosal homogenate: *one* activity affected
	Congenital lactase deficiency	
	Late-onset lactase deficiency	
	Congenital trehalase deficiency[98]	
	Congenital glucose-galactose malabsorption	Absence of glucose-induced short-circuit current in Ussing chamber
Intestinal biopsy: nonspecific inflammatory lesions	All causes of villous atrophy (cf Table 1–3), mainly	*All* saccharidase activities affected
	Celiac disease	Cf Table 1–3
	CMPI	Cf Table 1–3
	Postgastroenteritis syndrome	Cf Table 1–3

CMPI = cow's milk protein intolerance; CSID = congenital sucrose-isomaltase deficiency.

TABLE 1–5 CHRONIC DIARRHEA STARTING IN THE NEONATAL* PERIOD

CONDITION (IN ORDER OF DECREASING SEVERITY)	DISTINCTIVE CLINICAL FEATURES	KEY LABORATORY INVESTIGATION	THERAPEUTIC DECISION
Congenital microvillous atrophy[99]	Intractable† watery diarrhea	Intestinal biopsy (PAS stain)	Total parenteral nutrition
Tufting enteropathy[100,101]	Intractable watery diarrhea	Intestinal biopsy	Total parenteral nutrition
Intractable diarrhea with phenotypic abnormalities[102]	Intractable watery diarrhea Low birth weight	Immune system investigations	Total parenteral nutrition
Congenital glucose-galactose malabsorption	Acid diarrhea	Intestinal biopsy (Ussing chamber, brush border vesicles)	Replacement of glucose and galactose by fructose in the diet
Congenital lactase deficiency	Acid diarrhea	Intestinal biopsy (assay of activity)	Lactose-free diet
Congenital chloride diarrhea	Hydramnios, intractable watery diarrhea	Assay of electrolytes in stools	IV then oral Cl supplementation
Congenital defective jejunal Na^+/H^+ exchange[103]	Hydramnios, intractable watery diarrhea	Assay of electrolytes in stools	IV then oral Na^+ supplementation
Congenital bile acid malabsorption	Steatorrhea	Bile acid assay in plasma, stools	MCT, cholestyramine
Congenital enterokinase deficiency	Failure to thrive, edema	Intestinal biopsy (assay of kinase activity)	Protein hydrolysate

Cl = chloride; IV = intravenous; PAS = periodic acid–Schiff; MCT = medium-chain triglyceride.
*Neonatal = within the first week of life.
†Intractable = persisting despite nothing by mouth.

in the presence of any newborn presenting with a persistent diarrhea should improve the quality of the care offered to these neonates at risk.

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CHAPTER 2

MICROBIAL INTERACTIONS WITH GUT EPITHELIUM

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Increasingly, it is recognized that there is crosstalk between microbes and the environment in which they reside.[1] For pathogens causing human disease, this communication generally takes place first with epithelial cells lining mucosal surfaces. Study of the mechanisms underlying interactions between microbes and host epithelia has considered the gut as an excellent model system to test and delineate the responses of gut epithelial cells and M cells overlying Peyer patches to agents contained in the lumen. This review focuses on recent evidence elucidating the interaction of both bacterial commensals and enteropathogens with gut epithelia.

Although less work has been undertaken with viruses, protozoa, and helminths, it appears from evidence in initial studies that the general principles underlying host interactions with parasites are frequently shared features, even if the specific microbial gene products mediating the observed effects may differ. Understanding these common mechanisms, employed by agents coming into contact with the mammalian host, has implications for the design of new antimicrobial drugs, the use of prebiotics and probiotics in maintaining human health and treating human illnesses, and the development of vaccines to employ in the prevention of disease.

Study of the mechanisms underlying interactions between microbial products and the gut epithelium also provides insight into normal gut function. For instance, identification of guanylate cyclase C as the apical receptor on enterocytes to which a heat-stable enterotoxin is elaborated by some strains of enterotoxigenic *Escherichia coli* led to the search and subsequent discovery of an endogenous ligand. The protein, referred to as guanylin, changes levels of intracellular cyclic guanosine monophosphate, resulting in modulation of ion and water fluxes in the human intestine. In addition, guanylin may influence epithelial cell proliferation.[2]

The role of microbes, including organisms classically considered as commensals, in the pathogenesis of chronic inflammatory bowel diseases has been emphasized in animal models.[3] Knockout of genes involved in host immunity (such as interleukin-2, interleukin-10, and T-cell receptor expression) in mice causes relapsing and chronic inflammation involving the intestinal tract. However, gut inflammation does not occur in germ-free animals. Furthermore, *CARD15/NOD2*, a putative intracellular pattern recognition receptor for the bacterial product peptidoglycan, has been identified as the first susceptibility gene for Crohn disease in humans.[4,5] These findings emphasize the role of microbes or their products in modulating host responses. Such changes also may play a role in illnesses affecting the immature and developing host, for instance, in the pathogenesis of necrotizing enterocolitis and allergic gastroenteropathies. Thus, when one considers that the majority of cells that reside in the human gastrointestinal tract are, in fact, bacterial cells, understanding the diverse interactions between microbes and epithelial cells is highly relevant to clinicians because it provides insight into both physiology and pathophysiology.

VERSATILITY OF MICROBES

Increasingly, it is recognized that microbial pathogens frequently employ more than one virulence determinant to result in human disease.[6] This principle has important ramifications when considering the development of intervention strategies, including, for instance, the design of effective vaccine constructs.

For example, the development of attenuated *Vibrio cholerae* strains for use as oral vaccines to prevent epidemics in developing nations and to intervene in the eighth worldwide pandemic of cholera focused on deleting the gene encoding choleragen. In human volunteer studies, it became apparent that the *V. cholerae* mutant deficient in the production of cholera enterotoxin still caused diarrhea, albeit at much lower volumes than the wild-type parent strain producing cholera toxin. These observations led to the discovery of additional open reading frames in the bacterial genome adjacent to genes coding for choleragen (*ctxA*, *ctxB)* that result in the production of proteins with effects on the gut epithelium.[7] These proteins have been called zona occludins toxin (ZOT) for their effect on the intercellular tight junctions and accessory cholera enterotoxin (ACE). The level of complexity of the effects of this organism on gut epithelial responses is further emphasized by the observation that *V. cholerae* deficient in *CTXA*,

CTXB, *ZOT*, and *ACE* genes still causes diarrhea in human volunteers challenged orally with the isogenic mutant.[8]

Such evidence suggests the possibility of additional enterotoxins yet to be discovered. Other bacterial factors, such as lipopolysaccharide and outer membrane proteins (ie, components of the bacterial cell wall), may well participate in interactions with the host epithelium. These findings also indicate that vaccine constructs developed in consideration of a single bacterial virulence factor are likely to have diarrhea as a side effect. Alternatively, vaccines designed for future use in humans could require the use of more attenuated bacterial strains containing interruptions or deletions of multiple genes.

DELIVERY OF BACTERIAL ENTEROTOXINS

Noninvasive bacteria cause diarrhea in humans by the delivery of toxins that can effect fluid homeostasis in the gut. Cytotonic enterotoxins are characterized by massive volumes of nonbloody diarrhea without affecting the architectural integrity of the villus crypt axis in the small bowel. Many of these toxins, such as those elaborated by *V. cholerae*, enterotoxigenic *E. coli*, enterotoxigenic *Bacteroides fragilis*, and *Clostridium difficile*, are multimeric.[9] Such toxins are composed of a binding, or B, subunit and an active, or A, subunit that cause ion and water secretion by effecting second messengers in enterocytes lining the small intestine.

For instance, as outlined schematically in Figure 2-1, cholera toxin binds to the ganglioside GM_1 via a pentamer of B subunits and delivers the holotoxin by retrograde transport to the endoplasmic reticulum. The A subunit then dissociates from the B subunit and translocates to the cytosol, where it exerts its effects by irreversible activation of cyclic adenosine monophosphate and protein kinase A.[10] The net effect of these changes in intracellular second messengers is the opening of the cystic fibrosis transmembrane regulator (CFTR) chloride channel on the apical plasma membrane of enterocytes.

In contrast to the previously held notion that the plasma membrane is homogeneous, it is now clear that this membrane is heterogeneous, with specialized domains referred to as either cholesterol-enriched microdomains or lipid rafts. Lipid rafts are discrete domains in the plasma membrane that are enriched in cholesterol, glycosphingolipids, and glycosylphosphatidylinositol-anchored proteins.[11] Lipid rafts function in many aspects of cellular metabolism, including signal transduction.[12] Recent evidence indicates that binding of cholera toxin (CT) to GM_1 associates the toxin with lipid rafts. Alteration of lipid raft structure and function by cholesterol depletion alters the endocytosis, trafficking, and cytotoxicity of CT, indicating that cholesterol may function to associate CT with lipid raft domains.[11] Thus, lipid rafts are employed by the toxin to gain entry into the cell and thereby cause disease. Furthermore, a growing list of pathogens induces signal transduction responses through binding to and activation of constituents of lipid rafts. Accordingly, these microdomains are potential therapeutic targets that could be used to interrupt the infectious process.

The outline of events summarized in Figure 2-1 is very likely to be an oversimplification of events occurring in the in vivo setting. Many of the studies detailing the series of cellular responses to bacterial enterotoxins were undertaken employing reductionist models. Toxins purified from bacterial enteropathogens are incubated with epithelial cells of intestinal origin, grown in tissue culture or mucosal epithelium obtained from animals but stripped of the underlying muscle, nerves, and immune cells before use in in vitro studies. More recent studies employing intact gut epithelium indicate important differences from those identified previously.[13] For example, the effects of cholera enterotoxin on gut epithelial cells can be indirect, mediated by the activation of enterochromaffin cells and via secondary effects of the released secretagogue 5-hydroxytryptamine on enteric neurons and their neurotransmitters.[14]

It is also clear that in addition to choleragen,[10] other toxins elaborated by enteric bacteria, such as Shiga tox-

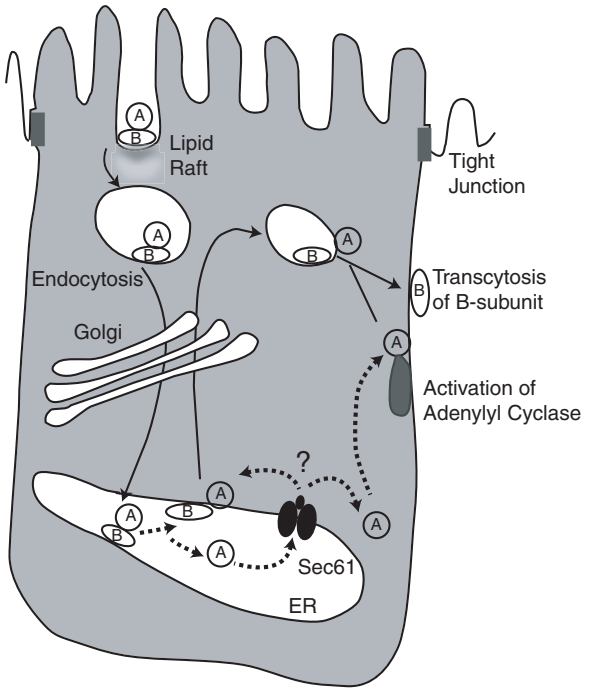

FIGURE 2-1 Working model for trafficking of cholera toxin (CT) into polarized cells. The CT holotoxin binds to ganglioside GM_1 in the apical membrane. After endocytosis, the CT-GM_1 complex trafficks retrograde through Golgi cisternae into the lumen of the endoplasmic reticulum (ER), where the A1 peptide is unfolded and dissociated from the B pentamer. The unfolded A1 peptide is probably dislocated to the cytosol through the sec61p complex. The A1 peptide may then gain access to adenosine diphosphate–ribosylate, its substrate, the heterotrimeric guanosine triphosphatase Gsα on the cytoplasmic surface of the basolateral membrane, by diffusion through the cytosol (if the A1 peptide breaks away from the membrane after translocation) or by membrane traffic back out the secretory pathway (if the A1 peptide remains membrane associated). The B subunit is not unfolded in the ER, remains membrane associated (presumably bound to GM_1), and moves to the basolateral membrane by trafficking back out the secretory pathway in anterograde vesicles in a process we have termed indirect transcytosis. Reproduced with permission from Lencer WI.[10]

ins,[15] can be transported intact across the cytosol of polarized gut epithelial cells (Figure 2-2). Paracellular delivery of the bacterial toxins across intact polarized gut epithelium through intercellular tight junctions appears to be less likely, even when there is enhanced transepithelial permeability to macromolecules of molecular mass smaller than that of the holotoxins.[15]

Intact toxin can be presented as antigen on the basolateral membrane of enterocytes. Delivery of holotoxin to other body organs where they may have systemic effects is a consequence of these series of events. This possibility still requires direct experimental confirmation because it could explain the systemic effects of orally ingested bacterial pathogens, including, for example, the renal and neurologic sequelae of infection with enterohemorrhagic (Shiga toxin producing) *E. coli*.

PATHOGEN-ASSOCIATED MOLECULAR PATTERNS

Innate immunity to bacterial pathogens is triggered by the binding of conserved structures, termed pathogen-associated molecular patterns (PAMPs), to specialized host cell receptors known as pattern recognition receptors.[16,17] Toll-like receptors (TLRs) are responsible for extracellular recognition of a variety of PAMPs, including lipopolysaccharide (TLR-4), peptidoglycan (TLR-2), lipoproteins, flagellin (TLR-5), double-stranded ribonucleic acid (RNA) (TLR-7), and CpG deoxyribonucleic acid (DNA) (TLR-9). These receptors trigger immune responses by stimulating secretion of proinflammatory cytokines mediated by activation of transcription factors such as nuclear factor (NF)-κB. Recently, members of a new group of cytoplasmic proteins, called CARD4 (Nod1 for nucleotide-binding oligomerization domain) and CARD15 (Nod2), that sense and respond to bacterial products within the cell have been identified.[18] With the discovery that mutations in CARD15 (Nod2) are associated with Crohn disease,[4,5] much interest has focused on understanding the role of CARD/Nod proteins in mediating gut epithelial interactions with bacteria and bacterial products.

Recent studies indicate that CARD4/Nod1 and CARD15/Nod2 recognize distinct bacterial peptidoglycan molecular motifs, resulting in the activation of transcription factors such as NF-κB.[19] In addition, current experimental evidence suggests that CARD4/Nod1 is solely responsible for recognition of these motifs in epithelial cells in steady-state condi-

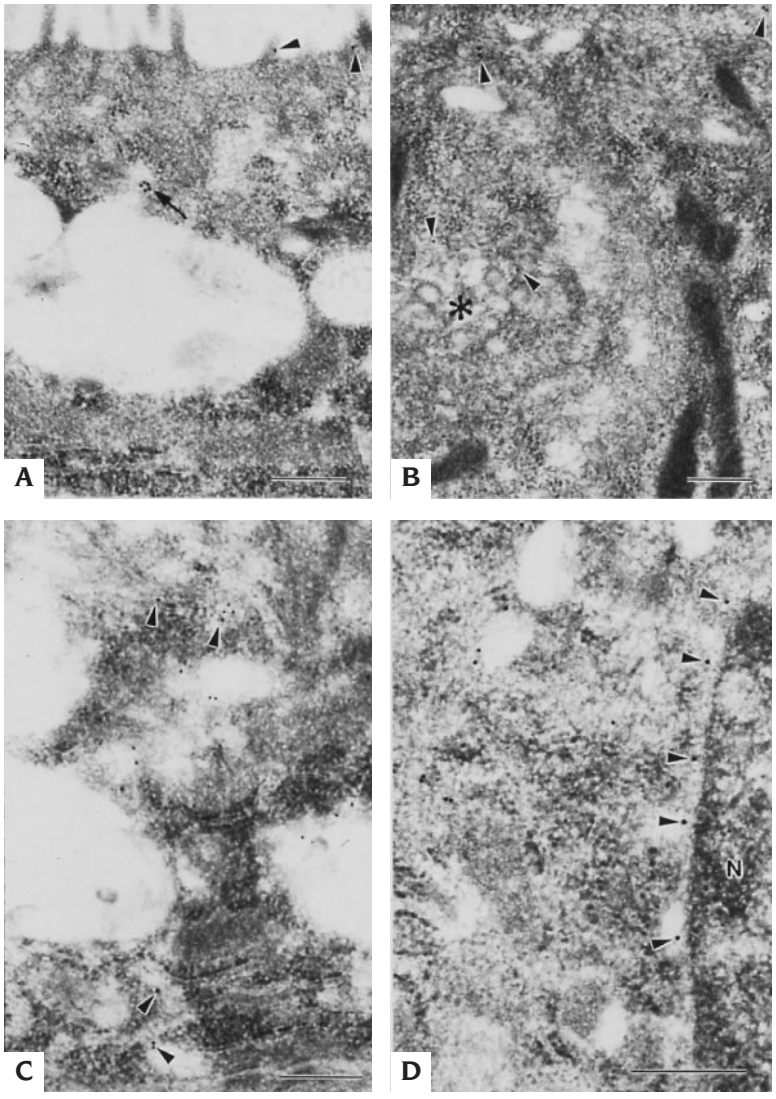

FIGURE 2-2 Intracellular trafficking of bacterial toxins. Transmission electron photomicrographs of immunogold-labeled Shiga toxin-1 present within T84 cells. Bars = 250 nm. *A*, The apical surface of Shiga toxin–treated T84 cells shows the immunogold label present at the plasma membrane of the cell (*arrowhead*), as well as in vesicles (*arrows*). *B*, An en face view of the Golgi apparatus (*asterisk*) in a Shiga toxin–treated T84 cell demonstrating gold particles (*arrowheads*) contained in Golgi-derived vesicles. *C*, Immunogold labeling of the endoplasmic reticulum and terminal cisternae (*arrowheads*) in a cell incubated in the presence of Shiga toxin. *D*, Cytoplasmic face of the nuclear envelope in a Shiga toxin–treated T84 cell showing gold labeling (*arrowheads*). Reproduced from Philpott DJ et al.[15]

tions, whereas CARD15/Nod2 appears to be nonfunctional. However, exposure to inflammatory cytokines results in the up-regulation of CARD15/Nod2 expression in intestinal epithelial cells.[20,21] Furthermore, intestinal cell lines engineered to express mutant CARD15/Nod2 display deficient clearance of an intracellular pathogen.[20] Taken together, these results suggest that CARD4/Nod1 and CARD15/Nod2 serve complementary roles in the host detection and response to intracellular bacterial pathogens.

Based on current understanding of the function of CARD15/Nod2, several hypotheses have been proposed to explain the molecular defect in Crohn disease. One attractive theory is that deficient CARD15/Nod2 in intestinal epithelial cells results in an abnormal NF-κB–mediated chemokine response to microbial products, thereby allowing the proliferation of bacteria and disruption of mucosal barrier function.[3] Alternatively, defective CARD15/Nod2 could result in persistent infection of intracellular pathogens owing to lack of their recognition. An additional hypothesis includes abnormal conditioning of antigen-presenting cells and resulting failure in the appropriate development of cellular immune responses.

TYPE III SECRETION SYSTEM AND PATHOGENICITY ISLANDS

Recent experimental evidence shows that multiple bacterial pathogens employ a specialized secretion system to deliver virulence determinants into the infected host.[22] Referred to as type III secretion, to distinguish it from previously identified secretion systems in prokaryotes (Figure 2-3), the protein effectors exported from bacteria are characterized by the absence of an obvious signature signal sequence. Many of these effectors are not expressed except when in the microenvironment exposed to host epithelia. In response to the specific environment (eg, bile salts, ambient pH, oxygen tension, and host cell markers), the bacterium turns on the expression of a secretory apparatus and produces proteins that are injected via the secretion system directly into the cytoplasm of the host cell.[23] In contrast to a marked heterogeneity in the sequence and structure of the injected effector proteins, the structural apparatus is more preserved. This has led Anderson and colleagues to propose that these conserved components of the secretory system recognize common signals that couple messenger RNA translation to the secretion of peptides.[24]

Role of Pathogenicity Islands in Transmission of Type III Secretion Systems

In pathogenic bacteria, these type III secretion systems frequently are encoded on a pathogenicity island. The term pathogenicity island refers to large pieces of DNA (often up to 40 kb) containing multiple open reading frames that can be characterized as distinct from the rest of the bacterial genome.[25] For instance, the guanine plus cytosine (G + C) content is usually lower than that found in the remainder of the bacterial genome. There is usually evidence of transmissibility of the large piece of DNA; for instance, features characteristic of transposable elements (referred to as transposases) may be present at one or both ends of the pathogenicity island. It is of interest that pathogenicity islands are not randomly inserted into the bacterial genome.[26] Rather, there are hot spots into which the pathogenicity islands are inserted. Frequently, these are at sites coding for transfer RNAs (Figure 2-4). In fact, the pathogenicity island encoding virulence determinants for uropathogenic *E. coli* is at precisely the same location (82 minutes) on the genome where the pathogenicity islands of enteropathogenic *E. coli* and some strains of enterohemorrhagic *E. coli* insert into selenocysteine transfer RNA.

Type III Secretion as a Method of Bacterial Delivery of Virulence Determinants

In enteropathogenic *E. coli*, host consequences of infection occur in response to products encoded on genes contained on a single pathogenicity island referred to as the locus for enterocyte effacement (LEE).[27] As shown in Figure 2-5, LEE codes a type III secretion system for the delivery of bacterial effectors into the host cell. Some genes code for structural proteins, such as EspA and EspcF, that form the needle or channel of the molecular syringe required to deliver proteins into the host epithelia (Figure 2-6).[28] Other proteins serve as chaperones that facilitate transport of effector proteins. In enteropathogenic *E. coli*, CesT is a 15 kD chaperone protein.[29] The CesT promotes the transfer of secreted proteins by binding to the aminoterminus, thereby forming a stable multimeric protein complex that is resistant to degradation.[30] Still other proteins serve as pores to permit the transfer of large proteins through the cell wall of the bacterial pathogen. Proteins EspB and EspD are encoded on the LEE of enteropathogenic *E. coli* and enterohemorrhagic *E. coli* that display homology to known pore-forming molecules.[31] An additional level of complexity is provided by the control of gene expression in the LEE of enteropathogenic *E. coli* by regulatory genes that are contained on a large extrachromosomal plasmid.[32]

Intact effector proteins are then injected through the molecular syringe into the cytosol of the eukaryotic cell to which the organism has adhered. In enteropathogenic *E. coli*, one of these injected proteins, translocated intimin receptor (Tir), is phosphorylated at the tyrosine residue.[33] Following integration into the host-cell plasma membrane, Tir functions as a receptor for intimate binding of the bacterium mediated by an outer membrane protein referred to as intimin. Intimin is itself encoded by a gene, *eae*, which is also present on the LEE. These recent studies provide the first evidence of a bacterium providing its own receptor for mediating attachment to the host.[33,34]

Moreover, the host signaling machinery is "hijacked" to modify the bacterial protein (ie, tyrosine phosphorylation). Deletion of genes contained in the LEE renders enteropathogenic *E. coli* unable to induce rearrangements of the cytoskeleton characteristic of the attaching and effacing lesion observed in infected epithelial cells. Conversely, transfer of the LEE locus from enteropathogenic *E. coli* into a nonvirulent laboratory *E. coli* strain is sufficient to result in all of the morphologic features of the attaching and effacing lesions in infected host epithelia.[35]

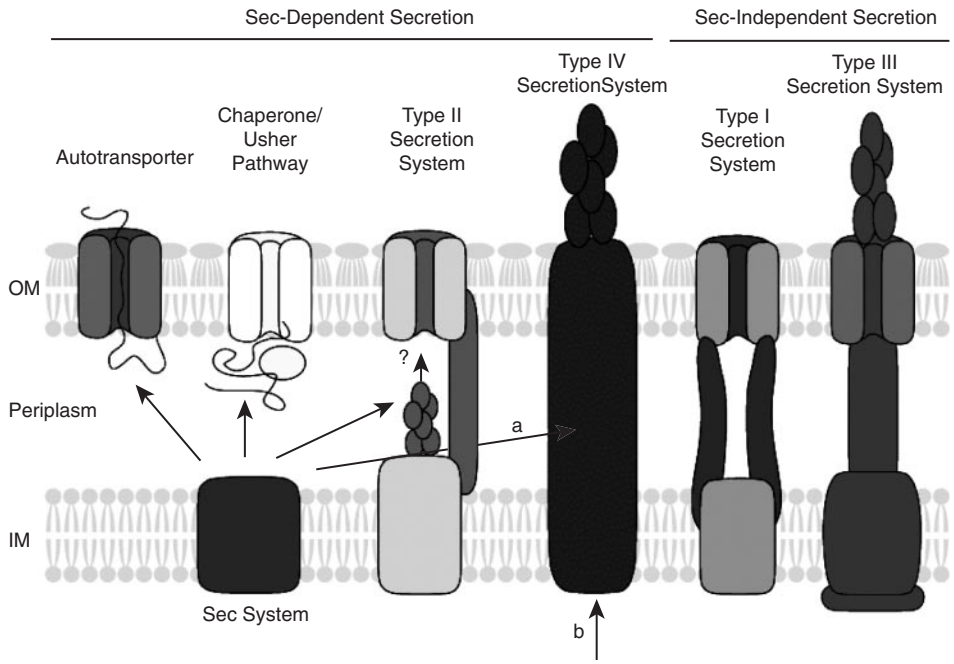

FIGURE 2-3 Protein secretion systems in gram-negative bacteria. Among the six major protein-secretion pathways of gram-negative bacteria, four depend on the Sec system for protein transport across the inner membrane (IM). Autotransporters (also known as type V secretion systems) mediate the transport of a passenger domain across the outer membrane (OM). Secretion by the chaperone/usher pathway requires a chaperone and an OM protein, termed an usher, and is dedicated to the transport of pilus subunits, which are assembled at the bacterial surface. The more complex type II secretion systems, which mediate transport of extracellular enzymes and toxins, involve 12 to 16 proteins, most of which are associated with the IM. Four IM proteins are proposed to form a pilus-like structure that could act as a piston to push proteins through the OM pore (*arrow*). Type IV secretion systems transport a variety of substrates, some of which, for example, pertussis toxin, require the Sec system for secretion (*a*), whereas others, such as the T-DNA-protein complexes of *Argobacterium tumefaciens*, are exported directly from the cytosol (*b*). The type I and type III secretion pathways are Sec independent. Type I systems secrete toxins, proteases, lipases, and S-layer proteins into the extracellular milieu, whereas type III secretion systems also mediate delivery of virulence proteins into the host cell. Extracellular appendages are associated with several type III and type IV systems. Reproduced from Buttner D and Bonas U.[23]

Enterohemorrhagic *E. coli* also contains the LEE pathogenicity island with most of the same genes, including *eae* encoding the outer membrane protein intimin, the bridging protein EspA, and the *espB* and *espD* genes coding for proteins injected into the infected host cell.[27,28] At least three sites of insertion of the pathogenicity island into the bacterial genome have been described using various clinical isolates. Another distinct feature of enterohemorrhagic *E. coli* is that the homologue to the intimin receptor EspE does not contain tyrosine residues. Thus, tyrosine phosphorylation is not a feature of host responses to infection by Shiga toxin–producing *E. coli*.[36,37] Moreover, tyrosine phosphorylation appears not to be an absolute requirement for the sequence of events leading to attaching and effacing lesion formation and to diarrhea.[38] In fact, recent evidence indicates that the outer membrane protein intimin not only binds to the bacteria-derived proteins that are injected into the host cell but also interacts with additional receptors present in the plasma membrane such as nucleolin that are of eukaryotic origin.[39]

Current experimental evidence indicates that the pathogenesis of infection by enterohemorrhagic *E. coli* differs from enteropathogenic *E. coli*.[40,41] The transfer of the LEE from *E. coli* O157:H7 does not confer the attaching and effacing phenotype to an avirulent, laboratory bacterial strain.[42] Thus, enteropathogenic *E. coli* and enterohemorrhagic *E. coli* use different mechanisms to trigger actin polymerization.[28] Enteropathogenic *E. coli* Tir recruits the adapter protein Nck to sites of actin assembly.[43,44] This recruitment is essential for pedestal formation. In contrast, enterohemorrhagic *E. coli* can form actin pedestals independent of Nck. Furthermore, the disruption of host signaling pathways by enteropathogenic and enterohemorrhagic *E. coli* appears to differ. For example, enterohemorrhagic *E. coli*, but not enteropathogenic *E. coli*, disrupts signal transducer and activator of transcription 1–mediated interferon-γ signaling in epithelial cells in vitro.[45]

In summary, pathogens that express these sophisticated secretion systems can alter many host cell functions, including signal transduction, cytokine production, and cytoskeletal structure. Furthermore, the components of the type III secretion apparatus tend to be conserved, leading to the suggestion that these specialized organelles may be attractive targets for the development of novel antimicrobial agents.

Horizontal Transmission of Pathogenicity Islands

The origin of virulence cassettes contained in the genome of pathogenic bacteria, including *Yersinia*, *Shigella*, and

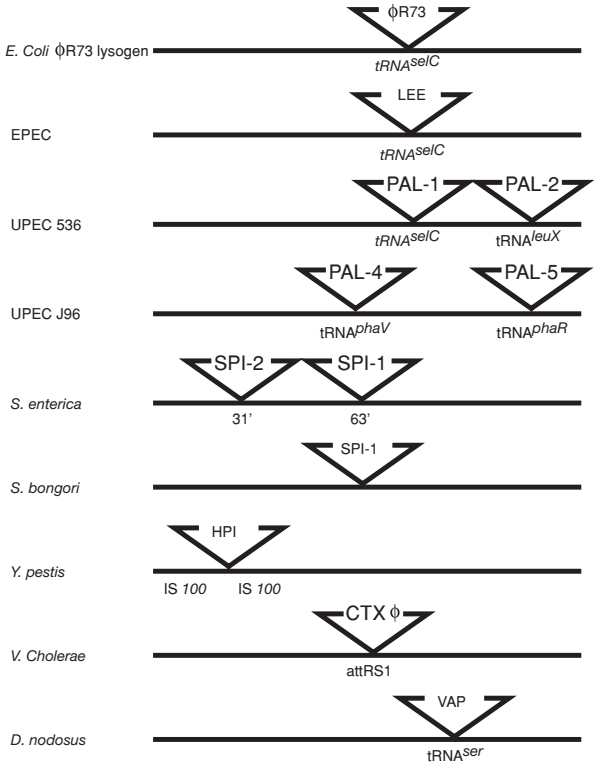

FIGURE 2-4 Location of selected pathogenicity islands and phages of gram-negative bacteria. Chromosomes and pathogenicity islands are depicted as the solid horizontal lines and triangles, respectively (but are not drawn to scale). The site of insertion is depicted below the pathogenicity island. Reproduced with permission from Groisman EA and Ochman H.[26] EPEC = enteropathogenic *Escherichia coli*; UPEC = uropathogenic *E. coli*.

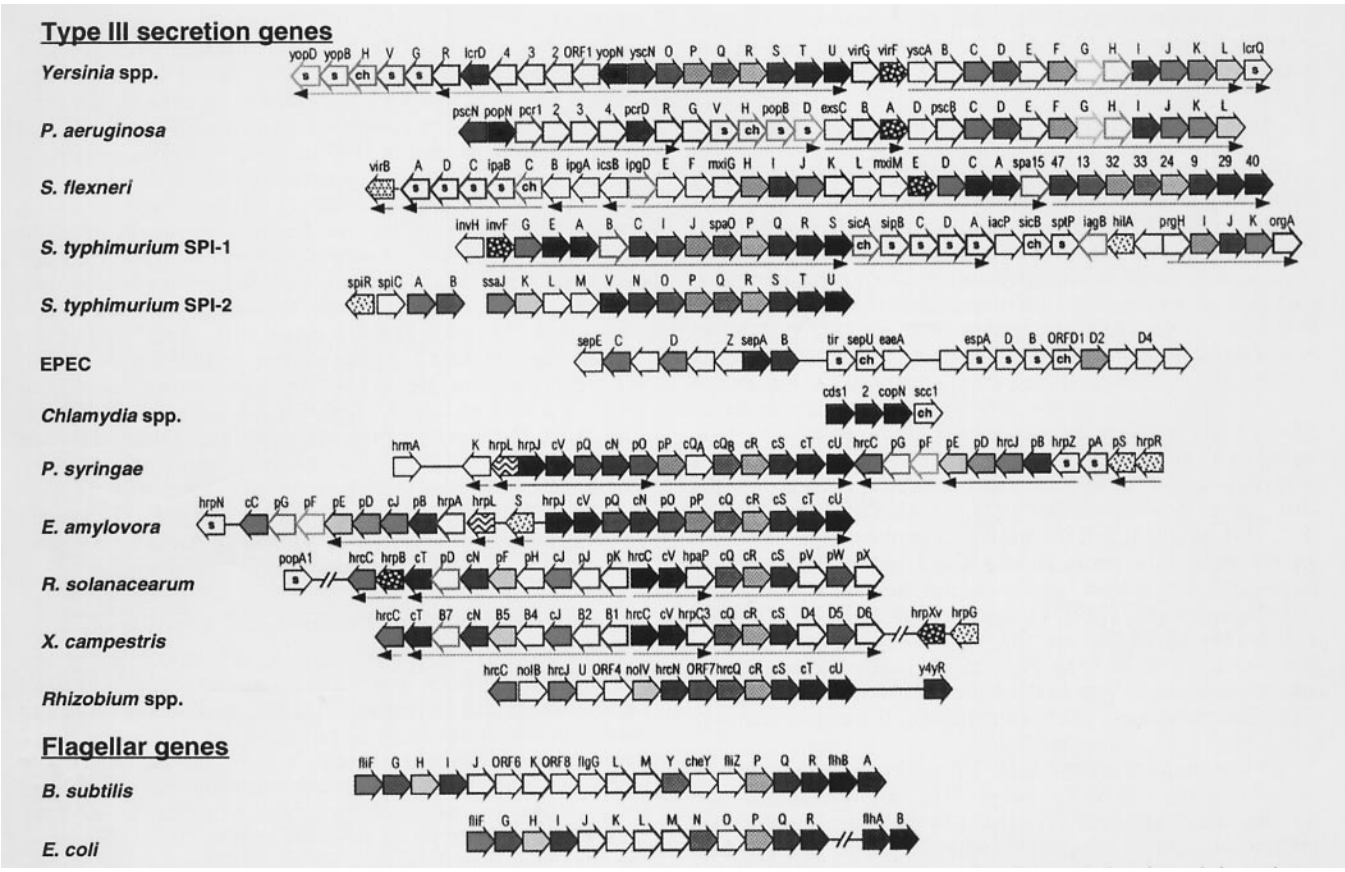

FIGURE 2-5 Genetic organization of the type III secretion system and of flagellum biosynthesis proteins. The type III secretion systems of animal and plant pathogens are grouped according to genetic similarities. Homologies of encoded proteins are indicated by the color code (see CD-ROM). Reproduced with permission from Hueck CJ. Type III protein secretion systems in bacterial pathogens of animals and plants. Microbiol Mol Biol Rev 1998;62:379–433. EPEC = enteropathogenic *Escherichia coli*

Salmonella species and pathogenic *E. coli*, remains uncertain. The natural transformation of enteric bacteria and their ability to take up and integrate foreign DNA[46] raise serious concerns about the safety of attenuated bacterial strains for use in humans as oral vaccine candidates. There is the potential that these strains, either in the complex environment of the microflora of the human large intestine or when excreted into environmental reservoirs, might acquire pathogenicity islands containing virulence genes from other bacteria in their immediate environment.

There is evidence that such concerns are more than theoretic. In the past decade, the first non-O1 *Vibrio* to cause epidemic cholera was identified in the Indian subcontinent. The *V. cholerae* strain is new, having acquired changes in the *rfb* gene cluster encoding a distinct bacterial polysaccharide, and is referred to as O139.[47] *V. cholerae* O139 appears to have acquired from *V. cholerae* O1, in the aquatic environment serving as a reservoir for the organism, the pathogenicity island containing genes encoding both toxin production and attachment factors to receptors in human small intestinal epithelium.[48] Evidence that the transmissible element is a bacteriophage[49] raises additional anxieties about the acquisition of virulence determinants by nonpathogenic organisms present in the environment, thereby transforming these microbes into pathogens capable of causing disease both in humans and in animals.[50]

Campylobacter jejuni is reported to secrete proteins that are injected into tissue culture epithelial cells even though the organism does not contain any genes with homology to those known to code for type III secretion proteins.[51] In addition, *C. jejuni* does not contain regions of G + C content lower than the rest of the bacterial genome, indicative of a pathogenicity island.[52] How then might the bacterium secrete proteins without signal sequences? It has been proposed that flagella may serve as an alternate structure for use in the delivery of bacterial proteins into the cytosol of infected epithelial cells. The biosynthesis of the motor apparatus of the flagellum is well characterized and appears to have many features in common with those described for the type III secretion system encoded on pathogenicity islands.[53] Indeed, it has been proposed that the genes encoding bacterial flagellum and those of the type III secretion system may well share a common ancestor.[53]

Loss of bacterial DNA is another potential mechanism for enhancing the virulence of microbes. In fact, recent evidence suggests that deletion of genomic DNA promotes the virulence of *Shigella* species and enteroinvasive *E. coli*.[54] How frequently such an event accounts for the emergence of new pathogens or promotes the virulence of known microbial enteropathogens requires clarification.

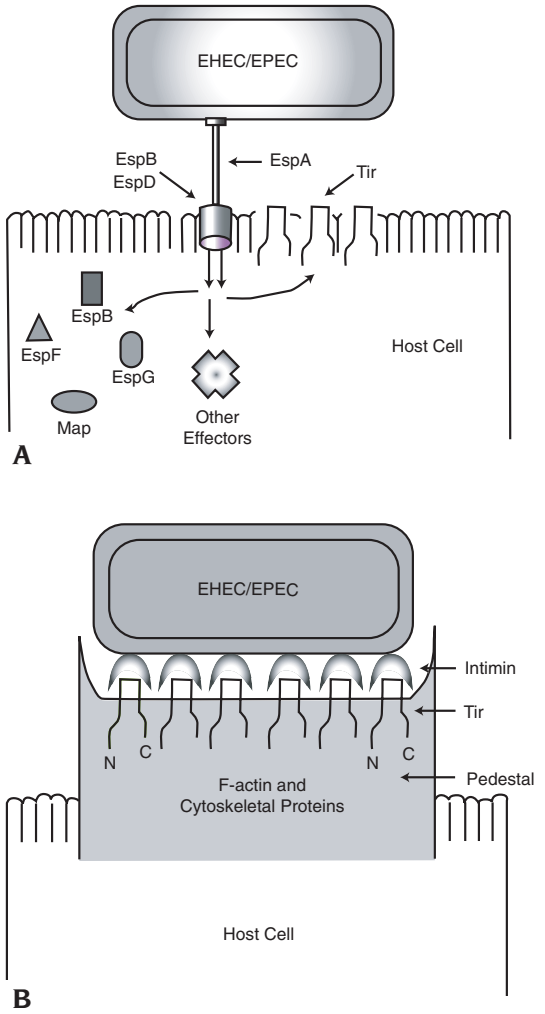

FIGURE 2-6 Model of translocation of bacterial effectors into host cells. *A*, Upon contact, enteropathogenic *Escherichia coli* (EPEC) and enterhemorrhagic *E. coli* (EHEC) use a type III translocation apparatus to inject bacterial effector proteins into mammalian cells. These bacteria translocate a number of proteins: EspB and EspD, which form a translocon in the plasma membrane; the cytoplasmic proteins EspF, EspG, and Map (there is also a cytoplasmic pool of EspB); the translocated intimin receptor Tir, which inserts into the plasma membrane; and other unidentified effectors. *B*, Membrane-localized Tir contains a central extracellular domain that binds to the bacterial outer membrane protein intimin and amino- and carboxyterminal cytoplasmic domains that interact with cytoskeletal elements. The interaction between Tir and intimin is the final bacterial signal to trigger the assembly of actin into pedestals within host cells. Reproduced with permission from Campellone KG and Leong JM.[28]

REARRANGEMENT OF THE HOST CYTOSKELETON

Changes in F-Actin following Microbial Infection

Both invasive and noninvasive enteric bacterial pathogens have developed mechanisms to disrupt and hijack the host cytoskeleton in a manner that enhances survival and transmission of microbes in the host.[55] Invasive pathogens such as *Listeria monocytogenes*, *Salmonella* species, and *Shigella* species have dramatic effects on the host cytoskeleton.[55,56]

Increasingly recognized as a foodborne pathogen causing intestinal disease, as well as severe systemic complications,[57] *L. monocytogenes* has developed unique mechanisms to invade into the host (Figure 2-7). During the course of infection, the organism produces a protein product, Act A, which recruits F-actin from the host cytosol to form a tail at one end, which then serves to propel the bacterium forward during intercellular spread.[58] The Arp 2/3 protein

complex, capping protein, vasodilator-stimulated phosphoprotein (VASP), profilin, α-actinin, and likely cofilin, but not myosin, are the host proteins that interact with actin filaments in response to the bacterial infection.[59] These proteins provide the driving force for moving *L. monocytogenes* in the cytosol and between epithelial cells.[60]

Other invasive bacteria employ host second messengers, such as Rac, Rho, and Cdc42, to effect changes in the host cytoskeleton to facilitate their own uptake by nonphagocytic cells, including intestinal epithelial cells.[61] For example, expression of mutant Cdc42 inhibits the internalization of *Salmonella typhimurium* into eukaryotic cells.[62] Through a type III secretion system, *S. typhimurium* injects effector proteins, referred to as SopE and SptP, that have opposing effects on the actin cytoskeleton via their actions on Cdc42 and Rac-1.[63] In contrast, the interaction of enteropathogenic *E. coli* with host cells does not involve these second messengers.[64] Such heterogeneity provides experimental evidence supporting the concept that different enteric pathogens have adapted a variety of strategies to interact with the cytoskeleton of host epithelial cells.

Classically noninvasive bacteria can also reorganize the host cytoskeleton. Attaching and effacing bacteria contain the locus for enterocyte effacement (including enteropathogenic *E. coli*, enterohemorrhagic *E. coli*, some strains of *Hafnia alvei*, and *Citrobacter rodentium*). Infection with these bacteria results in the recruitment of F-actin to the region of the plasma membrane below adherent bacteria where the integrity of the normal apical microvillus membrane has been disrupted. As shown in Figure 2-8, use of the mushroom-derived toxin phalloidin, which binds specifically to polymerized actin when conjugated to fluorescein, has been used to detect attaching and effacing lesions developing in response to bacterial infection. The rearranged F-actin contains additional cytoskeletal elements, including the actin bridging protein α-actinin, talin, ezrin, and myosin light chain, which is phosphorylated at serine and threonine residues.[28]

Precisely how the changes in cell morphology cause diarrhea is not known. The loss of microvillus membrane surface area is a theoretic possibility, but it is unlikely to be a predominant feature.[65] In most infections, attaching and effacing lesions are patchy in nature, with only a subset of enterocytes demonstrating morphologic changes. Moreover, when tested experimentally, brush border hydrolase activities are not markedly reduced.

Impact of Bacterial Infection on Intercellular Tight Junctions

The effects of the altered cytoskeleton on the integrity of intercellular tight junctions are another potential explanation that could account for passive transport of ions and water into the gut lumen.[44] Tight junctions seal the space between cells to limit the paracellular diffusion of solutes. Several groups have shown that the infection with attaching and effacing enteropathogenic *E. coli* alters phosphorylation of myosin light chain, ezrin, and occludin[66] and disrupts the morphologic integrity of the intercellular tight junction by causing dissociation of proteins such as zona occludens-1.[67] Such ultrastructural changes are accompanied by physiologic responses to infection, including a drop in transepithelial electrical resistance (Figure 2-9),[68] and increases in intercellular uptake of luminal antigens indicative of enhanced gut permeability (Figure 2-10).[69]

Another result of a disruption in the integrity of the epithelial cell monolayer overlying the gut surface could be the exposure of previously protected and unexposed host antigens and potential receptor-binding sites. For example, it is evident that microbes can attach to extracellular matrix proteins, such as fibronectin and vitronectin, and to sulfated polysaccharides, including heparin, and other glycosaminoglycans in vitro.[70] These findings now need to be extended to the in vivo setting, both in experimental animals and in humans, to clarify the biologic significance of these observations.

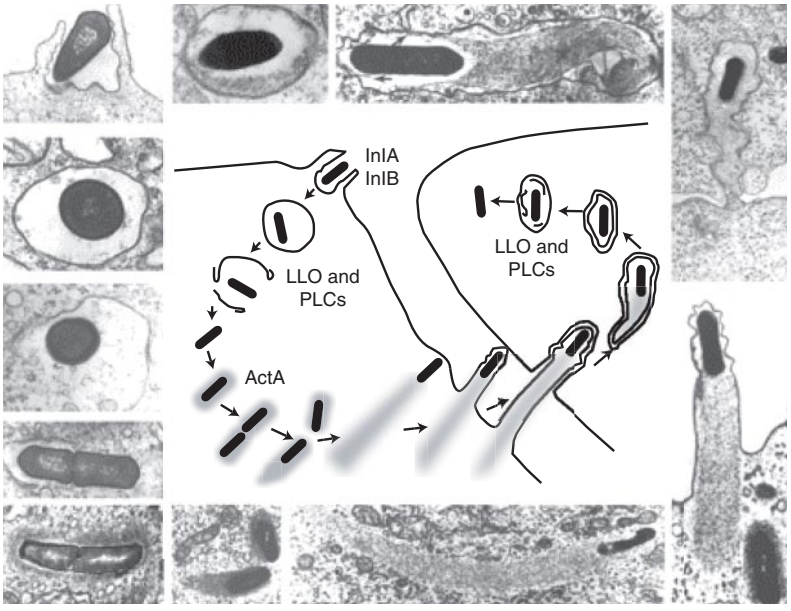

FIGURE 2-7 Stages in the intracellular life cycle of *Listeria monocytogenes*. *Center*, Cartoon depicting entry, escape from a vacuole, actin nucleation, actin-based motility, and cell-to-cell spread. *Outside*, Representative electron micrographs from which the cartoon was derived. The cartoon and micrographs were adapted from Tilney LG, Portnoy DA. Actin filaments and the growth, movement, and spread of the intracellular bacterial parasite, *Listeria monocytogenes*. J Cell Biol 1989; 109:1597–1608. InlA = internalin A; InlB = internalin B; LLO = listerio-lysin O; PLCs = phospholipases C.

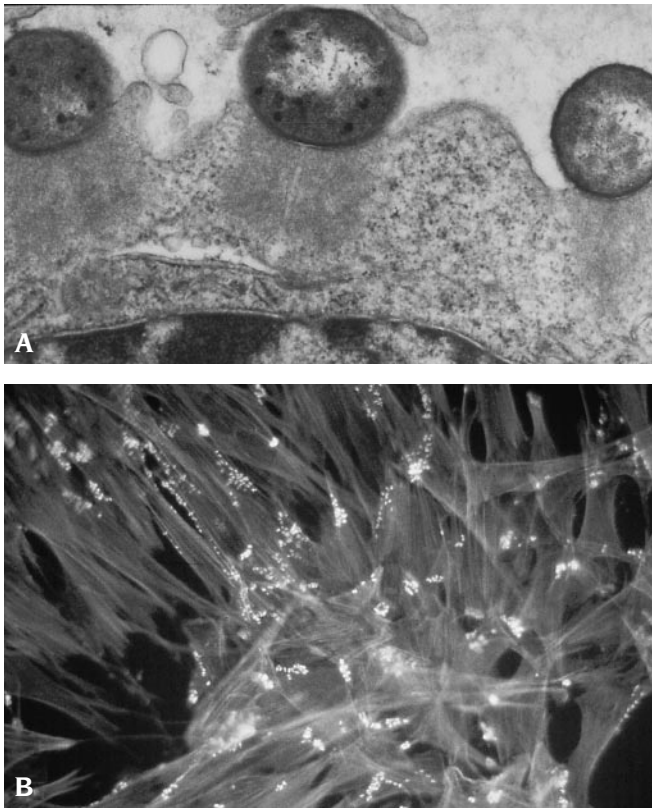

FIGURE 2-8 Attaching and effacing lesions in eukaryotic cells infected with attaching and effacing *Escherichia coli* for 6 hours at 37°C. *A*, Transmission electron photomicrograph showing three attaching and effacing lesions on tissue culture cells infected with enteropathogenic *E. coli*, strain E2348/69 (serotype O127:H6). There is loss of the apical microvillus membrane and intimate binding of bacteria to the plasma membrane of the eukaryotic cell. Beneath the bacteria are foci of electron-dense material (F-actin) contained in the cytoplasm of infected cells and the formation of cup-like pedestals to which the microbes have adhered (approximately ×30,000 original magnification). *B*, Fluorescent actin staining (FAS) assay showing fluorescein-labeled phalloidin binding to foci of F-actin accumulating in regions of tissue culture cells to which Shiga toxin–producing *E. coli*, strain Cl-56 (serotype O157:H7), have adhered (approximately ×100 original magnification). Reproduced from Philpott DJ, Sherman PM. Signal transduction responses in eukaryotic cells following verocytotoxin-producing *Escherichia coli* infection. Germs Ideas 1995;1:15–21.

Role of Cytosolic Free Calcium

Elevation in intracellular levels of calcium by incubation with calcium ionophores disrupts the apical microvillus membrane architecture of enterocytes and results in the formation of vesicles and membrane blebs. Changes in levels of calcium subjacent to the apical membrane serve as a second messenger mediating altered cytoskeleton following infection with enteric bacteria. Several groups have shown that increases in cytosolic free calcium, released from intracellular stores via activation of the inositol trisphosphate receptor, occur in response to infection with attaching and effacing bacteria, including both enteropathogenic *E. coli* and enterohemorrhagic *E. coli* (O157:H7). Inhibitors that block release of calcium from intracellular stores, or chelate intracellular calcium, disrupt the ability of these pathogenic bacteria to induce attaching and effacing lesions in tissue culture epithelial cells.[71]

Enteropathogenic *E. coli* infection also induces activation of protein kinase C in infected epithelial cells, with transfer of active isoforms from the cytosol to the plasma membrane.[72] These findings suggest that bacterial infection activates phospholipase C and the production of diacyl glycerol, but direct experimental proof has yet to be provided.

Potential Therapeutic Implications

If confirmed in in vivo experiments, these findings should have important therapeutic applications. For instance, DuPont and colleagues employed a calcium-calmodulin inhibitor, called zaldaride maleate, in human volunteers vis-

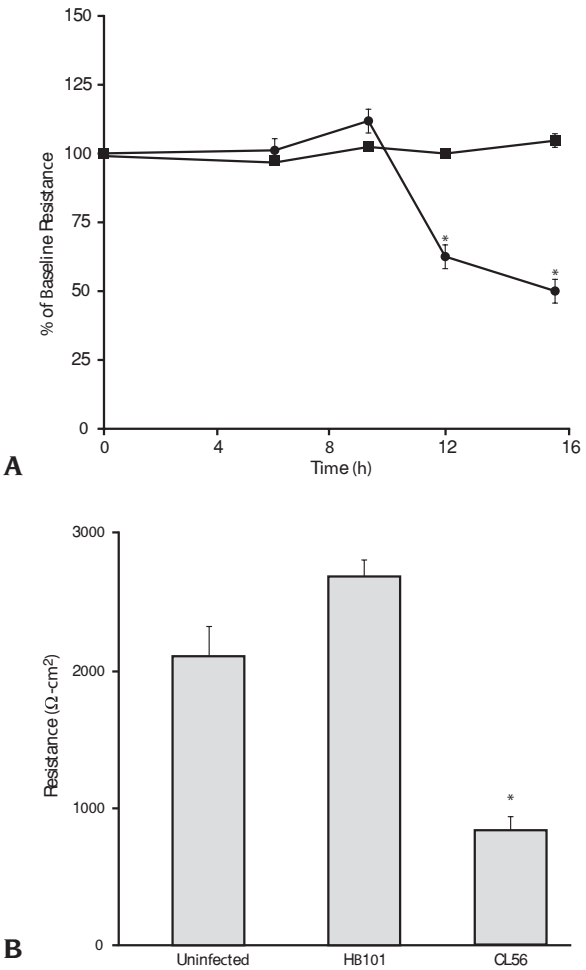

FIGURE 2-9 Transepithelial electrical resistance of T84 cell polarized monolayers following *Escherichia coli* infection. *A*, Time course of enterohemorrhagic *E. coli*–induced resistance decreases in T84 cells. In comparison with that of the uninfected cells (*solid squares*), the monolayer resistance of cells infected with bacteria (*solid circles*) first shows a decrease 12 hours after infection. Monolayer resistance was maximally decreased 15 hours postinfection. *B*, Monolayer resistance of uninfected T84 cells and cells infected for 15 hours with a nonadherent control *E. coli* strain (HB101) or a Shiga toxin–producing enterohemorrhagic *E. coli* (strain CL56). The pathogenic bacterium induced a significant decrease in monolayer resistance compared with that of both the uninfected and HB101-infected epithelial cells. Reproduced with permission from Philpott DJ et al.[67]

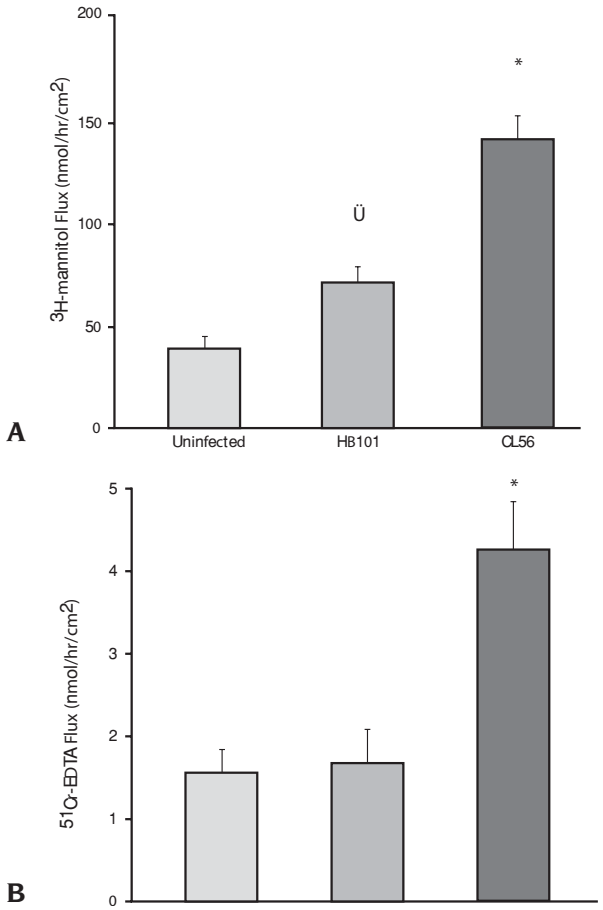

FIGURE 2-10 Permeability of polarized T84 cell monolayers as assessed by the transepithelial flux of radiolabeled probes. *A*, Flux of tritiated mannitol across uninfected T84 cell monolayers and monolayers infected for 15 hours with either the control *Escherichia coli* strain HB101 or enterohemorrhagic *E. coli* strain CL56 (serotype O157:H7). Infection with Shiga toxin–producing CL56 leads to a significant increase in the flux of radiolabeled mannitol. *B*, ^{51}Cr-EDTA flux across uninfected and *E. coli*–infected monolayers. Following 15 hours of infection with Shiga toxin–producing *E. coli* O157:H7 strain CL-56, the permeability of T84 monolayers to the radiolabeled probe increases compared with that of both uninfected and HB101-infected polarized epithelial cells. Reproduced with permission from Philpott DJ et al.[67]

iting Mexico.[73] As shown in Table 2-1, compared with the young adults receiving placebo, those taking the inhibitor demonstrated a reduction in both the severity and duration of diarrhea caused by enterotoxigenic *E. coli*, known also to elevate levels of cytosolic free calcium in the experimental setting. These findings need to be extended to an evaluation of efficacy following infection with other enteropathogens because other investigators describe attaching and effacing lesions occurring in response to enteropathogenic *E. coli* infection in the absence of detectable changes in cytosolic free calcium.[74] Thus, even though the use of selective and nontoxic inhibitors of second messengers holds promise as novel therapeutic agents, further evaluation is clearly required. The pleiotrophic effects of pathogenic bacteria on host epithelia may impair the therapeutic efficacy of highly selective inhibitors of signal transduction cascades.

Much of the experimental evidence cited in this review is from work undertaken in the in vitro setting. It is increasingly apparent that bacterial gene expression can vary widely depending on the environment in which they reside.[75] For example, a number of outer membranes are expressed in vivo in animals that are not identified when *V. cholerae* is cultured in the laboratory setting, either in broth culture or on agar plates. In contrast, other membrane proteins that are expressed in vitro are turned off when the organism resides in the gut lumen. New technologies, such as in vivo expression technology and signature tagged mutagenesis, have been devised to help to determine the biologic relevance of putative bacterial virulence factors.[76] Future studies using these complementary tools in appropriate experimental animals likely will provide data that are relevant to the human condition.

A variety of factors, including levels of iron, manganese, and other micronutrients, as well as ambient temperature, oxygen tension, pH, and the presence of bile salts, can each impact on gene expression in prokaryotes.[77] Whether or not a microbe is growing in the liquid phase or on a solid surface, as part of a biofilm, will also have profound effects on gene expression.[78]

QUORUM SENSING

Many gram-negative bacteria employ a regulatory system where the signal is produced by the organism itself.[79] Referred to as quorum sensing, the tightly controlled regulatory system permits the microbe to respond to the environment in which they reside. For example, *Pseudomonas aeruginosa* produces diffusible molecule *N*-acylated homoserine lactones, via the enzyme LuxI, in amounts that are proportional to the number of bacteria present in the immediate vicinity.[80] At a critical concentration, a second enzyme, LuxR, is activated and binds to regulatory DNA. Recently, a unique autoinducer termed AI-2, encoded by the *luxS* gene, has been identified.[79] AI-2 is widespread in the bacterial kingdom and is considered a universal signal because it can be used for interspecies cell-to-cell communication. AI-2 regulates a variety of functions, including expression of virulence factors in *V. cholerae*,[81] enteropathogenic *E. coli*, and enterohemorrhagic *E. coli*.[82] The crystal structure of AI-2 has been determined and found to contain a boron atom, which is interesting because the biologic functions of boron remain largely unknown.[83] Although it is not known how the diverse intestinal flora is established and maintained, communication between microbes of different species through quorum sensing could well play a role.

NUCLEAR TRANSCRIPTION RESPONSES IN INFECTED EPITHELIAL CELLS

Recent evidence points to the epithelial cell as an integral part of the host immune response to infection. For instance, epithelial cells produce chemokines and cytokines in response to a variety of bacterial pathogens to promote an influx of leukocytes, macrophages, T cells, and plasma cells to the site of infection.[84]

TABLE 2-1 IMPROVEMENT IN DIARRHEA DURING THE FIRST 24 HOURS OF THERAPY WITH ZALDARIDE MALEATE OR A PLACEBO FOUR TIMES DAILY AND FAILURE OF CLINICAL CURE 24 HOURS AFTER THERAPY*

	PLACEBO *n* (%)	ZM, 5 MG *n* (%)	ZM, 10 MG *n* (%)	ZM, 20 MG *n* (%)	*p* VALUE
Diarrhea improved in first 24 h	20/41 (49)	17/38 (45)	19/43 (44)	32/42 (76)	.013
Failure of cure after 24 h of therapy	5/39 (13)	6/36 (17)	0/38 (0)	0/40 (0)	.026

Zm = zaldaride maleate.
*Adapted from Dupont HL et al.[73]

As shown in Figure 2-11, enterocytes produce the polymorphonuclear leukocyte chemoattractant interleukin-8 in response to enteropathogenic *E. coli* and enterohemorrhagic *E. coli* O157:H7 infection.[71] Transcription of the chemokine occurs in response to the activation of NF-κB.[85] Activation of NF-κB is regulated by inhibitory proteins termed IκBs, which maintain NF-κB in an inactive state within the cytosol. Phosphorylation of IκB by IκB kinases leads to ubiquitination and degradation of IκB, resulting in release of NF-κB in the cytosol of infected epithelial cells. NF-κB can then translocate to the nucleus, where it binds to the promotor region of DNA upstream of the gene encoding interleukin-8.[85] Although originally considered to be a host response to invasive pathogens, it is now clear that polarized epithelial cells mount a vigorous chemokine response to the adhesion of pathogenic organisms to apical surfaces.[86,87]

Activation of NF-κB may also indirectly impact on ion and water secretion in response to bacterial infection through the activation of neuropeptide receptors. For instance, Hecht and colleagues have shown that enterohemorrhagic *E. coli* infection up-regulates NF-κB–dependent galanin-1 receptor expression in mouse intestine.[88] Short circuit current responsiveness to the neuropeptide galanin (Isc serving as an electrical marker of ion secretion in epithelia mounted into Ussing chambers) is also enhanced in the large intestine of mice challenged with Shiga toxin–producing *E. coli*. Human T84 cells also express the galanin-1 receptor, which, when activated by infection with enteropathogenic *E. coli* and enterohemorrhagic *E. coli*, enhances chloride secretion through a calcium-dependent mechanism.[89]

In addition to the conventional view that pathogens promote mucosal inflammation, recent evidence indicates that other bacteria are capable of limiting inflammatory responses. For example, infection with certain avirulent *Salmonella* strains abrogates the ability of proinflammatory strains and cytokines to activate NF-κB and thereby induce secretion of interleukin-8 from intestinal epithelial cells. This effect is mediated by the inhibition of ubiquitination and degradation of phosphorylated IκB. [90] Therefore, both pathogenic and nonpathogenic organisms can modulate host defenses.

THE ROLE OF COMMENSAL BACTERIA AS MEDIATORS OF GUT HOMEOSTASIS

It is clear that the complex interaction between the intestine and its microflora is dynamic and modulates gastrointestinal physiology.[1] For example, indigenous bacteria modulate the development of intestinal villus microvasculature by signaling through epithelial Paneth cells.[91] The small intestines of adult germ-free mice have arrested capillary network formation, which can be restarted following colonization either with microbiota from conventionally raised animals or with a predominant member of the murine gut microflora *Bacteroides thetaiotaomicron*. Furthermore, comparisons of mice lacking Paneth cells demonstrate that this lineage is responsible for the regulation of angiogenesis. In addition to regulating angiogenesis, recent evidence indicates that the gut microflora controls resident bacterial populations by modulating the induction of endogenous microbicidal proteins by epithelial cells. For instance, Hooper and colleagues demonstrated that intestinal colonization of germ-free mice with *B. thetaiotaomicron* stimulates Paneth cells to produce angiogenin 4.[92] Angiogen 4 displays a species-specific antimicrobial action against pathogens, but not commensals, thereby identifying this protein as a new class of antimicrobials.

Several groups have begun to sequence the genomes of members of the resident gut flora to provide additional insights into the complex interplay between the commensal flora and gut epithelia.[93,94] For example, the genome sequence of the mouse commensal *B. thetaiotaomicron* con-

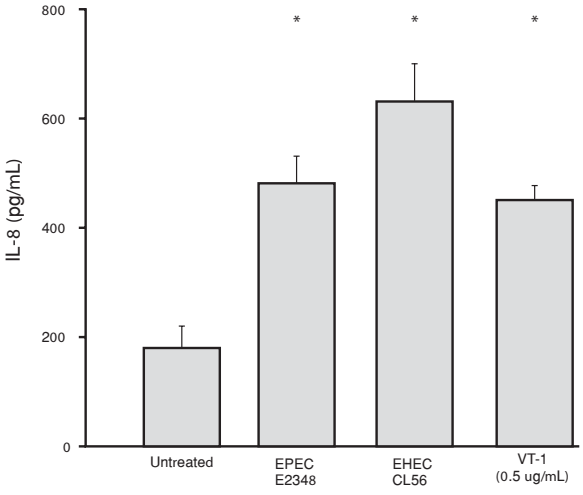

FIGURE 2-11 Nuclear transcription response in epithelial cells infected with attaching and effacing bacteria. Interleukin (IL)-8 production in T84 cells infected for 18 hours at 37°C with enteropathogenic *Escherichia coli* (EPEC) strain E2348/69, Shiga toxin–producing *E. coli* (STEC) strain CL56, or treated with 0.5 µg of Shiga toxin (Stx-1). Increases in the levels of the proinflammatory chemokine are greater than those observed in uninfected host cells. Reproduced with permission from Ismaili A et al.[71]

tains a large number of utilization pathways for breaking down undigestible carbohydrates, which can then be used as nutrients by both the bacterium and the host.[93]

SUMMARY

In the past several years, there have been great advances made in our understanding of the complex interplay between the commensal microflora and pathogenic microbes and the environment in which they reside. In the gut, it is clear that microbes modulate and impact on a wide variety of epithelial cell responses. Unraveling the communication strategies between microbes and their host has provided new understanding regarding both normal gut function and clinically relevant gastrointestinal diseases.

The study of host–parasite interactions bridges multiple scientific disciplines. The use of microbes and their products (eg, toxins and lipopolysaccharide) has had a major impact on our understanding of cell biology. Since the coining of the term "cellular microbiology,"[95] which describes the new discipline that has arisen from the marriage of microbiology and cell biology, our understanding of the relationship between prokaryotic and eukaryotic cells has increased greatly. Enhanced knowledge of the mechanisms underlying the diverse interactions between microbes and the gut epithelia holds exceptional potential for the future development of novel therapeutic interventions. As an example, administration of the probiotic bacterium *Lactococcus lactis*, engineered to produce interleukin-10 (an anti-inflammatory cytokine), reduces disease severity in a mouse model of inflammatory bowel disease.[96] Accordingly, we can now look forward to a continuing explosion of knowledge in this area in the years to come.

ACKNOWLEDGMENTS

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CHAPTER 3

INFLAMMATION

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Inflammation is the most common type of response that the body mounts when facing an assault from the surrounding environment. This is true for all tissues, organs, and systems, but in each one of these compartments, the inflammatory response varies depending on two key factors: the nature of the inciting agent(s) and the characteristics of the microenvironment in which inflammation ensues. The gastrointestinal tract is the ultimate example of how specific cellular and functional features shape the type, degree, and duration of inflammation. In this regard, the digestive system is unique owing to several specialized features: it is permanently exposed to the external environment, is constantly stimulated by a myriad of antigens, and harbors a luxuriant mix of bacteria, fungi, and viruses making up the endogenous enteric flora. Therefore, even under completely physiologic conditions, the intestinal tract contains enormous amounts of leukocytes, which are diffusely scattered in the lamina propria and the intraepithelial compartment or are organized in the Peyer patches of the terminal ileum and the isolated lymphoid follicles of the colon.[1] Combined, they form the anatomic basis of the gut- or mucosa-associated lymphoid tissue and functionally represent a tightly controlled form of self-contained inflammation termed "physiological inflammation."[2] The latter occurs in response to stimuli coming from the luminal surface of the mucosa and is found exclusively in the gut. In fact, other body surfaces exposed to alternate external or internal environments contain comparatively minimal amounts of lymphoid cells, as found in the lungs, skin, and urinary tract. In contrast to physiologic inflammation, which is anatomically restricted, tightly controlled, beneficial, and actually indispensable to health, pathologic inflammation in the gut is an injurious process that, particularly if severe and protracted, can lead to major functional and structural changes, causing clinical symptoms and impairing the quality of life of affected individuals. Fortunately, most forms of intestinal inflammation are transient and of limited impact on the general health of the patients, as are most acute viral and bacterial infections in children. Nevertheless, there is still a considerable number of other forms of gastrointestinal inflammation that result in serious clinical manifestations, as described in other chapters of this textbook. The focus of this review is on intestinal inflammation that induces tissue damage and functional derangements.

In addition to lymphoid cells, extremely diversified and highly specialized cell types of ectodermal, mesodermal, and endodermal origin compose the intestine. These include epithelial, mesenchymal, endothelial, and nerve cells, to which the extracellular matrix (ECM) must be added in view of increasing evidence for active participation of this acellular component in both immunity and inflammation.[3] A review of intestinal inflammation includes all of the above cellular and acellular elements because of accumulating evidence that an inflammatory response is not simply the consequence of a deranged immune response but a far more complex interplay of immune and nonimmune cell interactions.[4] How such interactions take place is incompletely understood, but at least two classes of elements are involved: one is represented by an enormous variety of soluble mediators released by immune and nonimmune cells such as cytokines, eicosanoids, neuropeptides, reactive metabolites, and proteases, and the other is represented by cell adhesion molecules, structures that are primarily, although not exclusively, involved in cell-to-cell adhesion events. Combined, soluble factors and cell adhesion molecules facilitate, allow, and amplify the exchange of signaling among cells that is essential to induce and mediate inflammation. Thus, in addition to the cellular components of inflammation, soluble factors and cell adhesion molecules are included in this review.

Because intestinal inflammation encompasses numerous and diverse factors, knowledge of the exact mechanisms and sequential interactions is far from complete. To provide the reader with a reasonably comprehensive overview of intestinal inflammation, information was derived from various sources, including human (pediatric and adult) and animal studies. An inherent caveat with this approach is that most human studies are based on chronic inflammatory conditions, whereas animal studies generally use acute models of intestinal inflammation. In addition, information is complemented by data derived from in vitro studies with single or multiple cellular systems. These sources of information are used to achieve functional and conceptual integration and generate a cohesive view of inflammation in the gastrointestinal tract. Finally, inflammation will be discussed as a broadly applicable response, keeping in mind that, in spite of a lack of direct supporting evidence, differences may exist between how this response is mediated in children and adults.

INFLAMMATORY CELLS

Plasma Cells

Immunoglobulin-producing plasma cells, which represent terminally differentiated B cells, are the most abundant type of lymphoid cell in the intestinal mucosa under both physiologic and inflammatory conditions. The majority of them normally produce dimeric immunoglobulin (Ig)A antibodies that are carried to the luminal surface by a well-defined transfer pathway involving a polymeric Ig receptor produced by the adjacent epithelial cells.[5] The expression of certain molecular determinants, such as the integrin $\alpha_4\beta_7$ on migrating B cells (and T cells) and of the mucosal addressin cell adhesion molecule (MAd-CAM) 1 on high endothelial venules, is believed to underlie the preferential migration of B cells to the mucosa, but cell differentiation and the functional status probably also play an important role.[6] In fact, normal intestinal B cells are in a higher state of activation compared with those in the peripheral circulation.[7] During inflammation, migratory patterns, cell proliferation, and state of activation are all drastically altered, resulting in major modifications in the distribution, number, class, and subclass of immunoglobulin-producing plasma cells. There is a consistent increase in IgG-secreting plasma cells that appears to be independent of the segment of the gastrointestinal tract involved, as observed in gastritis and inflammatory bowel disease.[8,9] In addition, the production of different subclasses of Igs is markedly abnormal, with predominance of IgG1- or IgG2-producing cells depending on the type of inflammatory process, as in ulcerative colitis versus Crohn disease.[10,11] Altered proportions of monomeric (systemic) and dimeric (mucosal) IgA or IgA1 versus IgA2 also can be found.[12,13] It is still uncertain what the consequences of an increased number of B cells in the inflamed mucosa might be because they are not, per se, pathogenic. Changes in IgA class could impair defenses against dietary and bacterial antigens, the production of complement-fixing antibodies, primarily of the IgG1 subclass, could contribute to or amplify tissue damage, and an increase in IgE-producing cells could mediate allergic reactions, leading to the release of a vast array of inflammatory mediators.

T Cells

The migration, distribution, and localization of T cells in the intestinal mucosa involve some of the same molecular mechanisms used by B cells.[6] However, these mechanisms tend to be more complex than those for B cells and are drastically altered during inflammation. In contrast to granulocytes, whose high cell number in an inflamed tissue is directly related to an increased emigration rate, changes in T-cell number and function involve multiple abnormalities in the entry, proliferation, exit, and death in the inflamed gut.[14] Expectedly, the number and state of activation of T cells are both increased in the inflamed intestine, but the proportions of CD4+ to CD8+ T cells in the mucosa are usually not remarkably shifted away from the normal CD4-to-CD8 ratio, regardless of the type and location of inflammation.[15–17] This is true for T cells infiltrating the lamina propria, which predominantly express the $\alpha\beta$ T-cell receptor. Alterations in the composition of intraepithelial T cells, which are predominantly CD8+ and tend to express the $\gamma\delta$ T-cell receptor in higher proportions, are less marked in some types of inflammation, such as inflammatory bowel disease.[18] In celiac disease, on the other hand, their number can increase, as well as the type of T cells that preferentially express the $\gamma\delta$ T-cell receptor.[19,20]

Because T cells control all aspects of cell-mediated immunity, their presence and abnormal state of activation suggest that they play a pivotal role in most, if not all, aspects of intestinal inflammation.[21] Their role likely includes antigen-specific responsiveness, immunoregulation, immunosuppression, cytokine production, and perhaps cytotoxic activity. With the exception of cytokine production (which is discussed in another section of this chapter), evidence that intestinal T cells exert all other functions is still fragmentary and incomplete. Mucosal T cells substantially differ from T cells circulating in the periphery in regard to their intrinsic ability to respond to receptor-mediated activation. They are preferentially stimulated through the CD2 pathways, in contrast to blood T cells that use the classic CD3 pathway for optimal activation,[22,23] an event perhaps conditioned by mucosal factors.[24] If true, one could also expect that when the mucosa is involved by inflammation, this may also influence and modify the behavior of local T cells. Mucosal T cells from inflammatory bowel disease display an enhanced proliferative response to bacterial antigens in both humans and experimental models,[25–27] as small intestinal T cells do in response to gliadin in celiac patients[28] and probably gastric T cells to *Helicobacter pylori* antigens.[29] Additional investigation is needed, however, to understand how these antigen-specific responses may trigger the cascade of events leading to the changes recognized as hallmarks of gastrointestinal inflammation. That these events actually occur in the mucosa is suggested by experiments in which nonspecific activation of local T cells induces an enteropathy manifested by anatomic adaptation of the mucosal architecture or even damage to the mucosa.[30,31] Additional supporting evidence comes from studies of celiac disease, in which the typical morphologic changes of flattened mucosa can be reproduced in vitro and in vivo by gliadin challenge,[32] an event associated with major histocompatibility complex (MHC) class II–restricted specific recognition of selectively modified gliadin peptides by gut mucosal T cells.[33] Detailed knowledge of the steps leading from antigen-specific T-cell recognition to overt tissue damage is nevertheless limited. In particular, evidence for the existence of classic cytotoxic T cells, which specifically recognize and destroy intestinal cell targets, is still missing. On the other hand, a study suggests that proteolytic injury mediated by broadly active proteases is active downstream of the T-cell activation events.[34]

The most recent type of T cells to attract considerable attention in intestinal inflammation is regulatory T cells (Treg). Previously called suppressor T cells, Treg cells represent a diverse group of cells with potent immunosuppressive activity capable of controlling and preventing autoimmunity and inflammation.[35] The best defined types are T regulator

1 (Tr1) cells, which mediate suppression primarily through the release of immunosuppressive cytokines such as interleukin (IL)-10 and CD4+CD25+ cells, which seemingly exert suppression by direct cell contact, and T helper (Th) 3 cells, which produce large amounts of transforming growth factor (TGF)-β. Tr1 and CD4+CD25+ cells have been shown to suppress intestinal inflammation in vivo in animal models of inflammatory bowel disease.[36,37]

Monocytic Cells and Toll-Like Receptors

Monocytes and macrophages are prominent inflammatory cells that, like neutrophils, lack immunologic memory but are extremely potent in their capacity to mediate tissue damage. The normal small and large bowel contain a moderate number of resident macrophages, which are made up of heterogeneous populations of monocytic cells identifiable by morphologic characteristics and expression of specific cell surface molecules that separate them into categories with preferential antigen-present or scavenger activity.[38,39] Depending on the type of inflammatory process, macrophage heterogeneity is amplified, underlying the recruitment of new cells types as well as diversification of function.[40] They undergo an enhanced respiratory burst activity with release of reactive radicals that contribute to inflammation and local tissue damage.[41] During inflammation, monocytes are actively recruited from the peripheral blood into the mucosa, where they differentiate into macrophages expressing the CD68 and L1 antigens, which differentiates them from preexisting RFD 7+ macrophages.[42] This recently recruited subset of macrophages with monocyte-like phenotype appears to be primed for release of several proinflammatory cytokines and to have greater pathogenic potential compared with macrophages normally residing in the gut.[43] Mucosal macrophages express several costimulatory molecules, including B7-1 and B7-2, which allow them to adhere to and activate T cells, an interaction that can further contribute to expand immune-mediated inflammation.[44]

Macrophages also express a newly described family of cell surface molecules termed pattern recognition receptors (PRRs). These PRRs recognize conserved, pathogen-associated molecular patterns (PAMPS) expressed by various microbes but not the host. One particular family of PRRs, the Toll-like receptors (TLRs), recognizes PAMPs and can influence the character of the inflammatory or immune response through synthesis of proinflammatory cytokines such as IL-1, IL-6, tumor necrosis factor (TNF)-α, and IL-12.[45,46] It is thought that TLRs use signaling components similar to the receptor for IL-1. Once a TLR recognizes the appropriate PAMP, this ultimately results in the activation of NF-κB inducing kinase and, subsequently, the phosphorylation of I-κB. Phosphorylation causes I-κB to physically dissociate from NF-κB, which is then free to translocate into the nucleus and initiate the gene transcription process that leads to the production of a variety of proinflammatory molecules.[47] Activation of NF-κB by bacterial products is not mediated exclusively by TLRs, and some members of the nucleotide-binding oligomerization domain (NOD) family of cytosolic proteins, particularly NOD1 and NOD2, also participate in the innate recognition of microorganisms and regulation of inflammatory responses.[48]

In humans, 10 TLRs have been described with different ligand-binding specificities, including flagellin, bacterial deoxyribonucleic acid (DNA), CpG motifs, peptidoglycan, and lipopolysaccharide (Table 3-1). The binding of specific TLRs dictates the role of the macrophage in promoting the inflammatory response. TLRs also can be found on other cell types, including mast cells and dendritic cells and, to a lesser extent, epithelial cells. In the intestinal epithelium, TLRs are predominantly expressed on the basolateral surface of the cell, but apical expression of TLR-4 is up-regulated during chronic inflammation.[49,50] Some reports have demonstrated that intestinal epithelial cells from patients with Crohn disease and ulcerative colitis expressed TLR-4, whereas intestinal epithelial cells from healthy individuals do not. TLR-3 and TLR-5 also have been identified in the small intestine.[51,52]

Other Inflammatory Leukocytes

A variety of other myeloid and lymphoid cells are found in inflamed gut, including neutrophils, eosinophils, mast cells, basophils, and dendritic cells. All of these cells are present in increased numbers in affected areas, but such an increase often represents a nonspecific response to inflammation. Less frequently, this represents a selective infiltration of cells that plays a specific role in the inflammatory reaction, such as in the case of eosinophilic gastroenteritis, allergic reactions, or helminthic infestations. Information about the contribution of these cell types is substantially less compared with what is currently known about T and B cells in gastrointestinal inflammation.

Polymorphonuclear neutrophils are virtually absent from the normal gut mucosa, and any increase in this cell type, no matter how minute, should raise suspicion that an inflammatory response is occurring. Because neutrophils are short-lived and quickly undergo apoptotic death once translocated into tissues, their numeric increase in the gut is exclusively due to and sustained by emigration from the circulation. This occurs through the expression of multiple adhesion molecules by the neutrophils and the mucosal microvasculature, a topic that is discussed in greater detail later in this chapter.[53] Once localized in the tissue, neutrophils mediate local injury by releasing broad-spectrum proteases and various free radicals,[54] as detailed in the section dealing with soluble mediators of inflammation.

TABLE 3-1 TOLL-LIKE RECEPTORS AND ASSOCIATED PATHOGEN-ASSOCIATED MOLECULAR PATTERNS

TLR	PAMP
TLR-2	Peptidogolycan, bacterial lipoprotein
TLR-3	Double-stranded RNA
TLR-4	Lipopolysaccharide
TLR-5	Flagellin
TLR-7	Small antiviral compounds
TLR-9	Cytosine-phosphorothioate-guanine

PAMP = pathogen-associated molecular pattern; RNA = ribonucleic acid; TLR = Toll-like receptor.

A moderate number of eosinophils are present in the normal intestinal mucosa, whereas mast cells and basophils are less common. All of these cells are increased in number during active inflammation, as seen in inflammatory bowel disease.[55] This is a nonspecific phenomenon, except in conditions in which each cell type can predominate. For example, eosinophils dominate the inflammatory infiltrate during allergic reactions[56] and in conditions of unknown etiology such as eosinophilic gastroenteritis. Like neutrophils, eosinophils are recruited from the circulation, but once localized in the mucosa, they release mediators specifically involved in allergic and parasitic responses, such as IL-4 and IL-5. This is in contrast to mast cells, which, although also involved in similar reactions, are heterogeneous and composed of at least two well-defined populations with distinctive features: connective tissue (typical) mast cells and mucosal (atypical) mast cells.[57] Mucosal mast cells are involved in food allergy, resistance to parasites, and inflammatory bowel disease.[58] Their action is mediated by several soluble products, prominent among which is histamine, an early messenger of inflammatory and immune reactions.[59]

Dendritic cells also need to be mentioned, even though their diversity, complexity, and function in normal mucosal immunity and inflammation have just begun to be investigated. Dendritic cells represent a heterogeneous group of cells with an interdigitating morphology, which are present in low numbers in most tissues of the body and express high levels of human leukocyte antigen (HLA) class II antigens, which make them highly efficient in antigen presentation. Dendritic cells work at the host-pathogen interface to sample antigens and influence the host response to bacterial antigens.[60] Their type and localization along the intestinal tract may provide key determinants of localized mucosal immune and inflammatory responses.[61]

ADDITIONAL CELLS INVOLVED IN INFLAMMATION

Until quite recently, mucosal inflammation was viewed as a response dominated by the action of classic immune cells, whereas all other cells had only a passive role as targets of immune cells and their products. This view is no longer tenable considering a growing body of information showing that multiple cell types display functional characteristics that make them active players in inflammation.[62] Multiple cell types composing the intestinal wall participate in inflammation, including epithelial cells, endothelial cells, nerve cells, and mesenchymal cells (fibroblasts and myofibroblasts).

Epithelial Cells

The involvement of intestinal epithelial cells in mucosal immune reactivity was initially suggested by the secretory, absorptive, and digestive adaptive changes that these cells undergo during immune and parasitic responses.[63] The identification of epithelial cell heterogeneity and the role of Paneth cell–derived defensins in innate immunity also indicate an active role of the epithelium in intestinal immunity.[64] Levels of defensin expression may vary in different types of intestinal inflammation.[65] Subsequent studies generated evidence for an active role of epithelial cells in normal mucosal immunity and inflammation. Immunohistochemistry detected MHC class II (HLA-DR) antigens on human intestinal epithelial cells,[66] and their capacity to function as antigen-presenting cells was documented in both human and animal studies.[67,68] The expression of HLA-DR antigens is enhanced during intestinal inflammation, as in inflammatory bowel disease, celiac disease, and gastritis,[69–71] indicating that it is a nonspecific response to inflammation unrelated to the type or location of disease. Enhanced expression of accessory molecules is also noted on gastric and colonic epithelial cells during inflammatory conditions, as in the case of the costimulatory molecules CD80 (B7-1) and CD86 (B7-2) in *H. pylori* gastritis and ulcerative colitis,[72,73] and intercellular adhesion molecule 1 (ICAM-1) is expressed during bacterial invasion.[74] Additional evidence for the participation of epithelial cells in inflammation comes from studies demonstrating their capacity to both produce and respond to proinflammatory and immunoregulatory cytokines[75] and to express chemokine receptors.[76] In response to bacterial invasion or inflammatory signals, intestinal epithelial cells produce a broad spectrum of bioactive molecules, including IL-1 receptor antagonist, IL-6, IL-7, IL-8, IL-15, monocyte chemotactic protein 1, granulocyte-macrophage colony-stimulating factor (GM-CSF), TNF-α, growth-regulated oncogene (GRO)-α, GRO-γ, macrophage inflammatory protein 2, nitric oxide (NO), and cyclooxygenase (COX) 2.[77–85] Some of these products act on intestinal cells, altering their function, such as the rate of proliferation and barrier function.[86–88] Finally, the normal ability to preferentially activate suppressor CD8+ T cells appears to be shifted to stimulation of helper CD4+ T cells in inflammatory bowel disease,[89] suggesting that epithelial cells may actually contribute to amplification or persistence of certain forms of chronic inflammation. Altogether, the number and variety of agents and functions mediated by intestinal epithelial cells combined with vigorous responses to multiple inflammatory stimuli provide irrefutable evidence of an essential role of intestinal epithelium in inflammation.

Endothelial Cells

Among the various cell types present in the intestinal wall, none is more directly involved in regulating inflammation than endothelial cells. In fact, the microvascular endothelium is a true "gatekeeper of inflammation" because translocation of leukocytes from the intravascular into the interstitial space, under both physiologic and inflammatory circumstances, is tightly controlled by the junctions between endothelial cells.[90] For translocation to occur, leukocytes must adhere to the endothelium through steps mediated by activation of both leukocytes and endothelial cells followed by the expression of adhesion molecules of the selectin, integrin, and Ig superfamily groups and their corresponding ligands in a highly orchestrated process regulated by multiple cytokines.[91] When inflammation ensues, all of these events occur at the level of the microcirculation

in each specific tissue and organ.[92] This is also true for the gastrointestinal tract, in which high endothelial venules, a specialized type of endothelium, play a central role in lymphocyte migration and extravasation.[93] Among others, MAd-CAM-1 is exclusively expressed by the microvascular cells of the gut mucosa, and its level of expression is enhanced during inflammation in both human and animals.[94,95] Thus, considering the importance of the mucosal microvascular endothelium, it is not surprising that leukocyte-endothelial interactions are critical to gastrointestinal inflammation,[96] when the selectivity of lymphoid cell binding to the vascular endothelium may be lost.[97] Studies of the intestinal microvasculature in intestinal inflammation are relatively few. Nonetheless, there is good evidence that inflamed mucosal endothelial cells are in a high state of activation, as indicated by enhanced expression of HLA-DR molecules and inducible nitric oxide synthase (NOS) in areas involved by inflammatory bowel disease.[98,99] More direct evidence of the importance of the microvasculature in inflammation is provided by in vitro studies with human intestinal microvascular endothelial cells (HIMECs). When these cells are obtained from inflamed mucosa of ulcerative colitis or Crohn disease patients, their capacity to up-regulate leukocyte adhesion is markedly increased.[100] This response is detected only when HIMECs are derived from the chronically inflamed but not the uninvolved mucosa of the same subject.[101] In addition, activated platelets interact with HIMECs via the CD40/CD40 ligand pathway and cause them to up-regulate adhesion molecule expression and chemokine production, inducing a proinflammatory response of the mucosal microvasculature.[102] These observations indicate that in the chronically inflamed intestine, the local mucosal microvascular bed undergoes important functional modifications, conditioned by prolonged exposure to a cytokine-rich milieu and other proinflammatory cellular elements, which are likely to contribute to the maintenance of the inflammatory response.

Nerve Cells

The participation of the nervous system in inflammation is well established.[103] In the gastrointestinal tract, the enteric nervous system forms a rich network of fibers regulating not only motility and secretion but also the local immune response.[104] A dramatic example of the critical role of enteric nerves on inflammation is illustrated by the development of jejunoileitis in animals whose enteric glia are specifically ablated.[105] The action of the enteric nervous system on inflammation is exerted through the release of a large number of neuropeptides that have both stimulatory and down-regulatory effects on the immune response.[106] These mediators play a modulatory role in many forms of intestinal inflammation, such as those induced by infectious agents,[107] or of idiopathic origin, such as in inflammatory bowel disease.[108] Perhaps a more important aspect of the nerve cells is to provide an anatomic basis for the effect of stressful events of life on intestinal immunity and inflammation, a connection proposed in both animal models and humans. Objective evidence supporting this connection is fairly convincing in models of experimental gut inflammation,[109] particularly with the recent demonstration that susceptibility to reactivation by stress requires CD4+ T cells and can be adoptively transferred by these cells.[110] The link between the enteric nerves and intestinal inflammation is more tenuous in humans, in whom the negative effects of stress on the outcome of inflammatory bowel disease are still a matter of controversy.[111] Various anatomic changes of enteric nerve fibers have been documented in both Crohn disease and ulcerative colitis,[112,113] but these are probably secondary to inflammation. Nevertheless, once established, they can alter the production and release of a variety of molecules involved in the mediation of inflammation.

Mesenchymal Cells

Mesenchymal cells have been traditionally viewed as simple structural cells, but during the last decade, abundant data demonstrate an active role of these cells in intestinal immunity and inflammation.[114] Distributed from immediately below the subepithelial basement membrane to the lamina propria, in the muscularis mucosae, submucosa, and in the muscularis propria, mesenchymal cells form a pleiotropic group of cells that respond to and secrete a large number of products that modulate the activity of surrounding epithelial and immune cells.[115] Under inflammatory conditions, mesenchymal cells display the intrinsic capacity of altering their phenotype and function evolving from pure fibroblasts to myofibroblasts, stellate cells, and muscle cells.[115,116] Resting and activated mesenchymal cells produce a variety of substances involved in inflammation, including IL-1, IL-6, TNF-α, GM-CSF, TGF-β, and prostaglandin E_2 (PGE_2), as well as adhesion molecules.[117,118] Through the specific action of these molecules, mesenchymal cells influence the response of epithelial cells to inflammation, such as enhancing their electrolyte secretory responses[119] or promoting wound healing by the stimulation of cell migration.[120] The recent demonstration that intestinal mesenchymal cells spontaneously express COX-2, leading to PGE_2 production, has been interpreted as evidence for their contribution to the maintenance of tolerance during intestinal immune responses.[121]

On the other hand, intestinal mesenchymal cells also modulate immune function by directly and indirectly interacting with T cells. Intestinal fibroblasts bind T cells through an ICAM-1–mediated pathway, and adherence is enhanced by proinflammatory cytokines such as IL-1, TNF-α, and interferon (IFN)-γ.[122] Intestinal smooth muscle cells can present antigens and activate T cells in a MHC class II–dependent fashion.[123] In addition to interacting with epithelial and immune cells, intestinal mesenchymal cells also interact with the local bacterial flora, as indicated by stimulation of IL-1, IL-6, and TGF-β expression in cultured myofibroblasts and induction of fibrosis and stenosis in normal and colitic animals.[124,125] Finally, all types of intestinal mesenchymal cells are the primary source of ECM proteins, whose production is dramatically enhanced under inflammatory conditions and is responsible for fibrosis and stricture formation, a topic that is addressed in more detail in a subsequent section.

INFLAMMATORY MEDIATORS

Cytokines

Cytokines represent one of the most numerous and complex group of secreted molecules. Cytokines are involved in multiple aspects of an inflammatory response, and an extensive body of literature exists with regard to intestinal inflammation.[126–128]

Within the cytokine network, a number of properties are attributed to these proteins.[129] Pleiotropy translates the observation that a single cytokine can be synthesized by multiple cell types, induce a response in multiple target cells, and mediate a number of stimulatory or inhibitory signals within one or multiple cell types. Redundancy indicates that multiple cytokines can elicit the same biologic response, whereas cross-regulation indicates that cytokines not only directly activate immune and inflammatory cells, but this activity is, in turn, modulated by the products secreted by the cells they activate. Owing to these various properties, the classification of cytokines is often arbitrary. Cytokines can be classified according to common characteristics to facilitate understanding of the role of individual molecules in gastrointestinal inflammation. A convenient classification groups IL-2, IL-7, IL-12, and IL-18 as immunoregulatory cytokines because they play a primary role in activating, modulating, and expanding the immunoregulatory T-cell population, although they also exert activities on other immune and nonimmune cells (Table 3-2). Some cytokines exert both immunosuppressive and immunoregulatory functions, such as IL-4, IL-10, and IL-13 (Table 3-3). Other immunoregulatory and effector cytokines, including IFN-γ, GM-CSF, IL-3, IL-5, IL-9, IL-11, and IL-15, act primarily during the effector phase of the immune response and impact on how immune and nonimmune cells function to eliminate a pathogen or perpetuate immunity and inflammation (Table 3-4). The last group includes the proinflammatory cytokines IL-1α, IL-1β, TNF-α, and IL-6, which are primarily produced by cells of monocytic and macrophage lineage (Table 3-5). Although classically categorized as a proinflammatory molecule, a number of anti-inflammatory activities have been attributed more recently to IL-6 because this molecule fails to induce activities traditionally associated with inflammation, such as enhancement of eicosanoid, NO, and metalloproteinase production and adhesion molecule expression.[130] An alternate way of classifying cytokines, which is restricted to cytokines produced by CD4+ Th cells, is following the Th1 and Th2 paradigm. According to this conceptual framework, a balance between Th1 (IL-2, IFN-γ, and TNF-α producing) and Th2 (IL-4, IL-5, IL-6, IL-10, and IL-13 producing) cytokines implies a physiologic immune response, as found in health, whereas an imbalance between Th1 and Th2 cytokines leads to conditions dominated by a delayed-type hypersensitivity/cell-mediated (Th1) or an allergic-type/antibody-mediated (Th2) pathologic response.[131]

The bulk of information on cytokine abnormalities in intestinal inflammation is based on studies of abnormalities found in human inflammatory bowel disease. The study of IL-2 has shown differential activity in Crohn disease and ulcerative colitis. Mucosal levels of IL-2 protein and messenger ribonucleic acid (mRNA) are consistently higher than in ulcerative colitis, as are levels of the IL-2Rα chain.[132] In both adult and pediatric Crohn disease patients, mucosal immune cells exhibit a hyperreactivity to IL-2 when compared with cells from ulcerative colitis patients.[133,134] The Th1 disease connotation presently attributed to Crohn disease is supported by the enhanced spontaneous production of IFN-γ by mucosal mononuclear cells.[135] In addition, both protein and mRNA for IL-12 and IL-18, cytokines essential for IFN-γ induction, are expressed at higher levels in Crohn disease– than ulcerative colitis–affected tissues.[136–138] Low production of IL-4 by lamina propria mononuclear cells and T-cell clones of Crohn disease patients also reinforces the concept that this is a condition with a prominent Th1-like profile.[139] The classification of ulcerative colitis as a Th2-like condition, however, is still in doubt. In support of this possibility are lower levels of IFN-γ produced by mucosal mononuclear cells and higher IL-5 levels in ulcerative colitis than Crohn disease mucosa.[140,141] Little information exists on mucosal levels of IL-10 in inflammatory bowel disease,[142] probably reflecting a nonspecific response to gut inflammation. Mucosal production of IL-15, a cytokine with many of the biologic activities of IL-2, is enhanced in both forms of inflammatory bowel disease.[143]

Levels of proinflammatory cytokines are elevated in tissues involved by inflammatory bowel disease. High concentrations of IL-1α and -β are found in both Crohn disease and ulcerative colitis,[144,145] but local effects are largely determined by the relative concentration of the natural antagonist IL-1RA. A mucosal imbalance between IL-1RA and IL-1 has been reported in inflammatory bowel disease, showing a relative deficiency of IL-1RA, which could con-

TABLE 3-2 IMMUNOREGULATORY CYTOKINES

CYTOKINE	MAIN CELLULAR SOURCE	MAIN TARGET CELL	DOMINANT FUNCTION
IL-2	T cells	T cells, all IL-2R–bearing cells	T-cell activation, proliferation, clonal expansion, and differentiation
IL-7	Stromal cells, epithelial cells	Leukocyte differentiation	T-cell proliferation and cytotoxicity
IL-12	Phagocytes, B cells, dendritic cells	T cells	Th1 differentiation, infectious responses, induction of IFN-γ
IL-18	Macrophages, dendritic cells, epithelial cells	T cells	IL-12–like

IFN = interferon; IL = interleukin; IL-2R = interleukin-2 receptor; Th = T helper.

TABLE 3-3 IMMUNOREGULATORY AND IMMUNOSUPPRESSIVE CYTOKINES

CYTOKINE	MAIN CELLULAR SOURCE	MAIN TARGET CELL	DOMINANT FUNCTION
IL-4	T cells, mast cells, basophils	Multiple cell types	Th2 differentiation, mediation of allergy, immunosuppression, and anti-inflammatory activity
IL-10	Monocyte, macrophages, T and B cells, nonimmune cells	Multiple cells	Anti-inflammatory activity and immunosuppression
IL-13	T cells	Multiple cells (except T cells)	IL-4–like

IL = interleukin; Th = T helper.

tribute to the chronicity of inflammation.[146] IL-6 is also consistently elevated in inflammatory bowel disease mucosa, where it primarily derives from macrophages and epithelial cells.[147,148] In contrast to IL-1 and IL-6, protein and mRNA levels of TNF-α have been inconsistently reported as both normal and elevated in inflammatory bowel disease. High TNF-α concentrations are found in the stools of children with Crohn disease and ulcerative colitis,[149] and production of TNF-α is higher in cultures of Crohn disease than in ulcerative colitis mucosal mononuclear cells.[150] In situ hybridization reveals elevated TNF-α mRNA in macrophages infiltrating inflammatory bowel disease tissues,[151] but some studies found no differences in TNF-α mRNA expression in normal and inflammatory bowel disease biopsies.[148,152]

Chemokines

Chemokines are cytokines that exhibit the ability to directionally attract leukocytes into sites of inflammation. They constitute a very large group of functionally related molecules usually divided into four families based on their content of cysteine (C) residues, which are separated by variable numbers of amino acids (X).[153] Chemokines are produced by most cells in the body, and each family displays relative selectivity in its capacity of attracting neutrophils, monocytes, macrophages, dendritic cells, T cells, natural killer cells, eosinophils, and basophils, depending on these cells' expression of multiple chemokine receptors (Table 3-6). Chemokines are involved not only in the recruitment of nonspecific inflammatory cells but also in the positioning and the preferential induction of Th1 and Th2 cells, making them active participants of cell-mediated immunity and Th1 and Th2 responses.[154] Therefore, it is not surprising that chemokines play a central role in intestinal inflammation,[155] in which their levels tend to be high and correlate with the histologic grade of inflammatory activity regardless of the organ involved, as observed in *H. pylori*–induced gastritis and colitis.[156,157] In actively inflamed intestine, multiple cell types are sources of chemokines, including macrophages, T cells, endothelial cells, and epithelial cells.[158,159] However, it is likely that most mucosal cells produce some type of chemokines, making it difficult to dissect out the relative contribution of each cell and chemokine to the initiation, amplification, and persistence of mucosal inflammation. In particular, the precise contribution of epithelial cells to chemoattraction in vivo is unclear, except for the production of the neutrophil chemokine epithelial cell–derived neutrophil activity peptide (ENA)-78 in active inflammatory bowel disease.[160] In addition to the type of chemokines produced during intestinal inflammation, it is also important to consider the expression of the various chemokine receptors by circulating and infiltrating leukocytes. This determines which cells are attracted into the mucosa and may explain the differences that exist among infiltrating cells in different types of gut inflammation and the preferential localization of particular cell subsets in distinct segments of the gut.[161,162]

Growth Factors

Intestinal inflammation has traditionally been considered as an excessively strong insult by activated immune cells and their products that ultimately results in a tissue-destructive process. An alternate and complementary view is that intestinal inflammation results from an inadequate

TABLE 3-4 IMMUNOREGULATORY AND EFFECTOR CYTOKINES

CYTOKINE	MAIN CELLULAR SOURCE	MAIN TARGET CELL	DOMINANT FUNCTION
IFN-γ	T cells, natural killer cells	Most cells	Induction of MHC class II antigens, monocyte activation, Th1 differentiation, and IL-4 suppression
GM-CSF	Phagocytes, B cells	Hematopoietic cells	Leukocyte differentiation
IL-3	Multiple cells	Hematopoietic cells	Leukocyte differentiation
IL-5	T cells, mast cells	Eosinophils	Mediation of allergic and parasitic diseases
IL-9	Th2 cells	T cells, mast cells	Undefined
IL-11	Hematopoietic stromal cells	Multiple cells	Stimulation of intestinal crypt cells
IL-15	Most cells	IL-2R–bearing cells	T-cell expansion, epithelial cell differentiation

GM-CSF = granulocyte-macrophage colony-stimulating factor; IFN = interferon; IL = interleukin; IL-2R = interleukin-2 receptor; MHC = major histocompatibility complex; Th = T helper.

TABLE 3-5 PROINFLAMMATORY CYTOKINES

CYTOKINE	MAIN CELLULAR SOURCE	MAIN TARGET CELL	DOMINANT FUNCTION
IL-1α, IL-1β	Monocytes, macrophages	Most cells	Mediation of infectious and inflammatory responses
IL-6	Multiple cells	Most cells	Enhancement of immunoglobulin production and immunoregulation
TNF-α	Macrophages	Multiple cells	Mediation of inflammatory and cytotoxic responses

IL = interleukin; TNF = tumor necrosis factor.

capacity of the gut mucosa to defend itself against infectious, immune, toxic, or ischemic injuries. Because cytokines and chemokines are generally considered mediators of injury, growth factors can be considered as mediators of defense and, once damage has occurred, of remodeling and healing. Like cytokines, growth factors derive from multiple cellular sources that are primarily nonimmune, such as mesenchymal cells, and also exert a great variety of diverse functions, essential among them being the ability to induce cell proliferation.[163] In addition to proliferation, other fundamental activities include cell differentiation, cell migration, angiogenesis, and ECM deposition, all of which are necessary to wound healing and resolution of inflammation.[163] Growth factors can be divided in families, including TGF-β, epidermal growth factor (EGF) and TGF-α, insulin-like growth factors, fibroblast growth factors, hepatocyte growth factor, and trefoil factors (Table 3-7).

In a reductionistic model of epithelial cell wounding in vitro, TGF-β is centrally important in reconstitution of epithelial integrity by stimulating cell migration, a response that is selectively enhanced by other growth factors and cytokines including TGF-α, EGF, IL-1, and IFN-γ.[164,165] Fibroblast growth factor also promotes epithelial cell restitution.[166] A similar protective effect is exerted by TGF-α in repair of acute gastric injury in animals.[167] Keratinocyte growth factor, a member of the fibroblast growth factor family, mediates a comparable healing effect in the inflamed colonic mucosa of rats exposed to trinitrobenzenesulfonic acid.[168] Increased expression of keratinocyte growth factor in the mucosa of inflammatory bowel disease patients can be interpreted as a defense against inflammatory damage by stimulating epithelial cell proliferation and promoting healing.[169] On the other hand, other growth factors, such as insulin-like growth factor I, may be more involved in the development of fibrosis.[170] Trefoil peptides represent a different class of growth factors whose main function is to reinforce the protective action of mucus by enhancing its physical resistance to mechanical injury.[171] This concept is supported by the beneficial effect of oral trefoil peptides in ethanol- and indomethacin-induced gastric inflammation and their enhanced levels in epithelial cells overlying areas involved by active inflammatory bowel disease.[172,173]

Eicosanoids

Eicosanoids include a group of substances derived from the metabolism of arachidonic acid resulting from the breakdown of cell membrane phospholipids by the action of phospholipases. Two main classes of enzymes are involved in the metabolism of arachidonic acid: the COXs and the lipoxygenases.[174] A large spectrum of vasoactive, pro- and anti-inflammatory, and immunomodulatory activities are mediated by various eicosanoids, whose main categories include prostaglandins, thromboxanes, and leukotrienes. An extensive literature exists on the various biologic functions of these eicosanoids in the gastrointestinal tract,[174–177] and their enhanced production in mucosa affected by various forms of inflammation is well documented.[178,179] COXs exist in constitutive (COX-1) and inducible (COX-2) forms and are intimately involved in the mechanisms of cytoprotection and destruction associated with various forms of gastrointestinal inflammation.[174–177] It is firmly established that prostaglandins primarily exert a cytoprotective action, which is lost when COXs are inhibited by the action of nonsteroidal anti-

TABLE 3-6 CHEMOKINES

GROUP	MAIN CHEMOKINES	MAIN CELLULAR SOURCE	MAIN TARGET CELLS
C-C	MCP-1/5, MIP-1α/β, RANTES, eotaxin-1, SDF-1, IP-10	Multiple immune and nonimmune cells	Monocytes, activated T cells, eosinophils, basophils
C	Lymphotactin, SDF-1	Multiple immune and nonimmune cells	Resting T cells
C-X-C	IL-8, MCP-1/5, MIP-1α/β, RANTES, eotaxin-1, SDF-1, GRO-α/β/γ, IP-10, ENA-78	Multiple immune and nonimmune cells	Neutrophils, dendritic cells
C-XXX-C	IL-8, MCP-1/5, MIP-α/β, RANTES, IP-10	Multiple immune and nonimmune cells	Natural killer cells

ENA = epithelial cell–derived neutrophil-activating peptide; GRO = growth-regulated oncogene; IL = interleukin; IP = IFN-γ–inducible protein 10; MCP = monocyte chemoattractant protein; MIP = macrophage inflammatory protein; RANTES = regulated on activation normal T cell expressed and secreted; SDF = stromal cell–derived factor.

TABLE 3-7 GROWTH FACTORS

FACTOR	MAIN TARGET CELL	PREDOMINANT EFFECT
TGF-α	Nonimmune cells	Enhancement of mucus protection, cell proliferation, differentiation, and migration and deposition of extracellular matrix
TGF-β	Immune and nonimmune cells	Enhancement of cell proliferation, differentiation, and migration; deposition of extracellular matrix; mediation of immunosuppression and tolerance
EGF	Nonimmune cells	Enhancement of mucus protection, cell proliferation, differentiation, and migration and deposition of extracellular matrix
IGF	Nonimmune cells	Enhancement of cell proliferation, differentiation, and migration and deposition of extracellular matrix
FGF	Nonimmune cells	Enhancement of cell proliferation, differentiation, and migration; deposition of extracellular matrix; collagenase production; and angiogenesis
Trefoils	Epithelial cells	Enhancement of mucus protection and cell migration and inhibition of proliferation

EGF = epidermal growth factor; FGF = fibroblast growth factor; IGF = insulin-like growth factor; TGF = transforming growth factor.

inflammatory drugs, a key step in the development of gastric ulceration.[180] This protective effect is mediated through multiple mechanisms, including stimulation of mucus and bicarbonate secretion, maintenance of mucosal blood flow, enhancement of epithelial cell resistance to cytotoxicity, inhibition of neutrophil recruitment and mast cell degranulation, and a broad immunosuppressive action (mostly by PGE_2) on macrophage and T-cell responses.[174] The anti-inflammatory activity of prostaglandins has been investigated more extensively in gastric injury and less in the rest of the intestinal tract. However, it appears that prostaglandins also mediate cytoprotective and anti-inflammatory activities in the small intestine and large bowel, as indicated by their mediation of enhanced survival of crypt stem cells in a model of radiation injury,[181] and the exacerbation of experimental colitis in animals receiving a selective COX-2 inhibitor.[182] A recent report suggests that the beneficial action of prostaglandins in the intestine may be even more fundamental than previously thought. This is based on evidence showing that PGE_2 produced through COX-2–dependent pathways is crucial to down-regulate physiologic immune responses to dietary antigens and, thus, maintains intestinal immune homeostasis by promoting immunologic tolerance.[183]

Leukotrienes are produced by the action of the enzyme 5-lipoxygenase, which is dependent on activation of 5-lipoxygenase activation protein. One of the main leukotrienes is LTB_4, which has a potent chemotactic effect for neutrophils and, as a result, acts as a strong proinflammatory substance. LTB_4 proinflammatory activity is probably exerted in both the upper and the lower gastrointestinal tract, as suggested by its elevation in the stomach of patients taking nonsteroidal anti-inflammatory drugs,[184] and in the colon of patients with inflammatory bowel disease.[185] Thromboxanes are COX-1–dependent products of platelets, and they are powerful vasoconstrictors believed to contribute to inflammation in various portions of the gastrointestinal tract, including the stomach[186] and small[187] and large bowel.[188]

Neuropeptides

Neuropeptides are small peptides released at nerve cell endings that influence the activity of immune and inflammatory cells, although many other cell types are also affected (Table 3-8). This effect can be stimulatory or inhibitory, depending on the type of neuropeptide and the target cells.[106] For instance, substance P tends to enhance immunity and promote inflammation, whereas vasoactive

TABLE 3-8 NEUROPEPTIDES

PEPTIDE	MAIN TARGET CELL	PREDOMINANT EFFECT
SP	T cells	Modulation of proliferation, enhancement of antigen-specific responses
	B cells	Enhancement of proliferation and antibody production
	Natural killer cells	Enhanced activity
	Macrophages	Enhancement of activity and chemotaxis
VIP	T cells	Inhibition of proliferation, enhancement of cAMP, enhancement of IL-2 and IL-4 and inhibition of IL-5 production, modulation of homing
	B cells	Inhibition of proliferation, modulation of antibody production
	Natural killer cells	Modulation of activity
	Macrophages	Inhibition of activity
SOM	T cells	Modulation of proliferation, inhibition of antigen-specific responses
	B cells	Inhibition of antibody production
	Natural killer cell	Modulation of activity
CGRP	T cells	Inhibition of proliferation, enhancement of cAMP
	Macrophages	Inhibition of activity
	Eosinophils	Enhancement of activity

cAMP = cyclic adenosine monophosphate; CGRP = calcitonin gene–related peptide; IL = interleukin; SOM = somatostatin; SP = substance P; VIP = vasoactive intestinal polypeptide.

intestinal peptide (VIP) has a predominant inhibitory action on the immune response. Consequently, changes in neuropeptide levels in the intestinal mucosal can modulate immunity and alter the degree of inflammation. Loss of VIP- and somatostatin-expressing fibers and decreased VIP tissue levels are reported in active Crohn disease and ulcerative colitis,[189–191] whereas substance P levels are generally increased, particularly in ulcerative colitis.[189,192] The exact meaning of these observations to disease pathogenesis is uncertain, but the data are compatible with a decrease of inhibitory and an increase of stimulatory peptides, with a net balance in favor of a proinflammatory response. In postproctocolectomy pouchitis, both VIP and substance P are increased.[193] When gut inflammation is induced by *Clostridium difficile* toxin A, both substance P and neurotensin contribute to neurogenic inflammation.[194,195] Not only neuropeptide levels are abnormal in inflamed tissue; so are the levels of specific receptors, such as those for substance P and somatostatin.[196,197] Owing to the large number and pleiotropic activities of the various neuropeptides, their overall impact on intestinal inflammation still remains to be defined but probably depends on the cause, type, and chronicity of the inflammatory process.

Reactive Oxygen and Nitrogen Metabolites

Although a plethora of different cells and secreted products are involved in inflammation, the vast majority of them act as initiators, mediators, or amplifiers of the inflammatory process, and few directly mediate tissue damage. Among the latter are molecules classified as reactive oxygen and nitrogen metabolites, both of which are abundantly produced during gut inflammation.[198]

Oxygen metabolites are highly reactive molecules that exert a direct cytotoxic effect on a broad scale by degrading amino acids, proteins, and biopolymers (eg, hyaluronic acid, mucin), oxidizing carbohydrates and sulfur-containing compounds, bleaching hemoproteins, causing lipid peroxidation, and inducing DNA strand scission.[54] Reactive oxygen metabolites are evanescent products released by activated polymorphonuclear leukocytes and include both radical and nonradical molecules (Table 3-9). All of them exhibit variable degrees of toxicity on multiple type cells, which explains, in addition to the release of proteolytic enzymes, the tissue-destructive capacity of neutrophils whose presence is the hallmark of inflammation.[199] The presence of neutrophils in the inflamed gut fluctuates depending on the type and phase of the disease process, but reactive oxygen metabolites are invariably produced and acquire particular importance in highly destructive conditions such as inflammatory bowel disease and necrotizing enterocolitis.[200,201] That active oxygen species are produced in heightened quantities by circulating neutrophils and monocytes of patients with intestinal inflammation is well documented,[202] but far more important is the demonstration that reactive oxygen metabolites are generated at the very sites of active inflammation, as observed in both humans and animals.[203,204]

A second group of reactive metabolites that has attracted intense attention in recent years is that of reactive nitrogen metabolites, formed by its major product NO and others resulting from its rapid oxidation, such as NO_2, NO_2^-, N_2O_3, N_2O_4, *S*-nitrosothiols, and peroxynitrite ($OONO^-$).[205] Essential to NO production are the enzymes responsible for its synthesis, NO synthesis (NOSs), which are produced by different cell types and are both constitutive or inducible.[206] NO has a broad range of activities in all tissues and organs of the body, and the gastrointestinal tract is no exception.[207] Elevated production of NO and the inducible form of NOS is extensively documented in the bowel of patients with inflammatory bowel disease and toxic megacolon,[83,208,209] as well as in various models of experimental gut inflammation.[210,211] Although there is general agreement that NO and NOS are intrinsic components of any intestinal inflammatory process, what is still unresolved is whether NO plays a protective and therefore beneficial role or whether its action is predominantly destructive and deleterious to gut tissue.[212] Examples of the noxious effects of NO are its ability to increase epithelial cell permeability and induce mucosal damage.[213,214] An explanation for the existing confusion is partly due to the multitude of actions mediated by NO but also the practical observation that the role of inducible NOS in inflammation varies in different settings and diseases, ranging from those in which the enzyme has a predominantly noxious effect to those in which it appears to benefit the host.[205] The exact roles of NO and NOS need to be better elucidated before modulation of their activity can be considered for their potentially therapeutic effects on intestinal inflammation.

Proteolytic Enzymes

ECM-degrading proteinases (endopeptidases) include aspartic, cysteine, and serine proteinases and metalloproteinases, each of them composed of several distinct enzymes synthesized and released by both immune and nonimmune cells (Table 3-10). The components of the ECM, including most collagens, fibronectin, elastin, laminin, entactin, and heparan sulfate proteoglycan, are susceptible to the destructive action of these proteases. This action is counterbalanced by the protective activity of a large number of endogenous inhibitors that include α_2-macroglobulin, serine protease inhibitors (serpins, kunins, and others), cysteine protease inhibitors (kininogens, stefin, cystatin, and calpastatin), and matrix metalloproteinase (MMP) inhibitors (tissue inhibitors of metalloproteinases (TIMP)-1, -2, and -3).

TABLE 3-9 REACTIVE OXYGEN METABOLITES

TYPE	NAME	STRUCTURE
Radicals	Superoxide anion radical	O_2^-
	Hydroperoxyl radical	HO_2^-
	Hydroxyl	$OH^\cdot$
	Alkoxyl radical	$RO^\cdot$
	Hydroperoxyl radical	$ROO^\cdot$
Nonradicals	Hydrogen peroxide	H_2O_2
	Hydroperoxide	ROOH
	Hypochlorous acid	HOCl
	N-Chloramine	RNHCl

TABLE 3-10 PROTEOLYTIC ENZYMES

ENZYME	MAIN SOURCE	SUBSTRATE
Aspartic proteinases	Lysosome	Collagens
Cysteine proteinases	Lysosome, cytosol	Aggrecan core protein, collagens, fibronectin, elastin
Serine proteinases	Plasma, neutrophils, fibroblasts, endothelial cells, mast cells	Collagens, fibronectin, elastin, heparan sulfate proteoglycan
METALLOPROTEINASES		
Interstitial collagenase (MMP-1)	Fibroblasts	Aggrecan core protein, collagens, fibronectin, elastin, laminin, entactin
Gelatinase A (MMP-2)	Fibroblasts	
Stromelysin (MMP-3)	Fibroblasts	
Matrilysin (MMP-7)	Macrophages	
Neutrophil collagenase (MMP-8)	Neutrophils	
Gelatinase B (MMP-9)	Neutrophils, macrophages, fibroblasts, other mesenchymal cells	

MMP = matrix metalloproteinase.

MMPs are attracting increasing attention as key molecules mediating injury in most tissues because of their ability to degrade all components of the ECM.[215] This is also true in the setting of intestinal inflammation.[216] Direct evidence of ECM degradation in intestinal inflammation is relatively limited, but abnormalities of ECM glycoaminoglycans and loss of glycoaminoglycans in the subepithelial basal lamina and from vascular endothelium are detected in tissues involved by ulcerative colitis and Crohn disease.[217] In these diseases, as well as in peptic ulcers, there is an enhanced expression of the MMPs matrilysin, collagenase, and stromelysin-1,[218] suggesting a cause-and-effect relationship among inflammation, release of proteases, and tissue injury. There is also mounting evidence that the mucosal immune system can trigger events leading to MMP-dependent tissue injury. In an intestinal organ culture model, activation of lamina propria T cells is accompanied by proteolytic degradation of ECM mediated by local release of MMPs.[219,220] These enzymes derive primarily from mucosal mesenchymal cells on stimulation by cytokines produced during the inflammatory reaction, such as TNF-α.[221] This event is inhibited by immunosuppressive cytokines, such as IL-10,[222] and exaggerated by amplifying the activity of Th1 cells with IL-12,[34] indicating that the extent of inflammatory damage is under the control of the strength and the type of ongoing immune responses. However, the degree of damage inflicted to gut tissue exposed to the action of MMPs depends not only on the absolute concentration of these proteases but also the relative proportion of MMPs and TIMPs. In inflammatory bowel disease, there is evidence for MMP overproduction, whereas the expression of TIMPs remains unaltered[223,224]; such an imbalance could well underlie the loss of mucosal integrity.

Extracellular Matrix

The ECM is recognized as an important player in mucosal inflammation because of its crucial role in leukocyte trafficking and activation, wound healing, and fibrosis,[225] as well as the ability to integrate (immune and nonimmune) cell-to-cell and cell-to-matrix interactions.[226] The ECM is a complex protein network secreted by various cell types and forms the intercellular space, where all cells reside.[227] The effects of the ECM are mediated primarily by integrins, a family of cell surface receptors that attach cells to the matrix and mediate mechanical and chemical signals from it.[228] In the gastrointestinal tract, the main components of the ECM are the basement membrane and the interstitial connective tissue matrix. The basement membrane is a specialized sheet-like ECM underlying and essential for adhesion and differentiation of epithelial and endothelial cells. It is composed of a number of multimeric glycoproteins and proteoglycans, including laminins, entactin/nidogen, type IV collagen, fibronectin, and perlecan. The interstitial connective tissue matrix serves as a working environment for all nonanchored cells and is the site of immune and inflammatory reactions. It is synthesized primarily by local mesenchymal cells and contains collagenous and noncollagenous glycoproteins such as the fibrillar collagens (collagen types I, III, and V); glycoproteins such as fibronectin, tenascin, and thrombospondin; and proteoglycans such as versican, decorin, lumican, fibromodulin; and the glycosaminoglycan hyaluronic acid.[227]

The participation of the ECM in intestinal inflammation comprises two domains. The first relates to the functional interaction of the ECM with leukocytes, resulting in the alteration of the migration, activation, and differentiation of these cells.[229] This interaction is influenced by cytokines and chemokines[226] and determines the type and strength of the resulting immune response.[3] There is a paucity of information in this area, but emerging evidence suggests that the ECM profoundly increases its capacity to adhere and retain T cells in inflammatory bowel disease[230] and that leukocyte-matrix interactions regulate mucosal inflammatory responses.[231] The second area of investigation is related to the quantitative and qualitative changes occurring in the intestine during inflammation. Information in this area is more abundant, although it is necessarily restricted to chronic inflammatory processes in which major structural changes are part of the natural history of the disease, as in ulcerative colitis and Crohn disease.[232] In both forms of inflammatory bowel disease, procollagen gene transcripts are increased in sites of inflammation, but they are more abundant in the subepithelial layers in ulcerative colitis and in the deeper layers in Crohn disease, sug-

gesting different regulatory mechanisms in each disease.[233] The same appears to be true in collagenous colitis, in which the subepithelial basement membrane deposit of ECM stain prominently for type VI collagen and tenascin.[234] In contrast, other ECM changes are not disease specific, such as the increased expression of tenascin in colons involved by ulcerative colitis or Crohn disease.[235] Regardless of specificity, changes in ECM translate to an active process of tissue remodeling in the inflamed intestine resulting from the action of agents that both promote and hinder ECM deposition. For instance, TGF-β1 selectively augments collagen synthesis by intestinal smooth muscle cells,[236] a response that contributes to intestinal fibrosis. On the other hand, inflammatory mediators, such as IL-1β, promote intestinal muscle cell proliferation while concomitantly down-regulating collagen synthesis and augmenting collagenase expression.[237] In addition to classic inflammatory mediators, the local intestinal flora also appears to promote intestinal fibrosis by directly stimulating local mesenchymal cells to secrete enhanced amounts of TGF-β1, IL-1β, and IL-6.[124,125]

Cell Adhesion Molecules

The involvement of cell adhesion molecules in inflammation depends on the organ affected and the nature of the inflammatory stimulus. In the gastrointestinal tract,[238,239] it is modulated by cytokines, chemokines, eicosanoids, bacterial products, and complement fragments.[240] Cell adhesion molecules, which can be both cell surface bound and secreted into the intercellular space, are a large number of structurally and functionally related and unrelated molecules forming four major families: the selectin family, which is primarily responsible for leukocyte-endothelial cell interactions; the integrin family, which mediates cell-cell and cell-ECM interactions; the immunoglobulin superfamily, which mediates homophilic adhesion between an identical cell adhesion molecule on another cell; and the cadherin family, which establishes molecular links between adjacent cells (Table 3-11).[241] In intestinal inflammation, cell adhesion molecules of all families are involved, but perhaps the most important are those regulating the adhesion of leukocytes to the vascular endothelial cells and their subsequent translocation into the interstitial space.[53] These include ICAM-1, vascular cell adhesion molecule (VCAM)-1, platelet–endothelial cell adhesion molecule 1, and MAd-CAM-1 of the Ig superfamily; CD11/CD18, very late activation antigen (VLA)-4, and $\alpha_4\beta_7$ of the integrin family; and L-, E-, and P-selectin of the selectin family (Figure 3-1).[242]

The bulk of information on the function and level of expression of cell adhesion molecules in intestinal inflammation derives from studies of chronic inflammatory processes such as inflammatory bowel disease or chronic gastritis.[94,243–246] As expected, there is an active recruitment of leukocytes by the microvascular mucosal beds in areas of active mucosal inflammation,[247] which is associated with a disruption of the normal selectivity of leukocyte-endothelial interaction.[97] This results in abnormal homing patterns of inflammatory cells to both intestinal and extraintestinal sites.[248] In addition, the level of expression of several cell adhesion molecules is increased on both leukocytes and vascular cells (CD11/CD18; VCAM-1; ICAM-1, -2, and -3; E-selectin; and VLA-4), further contributing to the inflammatory response.[94,243–246,249] Addi-

TABLE 3-11 CELL ADHESION MOLECULES

CELL ADHESION MOLECULE	MAIN CELLULAR SOURCE	MAIN LIGAND	MAIN TARGET
Selectin family			
E-selectin	Endothelial cells	L-selectin	Neutrophils, monocytes, T cells
L-selectin	Lymphocytes, neutrophils, monocytes	MAd-CAM-1	Mucosal HEV, endothelial cells
P-selectin	Platelets, endothelial cells	L-selectin	Neutrophils, monocytes, platelets
Integrin family			
LFA-1 (CD11a/CD18)	All leukocytes	ICAM-1, -2, -3	Lymphoid, nonlymphoid cells
LFA-3	All cells, some T cells	CD2	T cells, natural killer cells
Mac-1 (CD11b/CD18)	Neutrophils, monocytes	ICAM-1 and -3, fibrinogen	Lymphoid, nonlymphoid cells, ECM
VLA-1, -2, -3	Collagen, laminin	ECM	
VLA-4	Lymphocytes, monocytes	VCAM-1, fibronectin	Endothelial cells, fibroblasts, monocytes, ECM
VLA-5	Lymphocytes, monocytes	Fibronectin	ECM
VLA-6	Lymphocytes, monocytes	Laminin	ECM
$\alpha_4\beta_7$	Lymphocytes	MAd-CAM-1, fibronectin	Mucosal HEV, ECM
Immunoglobulin superfamily			
ICAM-1 (CD54)	Lymphoid, nonlymphoid cells	LFA-1, Mac-1	Neutrophils, lymphocytes, monocytes
VCAM-1 (CD106)	Endothelial cells, fibroblasts, monocytes	VLA-4, $\alpha_4\beta_7$	Neutrophils, lymphocytes, monocytes
MAd-CAM-1	Mucosal HEV	$\alpha_4\beta_7$, L-selectin	Lymphocytes, monocytes, neutrophils
PECAM-1	Endothelial cells, neutrophils	PECAM-1	Endothelial cells, neutrophils
Cadherin family			
Cadherin, α- and β-catenin	Adjacent cells	Cadherin	Same cell type
Other			
CD44 (Hermes)	Lymphoid, nonlymphoid cells	Hyaluronan, collagen	Endothelial cells, ECM

ECM = extracellular matrix; HEV = high endothelial venules; ICAM = intercellular cell adhesion molecule; LFA = leukocyte function–associated antigen; MAd-CAM = mucosal addressin cell adhesion molecule; PECAM = platelet–endothelial cell adhesion molecule; VCAM = vascular cell adhesion molecule; VLA = very late activation antigen.

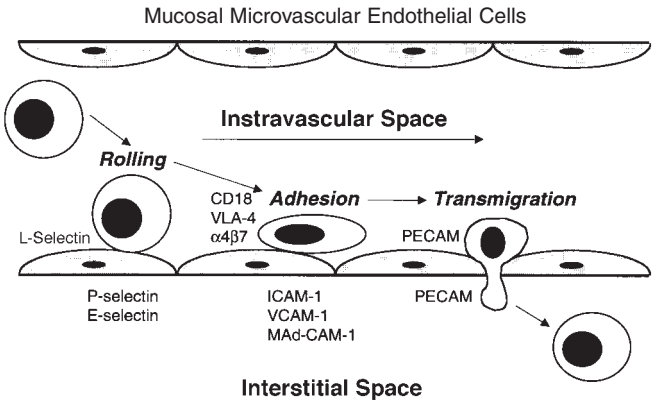

FIGURE 3-1 Major cell adhesion molecules involved in the various steps (rolling, adhesion, and transmigration) necessary to move leukocytes from the intravascular to the interstitial space. Molecules expressed by leukocytes are listed to their left, and molecules expressed by endothelial cells are listed below them. ICAM = intracellular adhesion molecule; MAd-CAM = mucosal addressin cell adhesion molecule; PECAM = platelet–endothelial cell adhesion molecule; VCAM = vascular cell adhesion molecule; VLA = very late activation antigen.

tional information derives from animal models of gastrointestinal inflammation, in which the administration of blocking antibodies confirms the importance of selected cell adhesion molecules in the mucosal inflammatory reaction. Representative examples are CD11/CD18 in rabbit gastritis,[250] ICAM-1 in rat colitis,[251] and $\alpha_4\beta_7$ and VLA-4 in monkey colitis.[95,252]

CONCLUSIONS

Intestinal inflammation is a highly complex phenomenon initiated by a myriad of different triggers and involving all of the components described in this chapter. For the sake of completeness, two additional components deserve mention. One is the endogenous enteric flora, whose capacity to control the overall function of the gastrointestinal tract under physiologic and inflammatory conditions has been underestimated.[253] The second is the phenomenon of apoptosis, which plays a central role in keeping the necessary balance between cell death and survival in the gastrointestinal tract.[254] If this balance is lost, defects of apoptosis can contribute to some forms of intestinal inflammation.[255,256] How these multiple components behave, how much each of them contributes to inflammation, and how they functionally interact among themselves will depend on the quality and quantity of the initial stimulus and the genetic makeup of the host. Together, they will ultimately determine the type, strength, and duration of the immune response and whether physiologic or pathologic inflammation will ensue (Figure 3-2). Each portion of the gastrointestinal tract displays specialized features that reflect adaptation to particular physiologic and metabolic requirements so that the outcome of an inflammatory process may vary in different segments of the intestine. In spite of diversity, however, gut inflammation is primarily a stereotypical event mediated by common pathways of tissue injury regardless of the initiating event.[257]

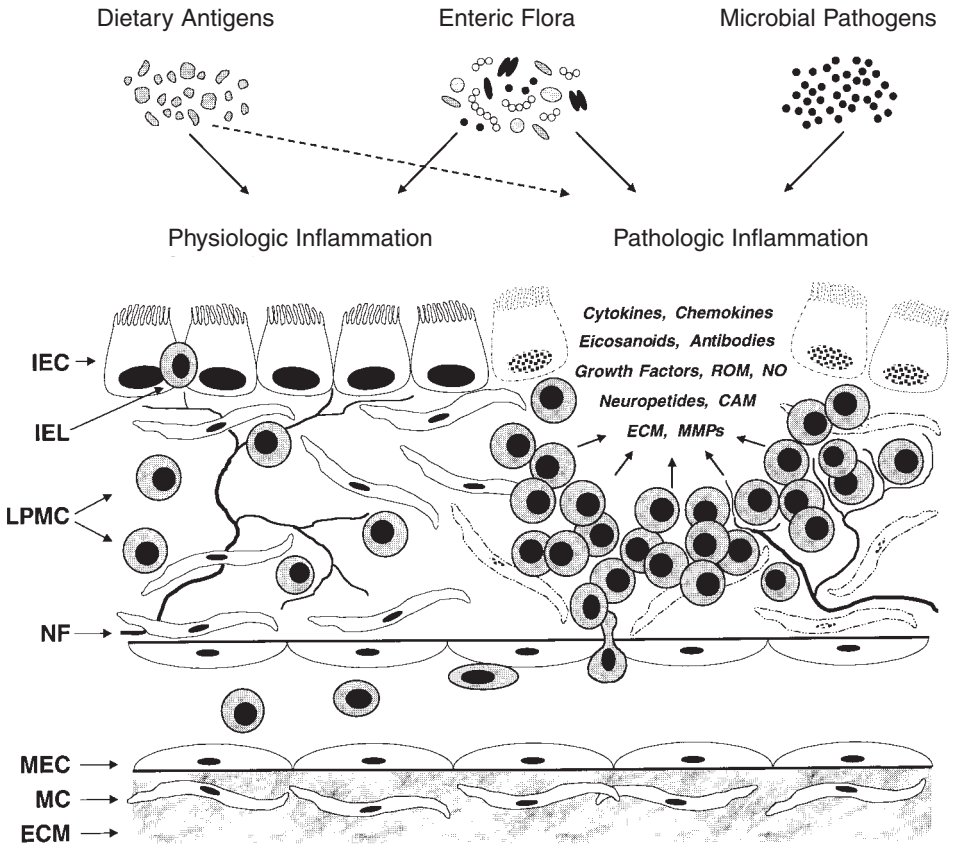

FIGURE 3-2 Key components of intestinal inflammation. In response to antigens derived from the diet and the normal enteric flora, a controlled physiologic inflammatory response is induced by the various cellular and soluble immune and nonimmune elements normally present in the intestinal mucosa. Depending on circumstances, microbial pathogens, the enteric flora, or selected dietary antigens induce a pathologic inflammatory response mediated by increased numbers of local immune and nonimmune cells and blood-derived immune cells and enhanced secretion of multiple soluble mediators. CAM = cell adhesion molecule; ECM = extracellular matrix; IEC = intestinal epithelial cells; IEL = intraepithelial lymphocytes; LPMC = lamina propria mononuclear cells; MC = mesenchymal cells; MEC = microvascular endothelial cells; MMPs = matrix metalloproteinases; NF = nerve fibers; NO = nitric oxide; ROM = reactive oxygen metabolites.

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CHAPTER 4

MOTILITY

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Gastrointestinal motility is the result of the muscular activity of the gut, responsible for mixing and propulsion of ingesta from mouth to anus. It consists of organized patterns of contractions and relaxations of enteric smooth muscles. Regional specialization gives rise to functions such as rapid propulsion, as in swallowing, vomiting and defecation, storage, mechanical grinding, controlled delivery, mixing, dispersion, and expulsion. Tonically contracted sphincters function to limit retrograde flow of ingesta. Unique biochemical and mechanical properties of smooth muscle myocytes reflect adaptation to these specialized functions.

Physiologic patterns of motility require the coordinated activity of enteric and autonomic nerves, interstitial cells of Cajal (ICC), and gastrointestinal smooth muscle cells and are modulated by different hormones. Contractions of gastrointestinal smooth muscle are initiated by specialized pacemaker cells. Propagation of contractions occurs as waves of membrane depolarization spread via gap junctions between adjacent smooth muscle cells. Regulatory neural input originates either from local reflexes within the enteric nervous system (ENS) or from the autonomic division of the central nervous system (CNS). Similarly, hormonal regulation occurs by both local paracrine and systemic endocrine mechanisms.

In this chapter, we review recent advances in knowledge about motility, including elucidation of the complex interplay between enteric neurons, smooth muscle, and the immune system and the role of the ICC as pacemaker cells of the gut. The many signaling molecules currently being explored for their physiologic roles and their potential as therapeutic targets are discussed. Further, the chapter outlines developing knowledge of learning and plasticity within the ENS. These processes are fundamental to common gastrointestinal conditions such as irritable bowel syndrome and to developmental and acquired lesions of the ENS. Although the control of motility is intimately linked with gastrointestinal sensation and the regulation of secretion, absorption, and blood supply, with changes in any one of these areas affecting all of the others, the current review focuses on motility. Familiarity with all of these new insights should facilitate understanding of gastrointestinal motility and of the many novel therapeutic strategies being developed to treat dysmotility syndromes.

This chapter discusses the structure and function of each of the neuromuscular components of motility and then outlines their integrated activity in normal and abnormal motility states. Detailed discussions of normal and disordered regional motility are given in Chapter 23, "Disorders of Deglutition"; Chapter 26, "Other Motor Disorders"; Chapter 31, "Motor Disorders including Pyloric Stenosis"; and Chapter 46, "Hypomotility Disorders." Diagnostic techniques (see Chapter 70, "Gastrointestinal Manometry: Methodology and Indications") and drug therapy for motility disorders (see Chapter 76.3, "Motility") are also covered elsewhere in this book in detail.

NEUROMUSCULAR COMPONENTS OF GASTROINTESTINAL MOTILITY

Gastrointestinal Smooth Muscle

The digestive tract from the posterior pharynx to the anus has two major muscular layers: the muscularis externa and the muscularis mucosae. In general, each layer consists of an inner circular and an outer longitudinal layer. Regional modifications of this schema include the addition of a third, oblique layer to the inner aspect of the muscularis externa in the stomach and the condensation of the outer longitudinal layer of the muscularis externa in the colon into three longitudinal bands, the tenia coli. Within each layer, smooth muscle fibers are oriented parallel to one another and communicate with one another via gap junctions, forming a functional syncytium, which transmits waves of depolarization and permits coordinated contraction.[1] Gastrointestinal myocytes are spindle-shaped cells 200 to 300 μm in length and 10 to 20 μm in diameter. Myocytes in neonates are up to 50% smaller. During contraction, gastrointestinal myocytes shorten up to 25%.

Within each smooth muscle cell, the contractile machinery consists of a complex of proteins: actin, myosin, and the regulatory proteins calmodulin and caldesmon. In contrast to the contractile proteins in cardiac and skeletal muscle, actin and myosin in smooth muscle are not arranged in regular arrays and cross-striations are not present. Instead, they are arranged in a net-like structure, with actin filaments anchored to dense bodies in the cytoplasm and on the cell membrane by α-actinin. Mechanical force

is transmitted at points where dense bodies on adjacent cells align, termed intermediate junctions.

Myogenic Control of Electrical and Mechanical Activity. Myogenic mechanisms control the rate and propagation of enteric contractions. Smooth muscle cells in the gastrointestinal tract exhibit spontaneous periodic depolarizations from a resting membrane potential of –50 to –60 mV. These depolarizations, also called electrical slow waves, govern both the maximum frequency and the direction of propagation of phasic contractions. The depolarizations are triggered by specialized pacemaker cells, the ICC. The morphology of these slow-wave depolarizations is similar to those in cardiac muscle (Figure 4-1), with a rapid upstroke, followed by a plateau phase owing to influx of extracellular calcium, and then a rapid repolarization.

Without additional stimulus, these spontaneous depolarizations increase membrane potential to only –40 to –50 mV and do not reach the excitation threshold necessary for cell contraction. If, however, an excitatory stimulus such as acetylcholine (ACh) from enteric nerves is present at the time of a spontaneous depolarization, the membrane depolarizes further. When it reaches the excitation threshold, depolarization spikes are superimposed on the plateau phase (see Figure 4-1). These spikes depolarize the membrane to –30 to 0 mV. Calcium influx is maximal at this voltage. Further calcium-induced calcium release occurs, with liberation of additional calcium from intracellular stores. This increase in intracellular free calcium initiates a cascade of signaling pathways, which cause myocyte contraction; this mechanism is termed electromechanical coupling. Thus, neural stimulation modulates but does not initiate smooth muscle depolarization. Neurotransmitters regulating smooth muscle contraction are listed in Table 4-1. The maximum frequency of phasic contractions at a given site is governed by the rate of slow-wave depolarization, but the actual frequency of contractions is determined by the number of slow waves that have concurrent excitatory stimulation and spike depolarizations.

Electromechanical Coupling. When the intracellular calcium concentration rises during depolarization, calcium binds to calmodulin, a regulatory protein, which has dual actions. Calcium-activated calmodulin binds to a second regulatory protein, caldesmon, which undergoes a conformational change to expose the myosin-binding domain of actin. The calcium-calmodulin complex also activates the enzyme myosin light-chain kinase. Phosphorylation of myosin light chains by this enzyme activates the adenosine triphosphate (ATP)-hydrolyzing region on the head portion of the myosin heavy chain, resulting in cross-bridge formation with actin. Cross-bridge formation and conformational change in the myosin head result in fiber shortening and muscle contraction, the so-called "myosin motor." When intracellular calcium subsequently falls during cell repolarization, myosin is dephosphorylated, and relaxation occurs. One phasic contraction occurs per slow wave when spike depolarizations are present.[4]

Conduction of Depolarization. Propagating contractions are generated when adjacent areas of gastrointestinal smooth muscle depolarize in sequence. Because adjacent myocytes are connected by gap junctions, waves of depolarization can spread rapidly through the tissue. Myocytes with faster cycles of depolarization and repolarization can entrain adjacent cells of lower intrinsic frequency, such that all myocytes at a given site contract almost simultaneously at the rhythm of the fastest cell. The cycling time for spontaneous membrane depolarizations increases along the intestine.[5] This allows for antegrade propagation of contractions along the

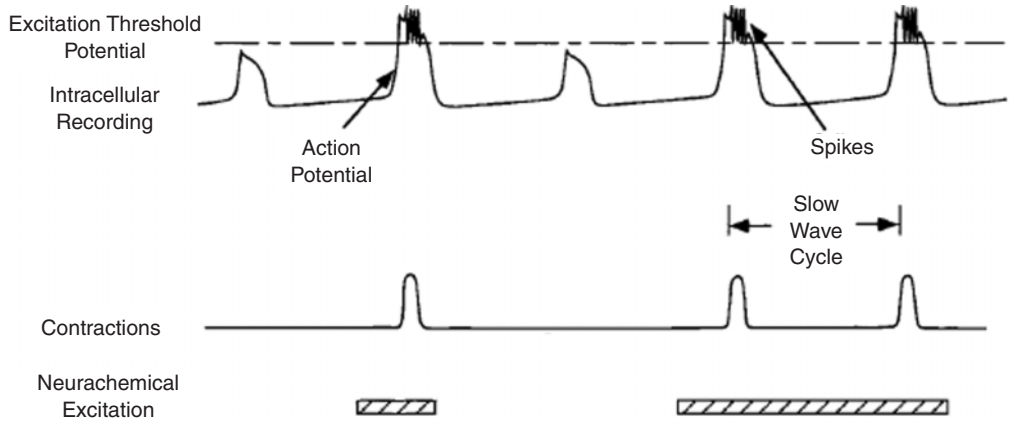

FIGURE 4-1 Illustration of regulation of rhythmic phasic contractions by slow waves and spikes. The resting potential of smooth muscle cells is negative with respect to the extracellular fluid potential. The depolarization during spontaneous slow waves does not exceed the excitation threshold; therefore, no contractions occur. The release of acetylcholine by neurochemical excitation depolarizes the plateau phase of the slow waves beyond the excitation threshold; spikes are superimposed on the plateau phase and the cell contracts. Adapted with permission from Sarna SK. In vivo myoelectric activity: methods, analysis and interpretation. In: Wood JD, editor. Handbook of physiology, section 6: the gastrointestinal system. Vol. 1. Motility and circulation. Bethesda (MD): The American Physiological Society; 1989. p. 817–63.

TABLE 4-1 PHYSIOLOGIC STIMULI AND INHIBITORS OF GASTROINTESTINAL SMOOTH MUSCLE CONTRACTION

AGENT	MECHANISM	INTRACELLULAR SECOND MESSENGER	RESULT
Stimuli			
Acetylcholine	Muscarinic receptors (GPCR)	Phospholipase C and IP_3 (M_3); ↓ cAMP (M_2)	↑ Ca
Substance P and neurokinin A	Neurokinin receptors (GPCR)	Phospholipase C and IP_3	↑ Ca
Mechanical stretch	Stretch-activated Ca channels[2]	None	↑ Ca
Inhibitors			
Nitric oxide	Diffusion into cells	↑ cGMP	↓ Ca
Vasoactive intestinal polypeptide (VIP)	VIP receptors (GPCR)	↑ cAMP	Hyperpolarizes membrane (activates K channels)
Adenosine triphosphate	P_2 purinergic receptors (GPCR)[3]	Phospholipase C and IP_3 and ↓ cAMP	Hyperpolarizes membrane (activates K channels)
Catecholamines	Beta receptor	↑ cAMP	↓ Ca

cAMP = cyclic adenosine monophosphate; cGMP = cyclic guanosine monophosphate; GPCR = G protein–coupled receptor; IP_3 = inositol 1,4,5-triphosphate.

gut as depolarization spreads from cells of faster intrinsic slow-wave rhythm to those with slower cycles. The maximum contraction frequency at any point is governed by the slow-wave frequency; in the duodenum, it is 11 to 12 per minute, decreasing to 7 to 8 per minute in the terminal ileum. The distance over which a contractile wave is propagated is determined by the length of gut receiving excitatory stimulation at the time of the contraction.

Specializations in Smooth Muscle for Continuous Contraction. In addition to the syncytial nature of gastrointestinal smooth muscle, other differences between skeletal and smooth muscle represent specializations that permit prolonged continuous contraction. Gastrointestinal sphincters have increased numbers of mitochondria and smooth endoplasmic reticulum compared with nonsphincter muscle, providing energy and calcium needed for prolonged contraction. The initiation of contraction in response to increased intracellular calcium is slower in smooth muscle myocytes, and the contraction duration is longer. Cycling of myosin cross-bridges, that is, the attachment of myosin to actin, release, and reattachment, occurs much more slowly in smooth muscle. Also, the fraction of time in which myosin is attached to actin is greater. As one molecule of ATP is required for each cross-bridge cycle, the longer cycle length considerably reduces the energy required to sustain contraction in smooth muscle cells. Once the smooth muscle contracts, full force can be maintained with little additional energy expenditure. This is called the "latch" mechanism. The latch mechanism permits the sphincter sustained contraction with little requirement for ongoing stimulation from nerves or hormones.

Gastrointestinal Myopathies. Disorders of smooth muscle that result in motility disturbance may be familial or sporadic, primary or secondary to a variety of conditions, such as autoimmune myositis, muscular dystrophies, Ehlers-Danlos syndrome, and connective tissue diseases, especially scleroderma. For a detailed account of visceral myopathies and neuropathies, the reader is referred to Chapter 46.3.

The effect of myopathy on gastrointestinal function is the reduction of the amplitude of contractions while preserving normal temporal and spatial coordination (Figure 4-2). Milder cases may present with abdominal pain, nausea, or constipation. More severe cases manifest as chronic intestinal pseudo-obstruction. Detection of concurrent extraintestinal involvement may assist in differential diagnosis. Urinary tract dysfunction, such as when megacystis is diagnosed, suggests hollow visceral myopathy; however, cases of hollow visceral disease may also be due to neural abnormalities.[6–9] Alternatively, the presence of progressive ptosis, external ophthalmoparesis, peripheral neuropathy, deafness, and lactic acidosis suggests mitochondrial neurogastrointestinal encephalomyopathy.[10,11] In this condition, a nuclear gene responsible for maintenance of mitochondrial deoxyribonucleic acid (DNA) is mutated, resulting in a mitochondrial disease phenotype that is inherited as an autosomal recessive trait.[12] Motility and pathologic features of both myopathy and neuropathy are present.

Enteric Neuromuscular Junction

Role of ICC in Enteric Neurotransmission. Previously, it was believed that enteric nerves controlled smooth muscle activity via diffusion of neurotransmitters released from varicosities located along nerves as they passed through the smooth muscle.[13,14] Neurotransmitters were

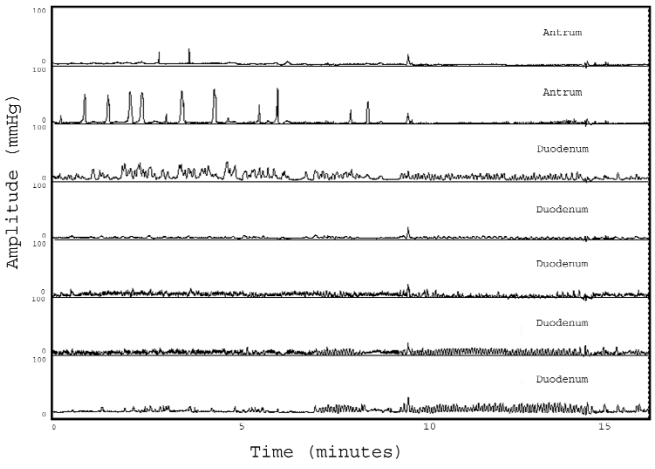

FIGURE 4-2 Fasting antroduodenal manometry in visceral myopathy: contraction amplitudes are reduced, but temporal organization is preserved.

believed to diffuse across relatively large spaces (up to hundreds of nanometers) to activate many nearby smooth muscles directly.[14] This was called volume theory or en passant innervation.[15] However, in recent years, it has become apparent that neural input is mediated by a specialized group of cells, the ICC,[16–20] which are the primary target of the ENS. ICC are critical components of the enteric neuromuscular junction (Figure 4-3), integrating and modulating neural input. Their presence is fundamental to normal motility.

Networks of ICC are intercalated between enteric nerve and smooth muscle cells.[16,17,21,22] Both excitatory[20,23,24] and inhibitory[18,19,24] enteric motoneurons are closely associated with ICC. Immunoelectron microscopy has revealed specialized synapse-like contacts between enteric neurons and ICC.[24] Far fewer contacts exist between enteric motoneurons and smooth muscle cells.[25]

Animals lacking ICC show severely impaired enteric neurotransmission, despite the presence of normal innervation and neurotransmitter release.[18–20] Such animals have a phenotype consistent with intestinal pseudoobstruction. The mediating effect of ICC at the enteric neuromuscular junction is also the likely reason for observed differences between responses to neural stimulation and to application of exogenous neurotransmitters such as ACh to enteric smooth muscle.[26] Current research aims to identify specific pharmacologic agents to modulate enteric neurotransmission at the level of the ICC.

ICC are modified smooth muscle cells expressing a receptor tyrosine kinase called kit,[27] which is important in the proliferation of ICC[28] and in their identification for scientific study. Apart from their critical role in enteric neurotransmission, ICC are now known to be the pacemakers of gastrointestinal smooth muscle.[27,29–31] Rhythmic depolarizations in ICC are the basis for enteric slow waves and phasic contractions throughout the gastrointestinal tract.[32–36] They are essential for coordinated peristaltic contractions.[32] In this sense, they resemble cardiac pacemaker (Purkinje) cells, which are also modified smooth muscle cells exhibiting spontaneous rhythmic depolarization. ICC depolarizations are the result of rhythmic inward currents that are unique to ICC.[34] Recent research demonstrates that high-conductance chloride channels are involved in spontaneous ICC depolarizations,[36] but other ion channels are also likely to contribute.

Although the ICC receive neural input via chemical neurotransmitters across synapse-like structures, they communicate with smooth muscle cells via gap junctions.[21,22,37] This permits rapid transmission of coordinated electrical activity and muscle contraction.

Diseases Associated with Abnormalities of ICC. Theoretically, ICC could be congenitally absent, displaced from their normal distributions, or damaged by infections, metabolic derangements (such as hyperglycemia or uremia), toxins, or autoimmune attack. Abnormal ICC networks have been identified in chronic constipation,[38] intestinal pseudoobstruction,[39–42] hypertrophic pyloric stenosis,[43] inflammatory bowel disease,[44] dysmotility associated with diabetes,[45,46] and paraneoplastic syndrome.[47] However, whether

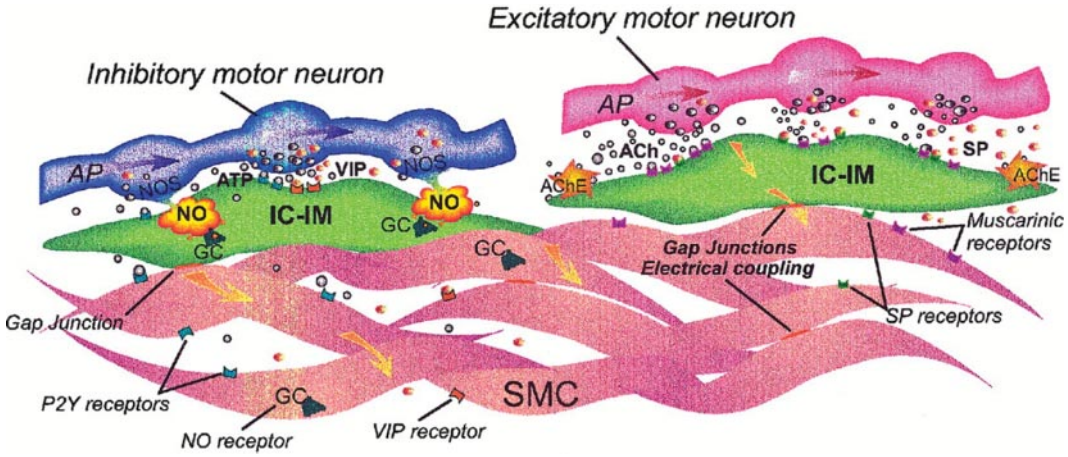

FIGURE 4-3 Diagram of the enteric neuromuscular junction. Neuromuscular junctions in gastrointestinal muscles are composed of enteric nerve fibers, interstitial cells of Cajal (ICC:IC-IM = intermuscular ICC in esophagus, stomach, colon, and sphincters; IC-DMP = deep muscular plexus ICC in the small intestine), and smooth muscle cells (SMC). When action potentials (AP) invade varicosities, stored transmitters are released and enzymes responsible for de novo transmitters are activated (ie, nitric oxide [NO] is made by nitric oxide synthase [NOS]). The close apposition between varicose nerve terminals and ICC facilitates rapid diffusion to ICC receptors. ICC are electrically coupled to smooth muscle via gap junctions, and electrical responses elicited in ICC are conveyed to smooth muscle cells via electrical conduction. ICC express receptors for major neurotransmitters: inhibitory transmitters NO, soluble guanylyl cyclase (GC), vasoactive intestinal polypeptide (VIP), and adenosine triphosphate (ATP) and excitatory transmitters acetylcholine (ACh) and substance P (SP). Extrajunctional receptors are also expressed by smooth muscle cells, but these may or may not be connected to the same cellular effectors as the receptors in ICC. Smooth muscle receptors may be "spare receptors" under most circumstances and may not receive stimulation from neurotransmitters released from neurons because diffusion distances are too great or metabolic enzymes (eg, acetylcholine esterase [AChE]) break down transmitters before effective concentrations can diffuse to extrajunctional receptor sites. Adapted from Ward SM, Sanders KM. Interstitial cells of Cajal: primary targets of enteric motor innervation. Anat Rec 2001;261:125–35.

the loss of ICC function is causative in the pathophysiology or a secondary phenomenon is unknown.[48] Delayed maturation of ICC may be a mechanism for transient dysmotility in infants.[39,43,49] Because of their role in neuromuscular transmission, the absence of ICC might result in a motility pattern similar to a neuropathic process.[42]

Nerves

Autonomic Innervation of the Gastrointestinal Tract. The gut has a rich and complex intrinsic nervous system, the ENS, which controls all gastrointestinal activities. However, the activities of the ENS are modulated by input from the CNS (Figure 4-4). Extrinsic innervation comprises parasympathetic and sympathetic neurons. Parasympathetic fibers from the vagus control motility, secretion, and absorption from the esophagus to the mid-transverse colon. Sacral parasympathetic fibers control distal colonic and rectal functions. Sympathetic nerve fibers from thoracic segments provide secretomotoneurons containing transmitters such as vasoactive inhibitory polypeptide (VIP), presynaptic cholinergic nerve endings, and nerves to submucosal blood vessels and sphincters.[50]

Enteric Nervous System. Congenital or acquired abnormalities of the ENS underlie many gastrointestinal diseases. Recent research has exponentially increased knowledge about this complex network of intrinsic neurons. Intramural enteric nerves are organized into two major plexuses of ganglia linked by bundles of axons: the myenteric (Auerbach) and submucous (Meissner) plexuses. For many years, it was believed that these ganglion cells were simply the postganglionic cells of the parasympathetic nervous system, acting as simple relay stations for signals from the CNS. However, recently, it has become clear that most ENS neurons receive no direct innervation from the CNS. The ENS contains complete neural circuits, which are capable of controlling many gastrointestinal functions independently of the CNS.[51–53] Programs for complex gastrointestinal motility patterns such as the interdigestive migrating motor complex (MMC) of the small intestine reside entirely in the ENS. This is evident after complete extrinsic denervation, with persistence of the MMC even after small bowel transplant.[54] Like the CNS, the ENS contains neurons, interneurons, and glial cells. There are more neurons in the ENS than in the spinal cord. All major neurotransmitters present in the CNS are also present in the ENS (Table 4-2). These facts have led to the ENS being termed "the little brain in the gut."

The ENS is composed of sensory and motoneurons as well as interneurons. The complex network of specialized enteric neurons is illustrated in Figure 4-5 and is reviewed in detail by Furness.[53] All gastrointestinal motor and secretory activity is controlled by the ENS; efferent neurons supply smooth muscle cells, mucosal secretory cells, neuroendocrine cells, blood vessels, and the immune and inflammatory cells involved in mucosal immunologic, allergic, and inflammatory responses.[55] Local reflexes control peristalsis,[56] fluid and electrolyte secretion[57,58] and absorption,[59,60] and mucosal blood flow.[61,62]

Origins of ENS Cells. ENS neurons arise from neural crest cells migrating from the vagal and sacral segments of the fetal CNS. A subset of foregut neurons arise from truncal somites.[63] Vagal cells predominate,[64] populating the entire length of the gut, with sacral neural crest cells comprising less than 20% of neurons in the distal bowel.[65] Migration of vagally derived neural crest cells occurs craniocaudally, with cells appearing in the rectum at 12 weeks gestation.[66] The myenteric plexus forms first, and then the submucous plexus is formed by myenteric neuroblasts migrating inward across the circular muscle layer.[66] Interruption of normal migration leads to distal aganglionosis

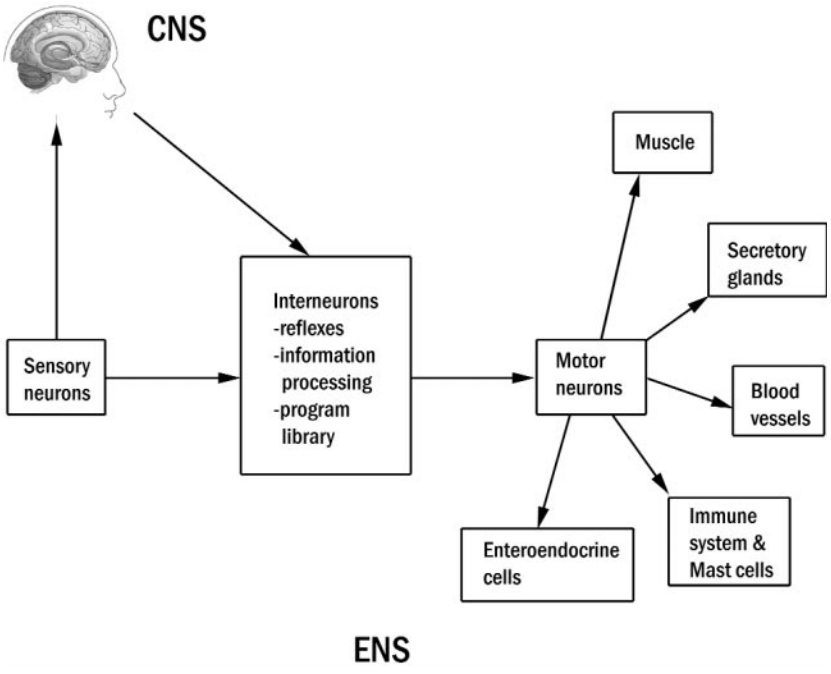

FIGURE 4-4 Relationship between the central nervous system (CNS) and the enteric nervous system (ENS) in control of gastrointestinal motility.

TABLE 4-2 PUTATIVE NEUROTRANSMITTERS IN THE ENTERIC NERVOUS SYSTEM

AMINES
Acetylcholine
Histamine
Norepinephrine
Serotonin (5-hydroxytryptamine)
AMINO ACIDS
γ-Aminobutyric acid
Glutamate
GASES
Carbon monoxide
Nitric oxide
PURINES
Adenosine triphosphate
PEPTIDES
Bombesin
Calcitonin gene–related peptide
Cholecystokinin
Galanin
Gastrin-releasing peptide
Neuromedin U
Neuropeptide Y
Neurotensin
OPIOIDS
Dynorphins
Enkephalins
Endorphins
PEPTIDE YY
PITUITARY ADENYLYL CYCLASE–ACTIVATING PEPTIDE
SOMATOSTATIN
TACHYKININS
Substance P
Neurokinin A
Neurokinin B
THYROTROPIN-RELEASING HORMONE
VASOACTIVE INTESTINAL POLYPEPTIDE

Adapted from Rolle U et al[55] with permission from Elsevier.

(ie, Hirschsprung disease). Mutations in several genes that control neural crest cell survival, migration, proliferation, differentiation, and ganglion formation have been described in patients with Hirschsprung disease. These include *RET*,[67] the endothelin B receptor gene,[68] glial cell line–derived neurotrophic factor,[69–71] and endothelin 3.[72] Interactions between susceptibility genes may explain the complex multigenic inheritance observed in this condition.[73,74] Genetic abnormalities in Hirschsprung disease are reviewed in Chapter 46.3, "Hirschsprung Disease."

Transmission in the ENS. Within the ENS, synaptic transmission occurs by the same mechanisms as elsewhere in the nervous system. Depolarization of the presynaptic cell results in calcium-mediated exocytosis of vesicles of stored neurotransmitters into the synaptic cleft. Neurotransmitters diffuse across the cleft to bind to receptors on the postsynaptic cell membrane. The resulting cellular response is mediated by two broad types of receptors. In ionotropic receptors, the receptor itself is an ion pore. Binding of neurotransmitter to ionotrophic receptors causes rapid changes in membrane potential by altering the permeability of the ion channel. For example, nicotinic ACh receptors are cation channels that become permeable to sodium and calcium ions when activated by ligand binding. In contrast, metabotropic receptors use intracellular second messengers, such as cyclic adenosine monophosphate, to mediate their effects. Many of these receptors belong to the G protein class of membrane receptors.[75] Cellular responses to metabotropic receptor stimulation are usually slower than those elicited by excitation of ionotrophic receptors. Examples of neurotransmitters that operate via metabotrophic receptors are ACh at muscarinic receptors, VIP, and most other neuropeptides. Neurotransmitters also stimulate presynaptic receptors. This occurs both on the cell of origin and on other adjacent neurons to produce feedback effects in the presynaptic cell and to modify the activities of nearby neurons. Eventually, the neurotransmitters are inactivated by enzymatic degradation or reuptake.

Neurotransmitters in the ENS. Currently, approximately 30 putative neurotransmitters have been recognized in the gastrointestinal tract (see Table 4-2). Excitatory motoneu-

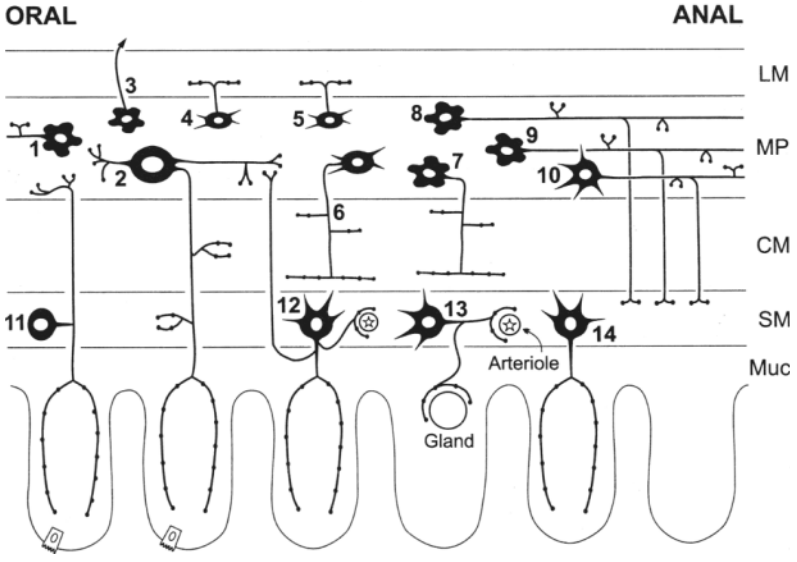

FIGURE 4-5 The types of neurons in the small intestine of the guinea pig, all of which have been defined by their functions, cell body morphologies, chemistries, and projections. 1 = ascending interneuron; 2 = myenteric intrinsic primary afferent neuron; 3 = intestinofugal neuron; 4 = excitatory longitudinal muscle motoneuron; 5 = inhibitory longitudinal muscle motoneuron; 6 = excitatory circular muscle motoneuron; 7 = inhibitory circular muscle motoneuron; 8 = descending interneuron (local reflex); 9 = descending interneuron (secretomotor reflex); 10 = descending interneuron (migrating myoelectric complex); 11 = submucosal intrinsic primary afferent neuron; 12 = noncholinergic secretomotor/vasodilator neuron; 13 = cholinergic secretomotor/vasodilator neuron; 14 = cholinergic secretomotor (nonvasodilator) neuron. CM = circular muscle; LM = longitudinal muscle; MP = myenteric plexus; Muc = mucosa; SM = submucosal plexus. Adapted from Furness JB.[53] Copyright 2000, with permission from Elsevier.

rons mostly release ACh, which is the predominant excitatory neurotransmitter throughout the gut. The constipating effect of anticholinergic medications demonstrates the importance of basal ACh stimulation for normal gastrointestinal function. Tachykinins such as substance P provide additional motor stimulation and are important in secretory and sensory function. Mast cells and enteric nerves participate in the regulation of substance P–induced intestinal secretion.[76] Enteric neurons often contain several neurotransmitters. Motoneurons are immunoreactive for both ACh and tachykinins. Similarly, inhibitory neurons may contain several inhibitory transmitters, including nitric oxide (NO), ATP, VIP, and pituitary adenylyl cyclase–activating peptide (PACAP).[53] Other ENS neurotransmitters include 5-hydroxytryptamine (5-HT)[77] and histamine, both of which have complex effects at various receptors. The following paragraphs will focus on recent developments in the understanding of some key enteric neurotransmitters: tachykinins, VIP, PACAP, NO, and 5-HT.

Tachykinins. Substance P and other tachykinins (neurokinins A and B) are important excitatory neurotransmitters in the ENS. ICC and smooth muscle cells have different neurokinin receptors, NK1 and NK2, respectively.[75] Although there is some cross-reactivity of different neurokinins for different receptors, the differential distribution of NK1 and NK2 likely confers differential responses to neurokinin released from excitatory motoneurons. In addition to their motility effects, tachykinins are important in the control of gastrointestinal secretion and sensation.

Nitric Oxide. NO is the major inhibitory neurotransmitter mediating neurogenic smooth muscle relaxation in the gastrointestinal tract. Nitrergic neurons make up 34% of all neurons in the myenteric plexus.[78] Deficiencies of nitrergic neurons have been associated with achalasia of the cardia,[79] pyloric stenosis,[80] Hirschsprung disease,[81] and internal anal sphincter achalasia.[82] Histochemistry for NO synthase activity has been used in the diagnosis of Hirschsprung disease.[83] It has recently been advocated as a simple, reliable, and rapid means for intraoperative determination of the extent of aganglionosis.[84,85] The inhibitory action of NO has been exploited clinically with the use of topical nitrates to relax the anal sphincter in conditions such as anal fissure.

VIP *and* PACAP. VIP- and PACAP-containing neurons are found in both the myenteric and submucosal plexuses. Although VIP was previously considered an endocrine hormone, it is now considered a neurocrine because all circulating VIP appears to be derived from neurons. VIP and PACAP are involved in the descending inhibitory reflex of peristalsis and in the regulation of secretion.

5-HT. Ninety-five percent of the body's 5-HT (serotonin) is present in the wall of the gut. It is present in enteric neurons, where it acts as a neurotransmitter, and in enterochromaffin cells, where paracrine release of 5-HT mediates sensory transduction. 5-HT elicits a wide range of responses throughout the gut owing to the presence of many different receptor types with varying mechanisms of action. In fact, the multiple different 5-HT receptor types make this the largest of all known neurotransmitter families. In general, 5-HT_1 receptors relax gastrointestinal smooth muscle, whereas 5-HT_2, 5-HT_3, and 5-HT_4 receptors are involved in contractile responses.[86]

As a neurotransmitter, 5-HT is synthesized, stored, and released and undergoes reuptake in enteric nerves. Reuptake is by the same mechanisms as in serotonergic neurons in the CNS and is antagonized similarly by antidepressant medications such as tricyclic antidepressants and selective serotonin reuptake inhibitors.[77] Slow excitatory postsynaptic potentials that depolarize myenteric neurons and increase their excitability are mediated by the 5-HT_{1P} receptor. Fast excitatory postsynaptic potentials can be mediated by 5-HT_3 receptors, although most fast excitatory postsynaptic potentials are due to cholinergic or purinergic mechanisms. Other receptor types on neurons include 5-HT_4, which facilitates cholinergic fast neurotransmission via presynaptic enhancement of ACh release. By contrast, stimulation of 5-HT_{1A} receptors inhibits some classes of neurons.

Enterochromaffin cells have important sensory functions (see below), releasing 5-HT to activate intrinsic and extrinsic primary afferent neurons. 5-HT then initiates a variety of responses, including peristalsis and secretion, mediated by intrinsic nerves, and nausea and vomiting via extrinsic afferents. Stimulation of intrinsic sensory nerves in the submucosal plexus occurs via 5-HT_{1P} and 5-HT_4 receptors, whereas 5-HT_3 is the predominant receptor type on extrinsic afferent nerves.

The presence of different receptor types for 5-HT on intrinsic and extrinsic afferent nerves allows selective pharmacologic manipulation, and this is currently the subject of intensive research. For instance, selective blockade of 5-HT_3 receptors on extrinsic afferent nerves by drugs such as ondansetron provides effective relief of nausea without interfering with intrinsic enteric reflexes. Drugs that stimulate or antagonize 5-HT_3 and 5-HT_4 receptors are currently being evaluated in the treatment of dysmotility and visceral hypersensitivity (see Chapter 76.3).

Sensation in the ENS. ENS control of gastrointestinal motility in response to the chemical and physical nature of the luminal contents relies on sensory input from chemical and mechanosensors within the gut wall. ENS sensation has recently been reviewed in detail.[87]

Three types of detectors are present: neurons, enteroendocrine cells, and immune cells. Sensory neurons include varieties with cell bodies and connections entirely in the gut wall, called intrinsic primary afferent neurons (IPANs), those with cell bodies in the ENS, and projections outside the gut, called intestinofugal neurons, and autonomic neurons with bodies in the brain or dorsal root ganglia.

IPANs are sensory nerves located in the myenteric and submucosal plexuses. Some project to the gut mucosa (see Figure 4-5) and respond to chemical stimuli, such as acid, alkali, and short-chain fatty acids, or to small movements of intestinal villi.[87] Projections from submucosal IPANs transmit information to the myenteric plexus to initiate local reflexes.

Mechanosensors, or stretch receptors, in the wall of the gut include three types of IPANs in the myenteric plexus,[88] as well as enterochromaffin cells (see below). There are

both slow-adapting and fast-adapting mechanosensitive neurons. Fast-adapting units signal the onset of a mechanical stimulus with a brief discharge and then cease firing even during prolonged stimulation. The slow-adapting neurons continue to fire for the duration of the mechanical stimulus, with a discharge frequency proportional to the intensity of the stimulus.[89] A third class of mechanosensors responds to mechanical stimulation with a prolonged burst of spike discharges that continues for a period after the cessation of the original stimulus.

Reflexes such as enteroenteric inhibitory reflexes are mediated by intestinofugal neurons and pass through the prevertebral sympathetic ganglia. CNS sensory pathways are involved in (1) control of organs distant to the site of a sensory stimulus (such as receptive gastric relaxation in response to fat in the duodenum), (2) integration of inputs from multiple sites and/or multiple different sensory modalities (such as the perception of satiety after a meal), and (3) coordinated control of several organs (such as swallowing and gastric receptive relaxation).

In addition to sensory neurons, mast cells and enterochromaffin cells perform sensory functions. Both cell types relay sensory information by paracrine means, secreting histamine and 5-HT, respectively. Mast cells provide the gut with specific immune memory for potentially damaging antigens. When mast cells in the epithelium encounter an antigen to which they have previously been sensitized, degranulation of histamine, prostaglandins, and leukotrienes occurs. Histamine signals to the ENS to increase secretion and to initiate motor activity in order to expel the noxious substance.[90–93]

Enterochromaffin cells function as pressure sensors, initiating the peristaltic reflex in response to pressure on the mucosa by food after a meal. In response to shear forces on the mucosa, enterochromaffin cells release 5-HT. 5-HT then stimulates intrinsic sensory nerves in the submucosal plexus, which initiate the peristaltic reflex by communication with neurons in the myenteric plexus.[77,94] Stimulation of peristalsis can also occur via mechanical stretch of the muscle wall by stimulating the mechanoreceptors in the myenteric plexus. This mechanism does not involve 5-HT.

Reflexes Mediated by the ENS. Local reflexes mediated entirely by the ENS include peristalsis and the rectoanal inhibitory reflex (RAIR). Peristalsis is defined as a migrating contraction proximal to an intraluminal bolus, with relaxation distal to the bolus, that causes rapid propulsion.[95] Giant migrating contractions such as swallowing and colonic high-amplitude propagating contractions (HAPCs) are typical peristaltic contractions. Orad stimulation is mediated by Ach and substance P. Receptive relaxation of the distal segment is mediated by descending interneurons in the ENS (see Figure 4-5). These activate inhibitory postsynaptic neurons to relax basal tone and inhibit phasic contractions using VIP and NO as the inhibitory transmitters. This descending inhibition is clearly evident at the lower esophageal sphincter, which relaxes at the onset of esophageal peristalsis (Figure 4-6). The antegrade propagation of peristaltic contraction is due to polarity of the neural circuits in the myenteric plexus. Thus, an intestinal segment that is isolated, reversed, and reanastomosed remains persistently antiperistaltic. The rhythmicity of peristaltic contractions in the gut is regulated by the underlying electrical slow-wave activity originating in the ICC.[96]

The RAIR consists of reflex relaxation of the internal anal sphincter in response to rectal distention by stool, balloon, or air during manometry testing. It is mediated by intramural nerves descending from the rectum to the internal anal sphincter, with NO being the putative inhibitory transmitter.[97–101] This reflex is absent in Hirschsprung disease, reflecting the absence of intramural ganglion cells of the ENS in the affected tissue.

Plasticity and Adaptation in ENS. Like the CNS, the ENS has considerable adaptive ability, enabling it to maintain gastrointestinal function in spite of injury and disease. There is also evidence that ENS function changes in response to variations in input. These changes can persist after discontinuation of the stimulus, a kind of enteric memory effect that may underlie the gastrointestinal hypersensitivity and hyperreflexia found in irritable bowel syndrome.[102] Remodeling of the ENS occurs throughout life, being most marked in the embryo, fetus, and young infant. All of these processes are examples of the phenomenon called neuronal plasticity.[103,104]

Mechanisms of ENS neuronal regeneration after injury are currently the subject of intense research activity, both for their potential relevance to the treatment of gastrointestinal disease and for their possible contribution to the understanding of disease in the CNS. Polypeptide nerve growth factors, called neurotrophins, are involved in recovery from injury, promoting differentiation and survival of neurons. They may also play roles in modulating the expression of neurotransmitters and in adaptive changes in response to inflammation.[104] Neurotrophins include nerve growth factor, brain-derived neurotrophic factor, and neurotrophin 3. They are released from neurons, glial cells, and fibroblasts. Pharmacologic agents may increase the potency of endogenous neurotrophins. In par-

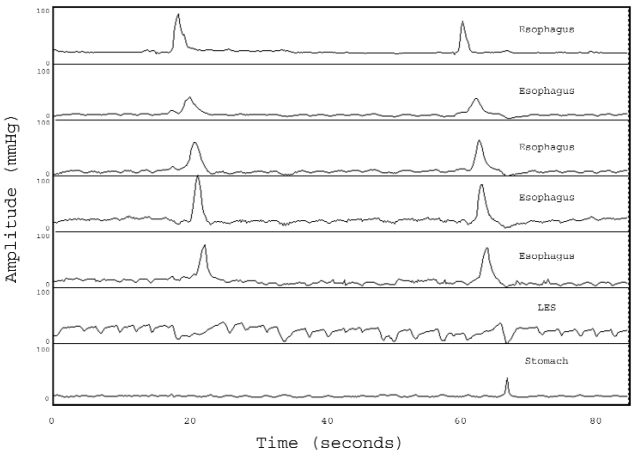

FIGURE 4-6 Esophageal manometry showing normal peristalsis during swallowing. LES = lower esophageal sphincter.

ticular, the recent finding that drugs such as cyclosporine and tacrolimus stimulate the regrowth of injured neurons has led to the development of experimental agents that stimulate neuronal repair without causing immunosuppression.[105] The concept that immunosuppressive medications may serendipitously assist neural recovery after small bowel transplant is tantalizing.

ENS and Inflammation. Inflammation alters ENS responses to mechanical and chemical stimulation.[106,107] There is extensive communication between enteric nerves and immune cells. Mast cells[108] and Peyer patches[109] receive direct innervation, and some enteric neurons release inflammatory mediators and cytokines. In turn, histamine and other substances released from mast cells alter neural function. Inflammation induces changes in neuron phenotypes, electrophysiology, neurotransmitter release, and receptor expression.[106] Substances such as nerve growth factor, released during inflammation, may alter the morphology of local intrinsic and extrinsic neurons, with resulting changes in function. Inflammation also damages ICC, impairing electrical slow-wave activity in smooth muscle.[110] The role of inflammation in the initiation of gastrointestinal hypersensitivity and motility disturbances in irritable bowel syndrome has recently been the focus of intense research activity.

ENS and Allergy. Acute allergic reactions to food proteins cause gastric and intestinal dysmotility in experimental animals.[111–115] Mast cell degranulation appears to activate ENS and extrinsic neural pathways via prostaglandins and serotonin rather than histamine release.[116,117] Similarly, gastric dysmotility has been demonstrated in children with cow's milk protein intolerance on exposure to the offending antigen.[118] Electrogastrography and manometry have even been proposed as objective tests for clarification of gastrointestinal symptoms in food allergy.[118, 119]

Response to Injury. Propagated peristaltic contractions may be interrupted by gut transection and anastomosis.[120,121] Rhythmic contractions appear distal to the anastomosis at the rate of the intrinsic slow-wave cycle for that location.[122,123] Local regeneration of ENS continuity is associated with return of peristalsis across the anastomosis in a proportion of cases.[122,124] Propagation of giant migrating contractions has even been reported from native to allograft intestine after small bowel transplant,[54] but the possibility that propagation in the distal segment may have been due to distention of the allograft by the received bolus, rather than neural continuity, must be considered. In general, transplanted bowel demonstrates persistent MMC activity asynchronous with the activities of the native gut.[54]

Diseases of ENS. In addition to abnormalities of neural crest cell migration discussed above, ENS pathology may be underlying a number of gastrointestinal diseases. Like visceral myopathies, visceral neuropathies may be congenital or acquired, familial or sporadic. A detailed discussion of visceral neuropathy is provided in Chapter 46.4, "Chronic Intestinal Pseudo-obstruction." From a functional perspective, neuropathy manifests as disordered, uncoordinated gastrointestinal motility (Figure 4-7). Unless the gut is abnormally dilated, the amplitude of contractions is normal. Pain may be a prominent symptom, especially in the presence of abnormally increased contractile activity. Because abnormal motility may affect gut development, a history of malrotation is frequently encountered in both myopathy and neuropathy and suggests antenatal disease onset.

The ENS is also a target for bacterial toxins. Enterotoxins from *Vibrio cholerae*, *Clostridium difficile*, and *Escherichia coli* are believed to activate local intrinsic neurons to produce motor and secretory responses by mechanisms involving substance P, mast cells, and NO.[106,125]

HORMONAL REGULATION OF GASTROINTESTINAL MOTILITY

The gut is the largest endocrine organ in the body. Enteroendocrine cells interspersed with mucosal epithelial cells throughout the gastrointestinal tract respond to changes in luminal contents by secreting over 30 different hormones. These act at endocrine, paracrine, neurocrine, and autocrine levels to complement myogenic, neuronal, and ICC mechanisms controlling gastrointestinal motility. There is close coordination between neural and hormonal mechanisms, and, in some instances, the same transmitters are released from both enteric neurons and endocrine cells. Enteroendocrine cells receive ENS innervation; conversely, the hormones secreted from endocrine cells modify neuronal function.

Microvilli on the luminal aspect of enteroendocrine cells allow them to "taste" the intestinal contents.[87] Specific responses to luminal stimuli include the release of cholecystokinin (CCK) from duodenal enteroendocrine cells in response to fat and protein from a meal. CCK acts locally by paracrine stimulation of vagal afferents, causing reflex delay of gastric emptying and contributing to the conscious perception of satiety. Systemically active CCK stimulates pancreatic secretion of digestive enzymes and acts on neurons in the gallbladder to trigger contraction and delivery of bile

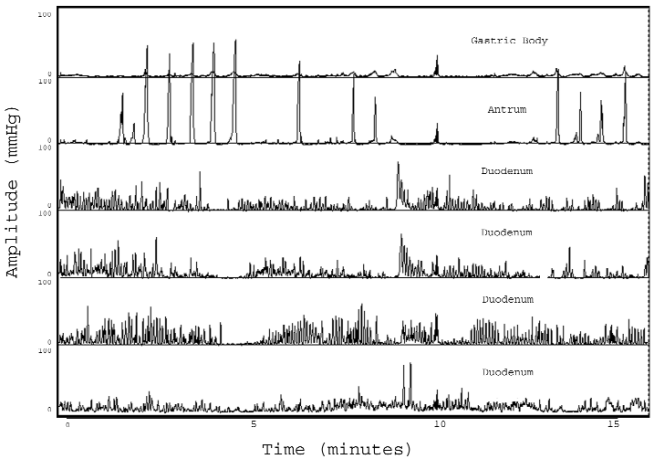

FIGURE 4-7 Fasting antroduodenal manometry in visceral neuropathy: contraction amplitudes are normal, but motility is disorganized, and normal patterns are absent.

to the duodenum. During fasting, another group of duodenal endocrine cells releases motilin, the physiologic stimulus for the interdigestive MMC.

NORMAL AND DISORDERED MOTILITY

Gastrointestinal contractions can be broadly divided into three basic types: phasic, tonic, and ultrapropulsive contractions. Ultrapropulsive contractions consist of giant migrating contractions in the antegrade direction and retrograde giant contractions in the orad direction. These peristaltic contractions move the luminal contents rapidly over relatively large distances. Giant migrating contractions include the esophageal phase of swallowing (Figure 4-6) and the HAPCs responsible for mass movements in the colon (see Figure 4-8). Retrograde giant contractions in the small intestine move the luminal contents back into the stomach prior to vomiting.

Phasic contractions are relatively brief and may be propagated, as in phase III of the MMC in the small intestine (Figure 4-9), or nonpropagated, such as most contractions observed in the small intestine after meals (Figure 4-10). Nonpropagated phasic contractions are also called segmenting contractions and serve to mix the intestinal contents, allowing maximum exposure of the mucosa to luminal contents.

Tonic contractions are prolonged contractions lasting minutes to hours. This type of contraction is typical of gastrointestinal sphincters (see Figure 4-6). The gastric fundus and colon also exhibit tonic contractions, whose function may be to reduce the luminal diameter, increasing the propulsive effects of superimposed phasic contractions, and to promote gradual transfer of luminal contents from areas of higher to lower intraluminal pressure. Tonic contractions are not associated with electrical slow-wave activity. They are sometimes initiated by continuous repetitive spike depolarizations, with greater spike frequency increasing the intensity of contraction. At other times, they are due to continuous partial depolarization or continuous calcium entry into cells.

ESOPHAGUS

The esophagus functions as a conduit, transferring food from the oral cavity to the stomach. Motility in the body of the esophagus is therefore characterized by high-amplitude peristaltic contractions (see Figure 4-6). Swallowing induces primary peristalsis, which originates at the pharynx. Secondary peristalsis, originating in the body of the esophagus, is stimulated by wall distention, such as by the presence of gastroesophageal reflux in the esophageal lumen. Its function is to return refluxed material to the stomach.

In contrast, the lower esophageal sphincter is tonically contracted to prevent the return of gastric contents. Vagally mediated transient relaxations occur during swallowing, vomiting, and burping to allow the passage of fluids. At other times, transient relaxations occur spontaneously, and these have been found to be the predominant mechanism for gastroesophageal reflux in both children[126] and adults.[127,128] Motility abnormalities of the esophagus may be primary or secondary and are detailed in Chapter 26. They may result in dysphagia, food bolus impaction, chest pain, or recurrent aspiration pneumonia.

STOMACH

This section focuses on postprandial gastric motility. Fasting motility is discussed in conjunction with small intestinal motility. The stomach has two functionally discrete regions. The fundus acts as a receptacle, relaxing to receive food, storing the meal, and controlling delivery of material to the antrum. With each swallow, there is receptive relaxation of the fundus, which is mediated by vagal efferents. Subsequently, the fundus exhibits tonic contractions, which transfer the luminal contents to the antrum. Strong propagated antral contractions then grind the solid components of the meal against the pylorus, which closes as each contractile wave reaches it. Consequently, only particles of less than 1 mm in size enter the small bolus of chyme delivered to the duodenum with each wave of contraction. This process is known as gastric sieving (Figure 4-11). The rate of delivery of chyme to the duodenum is

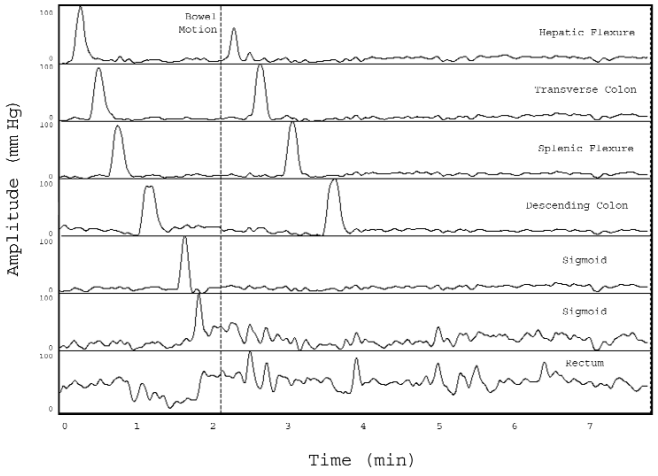

FIGURE 4-8 Colonic manometry showing high-amplitude propagated contractions (HAPCs).

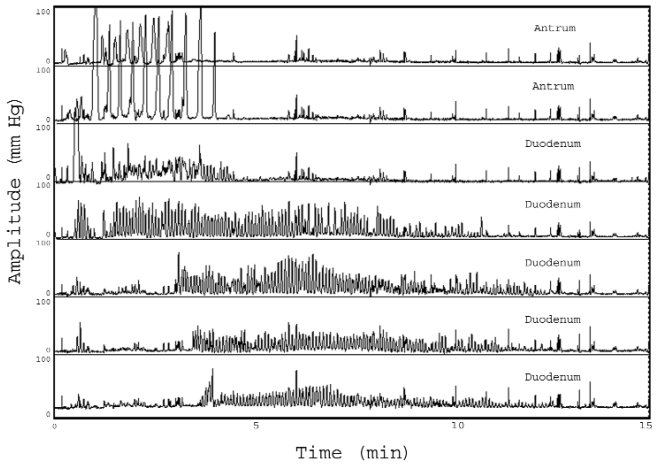

FIGURE 4-9 Antroduodenal manometry showing normal fasting motility: the migrating motor complex (MMC).

regulated both by occlusion of the antral lumen by advancing high-amplitude contractions and by the closure of the pylorus itself.

Whereas liquid meals tend to flow from the fundus to the antrum and begin to enter the duodenum without delay, gastric emptying of solid meals is delayed until food has been reduced to small particles. This accounts for the lag phase seen on gastric emptying tests performed using solid meals and explains why solids are superior for testing the "fitness" of the gastric antrum.

The physiology and pathophysiology of gastric motility are detailed in Chapter 31. Of note, the fundus lacks electrical slow waves and is electrically "silent," whereas the antrum exhibits regular contractions at three cycles per minute originating from the "pacemaker" region on the greater curvature.

Motility abnormalities in the stomach may be the result of damage to or dysfunction of muscle, nerve, or ICCs. Clinically, gastric dysmotility may result in sensations of fullness, early satiety, pain, nausea, and vomiting. Impaired fundic relaxation is a prominent feature of neuropathy states such as diabetic gastropathy and after vagotomy. Absence of antral contraction in response to a meal is typical in postviral gastroparesis but also occurs in idiopathic gastroparesis.

Small Intestine

The small intestine displays distinctly different patterns of motility in the fasting and postprandial states. Programs for both patterns reside in the ENS, and switching between the two states is regulated by neural and hormonal mechanisms. During prolonged fasting, such as overnight, motility of the stomach and small bowel consists of a stereotyped pattern known as the interdigestive MMC, whose function is to sweep the intestine clear of undigested food, sloughed enterocytes, and bacteria.[129] The MMC consists of three separate phases, illustrated in Figure 4-9, which occur in continuous cycles during fasting but are interrupted within minutes after the ingestion of a meal. There is a wide intra- and interindividual variation in the duration of the difference phases of the

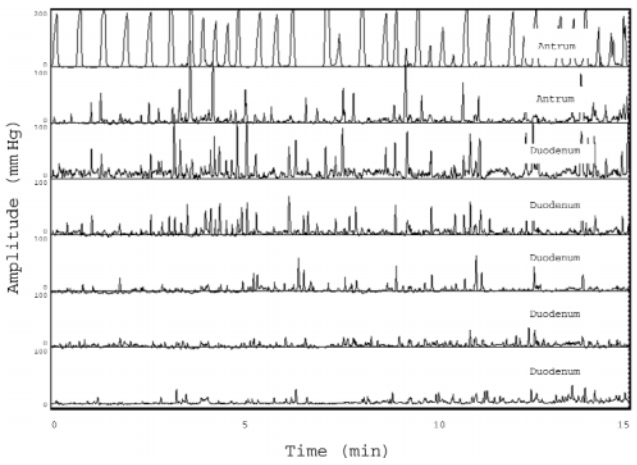

FIGURE 4-10 Antroduodenal manometry showing normal postprandial motility: apparently random contractions of varying amplitude and frequency.

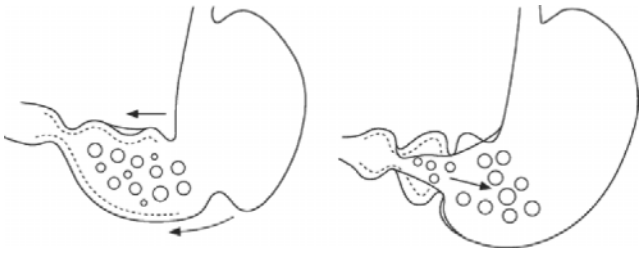

FIGURE 4-11 A diagram to show the nature of antral retropulsion or sieving. At first, particles of many sizes flow toward the pylorus under the influence of the shallow contractions of the body of the stomach. As these deepen and progress into the antrum, the narrowing lumen allows only smaller particles to proceed, whereas the larger ones return toward the gastric body. Copyright material used with permission of the author and the University of Iowa's Virtual Hospital, from Christensen J. The motility of the gastrointestinal tract. Virtual children's hospital, a digital library of health information. Iowa City (IA): The University of Iowa College of Medicine; 2000. http://www.vh.org/adult/provider/internalmedicine/motilitygastro/index.ht l (accessed Jan 14, 2003).

MMCs. Phase I typically lasts 10 to 15 minutes. Phase II lasts 50 to 80 minutes, whereas phase III lasts 3 to 5 minutes. Phase I always follows phase III. During phase I, there is almost complete motor quiescence. Although electrical slow waves are continually present in the smooth muscle, the lack of neural and hormonal excitation prevents contractions from occurring. Following phase I, there is a period of irregular contractions of variable frequency and amplitude. Although a proportion of contractions are propagating, the majority are nonpropagating segmenting contractions. This is phase II. At night and after vagotomy, there is a paucity of phase II activity. Phase III consists of migrating groups of regular propagating contractions occurring at the maximum amplitude and frequency for the site. Groups of phase III contractions normally originate either in the gastric antrum (70%) or duodenum (30%), and the pattern migrates distally along the intestine at a rate of 3 to 10 cm per minute to the ileum. The regular occurrence of the MMC is linked to cyclic secretion of motilin.

Within minutes of ingestion of a meal, the MMC is interrupted, and a pattern of seemingly random contractions results. This pattern of irregular contractions of varying amplitude, frequency, and propagation resembles phase II of the MMC. However, the overall contractile activity is greater after a meal (see Figure 4-10).

Disorders of small intestinal motility fall into two broad categories that may be defined manometrically. The myopathic type of dysmotility is characterized by contractions of reduced amplitude but normal spatial and temporal organization (see Figure 4-2). The second, neuropathic, type of small bowel dysmotility results in disorganized contractions that have normal amplitude unless the bowel becomes pathologically distended (see Figure 4-7). In the presence of distention, manometric recording of contraction amplitude is unreliable because contractions may not produce the occlusion of the lumen necessary for them to be recorded accurately.

Specific manometric patterns associated with disease include the absence of phase III of the MMC, abnormal migration of phase III, short intervals between phase III episodes, persistent low-amplitude contractions, and sustained tonic-phasic contractions.[130] Motility patterns have prognostic significance. For instance, the absence of MMC has been associated with inability to tolerate enteral feeding and prolonged requirement for parenteral nutrition.[131]

Colon

The colon functions to mix, store, and propel fecal matter. Two broad groups of contractions are present: segmental and propagated activity. Segmental contractions are predominant, occurring throughout the day as isolated contractions or bursts of contractions that may be rhythmic or arrhythmic. These serve to mix the contents, maximizing exposure to the intestinal mucosa to promote absorption of water, electrolytes, and bacterial products such as short-chain fatty acids. A special type of rhythmic contraction, the rectal motor complex, occurs several times per day and overnight. These cycles of three to six contractions per minute occur independently of small bowel MMCs and appear to have a role in the maintenance of fecal continence.[132] Propagated contractions have traditionally been divided into high- and low-amplitude propagating contractions. However, recent evidence suggests that there may not be a discrete group of HAPCs but rather a continuum of amplitudes.[133] The function of propagated contractions is to rapidly move feces and gas over large distances.

Disorders of colonic motility may result in diarrhea, constipation, bloating, and pain. They may be severe and acute, such as Ogilvie syndrome of acute colonic pseudo-obstruction, or subacute and chronic, as in functional constipation. Emerging research suggests that abnormal rectal motor activity may underlie some cases of chronic constipation.[134]

Anorectum

Anorectal function in continence and defecation results from the coordinated activity of the internal and external anal sphincters, pelvic floor, and abdominal muscles. The pelvic floor is critical to continence and is the true target of biofeedback exercises.[135] When stool is delivered to the rectum by colonic HAPCs, there is reflex relaxation of the internal anal sphincter (rectoanal inhibitory reflex) and contraction of the external sphincter and pelvic floor. Stool is retained until socially convenient and then is expelled in a coordinated series of contractions accompanied by pelvic floor relaxation. Although defecation is initiated voluntarily, it is controlled by spinal reflexes.

Disordered anal function is common in children. The most common disorder of defecation is functional fecal retention, where fear of defecation results in voluntary withholding of stool. More serious organic diseases, such as Hirschsprung disease and intestinal neuronal dysplasia, are rare but potentially life-threatening. Disorders of colonic and anorectal motility in children are discussed in Chapter 46.1, "Idiopathic Constipation," and Chapter 46.2, "Hirschsprung Disease."

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CHAPTER 5

LIVER FUNCTION AND DYSFUNCTION

1. *Bile Formation and Cholestasis*

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The liver, the largest organ in the body, has multiple functions, several of which depend on its ability to make and secrete bile. Bile secretion is the key means by which the liver controls cholesterol balance and toxin excretion, as well as lipid- and fat-soluble vitamin digestion and absorption in the gut. Bile is composed of many substances, including bile acids, cholesterol, phospholipids, heavy metals, and a variety of detoxified metabolites. Processes that impede the formation and secretion of bile lead to the retention of biliary constituents within the liver (cholestasis), which, in turn, can result in hepatic damage, and, if left uncorrected, ultimately lead to liver failure. Moreover, an absence of bile in the gut lumen leads to marked impairments in the absorption of fats and fat-soluble vitamins. This chapter reviews the main anatomic, physiologic, molecular, and genetic determinants of bile formation to provide the reader with an understanding of our current knowledge of how bile is made and the ramifications of cholestasis.

ANATOMIC DETERMINANTS OF BILE FLOW

To allow for simultaneous uptake and excretion functions of the liver, hepatocytes are highly polarized cells with distinct functions at their basolateral and apical surfaces. The basal surface faces the sinusoids and comes into direct contact with portal blood, allowing for secretion of hepatocyte-derived products, as well as the uptake of nutrients, hormones, drugs, bile acids, and other xenobiotics and endobiotics. The lateral surface is contiguous with the basal surface, also in full contact with portal blood, ending at the tight junctions (zonula occludens) that form between hepatocytes. The apical surface is demarcated by these tight junctions and is the cell's excretory pole, responsible for transporting biliary constituents into the canaliculus via its unique array of resident membrane transporter proteins. The space formed between two cells' apical membranes is the bile canalicular lumen, and the tight junctions serve as the anatomic barrier between bile and blood. The hepatocyte's apical membrane is characterized by microvilli that extend into the canalicular space, increasing the cell's excretory surface area.[1,2]

Within the hepatocyte, solutes pass from the basolateral to the apical surface by a variety of methods. Many of the details of intrahepatocyte transport are largely unknown but are under intense investigation.[3,4] Binding proteins play some role in the shuttling of bile acids, bilirubin, and other substances within the cytosol, directing detoxification and transport while preventing toxic interactions with organelles.[5,6] These substances may then move through the cell by diffusion, whereas other components of bile, such as plasma proteins and lipids, are mainly transported by vesicles that fuse with the canalicular membrane.[4,7] In brief, vesicles are trafficked along cytoskeletal actin-based microtubules that extend throughout the cell.[6,8,9] Vesicles may move either apically or basolaterally, depending on the intended destination of the substance being transported.[8,10]

The majority of hepatocyte bile formation activity occurs at the canalicular membrane and within the canalicular space that exists between two hepatocytes. Once biliary components have reached the apical surface of the hepatocyte, they are transported into the canalicular lumen to form bile. Bile canaliculi comprise only about 5% of the total hepatic membrane surface area, forming tiny conduits for bile and interconnecting in what is often called a "chicken wire"–type three-dimensional pattern.[11] These channels, approximately 0.75 μm in diameter, coalesce as they approach the portal triad into larger tubes, histologically the canals of Hering. These, in turn, feed into the bile ductules visible in the portal triad, which then drain into the increasingly larger branches of the biliary tree. The full system of bile ducts resembles an inverted tree root system, with the terminal bile ducts forming the smallest branches that, in sequence, join to form septal ducts, area ducts, seg-

mental ducts, right and left hepatic ducts, and, ultimately, the common hepatic bile duct.[11]

Bile flows countercurrently to sinusoidal blood within the hepatic lobule. Portal blood flows from the portal vein via sinusoids toward the central vein, whereas newly formed bile flows via the canalicular network beginning at the pericentral region and then toward periportal hepatocytes and into the bile ductules of the portal triads. As bile continues to flow through the biliary tree, it is modified by the secretions of cholangiocytes, epithelial cells that line the biliary system from the ductules to the common bile duct.[11–13] Although comprising only 3 to 5% of liver cells, these cholangiocytes play an important role in modifying bile via secretion of electrolytes and water in response to stimulation by secretin and other hormones.[12–15]

As bile exits the liver, it is stored in the gallbladder until a meal activates duodenal release of cholecystokinin, which causes gallbladder contraction and expulsion of bile into the common bile duct and into the duodenum after relaxation of the ampulla of Vater.[16] Bile acids pass through the upper and mid–small intestine, where they may be modified by bacteria to varying degrees (dehydroxylation and deconjugation) and are almost entirely reabsorbed in the terminal ileum and transported back to the liver via the portal vein.[17] Some bile acids may be absorbed passively in the jejunum and in the colon, an important mechanism in people with short-gut syndrome, but the vast majority are actively absorbed via an apical transporter found in terminal ileal enterocytes.[17,18] On reaching the liver, bile acids are taken up by hepatocytes and excreted again into the bile canaliculus. This process, called the enterohepatic circulation of bile acids, occurs approximately 8 to 10 times a day, approximately twice per meal. It is highly efficient, resulting in a loss of only 5% of circulating bile acids per day under normal physiologic conditions, and this fraction (400–600 mg/d in adults) is matched by hepatic bile acid synthesis to maintain a constant bile acid pool size.[17,19] The normal adult liver secretes approximately 600 to 800 mL of bile a day, but neither bile flow rates nor bile acid pool sizes in infants and children have been accurately quantified.[11,19,20] Given the known "physiologic cholestasis" of infancy, the enterohepatic circulation of bile acids is likely to be less efficient in infants and small children than in adults.[21]

BILE COMPOSITION

Bile is unique among bodily fluids in its high lipid and detergent bile acid content. Primarily an aqueous solution, solids constitute about 3 to 5% of bile by weight, and its osmotic activity is largely determined by inorganic salt, concentrations of which closely reflect plasma electrolyte levels.[11,22] Bile acids are the primary organic solute in bile, with a concentration of 20 to 30 mM; the concentration of phospholipids is approximately 7 mM and cholesterol is 2 to 3 mM (Figure 5.1-1).

Bile acids constitute the major organic solute of bile and serve as the most influential determinant of bile flow rates. Related to steroids by their basic ABCD ring structure, they differ from steroids in the length of the side chain (five carbon) that ends with carboxylic acid.[23,24] Bile acids may be categorized as either primary or secondary, and four different bile acids make up more than 95% of the bile acid pool. Cholic and chenodeoxycholic acids are the two primary bile acids in humans and are synthesized from cholesterol in the hepatocyte via two interacting biosynthetic pathways.[25–27] Most of the genes responsible for bile acid synthesis have been cloned.[28] Cholic acid is the major synthetic product of the "neutral pathway" of bile acid formation. This pathway is initiated by the enzyme cholesterol 7α-hydroxylase, which also serves as the pathway's rate-limiting step. The "acidic pathway" is initiated by sterol 27α-hydroxylase and leads primarily to the formation of chenodeoxycholic acid. Mutations in the bile acid biosynthetic pathways lead to significant liver disease and cholestasis (for more on bile acid synthetic disorders, see Chapter 55.4, "Bile Acid Synthesis and Metabolism").[29–33] These primary bile acids are conjugated in the liver to the amino acids glycine or taurine before being excreted into bile. Glycine conjugates outnumber taurine conjugates at about a 3:1 ratio.[34]

The conjugated primary bile acids are modified by bacterial enzymes into the secondary bile acids. Deconjuga-

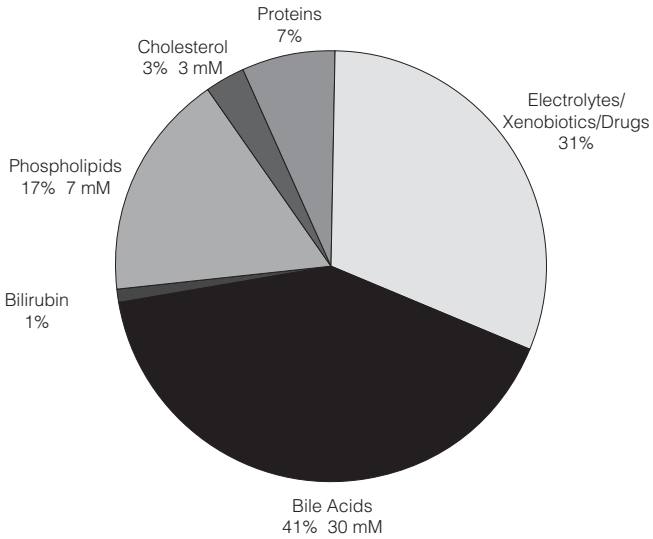

FIGURE 5.1-1 Human bile composition. Bile is predominantly water, with only 3 to 5% of its weight determined by solid solutes. Bile acids are the major solute of bile, and their micelle partners, cholesterol and phospholipids, contribute significantly to the solute composition as well. Xenobiotics, electrolytes, and proteins make up over a third of the biliary solute load, whereas conjugated bilirubin is the smallest component. Adapted from Vlahcevic ZR, Heuman DM, Hylemon PB. Physiology and pathophysiology of enterohepatic circulation of bile acids. In: Zakim D, Boyer J, editors. Hepatology: a textbook of liver disease. 3rd ed. Philadelphia: WB Saunders; 1996. p. 381.

tion, followed by 7α-dehydroxylation, occurs in the proximal jejunum and mid–small bowel, resulting in the more hydrophobic secondary bile acids deoxycholic acid and lithocholic acid.[35,36] Multiple enteric bacteria expressing the enzymes necessary for these reactions have been identified, particularly *Bacteroides*, *Clostridium*, *Bifidobacterium*, and *Escherichia coli*.[37,38] The hydrophobicity of bile acids relates to their detergent property and ability to solubilize phospholipids. This, in turn, translates into enhanced pathophysiology and hepatocyte and other cell membrane toxicity when they come into contact with secondary bile acids.[39] With progression of either liver or bowel disease, the proportion of hydrophobic bile acids increases, thereby altering the bile acid composition to become more hydrophobic and hence more hepatotoxic.[40]

Bile acids combine with phospholipids and cholesterol in the canalicular lumen to form mixed micelles. Phosphatidylcholines make up greater than 95% of the phospholipids in bile, and cholesterol is found in its free or unesterified form.[11,41,42] Plant-derived sterols (phytosterols) originating from the diet make up to about 10% of the total content of bile depending on the individual's intake.[41] Bile acids and phospholipids combine in a 1:2 ratio in bile to make mixed micelles, and these micelles form the primary route by which the body rids itself of cholesterol via the feces. Once in the gut lumen, the micelles exchange phospholipids for dietary lipids, allowing those lipids the contact with detergent bile acids and pancreatic enzymes necessary for digestion.[22] Finally, an imbalance between the ratios of bile acids, phospholipids, and cholesterol may lead to the precipitation of cholesterol in the gallbladder, which, in turn, may be a cause of biliary sludge and cholesterol gallstones (see Chapter 61, "Gallbladder Disease").[43,44]

Bile also contains a wide variety of other organic solutes, lipid-soluble compounds, proteins, drug metabolites, and heavy metals. Organic solutes include glutathione and glutathione conjugates, metabolized drugs, xenobiotics, and endobiotics such as bilirubin. Bilirubin is excreted primarily in its diglucuronide form, with less than 2% of total biliary bilirubin secreted as unconjugated bilirubin.[11,22,45] Lipid-soluble steroid hormones, vitamin D metabolites (25-hydroxyvitamin D), folic acid, pyridoxine, and transcobalamin all may be found in bile. Biliary proteins include those typically found in serum such as albumin and immunoglobulins M and G, as well as immunoglobulin A, which is modified by the liver to add a secretory component before being secreted into bile.[46–48] Other proteins include hepatocellular enzymes such as alkaline phosphatase and γ-glutamyl transferase, as well as bile-specific binding proteins for a variety of substances, such as copper and calcium.[49–51] Finally, bile is the major excretory route for many divalent heavy metals, such as copper, iron, manganese, and zinc.[52,53]

BILE FORMATION: CELLULAR AND BIOCHEMICAL DETERMINANTS

Two main determinates of hepatic bile flow have been designated as bile acid–dependent (BADF) and bile acid–independent (BAIF) flow. In addition, the cholangiocytes lining the biliary tree substantially contribute to bile, depending on the state of feeding and hormonal milieu.[54,55] There is wide variability in the relative contribution of each of these three components of bile formation in different species. In humans, BADF and BAIF contribute fairly equally to total bile flow, although BADF may be increased significantly with increases in bile acid pool size (Figure 5.1-2).[55,56]

Osmotic diffusion of water and electrolytes into bile is primarily determined by the concentration of bile acids in the fluid.[57,58] In this way, bile acid flux is a main determinant of bile flow, and the secretion of bile acids across the hepatocyte apical membrane is the rate-limiting step for BADF.[59–61] Bile acids are highly concentrated in bile, with a 100- to 1,000-fold increase over hepatocyte and portal vein concentrations. Postprandial portal vein bile acid concentration is 50 to 100 μM, whereas bile acid concentration in the canalicular lumen is 20 to 30 mM. By increasing transport of its large reserve of bile acids into bile, the liver can increase bile flow rates up to eightfold.[62] The particular species of bile acid that is transported significantly influences the rate of bile flow. Those bile acids that remain unincorporated into micelles have stronger osmotic properties, resulting in higher bile flow rates, whereas other bile acids have the same effect by enhancing bicarbonate secretion.[63,64] A bile acid's ability to increase bile flow rates is referred to as its choleretic potential and is described in microliters of bile produced per micromole of excreted bile acid.[65,66] Certain bile acids, such as ursodeoxycholic acid, have particularly high choleretic potential, making them attractive for pharmacologic applications.

BAIF is a relatively constant contributor to bile flow in humans at approximately 250 mL per 24 hours in adults.[54] It is mainly related to the liver cell mass, although it may be influenced by hormones and certain drugs.[56,67–70] The osmotic effects of transported glutathione, glutathione conjugates, and bicarbonate in bile are the primary determinate of BAIF.[56,71,72] Cholangiocytes contribute to bile flow both by secretion of water via water channels and by bicarbonate secretion.[13,73] The contribution of cholangiocytes in humans can be considerable, upward of 40% of total bile flow, depending on the state of feeding.[54,74] In

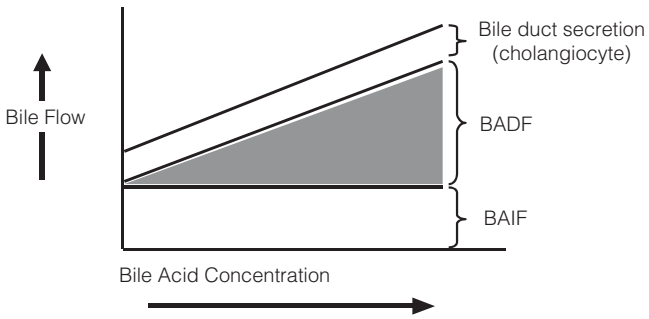

FIGURE 5.1-2 Bile flow determinants. Bile flow is primarily bile acid dependent (BADF) in humans, increasing as the bile acid pool size expands. Bile acid–independent flow (BAIF) remains relatively constant and is determined by glutathione, glutathione conjugates, and bicarbonate, among other solutes. Cholangiocytes secrete water and bicarbonate into bile and can contribute up to 40% of total bile flow, depending on the state of feeding.

response to feeding, the duodenum releases cholecystokinin, secretin, vasoactive intestinal peptide, and other hormones, which, in turn, stimulate cholangiocytes along the biliary ducts to increase water and electrolyte secretion and thus increase bile flow (see Figure 5.1-2).[75–80]

BILE FORMATION AND FLOW: MOLECULAR DETERMINANTS

Significant advances in the understanding of molecular control of bile formation have occurred in the last 10 years. Genes encoding critical transporters on both the basolateral and apical surfaces of hepatocytes have been identified (Figure 5.1-3).[14,81] These transporters are responsible for the uptake and excretion of bile acids, cholesterol, phospholipids, organic anions, xenobiotics, hormones, and other constituents of bile. Enzymatic pathways within the cell play crucial roles in cholesterol processing, bile acid synthesis, and xenobiotic metabolism.[14,15,82,83] These discoveries not only have contributed significantly to our understanding of basic metabolism and drug handling but also have provided a molecular explanation for many rare pediatric cholestatic disorders.[84,85]

BASOLATERAL MEMBRANE TRANSPORTERS

NTCP

The first sinusoidal bile acid transporter cloned was the rat Na+/taurocholate cotransporting polypeptide gene *NTCP* (SLC10A1).[86] Found only on the sinusoidal membrane of hepatocytes, *NTCP* is the primary mediator of hepatic uptake of conjugated bile acids from portal blood.[86–88] Subsequent to its isolation in rats, *NTCP* has been cloned in mice, rabbits, hamsters, zebrafish, and humans.[89–93] The liver extracts 75 to 90% of conjugated bile acids from sinusoidal blood on its first pass, and approximately 75% of this extraction occurs via *NTCP* in a Na+-dependent process.[94,95] Expression of *NTCP* is controlled at both transcriptional and post-translational levels. However, regulation of *NTCP* ribonucleic acid (RNA) expression appears to be the main means of governing its activity, with significant changes in molecular *NTCP* RNA expression during states of inflammation and cholestasis. Moreover, bile acids themselves act as negative feedback regulators of *NTCP* gene expression.[96,97] Mutations in the *NTCP* gene have yet to be described.

Organic Anion Transporting Polypeptide Family

Approximately 25% of bile acid extraction occurs in a Na+-independent process, and the primary transporters involved are the organic anion transporting polypeptides (OATPs).[14,81] It is thought that OATPs are responsible for the majority of BAIF, as well as Na+-independent BADF. These proteins are multispecific and bidirectional transporters of a variety of compounds, including bile acids, bilirubin, xenobiotics, drugs, and hormones. Many xenobiotics, steroids, and other compounds may rely entirely on OATPs for transport into and out of hepatocytes. Three members of the OATP family (Oatp 1 [Slc21a1], Oatp 2 [Slc21a5], and Oatp 4 [Slc21a10]) have been identified as significant Na+-independent transporters in rat liver, whereas primarily two OATPs (OATP-C [SLC21A6] and OATP-8 [SLC21A8]) play similar roles in human liver.[14,81] Human OATP-C, however, is unique in its ability to transport unconjugated bilirubin, a characteristic lacking in rat Oatp 1.[98,99] In fetal rat liver, Oatp 1 messenger RNA is detectable prior to that of *NTCP*, suggesting its importance in both bile acid and other molecular transport.[100] Similar to *NTCP*, rat Oatp 1, 2, and 4 are down-regulated in conditions of sepsis and cholestasis. Expression of human OATP-C and 8 in liver disease has not been determined, but OATP-C polymorphisms have been described that may alter drug pharmacokinetics.[101–106]

Multidrug Resistance Protein 3

Under normal circumstances, minimal efflux of bile acids occurs from the hepatocyte to sinusoidal blood

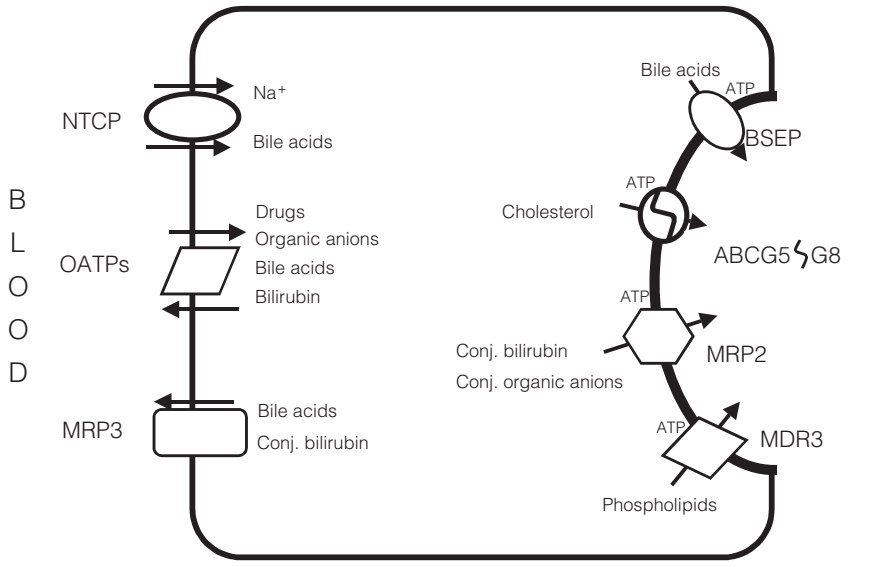

FIGURE 5.1-3 Hepatocyte transporters involved in bile formation. Hepatobiliary transporters on the sinusoidal (blood) and canalicular (bile) membranes of the cell are responsible for the importation of molecules from portal blood and export of biliary components into the canalicular lumen. Arrows connote direction of molecular flow across each transporter. ATP = adenosine triphosphate; BSEP = bile salt export pump; Conj. = conjugated; MDR = multidrug resistance; MRP = multidrug resistance–related protein; NTCP = Na+/taurocholate cotransporting polypeptide; OATP = organic anion transporting polypeptide.

across the basolateral membrane. In cholestatic conditions, however, serum bile acid levels rise, coincident with reduced *NTCP* gene expression as well as increased basolateral expression of the multidrug resistance protein 3 (MRP3 [ABCC3]).[107,108] This protein, also expressed by cholangiocytes, enterocytes, and renal epithelia, transports bile acids, conjugated organic anions, and conjugated bilirubin out of hepatocytes and into plasma.[14,109,110] Finally, although rat hepatocytes show Mrp3 up-regulation in response to cholestasis, the role for human MRP3 induction remains unclear because human liver biopsy samples from cholestatic patients have not shown as robust an activation of MRP3 RNA levels as in bile duct–ligated rats.[111] Patients with Dubin-Johnson syndrome (see below) have increased MRP3 expression, suggesting a compensatory means of unloading conjugated bilirubin into blood because these patients have a molecular defect in canalicular conjugated bilirubin excretion.[112]

CANALICULAR MEMBRANE TRANSPORTERS

Bile Salt Export Pump

The bile salt export pump (BSEP [ABCB11]) was identified in 1998 as the primary transporter responsible for bile acid export into bile.[113] An adenosine triphosphate (ATP)-binding cassette protein, BSEP uses ATP hydrolysis to provide the energy to export bile acids against the very high-concentration gradient found at the canalicular membrane.[114] Multiple mutations have been identified in the gene encoding for BSEP in patients with progressive familial intrahepatic cholestasis type 2 (*PFIC2*), and several of these mutations seem to result in either abnormal trafficking to the membrane or premature degradation of the transporter.[96,115] PFIC2 is a clinical syndrome characterized by progressive intrahepatic cholestasis, pruritus, usually low serum γ-glutamyl transpeptidase levels, normal serum cholesterol levels, and characteristic coarse appearance of canalicular bile on electron microscopy.[116–118] Progression to cirrhosis and liver failure in early childhood is the norm for most PFIC2 patients, resulting in early mortality without transplant.[15,85,116,117,119] Altogether, the rapid progression of liver disease in PFIC2 patients appears to be due to bile acid retention, supporting the notion that bile acids are the main hepatotoxins in cholestatic liver disease. (For more on PFIC2, see Chapter 55.6, "Disorders of Biliary Transport.")

Multidrug Resistance Protein 2

MRP2 (ABCC2) is likely responsible for the majority of bile salt–independent bile flow across the canaliculus.[120,121] The MRP2 transporter exports glutathione, sulfated and glucuronidated conjugates of drugs, toxins, bile acids, and bilirubin into bile. Mutations of the gene lead to reduced BAIF in rodent models and are responsible for the elevated serum levels of conjugated bilirubin seen in Dubin-Johnson syndrome.[120,122] Mouse data also suggest that this transporter may be involved in cholesterol gallstone susceptibility.[123]

Multidrug Resistance Protein 3

MDR3 (ABCB4) acts as the phopholipid translocator ("flippase") across the canalicular membrane in humans and rodents and is responsible for phospholipid secretion into bile.[124,125] Mutations or deletion of the *MDR3* gene results in PFIC3, a syndrome distinct from PFIC1 and PFIC2 in its elevated γ-glutamyl transpeptidase and indicative of more involved biliary tree damage.[126] In addition to PFIC3, cholestasis of pregnancy has been identified in heterozygous carriers of *MDR3* mutations.[127] Mutations may also predispose the patient to cholesterol gallstones given an imbalance of phospholipids to cholesterol and bile acids in the bile. Taken together, research to date displays a wide variation in the clinical manifestation of *MDR3* mutations, and much remains to be discovered about this transporter and its role in liver disease.[126,127]

Familial Intrahepatic Cholestasis 1 (ATP8B1)

This poorly understood protein is analogous to an ATP-dependent aminophospholipid transporter molecule that is found in the apical membranes of hepatocytes, cholangiocytes, pancreas, and small intestine enterocytes.[128–130] It gained attention and is named for its role in PFIC1, which, although clinically similar to PFIC2, is associated with familial intrahepatic cholestasis 1 (FIC1) mutations rather than BSEP.[128] As a consequence of these mutations, hydrophobic bile salts are markedly reduced in bile, suggesting that FIC1 may be involved in transporting highly hydrophobic bile acids such as lithocholate and chenodeoxycholate.[131] Patients with benign recurrent intrahepatic cholestasis also have mutations in the *FIC1* gene.[128,129,132] The clinical scenario in these patients, however, is markedly different from that of PFIC1 patients, characterized by normal liver function and life expectancy with only intermittent episodes of jaundice and pruritus. It is still not understood how mutations in the same region of the *FIC1* gene can result in quite divergent clinical syndromes.

ABCG5 and ABCG8

These two ATP-binding cassette proteins have recently been identified as the biliary cholesterol transporters.[133,134] Both are half-transporters expressed in the canalicular membrane and the apical membrane of enterocytes. Working together, they export sterols, including both dietary cholesterol and plant phytosterols. When mutated, they produce the rare disease of accumulated serum plant sterols, sitosterolemia, and recently have been implicated in cholesterol gallstone formation.[135–138]

MOLECULAR CONTRIBUTION TO CLINICAL CHOLESTATIC DISORDERS

As the molecular understanding of bile flow has advanced, new insight into various well-recognized clinical cholestatic conditions has emerged. The molecular mechanisms of both acute and chronic causes of cholestasis, such as sepsis and pregnancy, as well as congenital cholestatic disorders (eg, PFICs) may be explained in terms of alterations in membrane trans-

porter expression and mutations of critical transporter genes. Several congenital disorders associated with known membrane transporters have been mentioned previously, and their clinical profiles are more fully discussed elsewhere (see Chapter 55.6). In addition to these diseases, there are some prominent acquired and other congenital cholestatic conditions worthy of note.

Intracellular accumulation of bile acids is a key feature of cholestatic conditions, and these bile acids can be toxic to liver and biliary tissues.[39,139] Bile acid toxicity is due not only to its detergent properties (causing protein dissociation from the cell lipid bilayer) but also to its nondetergent mechanisms that alter intracellular signaling and lead directly to hepatocellular apoptosis.[140–143] Thus, situations of hepatic bile acid retention, whether congenital or acquired, intra- or extrahepatic, can lead to significant hepatobiliary damage.

Total Parenteral Nutrition–Associated Cholestasis

Total parenteral nutrition (TPN) has revolutionized the care of many chronically ill and intestinally devasted children over the past 30 years. With widespread use of TPN, however, a consequence of cholestasis has become apparent in some children, particularly premature and extremely low birth weight infants who develop necrotizing enterocolitis.[144–146] Several factors appear to contribute to TPN-associated cholestasis (TPNAC), including various components of the TPN, hydrophobic bile acids, prematurity, repeated bouts of sepsis, and poor bowel function.[145–149] Although most patients' TPNAC resolves following transition to enteral feeding, some infants will progress to end-stage liver disease within a few months.[144,149,150] The molecular explanation of TPNAC remains unclear, although emerging data on the developmental expression of hepatic transporters may help explain the particular vulnerability of premature infants to this disease. (For more on TPNAC, see Chapters 40, 56, and 76.4.)

Sepsis-Associated Cholestasis

Systemic bacterial infections have long been associated with jaundice, particularly in premature and young infants.[151] Recent research has shown the responsiveness of several hepatic transporters to endotoxin and inflammatory cytokines, particularly *NTCP* and the OATP family.[152–158] Expression of these basolateral transporters is significantly down-regulated in inflammatory and septic conditions, and *NTCP* and BSEP RNA expression is reduced in liver biopsies obtained from patients with inflammation-associated cholestasis.[111] Cumulatively, these changes in expression lead to decreased import of bile acids from the systemic circulation into the hepatocyte and into bile. Bile flow is thus reduced, leading to cholestasis and conjugated hyperbilirubinemia.[159] With treatment of the infection, cholestasis and any apparent jaundice typically resolve.

Disorders of Bile Acid Synthesis

In the last several years, at least six specific enzymatic defects in the bile acid synthesis pathways have been characterized.[23,29–33] Combined, these disorders account for approximately 2 to 3% of liver disease cases in the pediatric population. Atypical and often hepatotoxic bile acids accumulate in the liver, potentially leading to cholestasis, liver dysfunction, and end-stage failure if not treated. Cholestasis results from a decrease in the primary bile acids that stimulate bile flow.[23] These defects can be identified via urinalysis for abnormal bile acid species, and treatment with oral bile acid therapy may be curative if started early. (For more on bile acid synthetic disorders, see Chapter 55.4.)

Cystic Fibrosis

Cholangiocytes lining the bile ducts secrete chloride and water into bile via the cystic fibrosis transmembrane regulator (CFTR) transporter.[76] Found on the luminal side of cholangiocytes, CFTR is mutated in patients with cystic fibrosis (CF), resulting in thick, viscous secretions. In addition to the pulmonary and pancreatic complications characteristic of CF, cholestasis and biliary cirrhosis are significant problems for affected patients. The highly viscous bile forms bile duct plugs, which, in turn, can cause biliary obstruction, focal biliary fibrosis, and focal biliary cirrhosis.[160,161] With increased life expectancies in CF, liver disease has become the third leading cause of death in these patients.[162] The hydrophilic bile acid ursodeoxycholic acid has been used to improve the fluidity of bile in CF patients, although the long-term effects on survival or the need for liver transplant are unknown.[163–165] (For more on CF-related liver disease, see Chapter 65.1, "Cystic Fibrosis.")

SUMMARY

Bile production is a crucial function of the liver, constituting the body's primary mechanism for maintaining cholesterol balance, toxin excretion, and lipid- and fat-soluble vitamin digestion and absorption in the gut. The molecular understanding of bile formation, particularly in the handling of bile acids, has expanded considerably in the last several years. This insight has not only helped explain the mechanism of several congenital and acquired cholestatic conditions but also should lead ultimately to new therapies for cholestatic patients, who currently have few options.

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2. Fibrogenesis and Cirrhosis

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FIBROSIS AND CIRRHOSIS IN PEDIATRIC LIVER DISEASES

Almost all causes of chronic liver injury result in fibrosis and ultimately cirrhosis, from which most of the complications of chronic liver disease are derived. Fibrosis is a wound-healing response in which damaged areas of tissue are encapsulated by scar tissue or extracellular matrix. In the liver, this may lead to cirrhosis, which is a chronic diffuse disease characterized by irreversible widespread hepatic fibrosis with regenerative nodule formation. The prominent fibrous tissue contains vascular anastomoses, which cause hemodynamic alterations and portosystemic shunting. This diffuse pathology superimposes on the primary liver disease, often obscuring the nature of the original insult. The major pathophysiologic consequences[1] are the result of impaired hepatic function and portal hypertension, and some cases may be complicated by the occurrence of hepatocellular carcinoma.

In children, a wide range of causes of hepatocellular injury may result in cirrhosis. These include causes of cholestasis, with accumulation of hydrophobic bile acids toxic to hepatocytes (eg, biliary secretory disorders or obstruction), as well as infections, toxins, and metabolic, vascular, and nutritional disorders (Table 5.2-1). Irrespective of the cause of chronic injury, and in the absence of removal of the cause of injury, many complications can ensue (Table 5.2-2). Unfortunately, available therapies for many chronic liver diseases causing cirrhosis in children are ineffective, and treatment is limited to management of these secondary pathophysiologic complications, and when liver failure ensues, liver transplant is the only available definitive option. Advances in knowledge of the mechanisms leading to cirrhosis and its complications may impact on the outcome of this progressive pathologic process via development of new antifibrotic and other specific therapies. Such research may not only further develop traditional management approaches but, importantly, may also provide new directions in the prevention of progression and treatment of these diseases.

The pattern of progression to cirrhosis and its complications in pediatric liver diseases is highly variable. In some conditions, such as neonatal extrahepatic biliary atresia, a severe cholangiopathy of unknown etiology, the development of hepatic fibrosis is extraordinarily rapid, with cirrhosis occurring by 12 to 16 weeks of age and liver failure by as early as 24 weeks of age. Early diagnosis and surgical treatment by portoenterostomy improve outcome in some cases, but the degree of fibrosis at the time of portoenterostomy has been adversely related to poor outcomes in this disease. Other disorders, such as cystic fibrosis (CF)-associated focal biliary cirrhosis, can be compatible with normal liver function for many years, presenting with signs of portal hypertension only in the second decade of life. Despite this wide variation in occurrence and severity, it appears that the cellular mechanisms and factors responsible for the development of liver fibrosis in these two widely differing diseases, and indeed in most chronic liver diseases, are remarkably similar.[2–4]

TABLE 5.2-1 CAUSES OF FIBROSIS AND CIRRHOSIS IN CHILDREN

PEDIATRIC CHOLESTATIC DISEASES
Extrahepatic biliary atresia
Choledochal cyst, tumors, stones
Alagille syndrome, biliary hypoplasia
Progressive familiar intrahepatic cholestasis syndrome
Drug-induced cholestasis
TPN-associated cholestasis
Cystic fibrosis liver disease
Sclerosing cholangitis
Graft-versus-host disease
Histiocytosis X
HEPATOCELLULAR DISEASES
Neonatal hepatitis
Hepatitis B and hepatitis C
Autoimmune hepatitis
Drugs/toxins
Genetic/metabolic diseases
Carbohydrate defects
Galactosemia, fructosemia, glycogen storage III and IV
Amino acid defects
Tyrosinemia, urea cycle disorders
Metal storage defects
Neonatal hemochromatosis, Wilson disease
Lipid storage diseases
Gaucher disease, Niemann-Pick type C
Fatty acid oxidation defects
Peroxisomal disorders
Zellweger syndrome
Mitochondrial disorders
Respiratory chain defects
FIBROPOLYCYSTIC DISORDERS*
CHRONIC HEPATIC VENOUS OUTFLOW OBSTRUCTION
Hepatic vein thrombosis
Budd-Chiari syndrome
Veno-occlusive disease
Cardiac sclerosis

TPN = total parenteral nutrition.
*Does not cause cirrhosis.

TABLE 5.2-2 COMPLICATIONS OF CIRRHOSIS IN CHILDREN

Malnutrition and growth failure
Cholestasis with jaundice, pruritus, and impaired fat absorption
Portal hypertension and variceal bleeding, hypersplenism
Ascites
Encephalopathy
Coagulopathy
Hepatopulmonary syndrome
Hepatorenal syndrome
Bacterial infections, spontaneous bacterial peritonitis
Impaired hepatic drug metabolism
Hepatocellular carcinoma

Adapted from Shepherd RW.[1]

HEPATIC FIBROGENESIS

Liver fibrosis resulting in cirrhosis is a dynamic process in which environmental factors, such as the nature, duration, and intensity of the exposure to liver injury, interact with genetic factors, such as the nature of the immune response, to result in scar formation. Animal and adult human studies of various forms of liver injury suggest that the liver responds to injury in a stereotypic fashion involving programmed cell death (apoptosis), cell necrosis, and fibrosis.[4,5] Apoptosis involves polarization of mitochondria, cytochrome *c* release, nuclear fragmentation, and formation of apoptotic bodies without inflammation. Cell necrosis involves depolarization of mitochondria, depletion of adenosine triphosphate, and cell lysis with inflammation. In response to these cellular events, there is oxidant stress, release of cytokines, accumulation of collagens, increased turnover of components of the extracellular matrix, fibrosis, and replacement or regeneration of hepatocytes, resulting in nodule formation. This latter dynamic and complex series of events is called fibrogenesis. Until recently, little was known about the cells responsible for this increased hepatic extracellular matrix production. Technical advances in the isolation and characterization of hepatic cells[6] have led to the observations that a population of nonparenchymal liver cells, called hepatic stellate cells (HSCs), formerly known as lipocytes, Ito cells, or fat-storing cells, are the principal fibrillar collagen–producing cells in pathologic conditions of the liver.

Role of the HSC

HSCs are found in a perisinusoidal localization, in close contact with hepatocytes, sinusoidal endothelial cells, and Kupffer cells. In normal liver, they are vitamin A–storing cells that produce small amounts of extracellular matrix, which assist in maintaining the integrity of the basement membrane.[5] In conditions of hepatic injury, HSCs can be activated in vivo into a highly proliferative, "myofibroblast-like" cell. This activation process is associated with loss of intracellular vitamin A, increased production of the fibrillar collagen types I and III and other extracellular matrix components, expression of cytokines and cytokine receptors, and elaboration of a network of contractile microfilaments, chiefly α-smooth muscle action.

Mediators of Fibrogenesis

HSC activation is central to the development of hepatic fibrosis and appears to take place in two phases[4]: a phase of initiation followed by a phase of perpetuation (Figure 5.2-1). The earliest events are likely due to interactions with other hepatic cells or products derived from these cells, such as reactive oxygen species and lipid peroxides causing oxidant stress and sinusoidal endothelial cell–derived fibronectin.[6] There is then cytokine-induced perpetuation of the activation process, involving retinoid loss, proliferation, fibrogenesis, matrix degradation, chemotaxis, contractility, and leukocyte chemoattraction (see Figure 5.2-1). Key mediators include transforming growth factor-β_1 (TGF-β_1), which is the most potent stimulus for HSC-derived collagen type I production; platelet-derived growth factor BB (PDGF-BB), which is responsible for increased HSC proliferation; and the chemokine monocyte chemotaxis protein 1 (MCP-1), which sponsors white blood cell attraction.[7] These mechanisms and pathways of HSC contractility, chemotaxis, and chemoattraction are vital for the inflammatory processes associated with hepatic fibrogenesis. Finally, matrix remodeling is an essential facet of fibrogenesis. HSCs express many of the enzymes and inhibitors that mediate liver matrix remodeling, such as matrix metalloproteinases (MMPs) 2 and 3, which take part in the early degradation of basement membrane, which enhances HSC activation, as well as tissue inhibitors of MMPs (TIMPs) 1 and 2, which inhibit protease activity, leading to unhindered matrix accumulation during fibrillar collagen deposition.[8]

Fibrogenesis in Pediatric Liver Diseases

The precise mechanisms responsible for the activation of HSCs and the subsequent development of hepatic fibrosis in most pediatric liver diseases are not well characterized to date. A composite pattern is, however, emerging from recent studies of pediatric cholestatic diseases,[9] as depicted in Figure 5.2-2. Activation of HSCs and associated increased production of type I collagen have been

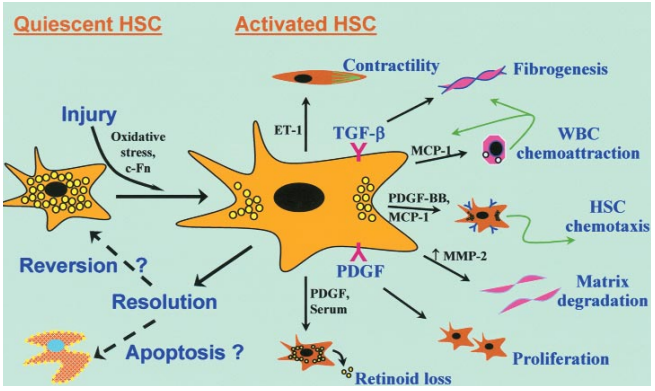

FIGURE 5.2-1 The hepatic stellate cell (HSC) in the quiescent and activated states following liver injury. Adapted from Friedman SL.[4] cFN = cell-associated fibronectin; ET = endothelin; MCP = monocyte chemotaxis protein; MMP = matrix metalloproteinase; PDGF = platelet-derived growth factor; TGF = transforming growth factor; WBC = white blood cell.

documented in biliary atresia[2] and in the focal biliary cirrhosis associated with CF.[3] Myofibroblast-like cells were seen in the extracellular matrix surrounding expanded bile ducts and within fibrosis septa bridging between portal tracts. Activated HSCs, demonstrated by the expression of procollagen, smooth muscle actin, and their stellate cell morphology, were particularly evident at the interface between scar and normal tissue (see Figure 5.2-2). This growing margin of scar tissue formation appears to be the site of maximal HSC activation and type I collagen messenger ribonucleic acid (mRNA) expression, although some evidence of collagen gene expression is also seen in myofibroblast-like cells within established fibrous septa. Whether the myofibroblast-like cells that surround expanded bile ducts within the fibrotic septa are derived from activated HSCs or portal fibroblasts is unknown. Possible mechanisms of injury to bile duct cells and/or hepatocytes are shown in Figure 5.2-2. Notably in biliary atresia, bile duct hyperplasia appears to be an early pathologic event, possibly attributable to, as suggested by studies in bile duct-ligated rats, increased intraductal pressure,[10] circulating cholangiotrophic factors,[11] or depletion of hepatic antioxidants, such as vitamin E or glutathione.[12] Oxidative stress, a result of increased free radical generation and depletion of antioxidant defenses, is thought to play an important role in the development of hepatic injury. In cholestasis, there is an increase in the levels of glycine-conjugated hydrophobic bile acids, which occurs in response to biliary obstruction and decreased hydration of bile.[13] Indeed, hydrophobic bile acid–induced oxidant stress may play a major role in the viability and function of Kupffer cells, hepatocytes, bile duct cells, and HSCs, all of which are capable of producing cytokines and growth factors that may drive inflammation and fibrogenesis (see Figure 5.2-1).

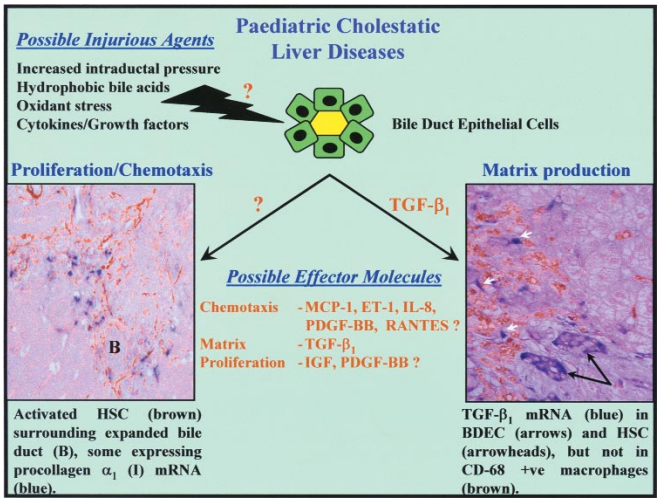

FIGURE 5.2-2 Schema representing mechanisms of liver fibrogenesis in pediatric cholestatic disorders. Adapted from Ramm GA.[9] BDEC = bile duct epithelial cell; ET = endothelin; HSC = hepatic stellate cell; IGF = insulin-like growth factor; IL = interleukin; MCP = monocyte chemotaxis protein; mRNA = messenger ribonucleic acid; PDGF = platelet-derived growth factor; TGF = transforming growth factor.

The most potent fibrogenic mediator released during liver injury appears to be TGF-β, which is responsible for the transactivation of the procollagen α_1 gene in both myofibroblast-like cells and activated HPCs in these same areas of scar tissue formation (see Figures 5.2-1 and 5.2-2). TGF-β_1 is expressed at the scar interface in both biliary atresia and CF liver disease (see Figure 5.2-2), suggesting a spatial and temporal association with the generation of scar tissue. In CF liver disease, TGF-β_1 protein expression in bile duct epithelial cells is significantly correlated with the percentage of portal tracts, and TGF-β_1 mRNA expression correlates with disease progression.[3] Possible effector molecules for activation, proliferation, and chemotaxis include PDGF-BB, at least in biliary atresia.[14] In animal models using bile duct–ligated rats, bile duct cells also express PDGF-BB,[11] and in vitro studies have shown that HSCs are recruited to bile duct segments by PDGF-BB.[15] HSCs also respond to numerous other chemokines in vitro, such as MCP-1, interleukin-8, and endothelin-1 (see Figure 5.2-1).[16] Marked hepatic expression of MCP-1 has been reported in biliary atresia and CF liver disease, although the role of MCP-1 in stellate cell recruitment in vivo remains to be determined.[17]

Genetic Polymorphisms and Fibrogenesis

The importance of genetic influences on the outcomes and progression of chronic liver diseases has recently been a topic of scientific interest, with specific reference to genetic modifiers in the occurrence of and progression of hepatic fibrosis.[18] Although most of the evidence for this relates to adult liver diseases and animal models,[18] epidemiologic studies have identified possible polymorphisms in a number of candidate genes that may influence the progression of liver fibrosis in some pediatric liver diseases. For example, liver disease in pediatric patients with CF has been associated with glutathione *S*-transferase P1 polymorphism.[19] This may be important because the glutathione *S*-transferases play a key role in the protection against oxidative stress. As indicated above, oxidant injury contributes to the development of hepatic fibrosis. Similarly, genetic variations may explain susceptibility to the development of progression to cirrhosis in some patients with nonalcoholic steatohepatitis.[19] In hepatitis C, epidemiologic studies have suggested the presence of rapid and slow fibrosers, and many candidate genes that might explain this phenomenon are being actively researched. Theoretically, any gene involved in the pathogenesis of hepatic fibrosis may potentially modify progression.

Serum Markers of Fibrosis

It is possible to evaluate hepatic fibrosis by measuring serum levels of the components of matrix degradation and remodeling. Numerous studies have examined the utility of a series of potential markers of hepatic injury progression to cirrhosis in adult human liver disease, and there appears to be a disease-specific pattern of the various markers, which also varies with progression of the degree of fibrosis. For example, elevated levels of collagen type III, collagen type IV (CL-IV), prolyl-hydroxylase (PH),[20]

laminin and CL-IV,[21] and MMP-1 have been observed in different cohorts of patients with differing causes of cirrhosis.[22] Levels of hyaluronic acid (HA),[23] TIMP-1, and TIMP-2[24] are elevated in patients with chronic hepatitis C virus infection. In hemochromatosis, levels of CL-IV and MMP-2 are correlated with hepatic fibrosis.[25]

A few studies have assessed the efficacy of serum markers in evaluating liver injury in pediatric liver diseases. Elevated serum levels of procollagen III peptide and CL-IV and/or HA may indicate progression of fibrosis after Kasai portoenterostomy. Serum HA may be of value for monitoring postoperative biliary atresia patients as a noninvasive indicator of progressive hepatic fibrosis.[26–28] In established CF liver disease, collagen type VI,[29] collagen type III, PH,[30] and HA[31] levels are elevated. In one study, serum concentrations of CL-IV, PH, HA, MMP-2, and TIMP-1 were measured in patients with CF liver disease and well-characterized hepatic fibrosis versus age- and sex-matched patients with CF but no liver disease and non-CF controls.[32] Elevated levels of serum TIMP-1, CL-IV, and PH were found in CF liver disease, and higher levels of TIMP-1 and PH were associated with early fibrosis. These may thus be useful markers for the early detection of CF liver disease.[32]

Prospects for Antifibrogenic Therapy

Except for a few therapies for causes of chronic liver disease, such as chelation therapy in Wilson disease and the use of antiviral agents in hepatitis B and C, removal of the cause of the chronic hepatic injury is usually not possible, and therapies targeting the fibrogenic response to mediate progression of chronic liver disease and its complications are being actively researched, holding some promise for the future.[33] These include inhibition of HSC activation (eg, antioxidants), antifibrogenic agents (eg, TGF-β antagonists, inhibitors of the endothelin receptor), and agents that increase degradation of scar (eg, metalloproteinases).

CIRRHOSIS AND ITS COMPLICATIONS IN CHILDREN

Definitions

Cirrhosis may be either active or inactive, depending on the presence of biochemical or histologic evidence of hepatocellular necrosis, apoptosis, and inflammation, and either compensated, where there are no clinical or laboratory features of liver failure, or decompensated, where such features are evident. In general, morphologic and histologic classifications are often unhelpful in clinical settings, although certain features may help in determining the cause of the cirrhosis, as in biliary disease, hepatic venous outflow obstruction, and features specific for particular inherited or infective conditions. Grouping disorders that progress to cirrhosis by etiology is helpful because of the framework this provides for diagnosis, prognosis, treatment, and genetic counseling. Ultimately, the presence or absence of liver failure provides the basis for transition from supportive therapy to considerations of liver transplant when the condition of end-stage liver disease has been realized.

Pathology

Classification of liver pathology is based on morphology, histology, etiology, and degree of activity. Fibrosis is not synonymous with cirrhosis, and fibrosis without nodules occurs typically in congenital hepatic fibrosis and granulomatous liver disease. The *morphologic classification* divides cirrhosis into micronodular, macronodular, and mixed-type disease. Micronodular cirrhosis is characterized by fibrous septa separating small (< 3 mm) regeneration nodules of almost uniform size, present throughout the liver. This is most commonly seen in the early stages of extrahepatic biliary atresia. Macronodular cirrhosis is characterized by nodules up to 5 cm in diameter, separated by irregular septa of varying widths. Regenerative nodules larger than 2 cm in diameter are evidence that the cirrhotic process has persisted for a number of years. This pattern is usually seen in α_1-antitrypsin deficiency, autoimmune hepatitis, late CF liver disease, and Wilson disease. Many cases, however, have characteristics of both types of cirrhosis (mixed), and it is known that micronodular cirrhosis can mature into macronodular or mixed cirrhosis. Macronodular cirrhosis may be suggested on ultrasonography. Care in interpretation of percutaneous needle liver biopsies is needed because of small samples, because of fragmentation of the specimen, or if the specimen is taken from a macronodule. The latter may be suggested by hyperplasia of the hepatocytes or a relative excess of hepatic vein branches. The *histologic classification* is generally more helpful in defining etiology and in management, defining cirrhosis as postnecrotic, biliary (periportal), or hepatic venous outflow (cardiac) cirrhosis. Postnecrotic cirrhosis is the result of liver cell damage and is most commonly seen in chronic hepatitis owing to viral autoimmune factors or drugs. This type of histology is a common sequela of neonatal hepatitis. Features include piecemeal necrosis, bridging fibrosis, collapse of the hepatic lobules, and regeneration, with the development of macronodular cirrhosis. In biliary cirrhosis from cholestatic disorders, there is fibrosis developing from within the portal tracts extending out into the parenchyma linking adjacent portal tracts, with little change in the hepatic parenchyma and preservation of the lobular architecture. In infant cholestasis, bile duct proliferation is a feature of extrahepatic biliary atresia, and bile duct paucity or hypoplasia is a feature of certain intrahepatic cholestatic syndromes. Obstruction to hepatic venous outflow causes centrilobular hemorrhagic necrosis, with fibrosis extending from central veins to portal tracts, and is caused by cardiac lesions, resulting in increased right atrial pressure and vaso-occlusive disorders. In chronic cases, cirrhosis eventually develops, and the initial distinguishing features may be obliterated. Specific histologic patterns occur in Wilson disease (copper pigment deposition), α_1-antitrypsin deficiency (intracellular periodic acid–Schiff–positive, diastase-resistant inclusions), and certain storage disorders.

Pathophysiology

As fibrosis and regenerative nodule formation advances, there is distortion of the liver architecture with compres-

sion of hepatic vascular and biliary structures, resulting in altered hepatic blood flow, and the development of portal hypertension and shunting of blood from hepatic artery to portal vein branches and to hepatic vein tributaries. These hemodynamic disturbances lead to irregular delivery of oxygen and nutrients to the hepatocytes, perpetuating a vicious cycle of events, which may persist even if the original process causing liver injury has ceased. The complications of chronic liver disease and cirrhosis (see Table 5.2-2 and below) are primarily due to impaired hepatic function, cholestasis, and altered hemodynamics.

Malnutrition and Growth Failure

The liver has a central role in regulating fuel metabolism, nutrient homeostasis, and absorption of a number of nutrients. Malnutrition itself may induce further derangements of liver function because the liver requires energy for a number of synthetic, storage, and detoxification functions. These factors, combined with the common symptoms of anorexia and a poor dietary intake, make malnutrition common in chronic liver disease, particularly if the onset is in infants, who are more vulnerable to the debilitating effects of malnutrition because of their higher energy and growth requirements. The body composition of malnourished children with pediatric liver disease is similar to that of protein-energy malnutrition,[34,35] with growth failure and depletion of metabolically active cell mass and body fat stores, resulting from combined disturbances of intake, absorption, and metabolism of nutrients.

Importantly, because many of the above changes are energy linked, overall energy metabolism is compromised in children with chronic liver disease.[35] Studies in biliary atresia show that most patients, compared with controls, are hypermetabolic and catabolic during fasting, with a reduced respiratory quotient (an index of altered substrate oxidation). Protein synthesis is clearly impaired, as evidenced by decreases in circulating protein such as albumin, transferrin, and clotting factors. An abnormal serum amino acid profile is seen, with elevations in plasma aromatic amino acids and depression in branched-chain amino acids.[34] Branched-chain amino acids comprise 40 to 50% of the minimum daily requirement for essential amino acids in humans, playing an important regulatory role in protein synthesis. Neither growth nor positive nitrogen balance is possible without these amino acids. Carbohydrate metabolism is abnormal and is characterized by carbohydrate intolerance, peripheral insulin resistance, hyperinsulinemia, and reduced hepatic glycogen stores. Chronic liver disease also alters the synthesis of lipids, including very-low-density lipoproteins and cholesterol, and hypocholesterolemia has been found to be an adverse factor in the outcome of liver transplant. Disturbances of the growth hormone–insulin-like growth factor 1 axis may also contribute to wasting and growth failure in children with liver disease by virtue of insulin-like growth factor 1 deficiency[36] and growth hormone resistance.[37]

Vitamin K is a necessary cofactor for the conversion of inactive precursors of prothrombin and factors VII, IX, and X into their active forms. Dietary vitamin K requires bile and pancreatic juice for uptake; thus, in cholestasis, vitamin K malabsorption is the primary cause of prolonged prothrombin time. In parenchymal liver disease, there is decreased synthesis of liver-dependent clotting factors. Where vitamin K deficiency results from malabsorption, parenteral vitamin K normalizes the prothrombin time, but in advanced liver disease, vitamin K only partially corrects the prolonged prothrombin time, a useful clinical feature suggesting poor liver synthetic function in advanced chronic liver disease. Vitamin E deficiency is also common, particularly in cholestatic liver diseases and in infants, resulting in a distinctive progressive but preventable neurologic disorder associated with peripheral neuropathy, ophthalmoplegia, and ataxia. Patients with cholestatic syndromes, particularly infants with biliary atresia who have little or no exposure to sunlight, depend primarily on dietary vitamin D to maintain body stores and are particularly likely to develop rickets (defective mineralization) and osteopenia (reduced formation of matrix), with low serum 25-hydroxyvitamin D levels. These have been thought to result mainly from vitamin D and calcium malabsorption, with secondary hyperparathyroidism. However, vitamin D treatment does not easily reverse bone disease, and although there are usually low total 25-hydroxyvitamin D levels, osteocalcin levels are also low, suggesting that decreased bone formation and not increased bone resorption is the main determinant of bone disease.[38] Signs of vitamin A deficiency are not common, but abnormalities of the regulation of metabolism of retinol-binding proteins and biochemical vitamin A deficiency have been reported in infants with biliary atresia.[39] Biochemical deficiencies of water-soluble vitamins, including thiamine and pyridoxine, may occur, and cases of nutritional cardiomyopathy and peripheral neuropathy have been reported. Of the trace elements, iron, zinc, and selenium deficiencies have been reported in children with end-stage liver disease.[34] These can be associated with growth failure and poor protein synthesis.

Ascites and Edema

Ascites and edema are common major complications of decompensated cirrhosis. Although there are differences in the spectrum of etiologies causing end-stage liver disease in pediatric patients, the principles and practices are similar to those in adults. Extravascular fluid accumulation may develop insidiously or be precipitated by events such as gastrointestinal bleeding, infection, or the development of hepatoma, manifest as peritoneal ascites, as peripheral edema, or in the pleural effusion. The two important factors in extravascular fluid accumulation are portal venous pressure and plasma oncotic pressure owing to hypoalbuminemia, both of which interact in chronic liver disease, resulting in fluid redistribution between intra- and extravascular spaces.

Several theories as to the formation of ascites exist. The underfilling hypothesis suggests that increased sinusoidal pressure leads to a cascade of events, resulting in fluid retention owing to elevated portal venous pressure, increased splanchnic volume, decreased systemic vascular resistance, and decreased effective plasma volume. The decreased

plasma volume results in increased activity of plasma renin and aldosterone, resulting in avid renal retention of sodium and water, leading to the accumulation of fluid that is ascites. This hypothesis is supported by the fact that expansion of the plasma volume by methods such as albumin infusion commonly reverses ascites, decreases levels of renin and aldosterone, and results in a diuresis. The overflow hypothesis speculates that inappropriate renal sodium and water retention is the primary abnormality triggered by possible hepatorenal reflex. This hypothesis is supported by some animal models but is mitigated by the observation that the renin-angiotensin-aldosterone system is activated in decompensated cirrhosis. These systems should be suppressed and not activated with sodium retention and volume expansion. The peripheral arterial vasodilation hypothesis suggests that peripheral arterial vasodilation is the initiating event in ascites formation. The fact that patients with chronic liver disease are prone to the development of arteriovenous connections implies the presence of vasoactive hormones. These connections are known to be associated with peripheral vasodilatation and renal sodium retention.

These various models for the development of ascites are not necessarily mutually exclusive. Early overflow secondary to renal sodium retention may be the initiating factor, but in later phases of chronic liver disease, diminished effective plasma volume, with its accompanying hormonal changes, may predominate, leading to peripheral arterial vasodilatation and a further increase in sodium and water retention. Thus, sodium retention seems to be fundamental to the occurrence of ascites and edema. In some patients, sodium retention occurs despite normal activity of the renin-aldosterone and sympathetic nervous systems and increased circulating plasma levels of natriuretic peptides and activity of the so-called natriuretic hormone. This impairment in circulatory function, although less intense, is similar to that in patients with increased activity of the renin-aldosterone and sympathetic nervous systems, suggesting that antinatriuretic factors are more sensitive to changes in circulatory function and that these systems may be important in the pathogenesis of sodium retention. The development of drugs that inhibit the tubular effect of antidiuretic hormone and increase renal water excretion without affecting urine solute excretion has opened a field of great interest for the management of water retention and dilutional hyponatremia in cirrhosis. Two families of drugs, the V_2 vasopressin receptor antagonists[40] and the κ-opioid agonists,[41] have been shown to improve free water clearance and correct dilutional hyponatremia in human and experimental cirrhosis with ascites.

Portal Hypertension and Variceal Bleeding

Portal hypertension is one of the major causes of morbidity and mortality in children with chronic liver disease. It is the result of a combination of increased portal blood flow and increased portal resistance and occurs when portal pressure rises above 10 mm Hg. Signs and symptoms are primarily the result of decompression of this elevated portal blood pressure through portosystemic collaterals. The main clinical features are splenomegaly; the occurrence of esophageal, gastric, and rectal varices; and the development of ascites. Splenomegaly and hypersplenism rarely require specific intervention. The major problems are bleeding from esophageal and other varices, ascites, and nutritional disturbances.

An understanding of portal hypertension requires knowledge of the anatomy of the portal system. Portal capillaries originate in the mesentery of the intestine and spleen and in the hepatic sinusoids. Capillaries of the superior mesenteric and splenic veins supply the portal vein with nutrient-rich and hormone-rich blood supply. At the hilum of the liver, the portal vein divides into two major trunks supplying the right and left lobes of the liver, and these trunks undergo a series of divisions supplying segments of the liver terminating in small branches that pierce the limiting plate of the portal tract and enter the sinusoids through short channels. The partly oxygenated portal venous blood supplements the oxygenated hepatic arterial blood flow to give the liver unique protection against hypoxia. Blood flow from both the hepatic artery and the portal vein is well regulated, allowing the liver to withstand thrombosis of either one of these major vessels. Portal hypertension owing to chronic liver disease may arise because of a prehepatic or intrahepatic block, where the block may be presinusoidal, sinusoidal, or postsinusoidal.

The major pathologic effect from portal hypertension is the development of collaterals carrying blood from the portal venous system to the systemic circulation in the upper part of the stomach, the esophagus, and the rectum and in the falciform ligament and may drain into the inferior vena cava or the left renal vein. Only the submucosal collaterals, such as in the esophagus, stomach, and, rarely, other parts of the intestine, are associated with gastrointestinal bleeding. Collaterals in other parts of the intestine are more frequently likely to occur at sites of surgery along the gastrointestinal tract, particularly from stoma and anastomotic sites. Portal hypertensive gastropathy, which is suggested by dilated mucosal veins and capillaries and mucosal congestion in the stomach, develops particularly in patients who have had variceal obliteration.

Although changes in vascular resistance to flow of blood between the splanchnic bed and the right atrium appear to be the initial events in the development of portal hypertension, a number of other hemodynamic changes contribute to and amplify the increased portal blood pressure. There is a hyperdynamic circulatory state with increased cardiac and decreased splanchnic arteriolar tone, both of which increase portal inflow. Studies in animal models indicate that a number of humoral mediators are involved, including glucagon, prostaglandins, nitrous oxide, and endothelium-derived relaxing factor. Changes in intravascular volume also play an important part in pathophysiology of the hyperdynamic circulation, as do alterations in adrenergic tone in the splanchnic system. All of these observations have led to new experimental and clinical studies, suggesting possible pharmacologic treatments for portal hypertension,[41] although because the major clinical effect is that of bleeding from esophageal

varices, direct treatment of variceal hemorrhage remains the major approach.

Hepatic Encephalopathy

Hepatic encephalopathy is difficult to recognize in children, particularly in infants. Early symptoms of encephalopathy in children are subtle and include neurodevelopmental delay, school problems, and lethargy or sleep reversal. Intellectual impairment and personality change may occur in older children, whereas clouding of consciousness, progressing to stupor and coma, is a late sign. Clinical signs such as ataxia, tremor, and dysdiadochokinesia are difficult to determine in small children. Precipitating factors are common and include an oral protein load, gastrointestinal bleeding, the use of sedatives, infections, and following shunt surgery for portal hypertension.

The pathophysiology appears to be determined by four events: portosystemic shunting, hepatocellular dysfunction, interaction of nitrogen metabolites from the intestine with the central nervous system, and altered neurotransmitter function. From clinical observations and from studies in experimental models, nitrogenous products such as ammonia derived from the gastrointestinal tract appear to be of primary importance. Hepatic encephalopathy is rare when liver function is able to remove nitrogenous intestinal metabolites, although portosystemic shunting alone can result in encephalopathy if the subject is given a high-protein diet. However, serum ammonia levels, which are usually elevated in encephalopathy, do not directly correlate with cerebral state, and experimental evidence suggests that such changes are not the only ones responsible for the encephalopathy. Neuropathologically, there is astrocytic (rather than neuronal) alteration. Magnetic resonance imaging reveals bilateral signal hyperintensities, particularly in the globus pallidus on T_1-weighted imaging, a phenomenon that may result from manganese deposition. Proton (^1H) magnetic resonance spectroscopy shows increases in the glutamine resonance in brain, reflecting increased brain ammonia removal. Although the exact molecular mechanisms are not known, excitatory or inhibitory neurotransmitter imbalance leading to dysfunction of the glutamate–nitric oxide (NO) system is thought to play a major role. Activation of glutamate receptors leads to an increase in intracellular calcium, which initiates several calcium-dependent processes, including NO formation. NO is a gaseous, highly reactive, freely diffusible molecule with a short half-life. Increased expression of the neuronal isoform of NO synthase and the uptake of L-arginine (the obligate precursor of NO) has been demonstrated. Hyperammonemia associated with liver dysfunction results in increased NO, which may lead to learning and memory impairments. Other metabolic factors that have been implicated are short-chain fatty acids such as butyrate, valerate, and octanoate, which are increased in the plasma and cerebrospinal fluid in encephalopathy and may act synergistically with ammonia.

Hepatic encephalopathy may also be affected by the accumulation of inhibitory neurotransmitters in the brain. Neurotransmitters mediate the postsynaptic action of neurons. Inhibitory neurotransmitters may be false (not ordinarily present in the brain) or true, such as the amino acid γ-aminobutyric acid (GABA), which is produced in the brain by the decarboxylation of glutamic acid. False transmitters may be produced by metabolism of amines by gastrointestinal tract bacteria and are normally removed by the portal circulation by the liver. False neurotransmitters have not been found to induce encephalopathy in experimental animals. In contrast, GABA has an important role in central nervous system inhibition, and GABA-like activity has been found in portal blood in both animal models and subjects with chronic liver failure after gastrointestinal hemorrhage. In experimental models, GABA may produce coma. The GABA receptor is activated not only by GABA but also by benzodiazepines. The reversal in some patients of encephalopathy after the administration of a benzodiazepine antagonist supports this hypothesis, although the effect is not entirely consistent. Alterations in neurotransmitter function may also occur as a result of disturbances of amino acid metabolism, particularly deficiency of branched-chain amino acids and excess of aromatic amino acids.

Further alterations in brain function can be induced by hypoglycemia, which is common even with fasting in young children, respiratory alkalosis leading to a decrease in cerebral perfusion, or hypoxemia owing to hemodynamic changes. Aggravating factors include gastrointestinal hemorrhage, hypovolemia, hypokalemia, sedatives, anesthetics, sepsis, and high protein intake, which increase the endogenous nitrogen load, thus precipitating overt encephalopathy.

Coagulopathy

The liver plays an important role in hemostasis by a complex balance between the production of coagulation proteins and inhibitors of coagulation and the removal of fibrin degradation products and coagulation factors. Thus, coagulation disorders are common in chronic liver disease owing to a combination of vitamin K malabsorption and deficiency, reduced synthesis of coagulation factors and inhibitors of coagulation, thrombocytopenia secondary to hypersplenism, or intravascular coagulopathy. These disturbances are particularly important in prognostic assessment and in the genesis and management of gastrointestinal bleeding and may lead to serious complications, such as intracerebral bleeding and intravascular coagulopathy.

Hepatopulmonary Syndrome

This syndrome is defined as a triad of liver dysfunction, intrapulmonary arteriovenous shunts, and arterial hypoxemia and is relatively common in childhood cirrhosis. Oxygen saturations of less than 90% and cyanosis appear unrelated to the severity of liver damage but are associated with the occurrence of clubbing. The pathogenesis appears to be multifactorial, including intrapulmonary shunts, arteriovenous shunts, ventilation-perfusion mismatch, and portopulmonary venous anastomoses. In some cases, the degree of cyanosis may be proportionally greater than the hypoxemia. In infants, hypoxemia can be aggravated by poor respiratory effort related to ascites or hepatomegaly.

Such patients have dyspnea at rest, particularly when upright, which is often relieved by lying down.

The diagnosis is established by ensuring that there is no underlying cardiac defect (echocardiography, electrocardiography, or cardiac catheterization). Simple transcutaneous techniques of oxygen monitoring can be of most diagnostic value.[42] Ventilation-perfusion scans will demonstrate the presence of extrapulmonary isotope in the cerebral blood and other organs; lung function tests are usually normal. Although no specific treatment for this syndrome has been proven, the degree of hypoxemia is improved by oxygen administration, attention to nutrition support, and control of ascites. The hepatopulmonary syndrome may seriously limit tolerance to anesthesia. During or after transplant, worsening hypoxemia may be improved by using inhaled NO. Liver transplant will reverse the systemic and pulmonary vascular changes, although recovery may be slow.

Pulmonary Hypertension

This may occur in cirrhosis as a result of failure of degradation of vasoactive substances in the splanchnic circulation associated with portopulmonary venous anastomoses and paraesophageal portosystemic collaterals within the pulmonary venous system. The presenting feature is cyanosis, but early signs include right ventricular hypertrophy or accentuation of the pulmonary vessels on chest radiography.

Hepatorenal Syndrome

This syndrome is a functional progressive renal failure of unknown cause occurring in patients with severe liver disease. It is a serious complication of cirrhosis and carries a poor prognosis. Although the pathogenesis is not understood, abnormalities of renal cortical blood flow appear central to the pathogenesis. A high incidence of glomerulosclerosis and membranoproliferative glomerulonephritis has been documented in children with end-stage liver disease at the time of liver transplant,[43] probably secondary to chronic reduction in renal cortical blood flow. Studies in adult cirrhotics have suggested that those without hepatorenal syndrome have systemic vasodilatation, whereas those with hepatorenal syndrome have evidence of peripheral vasoconstriction, leading to the hypothesis that patients with the hepatorenal syndrome have increased splanchnic blood pooling, resulting in decreased renal blood flow, possibly related to up-regulated endothelial NO synthase. Administration of vasopressin, which causes splanchnic vasoconstriction, has been shown to increase glomerular filtration and renal blood flow[44] and forms the basis of current approaches to medical therapy. Renal vasoconstriction is also possibly related to an increase in the production of thromboxane, a potent vasoconstrictor, and a decrease in prostaglandin 2, a dilatory metabolite.[45]

Bacterial Infections

Bacterial infections are common in chronic liver disease and may precipitate other complications, such as encephalopathy, ascites, and hepatorenal syndrome. Urinary and respiratory tract infections are frequent, and bacteremia commonly results from invasive investigations. Spontaneous bacterial peritonitis is a common serious complication of ascites and should always be excluded in all children with sepsis. Immune deficits associated with chronic liver disease include abnormalities of complement and opsonization, impaired function of Kupffer cells, neutropenia, and alterations in mucosal barriers, particularly the gastrointestinal tract. Portal hypertension makes patients susceptible to frequent bacteriemia, perhaps by inducing bacterial translocation of the gut. The specific risk factors for infection are low serum albumin, gastrointestinal bleeding, intensive care unit admission for any cause, and therapeutic endoscopy. Certain infectious agents are more virulent and more common in patients with liver disease. These include *Klebsiella*, *Escherichia coli*, *Vibrio*, *Campylobacter*, *Yersinia*, *Plesiomonas*, *Enterococcus*, *Aeromonas*, *Capnocytophaga*, and *Listeria* species, as well as organisms from other species.

Hepatocellular Carcinoma

Hepatocellular carcinoma may occur in the setting of cirrhosis in childhood. The mechanisms for development are unknown. Associations include hepatitis B, in which children with neonatally acquired hepatitis B have developed hepatocellular carcinoma from the age of 7 or 8 years.[46] There is also a close association of the development of hepatocellular carcinoma with tyrosinemia type I, with one study suggesting an occurrence rate of 37% in patients surviving beyond 2 years of age.[47] Patients may present with abdominal pain and/or abdominal mass or an increase in α-fetoprotein, but hepatocellular carcinoma may be found incidentally at liver transplant.

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3. Normal Hepatocyte Function and Mechanisms of Dysfunction

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The hepatocyte is the most abundant cell type in the liver. It is responsible for most of its metabolic functions and is the target cell of many diseases. Both acquired and congenital diseases of the liver affect hepatocytes. Liver-based metabolic diseases are numerous and often result from the abnormal expression of a single gene in hepatocytes. This chapter presents important structural aspects of hepatocyte function; discusses the role of hepatocytes in the metabolism of carbohydrates, proteins, fat, and other molecules; and focuses on mechanisms of dysfunction and injury, such as apoptosis, necrosis, and regeneration. Hepatocyte transplant is also discussed as a model of function and dysfunction. Biliary excretory function and injury are discussed in Chapter 5.1, "Bile Formation and Cholestasis."

STRUCTURAL BASIS FOR HEPATOCYTE FUNCTION

The liver, the cradle of the soul according to the ancient Greeks, is the largest organ in the body, weighing 2 to 2.5% of total body weight. A closer approximation for liver weight for transplant has been developed as 772 × body surface area (–38 if less than 1 m^2).[1] If hepatocytes are isolated from the liver, each gram of tissue yields an average of approximately 50 million hepatocytes.[2] A human left liver lobe, for example, contains, in general, over 10 billion hepatocytes. Hepatocytes constitute approximately 60% of the total cells in the liver. The other 40% are called nonparenchymal cells and include macrophage-derived Kupffer cells, which are important in host defense and mediators of the inflammatory response; fenestrated endothelial cells; lymphocytes; and the stellate cell, which is responsible for the synthesis of extracellular collagen in response to liver and hepatocyte injury. Hepatocytes provide a selective barrier between the external and internal milieu by cementing themselves with gap and tight junctions, which, in turn, provide polarity and restrict distinct activities to three separate membrane domains: basolateral, apical, and lateral (Figure 5.3-1).[3] At the basolateral (sinusoidal) membrane, hepatocytes exchange metabolites with the blood. At the apical (canalicular) membrane, hepatocytes secrete bile, detoxified waste products, cholesterol, and phospholipids. The bile canaliculi are formed by the tight junction–bound apical membranes and are the earliest component of the bile drainage system. Disruption of tight junctions can permit leakage of bile from canaliculi into the sinusoids and circulation. The lateral membrane is the surface between adjacent hepatocytes. Gap junctions permit attachment between hepatocytes and nerve impulse transmission between hepatocyte acinar zones.

HEPATOCYTE LOBULE

Two models exist of hepatic organization: the lobule and the acinus. The lobules have a central vein, a portal area, and liver plates that converge from portal area to central vein. The portal space at the periphery of the lobule contains a hepatic arteriole, a portal venule, a bile ductule, nerves, and lymphatics. Lobules are cylindrical structures measuring several millimeters in length and 1 to 2 mm in diameter. The human liver contains approximately 50,000 individual lobules. Blood enters the lobule from the portal area, traverses the hepatic sinusoids, and is collected into the central veins toward which the hepatic cellular plates converge (Figure 5.3-2). Central veins join and drain into the hepatic veins and subsequently into the right atrium of the heart.

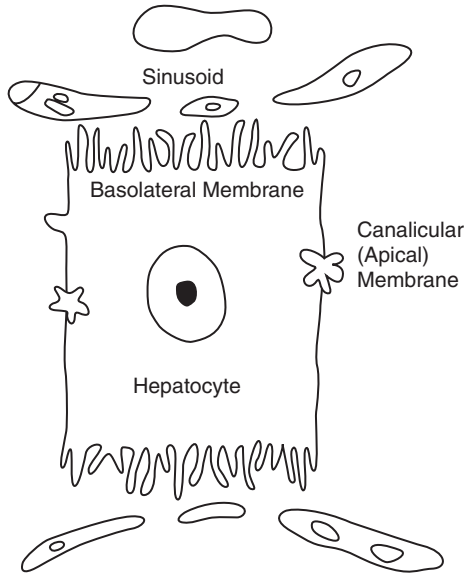

FIGURE 5.3-1 Hepatocytes are polarized and bound by three membrane domains: the lateral membrane between adjacent hepatocytes, the basolateral (sinusoidal) membrane that abuts the sinusoidal space, and the apical or canalicular membrane that forms the bile canaliculi.

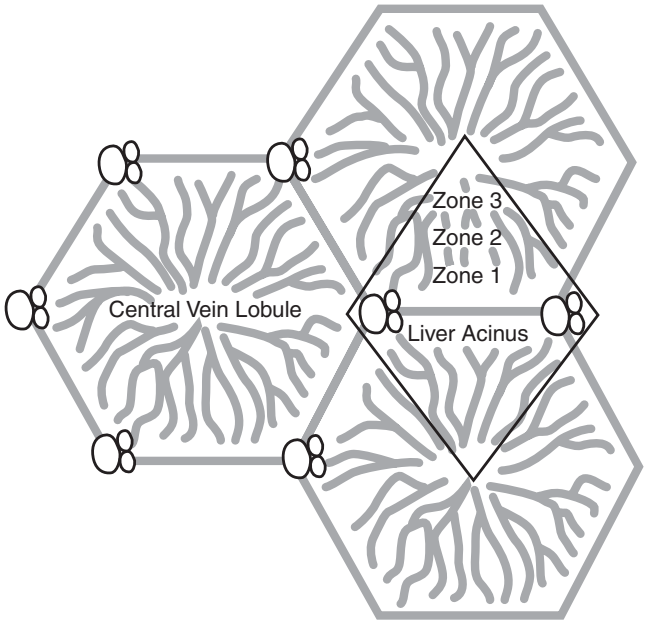

FIGURE 5.3-2 Hepatocyte lobule: blood flow goes from portal triads toward the central vein. Bile is collected in the opposite direction into the portal ducts. Sinusoids and hepatic cords are arranged as trabeculae between portal and central vein areas.

Acinar Zonal Differentiation

The simple liver acinus is arranged around an axis containing the hepatic arteriole, portal venule, and ductule that grow out from one portal area (see Figure 5.3-2, diamond area). Hepatocytes vary in their metabolic functions depending on their location within the hepatic lobule. Periportal hepatocytes (zone 1) receive blood rich in oxygen and nutrients from the portal venules and hepatic arterioles. Pericentral hepatocytes (zone 3) receive blood that has already traversed most of the sinusoid and is thus lower in nutrients and oxygen and higher in waste products. These differences result in variations in hepatocyte synthetic function, proliferative potential, ability to detoxify substances, and susceptibility to drug or ischemia injury. Periportal hepatocytes specialize in oxidative metabolism, whereas pericentral hepatocytes detoxify drugs. The periportal hepatocytes are also predominantly responsible for converting ammonia to urea by the concerted action of the urea cycle enzymes. This is a high-capacity, low-affinity system, and because periportal cells also generate ammonia from deamination of amino acids, ammonia reaches the pericentral hepatocytes. Pericentral hepatocytes exclusively express glutamine synthetase and can uptake this ammonia to synthesize glutamine. Thus, pericentral hepatocytes scavenge ammonia with high affinity, convert it to glutamine, and prevent toxic ammonia from reaching the systemic circulation.

Liver Cellular Structure

Hepatic plates are usually two cells thick and are bound by tight junctions that separate the sinusoidal space from the bile canaliculi. Endothelial cells line the sinusoids. Between adjacent hepatocytes lie the bile canaliculi that empty into bile ductules located in the portal spaces. Hepatocytes are thus polarized and bound by three membrane domains: the lateral membrane between adjacent hepatocytes, the basolateral membrane that abuts the sinusoidal space, and the apical or canalicular membrane (see Figure 5.3-1). Endothelial cells in the liver are very specialized. They have pores measuring almost 1 μm in diameter. This is a very large area considering that a red blood cell measures, on average, 6 μm in diameter. These fenestrated endothelial cells that lack basement membranes facilitate rapid exchange of substances between plasma and hepatocytes. Hepatocytes have microvilli on the sinusoidal plasma membrane, which facilitate the exchange of nutrients. In addition, the low pressure and slow blood flow further enhance bidirectional transfer of solutes. Between endothelial cells and the hepatocytes are narrow spaces called the spaces of Disse, which interconnect and drain into the lymphatic vessels that are located in the portal areas. Hepatic lymph is formed when there is increased sinusoidal pressure, especially with obstruction to the outflow of blood from the liver. This lymph may accumulate as ascites. Two other cell types found around the sinusoidal space include the Kupffer and the stellate cells. The stellate cell, also known as a fat storage or Ito cell, is a major site for vitamin A storage and can be identified by its high lipid content. Relevant to disease, the stellate cell is the major cell type associated with the development of hepatic fibrosis in response to liver injury. With liver injury, stellate cells become activated to a myofibroblast-like state, which is associated with collagen gene expression, reduction of vitamin A content, and morphologic changes. Kupffer cells are prominent in the sinusoids, macrophage derived, and the principal phagocytic cells of the liver. Kupffer cells are important mediators in the inflammatory response in the liver.

Liver's Unique Blood Flow

To understand hepatocyte physiology, it is necessary to know the significance of the liver blood flow. It is surprising how frequently physicians are not aware of the portal circulation's unique characteristics. The liver is the first organ to receive the nutrient-enriched blood from the gut's venous system, which converges via the portal vein and branches into the liver sinusoids.

Whereas blood from the extremities and the rest of the body returns directly to the heart, blood from most abdominal organs collects into the portal vein and its branches within the liver and is distributed into the hepatic sinusoids. About 80% of the liver blood supply comes via the portal vein from the stomach, small and large bowel, spleen, and pancreas. The portal system has high flow (approximately 1 L/min in adults), but low vascular resistance allows the portal pressure to be low, around 9 mm Hg, whereas the pressure in the hepatic vein leading from the liver into the vena cava is close to 0 mm Hg. During cirrhosis, the increase in fibrous tissue results in increased vascular resistance and portal hypertension of 15 to 20 mm Hg above normal. Acute liver failure, possibly owing to inflammation and necrosis in the liver parenchyma, also results in increased portal pressures.

HEPATOCYTE METABOLIC FUNCTIONS

Hepatocytes are key cells in the synthesis and catabolism of carbohydrates, proteins, and lipids.[4] The liver is thus a primary site for the metabolism of organic and inorganic substrates. Such functions allow hepatocytes to prevent disease by detoxifying endogenous and exogenous toxins. Bile formation and secretion into the bowel are dealt with in Chapter 5.1.

Hepatocyte Carbohydrate Metabolism

Normal hepatocyte function is critical to maintain the body's glucose homeostasis and carbohydrate metabolism. Glucose is the primary source of energy for the brain, muscle, and kidney. The liver can store and modulate the availability of ingested nutrients according to the requirements of peripheral organs for energy sources. The ability to fast relies on the ability of hepatocytes to store glycogen and synthesize glucose from amino acids, glycerol, and, principally, glycogen degradation. These metabolic functions are, in turn, regulated by the functions of the pancreas, adrenal glands, and thyroid. Although peripheral need for glucose is met by gluconeogenesis and glycogenolysis, excess glucose is converted by the hepatocytes into amino acids, fatty acids, and glycogen, which is the major form of glucose storage. Glucose entry or exit from the hepatocytes occurs via the glucose transporter 2, a facilitative low-affinity, high-capacity glucose transporter that responds directly to local plasma glucose concentrations.[5,6] During hepatocyte injury such as in acute liver failure, glucose homeostasis ability is impaired and hypoglycemia results, which is occasionally severe enough to cause generalized seizures.

Hepatocyte Protein Metabolism

Synthesis. All proteins in plasma, except the immunoglobulins, are synthesized by the hepatocytes. Immunoglobulins are synthesized in the reticuloendothelial tissues throughout the body by B lymphocytes. Hepatocytes make about 90% of all of the plasma proteins at a rate of 15 to 50 g/d. This is a rapid rate considering that an adult has approximately 350 g of plasma proteins. Indeed, when there are important protein losses, hepatocytes undergo mitosis, the liver size grows, and the plasma protein synthetic rate increases dramatically until the plasma concentration returns to normal. The most prominent of the serum proteins is albumin, and its serum concentration is used to evaluate the liver's synthetic capacity. In cirrhosis, for example, in part owing to decreased synthesis, plasma protein and albumin concentrations can decrease significantly.

Among the proteins synthesized by hepatocytes, clotting factors are of special significance. Other than factor VIII, which is also platelet dependent, all of the coagulation factors are synthesized in the liver. Factors II, VII, IX, and X are also dependent on vitamin K sufficiency. During liver failure, bleeding owing to impaired coagulation occurs often and coagulation factor replacement may be necessary. Hepatocytes are also responsible for the interconversion of the various amino acids through several stages of transamination. All of the nonessential amino acids can be synthesized in this way from other amino acids and the corresponding keto acid.

Catabolism. Hepatocytes are the main cells responsible for the deamination of amino acids in the process of their conversion into energy or for the synthesis of carbohydrates and lipids. Other organs in the body, such as the kidneys, can perform a small amount of deamination.

Detoxification. Hepatocytes are also responsible for converting ammonia into urea for excretion by the kidneys. Ammonia is the principal by-product of amino acid metabolism. In addition, ammonia is continually formed in the bowel by bacteria and absorbed via the portal circulation. During liver failure, as the hepatocyte's detoxifying function is impaired, serum ammonia and several other toxic metabolites accumulate in the bloodstream, resulting in progressive encephalopathy, cerebral edema, and, eventually, death.

Hepatocyte Lipid Metabolism

Most cells in the body metabolize lipids. However, some aspects of lipid metabolism are carried out mainly by hepatocytes. These include fatty acid oxidation to supply energy for other parts of the body; synthesis of cholesterol, phospholipids, and lipoproteins; and synthesis of lipids from excess carbohydrates and proteins.

Triglycerides are broken down in the liver into glycerol and three fatty acid molecules. The fatty acid chains are split into two-carbon units and bound to coenzyme A (CoA) in the form of acetyl CoA. This molecule can enter the Kreb citric acid cycle in the common terminal pathway of carbohydrate metabolism to liberate a large amount of energy. The hepatocytes, however, cannot use all of this energy, and acetyl-CoA molecules are combined to form acetoacetic acid, a highly soluble ketone body, which is transported by the circulation to other parts of the body. In these tissues, acetoacetic acid is transformed back into acetyl CoA and is oxidized for energy.

Hepatocytes uptake cholesterol and lipoproteins from the bloodstream using specific receptors. Genetic diseases of these receptors can lead to severe hypercholesterolemia. The liver also synthesizes cholesterol. About 80% of it is converted into bile salts, which are secreted into the bile. Other cholesterol and phospholipids circulate as lipoproteins to cells everywhere in the body and are used to form membranes, intracellular structures, and other chemical compounds important to cellular function. In chronic cholestatic diseases, cholesterol excretion can be impaired, resulting in hypercholesterolemia.

Most excess carbohydrate and proteins are converted into lipids by the hepatocytes. Lipids thus synthesized are transported as lipoproteins into adipose tissue, where they are stored as fat.

Other Metabolic Functions

Vitamin Storage. The liver stores large amounts of vitamins, in particular vitamins A, D, and B_{12}. Storage of vitamins occurs in nonhepatocyte cells. For example, vitamin A is

stored in perisinusoidal stellate cells. Stellate cells, when activated, produce collagen and contribute to the pathogenesis of cirrhosis. It has been estimated that in a healthy individual, storage of these vitamins is sufficient to prevent deficiencies in spite of no intake for as long as 10 months for vitamin A, 3 months for vitamin D, and 1 year for vitamin B_{12}.

Iron Storage. Hepatocytes contain large amounts of apoferritin. When excess iron is available, it is taken up by hepatocytes and combined with aproferritin to form ferritin. Other than the iron in the hemoglobin, ferritin is the most important iron storage site in the body. Interestingly, several liver disorders, as well as most chronic liver diseases, are associated with increased iron storage in the hepatocytes.

Excretion of Exogenous Substances. Several important substances are excreted by hepatocytes into the bile. For example, the role of hepatocytes in the excretion of calcium via the bile and into the gut and feces is often overlooked. Many drugs are metabolized and excreted by the hepatocytes in the liver. Some of these include penicillins, sulfonamides, erythromycin, cyclosporine, and most anticonvulsants. Hormones, such as thyroxine, estrogen, and cortisol, are also metabolized or excreted by the liver. Liver failure often leads to accumulation of these hormones. For example, excess estrogen effects can be seen in people with chronic liver disease.

LIVER REGENERATION AND HEPATOCYTE PROLIFERATION

Although the conventional wisdom only 20 years ago placed the liver and the brain as organs exhibiting little proliferation, remarkable studies in liver regeneration after hepatectomy, living-related liver transplant, and seminal work in hepatocyte transplant have shown that hepatocytes can proliferate rapidly and are able to undergo over 20 cell divisions.[7] Such remarkable proliferation was already hinted at by the ancient Greeks in the myth of Prometheus, who had his liver eaten daily by an eagle only to regenerate it by night. Hepatocyte regeneration after liver injury is closely regulated by molecular and biochemical processes.[8] Most liver injuries resulting in significant reduction of the liver mass are followed by transcription of immediate early genes and result in key growth factors and mediator release, including hepatic growth factor (HGF), epidermal growth factor, and transforming growth factor-α. The serum concentrations of these factors, in particular that of HGF, vary according to the type and degree of liver injury. HGF promotes proliferation by binding to its receptor, the cellular homologue of the *met* oncogene, in activated cells. HGF is a heterodimer glycoprotein produced by nonparenchymal liver cells as a proprotein. Transcription of the HGF gene increases 12 to 24 hours after surgical resection in rodents. However, infused HGF in the normal rat does not cause hepatocyte proliferation. For hepatocytes to proliferate, initial signals must have moved hepatocytes from their resting G_0 cell-cycle stage to the G_1 stage, where they are committed to undergo regeneration. Deoxyribonucleic acid (DNA) synthesis begins a few hours after hepatectomy or injury, resulting in a reduction of liver mass. A coordinated cascade of events occurs prior to DNA synthesis involving unique genes that are highly expressed early during this process. These genes encode a variety of proteins, such as transcription factors, tyrosine phosphatases, tyrosine kinases, membrane receptors, and, ultimately, enzymes and proteins involved in proliferation. The highest concentrations of liver-related growth factors are seen during acute liver failure and after major hepatic resection. Changes are modest in acute hepatitis and in chronic liver disease. These growth factors directly affect hepatocyte renewal and, provided that the liver environment is permissive, whether regeneration will keep up with cell death. The hope is that in understanding the regulation of these processes, new strategies for the treatment of diseases that injure the hepatocyte are developed.

APOPTOSIS IS A COMMON PATHWAY OF CELL DEATH DURING LIVER DISEASE

Apoptosis is an important component of hepatocyte physiology during organogenesis, regeneration, and liver disease.[9] Apoptosis is the process by which hepatocytes "tagged" for death undergo orderly cell disassembly and removal by macrophages without tissue inflammation. Apoptosis is regulated by a variety of internal and external factors or signals. Cells undergoing apoptosis include those injured by disease but also senescent cells or cells during regeneration, remodeling, and organogenesis. A classic example of apoptosis during organogenesis occurs in the hand during digit formation, which is accomplished by apoptosis of the budding hand tissue between the fingers. Similarly, apoptosis occurs during liver remodeling. During disease, for apoptosis to occur, cell injury has to be severe enough to overwhelm the cell's repair mechanisms. Sudden or too severe injury might cause cell destruction or necrosis instead.[10] At a molecular and ultrastructural level, apoptosis is characterized by internucleosomal cleavage of the DNA into fragments of 180 to 200 bp, chromatin condensation, shrinkage of cell volume, formation of apoptotic bodies, and blebbing of the plasma membrane. Because cleavage occurs at specific internucleosomal or unprotected sites, DNA fragments are formed that are a multiple of 180 to 200 bp, creating a ladder in an agarose gel after electrophoresis (Figure 5.3-3). In contrast, necrosis occurs as the result of rapid cell poisoning and is characterized by immediate loss of plasma membrane integrity.[11] In normal human liver, few apoptotic cells are seen because of the long half-life of hepatocytes. Examples of diseases during which apoptosis plays an important role include ischemia-reperfusion injury, viral hepatitis, autoimmune hepatitis, and toxic injury. FasL binding of the Fas receptor on the hepatocyte cell membrane has been shown to mediate cell death in many of these liver diseases.[12–16] Drugs that inhibit apoptosis have been postulated as potential therapies of some of these diseases. For example, a common medication used in people with liver disease, ursodeoxycholic acid, has antiapoptotic actions in addition to its choleretic effects. Strategies have been suggested for the treatment of liver disease via inhibi-

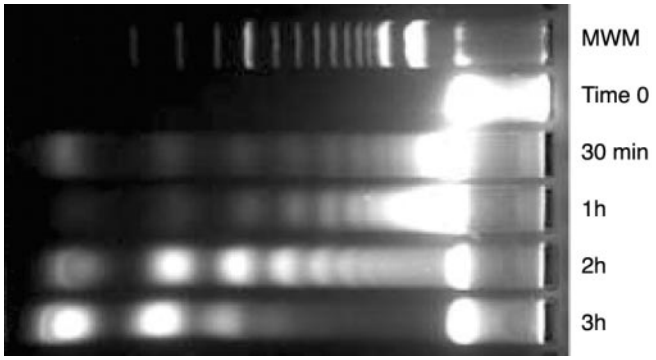

FIGURE 5.3-3 Deoxyribonucleic acid (DNA) fragmentation in detached hepatocytes was confirmed by DNA laddering after 30 minutes in detached culture. The degree of fragmentation, from oligo- to mononucleosomes, increased with time. MWM = molecular weight marker.

tion of tumor necrosis factor (TNF) family pathways and possibly the activation of nuclear factor-κB, a signaling molecule that might inhibit hepatocyte apoptosis.[17]

Specific pathways exist that can trigger apoptosis in hepatocytes. Apoptosis by activation of death receptors of the TNF superfamily, such as CD95/Fas, has been well characterized.[18] Soluble or cell-bound ligands, such as FasL, bind Fas on the surface of hepatocytes and promote formation of a death-inducing signaling complex. This complex, via the Fas-associated death domain protein, activates procaspase 8. In liver cells, caspase 8 activation leads to cleavage of Bid, a proapoptotic Bcl-2 family member.[19] Cleaved Bid translocates into the mitochondria, where it promotes cytochrome *c* release. In the cytoplasm, cytochrome *c* associates with Apaf-1 and then procaspase 9, forming the apoptosome. The apoptosome activates procaspase 3 and the downstream cascade of effector caspases 3, 6, and 7.[18] Effector caspase activation leads to a coordinated program of cell disassembly that results in apoptosis. It includes the selective cleavage of target proteins, DNA fragmentation, phosphatidyl serine translocation to the external aspect of the cell membrane, and characteristic morphologic changes, such as nuclear shrinkage, loss of cell shape, and membrane blebbing.[20] FasL binding of the Fas receptor on the hepatocyte cell membrane often mediates hepatocyte cell death during liver disease and can be readily reproduced in vitro (Figure 5.3-4). One described mechanism of alcohol-induced liver injury involves oxidative stress induced by overexpression of FasL on the surface of hepatocytes. Because hepatocytes constitutively express Fas, the receptor for FasL, overexpression of FasL results in self-induced death or "fratricide."[12,13] Another example of Fas-mediated liver cell death occurs during hepatitis.[15] Indeed, increased Fas antigen is detected in the liver tissue of patients with hepatitis C, and Fas pathways are a major mechanism of T cell–mediated cytotoxicity.[14] Additionally, Fas-mediated apoptosis also occurs in liver allograft rejection.[16] Apoptosis occurs in isolated and transplanted hepatocytes.[21] In liver cell transplant, Fas-FasL interactions have been studied as a way to enhance engraftment of transplanted hepatocytes.[22]

A delicate balance between pathways that promote and inhibit apoptosis exists. Bcl-2 is an example of a molecule that inhibits apoptosis in the liver. Bcl-2, a signaling molecule in the Fas pathway, inhibits Bid-mediated mitochondrial cytochrome-*c* release, a key event during Fas-induced apoptosis. Bcl-2 transgenic hepatocytes are protected from Fas-mediated liver apoptosis. Bcl-2 transgenic hepatocytes were transplanted into wild-type livers of mice treated with nonlethal doses of Jo2 antibody, an activator of Fas. Because of selective death of the recipient's hepatocytes by Fas activation, the transplanted Bcl-2 hepatocytes had a survival advantage and repopulated up to 16% of the host liver.[22] A similar strategy was used to achieve therapeutic liver repopulation in a mouse model of hypercholesterolemia.[23] Genetic manipulation has been used to enhance hepatocyte survival in liver transplants in rodents. Whole-organ liver transplants of mutant graft livers expressing an inactive form of Fas (MRC-lpr/lpr) were resistant to apoptosis induced by FasL overexpression after adenoviral transfection.[24] Although these data illustrate

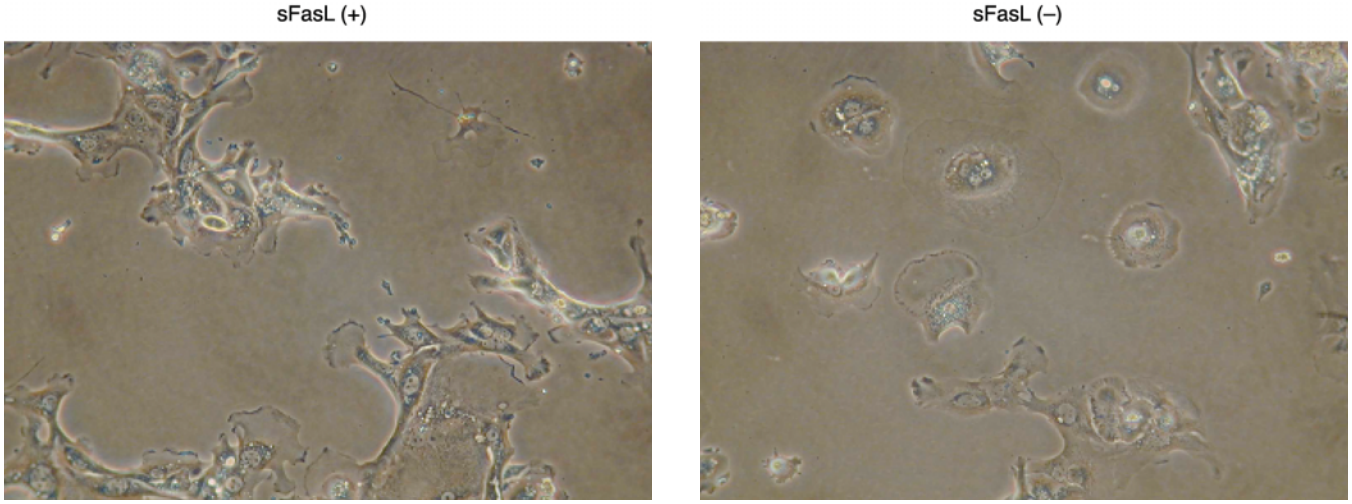

FIGURE 5.3-4 Phase-contrast microscopy of primary mouse hepatocytes in culture incubated with or without sFasL (50 ng/mL) for 12 hours. Changes in cell shape, loss of cytoplasm, and decrease in nuclear size are apparent in sFasL-treated cells (×200 original magnification).

important mechanisms, there is still much to understand regarding liver- and cell-specific signaling pathways. Bcl-2, an inhibitor of apoptosis signal transduction, for example, is actually not expressed normally in hepatocytes but in small bile ducts and in endothelial cells.

HEPATOCYTE NECROSIS

Hepatocyte necrosis occurs in acute liver failure by toxic or ischemic injury. Necrosis results in leakage of hepatocyte cytoplasmatic enzymes into the systemic circulation, which can be detected by elevation of alanine aminotransferase and aspartate aminotransferase. Other enzymes, such as sorbitol dehydrogenase and lactic dehydrogenase, also leak into the systemic circulation. In chronic liver diseases in which apoptosis is a predominant mechanism of injury, such as in hepatitis C, alanine aminotransferase elevation correlates only moderately well with inflammation. Cell death by necrosis occurs when the injury is intense or sudden; the mechanisms of defense and response to injury are overwhelmed. Rather than the organized cell disassembly that occurs with apoptosis, necrosis involves inflammation, accumulation of toxic metabolites, and activation of the liver's reticuloendothelial cells. Necrosis involves coagulation of the cell's proteins and disorganized loss of cell structure. During necrosis, the nucleus is often marginated, the cell swells, and histologic changes persist for days. Different toxins can produce necrosis in different areas of the hepatic lobule. Injury by carbon tetrachloride, for example, affects the centrilobular, less oxygenated hepatocyte zone 3 and is followed by hepatocyte proliferation (Figure 5.3-5). During acetaminophen overdose, an excess of the metabolite *N*-acetyl-*p*-benzoquinoneimine is produced that covalently binds to proteins and macromolecules, causing cellular damage. Under normal circumstances, this metabolite would be inactivated by binding to glutathione. During acetaminophen overdoses, however, free glutathione is quickly depleted, resulting in necrosis. Mechanisms of hepatocellular necrosis involve lipid peroxidation, mitochondrial damage, enzyme inhibition, and disruption of the cytoskeleton. Ceramides, for example, induce liver cell necrosis by causing adenosine triphosphate depletion and mitochondrial depolarization, leading to permeability transition and mitochondrial failure.[25] Other mediators can both protect or promote damage depending on the context. Nitric oxide, for instance, can protect from apoptosis, but it potentiates oxidative damage from warm ischemia reperfusion.[26] The hepatocyte is not the only cell involved during liver necrosis. Cellular events that occur following hepatocyte injury include sinusoidal cell activation, activation of Kupffer cells, and migration of inflammatory cells to the injury site that enhance hepatocyte damage.

MODELS OF HEPATOCYTE TRANSPLANT, PROLIFERATION, AND INJURY

Hepatocyte Transplant and Proliferation

Liver cell isolation has been possible for over 25 years by using calcium chelators that disrupt cell-cell desmosomal adhesion, followed by collagenase enzymes that break

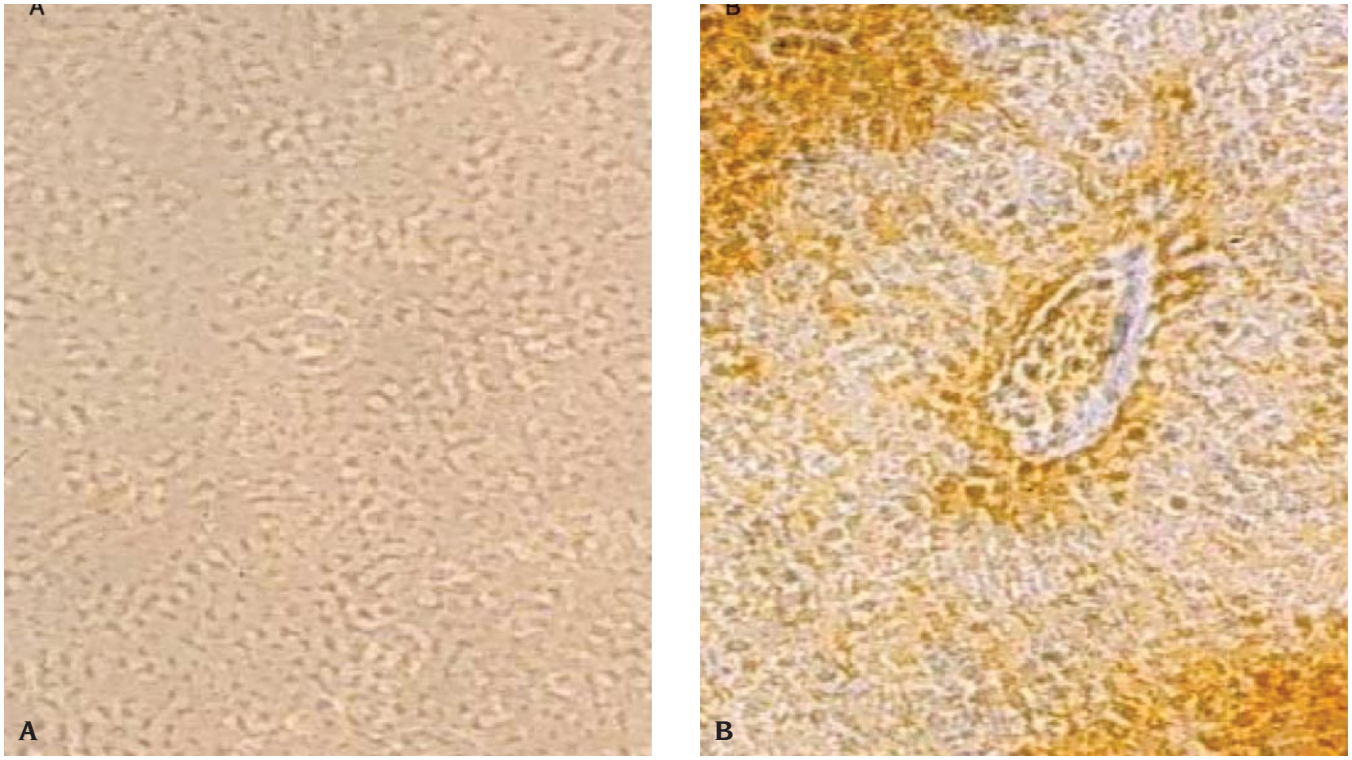

FIGURE 5.3-5 Proliferation and necrosis are evaluated in mouse liver sections 2 days after 0.5 mL/kg CCl_4 (*A*, control; *B*, CCl_4 treated). PCNA immunohistochemistry revealed that over 60% of hepatocytes, mostly periportal, showed positive staining, indicating proliferation. Necrosis is seen in pericentral hepatocytes. Untreated control mouse livers were unaffected. Immunohistochemistry; ×100 original magnification.

apart the organ's supporting structure.[27] It has been shown in animal models and in people with liver disease that hepatocytes can be transplanted into the liver parenchyma via the portal circulation, take permanent residence in the recipient liver, and provide metabolic function for the correction of acute, chronic, and genetic liver insufficiency.[7,28–33] Although the idea of liver cell transplant for the treatment of liver disease was first touted in 1977,[34,35] many hurdles remain before this technique can achieve clinical relevance, including massive cell loss and poor engraftment during liver cell transplant. In part because of poor initial engraftment, the potential of hepatocytes to proliferate after engraftment has been explored in transgenic and knockout animal models.[7,36] The concept that a survival advantage by transplanted cells could repopulate a diseased liver with healthy cells was first developed in a urokinase-type plasminogen activator transgenic mouse model.[36] This repopulation is feasible thanks to a high proliferative potential of hepatocytes, as demonstrated in serial transplant studies of mice with tyrosinemia.[7] More recent advances have shown the ability of non–liver-derived stem cells, such as bone marrow cells, to repopulate the diseased liver with functional hepatocytes.[37–39] However, in spite of this promising work on proliferation, it has been difficult to find clinically applicable methods that will reproduce these "survival advantage" conditions for transplanted liver cells or to create a safe hepatocyte stem cell line.

Transplanted Hepatocyte Function and Death

Hepatocytes can be transplanted and exhibit hepatocyte-specific function in an allogeneic recipient liver. Potential metabolic clinical relevance was suggested by results in a patient with Crigler-Najjar syndrome who had a significant reduction in serum bilirubin concentrations after repeated liver cell transplant.[29] In support of a clinical cure after cell therapy, a recent breakthrough in the field of solid organ cell transplant was recently reported. Patients with insulin-dependent diabetes mellitus were cured after pancreatic islet transplant into the portal vein.[33] A principal reason given for this cell therapy trial's success was the improved cell quality and preparation procedures that prevented cell injury and death. Engraftment efficiency is low after liver cell transplant. Although permanent engraftment and function of transplanted hepatocytes in mice were shown over 10 years ago using transgenically tagged hepatocytes,[40,41] only 0.03 to 0.5% of hepatocytes in the recipient liver could be identified as transplanted cells after a single-cell infusion. This is surprising because, in mouse studies, typically 2 million cells, or approximately 5% of the total recipient liver cells, are infused. This implies that 90 to 99% of the donor-infused hepatocytes are not detected and do not engraft in the recipient liver after cell infusion. Several studies show that ectopic engraftment after splenic or portal infusion in rodents is minimal and could thus not account for this large cell loss.[42,43] Because these studies occur in syngeneic or inbred animals, cell death by rejection is also not likely to occur. However, cell damage related to the isolation procedures and mediated by apoptosis might account for part of this cell loss.[21] Others have also described spontaneous apoptosis in freshly isolated hepatocytes in primary culture.[44] Potential mechanisms for this apoptosis in hepatocytes are being investigated and could involve loss of survival signals induced by collagenase perfusion, activation of Fas pathways, and loss of anchorage-induced apoptosis, termed "anoikis."[45] Attention to the molecular mechanisms that mediate this cell loss might provide information to improve the quality of transplanted liver cells and ultimately enhance engraftment efficiency, as suggested by the use of apoptosis inhibitors during cell transplant (Figure 5.3-6).

Liver Collagen Digestion and Cell Injury

Collagen, as a ligand for both integrins and tyrosine kinase receptors, plays an important role in cell differentiation, proliferation, and survival.[46–48] Collagenase perfusion is known to produce changes on the expression or the activity of several membrane receptors[49–51] and to result in reversible DNA damages.[52] Collagenase perfusion has been described to trigger apoptosis in cells prepared from non-regressing corpus luteum.[53] However, whether this induction is related to the detachment from the extracellular matrix, to the suppression of a survival factor, or to the induction of death signals remains unclear. Possible membrane proteins involved in this collagenase-induced apoptosis are the integrins family, the tyrosine kinase receptors family, and the death-associated domain such as Fas-

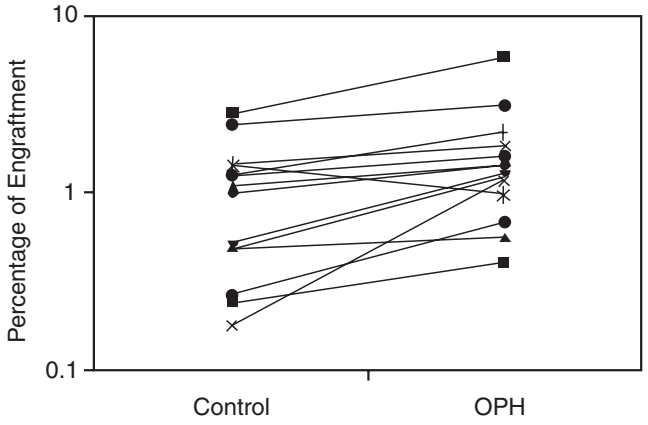

FIGURE 5.3-6 By inhibiting injury related to the hepatocyte isolation and transplant procedure, engraftment efficiency is increased by 60%. The figure shows the percentage of engraftment of transplanted hepatocytes in mice with and without the use of an apoptosis inhibitor, Q-VD-OPH. Two million male hepatocytes were transplanted in female mice. Fifteen paired mice were included in both the control group and the OPH group. In this later group, the broad-spectrum caspase inhibitor Q-VD-OPH was used at 20 μM in all of the solutions and 10 mg/kg was injected intraperitoneally in the recipients after the transplant and 3 hours later. The recipient livers were harvested after 3 days and homogenized, and deoxyribonucleic acid (DNA) was extracted. Donor-related DNA was quantified by real-time polymerase chain reaction for the Y chromosome. The percentage of male DNA in female DNA was 1.0 ± 0.06% and 1.7 ± 0.1% (mean ± SEM) for the control and the OPH group, respectively, with a significant increase of 60% in the OPH group compared with the control group (paired *t*-test, $p < .01$).

associated death domain. The integrins are the major receptors of collagen and extracellular matrix,[54] which maintain downstream transduction of survival signals. Cleavage or loss of signaling through the integrins could induce loss of survival signals such as FAK, PI-3K, and PKB/Akt.[55–57] Often in coordination with integrins, the tyrosine kinase receptors, which are mainly known to bind growth factors, also play a role in cell survival.[58] Collagenase action on liver cells could interact with survival signals mediated by those tyrosine kinase receptors because collagen is known to bind them.[48,59] Moreover, a crude preparation of collagenase also contains other proteases that could be involved in the death signals by nonspecific proteolysis of known receptors.[58–60] For example, the HGF receptor Met has been described to promote survival of a hepatocellular cell line by sequestration of Fas,[61] and its cleavage could lead to decreased activation, overexpression of Fas-associated death domain, and increased apoptosis. Additionally, although cell polarity is conserved immediately after isolation,[62] freshly isolated hepatocytes quickly demonstrate loss of cell polarity in culture, except if they are kept in collagen sandwich or tridimensional conditions.[63–65] Whether this loss of cell polarity could trigger apoptosis is currently unknown.

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CHAPTER 6

PANCREATIC FUNCTION AND DYSFUNCTION

Mark E. Lowe, MD, PhD

The mature pancreas has two morphologically and functionally distinct populations of cells required to maintain nutritional balance: the endocrine cells responsible for producing the hormones and the exocrine cells that make the digestive enzymes. The endocrine pancreas, about 2% of the pancreatic cell mass, consists of islets populated by four principal cell types defined by the hormones they secrete. The exocrine pancreas, the portion responsible for producing and secreting digestive enzymes and bicarbonate-rich fluid, makes up the bulk of the gland. The capacity of the exocrine pancreas to synthesize proteins exceeds that of any other organ. Each day, the human pancreas delivers into the duodenum between 6 and 20 g of protein mixed in approximately 2.5 L of fluid. This productivity provides sufficient enzymes to digest dietary nutrients, including complex carbohydrates, proteins, and fats, and ensures an optimal pH for the activity of the enzymes.

Diseases of the exocrine pancreas range from acute pancreatitis, the self-limited inflammation of the gland, to pancreatic insufficiency, the inability to synthesize adequate quantities of digestive enzymes, resulting in the malabsorption of nutrients. In childhood, the first accounts of exocrine pancreatic disease were published in the 1930s with the description of cystic lesions and fibrosis of the pancreas.[1,2] Over time, this entity, cystic fibrosis, was documented as the most common cause of pancreatic insufficiency in childhood. Other causes of pancreatic insufficiency, mostly inherited diseases, are seen infrequently. Children can also develop acute pancreatitis, albeit at lower rates than in adults. Still, acute pancreatitis is seen regularly in large pediatric referral centers, and its incidence in childhood may be increasing.[3] Anomalies of pancreatic development, although rare, can cause acute or chronic pancreatitis or pancreatic insufficiency. This chapter first discusses the normal function of the exocrine pancreas and then describes current knowledge about the pathophysiology of exocrine pancreas dysfunction.

EXOCRINE PANCREATIC FUNCTION

Pancreatic Development

During embryogenesis, the pancreas develops from distinct dorsal and ventral outpouchings from the duodenum.[4] Recognizable pancreatic buds first appear at 4 to 5 weeks gestation. The buds proliferate, with the ventral remnant remaining smaller than the dorsal bud. Differential growth of the duodenum and axial rotation of the gut bring the ventral pancreas below the dorsal pancreas to the left of the duodenum. After the rotation finishes during the seventh week of gestation, the two buds fuse to form the pancreas. Concomitantly, the ductal systems of the two buds anastomose. By 9 weeks of gestation, groups of endocrine cells can be identified, and by 12 weeks of gestation, exocrine cells containing secretory granules are apparent. Genetic and metabolic mechanisms must underlie the commitment of a specific region of gut epithelium to a pancreatic fate, the convergence of the initially separate dorsal and ventral bud development, and the differentiation of specific pancreatic cell types.

To date, investigations, predominantly in mice, have identified only a few genes that influence pancreatic development.[5–8] Even fewer have been shown to function during the development of the exocrine pancreas. Several homeobox and basic helix-loop-helix transcription factors contribute to pancreatic development. Most of the identified factors influence endocrine cell differentiation, but one basic helix-loop-helix transcription factor, p48, is required for the development of exocrine cells.[9] Mice lacking p48 do not develop any exocrine pancreatic tissue. Two genes encoding homeobox proteins have also been implicated in development of the exocrine pancreas. One, *Pdx1*, acts prior to the commitment of the gut endoderm to a pancreatic fate.[10] *Pdx1*-deficient mice and humans fail to develop a pancreas.[10–13] They do, however, undergo evagination of the gut epithelium and form the dorsal and ventral buds. The second homeobox gene, *Hlxb9*, predominantly affects development of the dorsal pancreas, demonstrating a distinct difference in the developmental programs of the ventral and dorsal pancreas. In mice lacking *Hlxb9* function, only the ventral pancreas develops, although it has abnormal spatial organization and a disproportionate number of endocrine cells.[14,15] These observations implicate *Pdx1*, *Hlxb9*, and *p48* in early pancreatic development and also imply the existence of other genes that regulate even earlier stages of pancreatic development.

Multiple investigations have identified several signaling pathways that govern interactions in the developing

pancreas (Figure 6-1). Early experiments established the importance of cellular and humoral interactions between pancreatic epithelium and the adjacent mesenchyme on pancreatic organogenesis.[16,17] Both mouse and tissue culture experiments implicate epidermal growth factor, fibroblast growth factor, activin-β, a transforming growth factor (TGF)-β ligand, and the Notch signaling pathways as mediators of pancreatic growth and morphogenesis. Genetic studies in mice demonstrate that Notch signaling regulates pancreatic endocrine and exocrine cell fate.[18,19] Inhibition of Notch signaling promotes early endocrine cell differentiation at the expense of exocrine cell proliferation. Activin and fibroblast growth factor may act by repressing the expression of a transcription factor, Sonic hedgehog.[20] In mice that ectopically express Sonic hedgehog in pancreatic epithelium, the epithelium develops into gut instead of pancreas.[21] Mice deficient in Sonic hedgehog have overgrowth of the ventral pancreas.[22–24] Thus, repression of Sonic hedgehog in the posterior foregut endoderm prevents intestinal differentiation and promotes differentiation of the pancreas. Several lines of evidence suggest that the Hedgehog and Notch signaling pathways interact or act in parallel during pancreatic development.[5] All of these signaling pathways permit rather than instruct pancreatic development.

Even less is known about the genetic factors that control the specification of pancreatic duct cells. Evidence in mice suggests that a critical event for duct formation occurs in the first 2 weeks of gestation.[25,26] The surrounding mesenchyme influences the formation of pancreatic ducts, but the factors involved have not been identified.[27] One potential candidate is the basement membrane glycoprotein laminin-1.[28] Including laminin-1 in the culture medium of embryonic pancreatic epithelium permits the differentiation of ducts and acini. In contrast, epithelium cultured in the absence of laminin-1 develops into islets.

Exocrine pancreatic development continues after birth with the postnatal maturation of specific digestive enzymes.[29] Many enzymes, particularly the proteases, are produced at adult levels at birth, but there are several notable exceptions. Pancreatic amylase expression is low at birth and rises slowly, reaching adult levels by 2 to 3 years of age.[29] In healthy infants, the amylase deficiency has no clinical consequences, perhaps because infants have limited amounts of complex carbohydrates in their diet. The other major enzyme deficiency is of pancreatic lipase, the enzyme responsible for digesting dietary triglycerides.[29–32] A homologue of pancreatic lipase, pancreatic lipase–related protein-2, may compensate for the low levels of pancreatic lipase expressed during the first months of life, as may

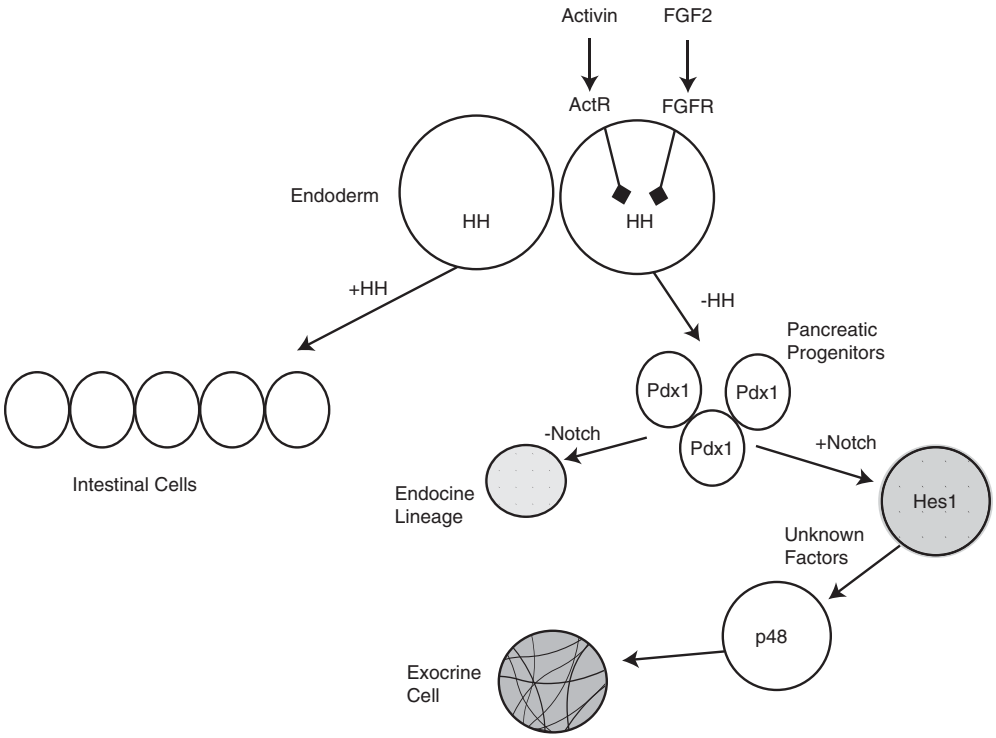


FIGURE 6-1 Differentiation pathway of pancreatic development. In contrast to intestinal progenitors, endodermal pancreatic progenitors do not express hedgehog (HH) signaling molecules. HH signaling is controlled by activin and fibroblast growth factor 2 (FGF2) through interactions with their respective receptors, ActR and FGFR. Evagination and bud formation are independent of the transcription factor Pdx1, but growth and differentiation of the pancreas require Pdx1 expression. Notch signaling pathways appear to control the divergence of the endocrine and exocrine pathways. Active signals through the Notch pathway stimulate cells that express high levels of the transcription factors Hes1 and p48. Other as yet unidentified factors control progression of cells toward the mature exocrine cell. Adapted from Edlund H.[7]

another pancreatic protein, carboxyl ester lipase.[33–36] Consequently, premature and newborn infants absorb large dietary fat loads with only a small increase in steatorrhea compared with that of older infants.[37] The molecular mechanisms that regulate the temporal changes in amylase and pancreatic lipases after birth are unknown but may respond to changes in dietary intake.

Functional Anatomy

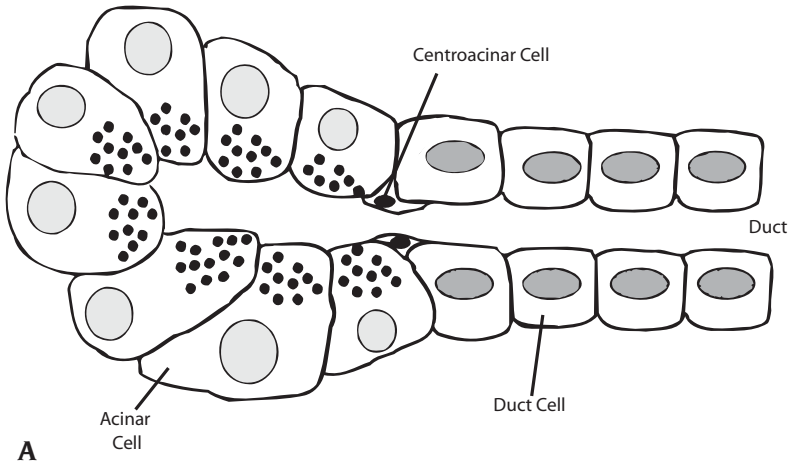
The exocrine pancreas contains lobules of parenchyma bound together by connective tissue. Each lobule contains a complex structure of acini, which account for greater than 80% of the parenchyma, and a ductal system, which makes up about 5% of the gland mass.[38] An acinus may be the terminal structure of a duct, or acini may form on the sides of a duct as it traverses a lobule.[39] In general, each acinus consists of six to eight pyramidal acinar cells with their apical poles facing a lumen that leads into a duct (Figure 6-2A). The acinar cells possess the features of specialized secretory cells, an elaborate network of rough endoplasmic reticulum, a prominent Golgi complex, and large numbers of secretory vesicles called zymogen granules (Figure 6-2B).

Each acinus drains into intercalated ducts, which fuse to form intralobular ducts that eventually drain into interlobular ducts. The interlobular ducts empty into the main duct as it courses through the tail and body of the pancreas. In the pancreatic head, the main pancreatic duct enters the duodenum at the ampulla of Vater. Additional drainage can occur via the accessory duct, which joins the main duct in the pancreatic head and enters the duodenum through a minor papilla in about 33% of people and ends blindly in about 8% of people. Nearly half of individuals do not have an accessory duct.[40] The shape of the ductal cells varies in the different regions. Centroacinar cells, the terminal cells of the duct, are squamous to low cuboidal cells with a sparse cytoplasm containing many mitochondria and no zymogen granules. Further down the ductal tree, the cells become cuboidal and then columnar. In addition to the main ductal cells, the ducts contain a number of specialized cells such as goblet cells.

Composition of exocrine secretions

Secretion of Inorganic Constituents. Pancreatic juice, a clear and colorless alkaline fluid, contains electrolytes and proteins.[41] Water, sodium, potassium, chloride, and bicarbonate are the principal inorganic constituents in pancreatic secretions. Pancreatic juice remains isotonic at all secretion rates, ranging from 0.2 mL/min to 4.0 mL/min, but the concentrations of bicarbonate and chloride change with secretin stimulation, the major regulator of volume output. At low secretion rates, the bicarbonate concentration equals that of plasma. As the secretion rate increases in response to secretin stimulation, the bicarbonate concentration increases to around 140 mEq/L in humans, which makes the pH of the juice about 8.2. Concomitantly, there is a reciprocal decrease in the chloride concentration such that the sum of bicarbonate and chloride remains constant at all secretion rates. These changes in anion concentrations take place because secretin stimulates water and bicarbonate secretion from pancreatic duct cells to a level that overwhelms the relatively small fluid and electrolyte flow from acinar cells. The net result produces pancreatic juice with a concentration of ions nearly identical to the concentrations found in pure ductal fluid.

Secretion of Organic Constituents. The pancreas synthesizes and secretes a variety of proteins, mainly hydrolases.[42,43] Twenty to 25 different proteins can be identified in pancreatic juice from all species, including humans (Table 6-1). Proteolytic, amylolytic, and lipolytic enzymes comprise the majority of the digestive enzymes. Many of these are synthesized and stored as inactive precursors or zymogens prior to secretion into the pancreatic duct. Consequently, there is little to no detectable activity of digestive enzymes in pancreatic fluid. Activity first appears in the duodenum when enteropeptidase, an intestinal brush



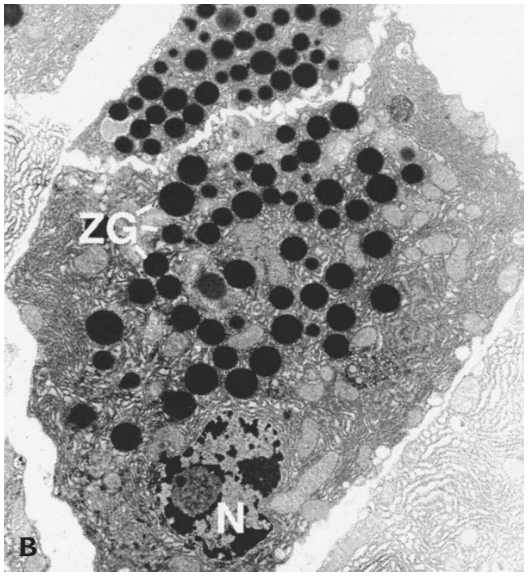


FIGURE 6-2 Anatomy of a pancreatic acinus and acinar cell. *A*, Schematic representation of a pancreatic acinus. *B*, Electron micrograph of an acinar cell (×10,000 original magnification). N = nucleus; ZG = zymogen granules.

border enzyme, triggers an activation cascade by converting trypsinogen to trypsin.[42] Trypsin, in turn, activates additional trypsinogen as well as the other zymogens (Figure 6-3). Through this activation cascade, the inactive zymogens convert to active enzymes.

The synthesis, packaging, and secretion of digestive proteins follow a well-established pathway.[44] Nascent protein chains are produced in the rough endoplasmic reticulum and then are moved into the Golgi apparatus for additional processing. In the Golgi apparatus, the digestive and lysosomal enzymes are efficiently sorted into separate compartments: the lysosomal enzymes into lysosomes and the digestive enzymes into condensing vacuoles. The latter then form mature zymogen granules, which are stored in the apical region of the acinar cell until the contents are secreted.

Protective Mechanisms. Inherent in the process of synthesizing and secreting digestive enzymes are mechanisms to protect the acinar cell from the premature activation of digestive zymogens.[45] The proteases and lipases produced in the acinar cell all have the capability to damage the pancreatic parenchyma if they are activated within the acinar cell or pancreatic duct. Producing inactive zymogens provides one important protective mechanism. Packaging the zymogens in a compartment separated from lysosomal enzymes, some of which can activate trypsinogen, provides another. Once trypsinogen is activated, pancreatic secretory trypsin inhibitor, SPINK1, provides a first line of defense by binding and inactivating trypsin. SPINK1 can inhibit only about 20% of potential trypsin, and if there is excessive trypsin activation, then free trypsin increases and can start activating other digestive enzymes. To protect against this situation, trypsin can be degraded by autolysis and by other proteases.

TABLE 6-1 HUMAN PANCREATIC EXOCRINE PROTEINS

PROTEASES
Trypsinogen 1, anionic
Trypsinogen 2, mesotrypsin
Trypsinogen 3, cationic
Chymotrypsinogen A
Chymotrypsinogen B
Procarboxypeptidase A_1
Procarboxypeptidase A_2
Procarboxypeptidase B_1
Procarboxypeptidase B_2
Proelastase
LIPASES
Triacylglycerol lipase
Pancreatic lipase–related protein 1
Pancreatic lipase–related protein 2
Prophospholipase A_2
Carboxyl ester lipase
Procolipase
GLYCOSIDASES
Amylase
NUCLEASES
Ribonuclease
Deoxyribonuclease

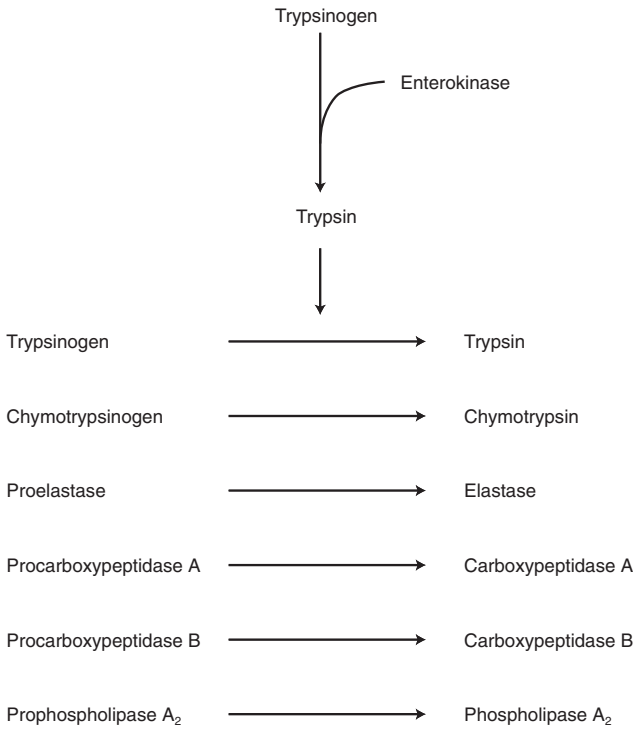


FIGURE 6-3 Activation cascade of pancreatic digestive zymogens.

DIGESTION OF NUTRIENTS

Carbohydrates. Carbohydrates, in the form of starch or simple sugars, account for 40 to 50% of the calories in the Western diet. Starch, polymers of glucose, is the storage form of carbohydrate in plants, accounting for 10 to 80% of the plant volume. Amylose, a straight-chain α-1,4-linked glucose polymer, and amylopectin, a branched starch with a backbone of α-1,4-linked glucose with α-1,6-linked glucose branches about every 20 to 25 residues, are the major dietary starches. About 20% of dietary starch is amylose, and the remainder is amylopectin. Because the intestinal epithelium only absorbs monosaccharides, dietary starch must be hydrolyzed into glucose by the action of α-amylase.

α-Amylase is produced in the salivary glands by the parotids and in the pancreatic acini.[42] About 5 to 6% of the total protein in pancreatic secretions is α-amylase, an enzyme that preferentially cleaves interior α-1,4-glucose linkages. Neither terminal glucose residues nor α-1,6-linkages can be cleaved by α-amylase. The resulting products of α-amylase digestion are called dextrins, a mixture of maltose, maltotriose, and branched oligosaccharides of six to eight glucose units that contain both α-1,4 and α-1,6 linkages. Intestinal brush border enzymes, maltase and isomaltase, finish the digestion of dextrins.

Lipids. Dietary lipids provide an important source of energy in Western diets.[46] Triglycerides account for greater than 95% of the 100 to 150 g of fat consumed by adults each day. Before dietary fats can be absorbed by enterocytes, they must be digested into fatty acids and monoacylglycerols. In humans, the process of fat digestion begins in the stomach with the action of gastric lipase.[47] About 15% of

fatty acids are released in the stomach. Digestion then continues in the proximal small intestine with the addition of several different lipases from the pancreas.[48]

The contribution of two lipases, phospholipase A_2 and carboxyl ester lipase, to dietary fat digestion remains unresolved despite studies in mice deficient in either enzyme. Phospholipase A_2 hydrolyzes the fatty acid from the sn-2 position of phospholipids.[42] Although phospholipase A_2 has the potential to digest luminal phospholipids, phospholipase A_2–deficient mice absorb phospholipids from a test meal at a normal rate.[49] Either phospholipase A_2 makes no contribution to dietary phospholipid digestion or other enzymes compensate for the absence of this enzyme in phospholipase A_2–deficient mice.

Carboxyl ester lipase is one enzyme that could contribute to dietary phospholipid digestion and to the digestion of other dietary fats and fat-soluble vitamins.[50,51] All species from fish to humans produce carboxyl ester lipase, which constitutes up to 5% of the protein in pancreatic juice. In vitro, carboxyl ester lipase demonstrates broad substrate specificity, cleaving cholesterol esters, fat-soluble vitamin esters, triglycerides, ceramides, and phospholipids. Despite many years of research interest in this enzyme, the role of carboxyl ester lipase in dietary fat digestion remains enigmatic. Work with carboxyl ester–deficient mice demonstrates a role for this enzyme in the digestion of cholesterol esters, a minor dietary component, and in the absorption of vitamin A.[52] The contribution of carboxyl ester lipase to the digestion of other vitamin esters, dietary phospholipids, and dietary triglycerides remains speculative.

Pancreatic triglyceride lipase cleaves the majority of fatty acids from dietary triglycerides, as evidenced by patients with congenital deficiency of this enzyme who malabsorb 50 to 65% of dietary triglycerides.[53,54] A carboxyl esterase that prefers acylglycerides over other lipids, pancreatic triglyceride lipase hydrolyzes acyl chains of varying lengths and saturation from the sn-1 and sn-3 positions of tri- and diglycerides to produce fatty acids and 2-monoacylglycerols. Interestingly, the bile salts, dietary proteins, and phospholipids in the duodenum inhibit pancreatic triglyceride lipase. Colipase, a pancreatic protein with no enzymatic activity, restores activity by forming a complex with pancreatic triglyceride lipase.[55]

Proteins. Most adults in the Western world consume 70 to 100 g of protein daily. Protein digestion begins in the stomach with the action of pepsin and reaches completion in the intestine with digestion by pancreatic proteases.[56] The pancreas secretes various proteases, all as inactive enzymes. Trypsin, the predominant protease, plays a central role in the activation of the other zymogens. Three of the proteases, trypsin, chymotrypsin, and elastase, cleave internal peptide bonds, and two proteases, carboxypeptidases A and B, cleave amino acids from the carboxy-terminal of peptides. The combined action of gastric and pancreatic proteases digests dietary proteins into oligopeptides and free amino acids. Intestinal brush border enzymes further digest the oligopeptides prior to the absorption of both amino acids and small peptides by sodium- and hydrogen-coupled transporters.[56]

Control of Secretion

In humans, the exocrine pancreas secretes enzymes and fluid during fasting, the interdigestive period, and after eating, the digestive period.[57,58] The interdigestive period begins when food has cleared the upper gastrointestinal tract, mainly at night. In an individual who eats three meals a day, the digestive period starts after the first meal and continues until the evening meal clears the upper intestine, usually late in the day.

Interdigestive Secretion. The interdigestive secretory pattern cycles every 60 to 120 minutes with the three phases of the migrating myoelectric complex of the stomach and duodenum.[57,58] Phase I is a period of no motor activity and negligible pancreatic secretion. As motor activity increases in phase II, pancreatic secretion increases and reaches a maximum rate just before the onset of phase III motor activity. Both hormonal and neural mechanisms control interdigestive pancreatic secretion. The neural regulation involves vagal cholinergic and parasympathetic inputs. Of the gastrointestinal hormones, motilin and pancreatic polypeptide are the most likely candidates to influence interdigestive pancreatic secretion. Both hormones cycle during the interdigestive period and regulate the migrating myoelectric complex, and motilin stimulates and pancreatic polypeptide inhibits pancreatic secretion. Most likely, interdigestive secretions help clear the gastrointestinal tract of residual food particles, cellular debris, and bacteria.

Digestive Secretion. Exocrine pancreatic secretion during a meal occurs in three phases: the cephalic, gastric, and intestinal phases. Vagal nerves mediate the cephalic phase in response to seeing, smelling, chewing, swallowing, or thinking about food. Cephalic stimulation specifically stimulates acinar cell secretions without affecting ductal cell secretions.[59] The gastric phase begins when food enters and distends the stomach.[60] Gastric distention activates mechanoreceptors located in the body of the stomach and results in a low-volume, enzyme-rich secretion mediated by a vagal reflex. When gastric juice and food enter the duodenum, the intestinal phase of digestion begins, and a variety of hormones and enteropancreatic vagovagal reflexes stimulate pancreatic secretion. Now both ductal and acinar secretions increase. The primary mediator of ductal secretion is secretin, but maximum secretion requires cholecystokinin and cholinergic input as well.[61,62] Cholecystokinin is the major mediator of enzyme secretion, but a vagovagal reflex also contributes input. Other hormones, such as gastrin-releasing peptide, bombesin, and neurotensin, can stimulate pancreatic secretion in experimental models, but the effects may not be physiologically significant.

Exocrine Pancreatic Dysfunction

Exocrine pancreatic dysfunction occurs in a number of diseases, including congenital anomalies, inherited disorders,

and pancreatitis, both acute and chronic. In acute pancreatitis, the dysfunction reverses after resolution of the disease, but in diseases that cause irreversible damage to the gland, important clinical consequences result from malabsorption of nutrients. Because the pancreas secretes a large excess of digestive enzymes, much of the pancreas must be destroyed before malabsorption results. For instance, steatorrhea occurs only after greater than 95% of the capacity to secrete pancreatic lipase has been lost.[63] This section reviews recent studies that have provided insight into the molecular mechanisms of childhood diseases that cause exocrine pancreatic dysfunction.

Congenital Anomalies. Although much remains to be learned about the control of pancreatic development, misregulation of development probably gives rise to congenital anomalies of the pancreas and to some of the inherited disorders of the pancreas. Genetic studies of mice provide support for this concept and have identified candidate genes for many human diseases. Some of these studies have identified pathways involved in normal pancreatic development and suggest mechanisms for the development of pancreatic congenital anomalies. Inactivation of the genes encoding Indian hedgehog or Sonic hedgehog in mice causes overgrowth of ventral pancreatic tissue to produce a phenotype resembling the human disorder annular pancreas.[23,24] Ventral pancreatic ductal abnormalities similar to pancreas divisum occur in mice heterozygous for null alleles of genes encoding Indian hedgehog or Sonic hedgehog, as well as in mice deficient in *Smad2*, a component of the TGF-β signaling pathway.[5] Pancreatic hypoplasia in mice results from inactivation of *Hes-1* and *Jag-1*, genes producing proteins in the Notch signaling pathway.[64,65] Targeted disruption of the gene encoding a transcription factor, *Pdx1*, in mice results in the failure of the pancreas to develop, a rare disorder in humans called aplasia or agenesis of the pancreas. These findings led Stoffers and colleagues to identify a single homozygous nucleotide deletion in codon 63 of the human gene encoding *PDX1* in a patient with pancreatic aplasia.[13] The resultant frameshift mutation in *PDX1* caused the synthesis of a truncated and functionally inactive protein. Analysis of the *PDX1* gene in a second patient with pancreatic aplasia failed to find any abnormalities in the coding region of the gene, and immunohistochemistry showed normal distribution of *PDX1* in gastrointestinal endocrine cells.[66] These findings suggest, as would be expected, that other genes also influence the early development of the human pancreas.

Developmental disorders

Even though many of the molecular pathways controlling development of the exocrine pancreas remain to be identified, genetic defects in these pathways probably result in many of the conditions causing pancreatic dysfunction in pediatrics. Some of these disorders appear to be secondary to defects in early pancreatic development, even before the speciation of the acinar cells, and others involve regulation at later stages of development. Thus, defects of development range from a complete lack of the pancreas to defects in individual cell types of the pancreas, with relative preservation of the other cell types.

Shwachman-Diamond Syndrome. Shwachman-Diamond syndrome is the most common inherited cause of exocrine pancreatic dysfunction after cystic fibrosis. Histology of the pancreas from patients with Shwachman-Diamond syndrome reveals acinar cell depletion and replacement with fat.[67] The ductal system appears to be preserved, and physiologic studies show normal anion and fluid excretion with impaired production of digestive enzymes.[68] Recently, mutations in the *SBDS* gene were associated with this syndrome.[69] The gene resides on chromosome 7q11 and contains five exons, which encode a predicted 250–amino acid protein of unknown function, although the structure of the predicted protein suggests that the protein may be involved in ribonucleic acid processing. Sequence analysis of 316 alleles from affected individuals revealed that 82% had mutations in the *SBDS* gene. Most of the mutations resulted from gene conversion owing to recombination between the gene encoding *SBDS* and a pseudogene of the *SBDS* gene. A few mutant alleles from affected individuals did not have conversion mutations and, instead, had frameshift or missense mutations. None of the mutations were present in the alleles of 100 controls.

Johansson-Blizzard Syndrome and Jeune Syndrome. Several other rare genetic syndromes in which the genetic defect is not known also include pancreatic exocrine dysfunction as part of the phenotype. Patients with Johansson-Blizzard syndrome have absent pancreatic acini with replacement by connective tissue.[70] Their ducts and islets appear normal and, similar to those in Shwachman-Diamond syndrome, function normally.[71] In Jeune syndrome, pancreatic fibrosis and cyst formation produce exocrine pancreatic insufficiency associated with skeletal, renal, and liver abnormalities.[72] Both of these syndromes may well result from the failure of pancreatic acinar cell development.

Pearson Syndrome. In 1979, Pearson and colleagues reported four children with bone marrow failure and exocrine pancreatic disease characterized by both acinar and ductular deficiencies.[73] Histology shows acinar cell loss, fibrosis, and siderosis but no fatty infiltration. The syndrome is caused by deletions in mitochondrial deoxyribonucleic acid (DNA), leading to defects in oxidative phosphorylation and energy production.[74,75] Presumably, the inability to meet the energy demands of the acinar cells leads to their injury and death by apoptosis.

Isolated Enzyme Deficiencies. Several articles report patients with deficiencies of individual pancreatic digestive enzymes and of enterokinase, an intestinal brush border enzyme that activates pancreatic proenzymes.[76] Inherited deficiencies of pancreatic lipase, colipase, amylase, trypsinogen, and enterokinase have all been reported.[76] These entities are extremely rare, and no gene defects have been described in patients with any of these reported deficiencies.

PANCREATITIS

Acute Pancreatitis. The pathophysiology can be conveniently divided into three phases.[77] A variety of extrapancreatic triggering events initiate the onset of pancreatitis (Figure 6-4). In adults, the most important triggers are the passage of gallstone or ethanol ingestion. In children, systemic illness, trauma, and congenital anomalies predominate, although gallstone pancreatitis occurs in childhood. Next, a series of intra-acinar cell events produce cellular injury and local tissue damage. Studies in animals suggest that the normal secretion of digestive enzymes is altered during pancreatitis, and these changes induce activation of trypsinogen and other zymogens within the acinar cell. Lastly, acinar cell damage induces a variety of changes, including the production of proinflammatory cytokines, the generation of reactive oxygen species, and abnormalities in the local circulation. The severity of the clinical course depends on the magnitude of these changes and on the induction of a systemic inflammatory response.

Initiating Events. The triggers of acute pancreatitis fall into several broad categories.[78] Obstructive causes are common in adults but less common in children. Gallstones, pancreas divisum, choledochal cysts, gastric or duodenal duplication cysts, and periampullary lesions such as Crohn's disease or duodenal ulcer disease can obstruct pancreatic flow and lead to acute pancreatitis in children. Several theories have been proposed to explain gallstone pancreatitis, including obstruction of the ampulla with a stone and subsequent reflux of bile into the pancreatic duct, repeated passage of small stones to produce fibrosis, and incompetence of the ampulla of Vater, which allows reflux of duodenal content into the pancreatic duct, and transient obstruction of the pancreatic duct with increasing pressure in the duct, leading to extravasation of pancreatic juice into the interstitium of the gland. Increased ductal pressure secondary to relative stenosis of the minor papilla may be the mechanism of pancreatitis in pancreas divisum. Similarly, periampullary lesions can increase ductal pressure by causing edema or fibrosis around the ampulla of Vater that interferes with drainage of the duct.

The mechanism of other triggers is similarly speculative.[78] Drugs or their metabolites may interfere with cellular metabolism in a variety of ways. For instance, ethanol stimulates pancreatic secretion through the effects on gastric acid secretion, and the metabolites of ethanol are cytotoxic. Thiazides may alter calcium metabolism. In lipid disorders, which may be induced by drugs or associated with familial hyperlipidemias, high local fatty acid concentrations, from the action of either pancreatic lipase or lipoprotein lipase, may overwhelm the capacity of albumin to bind fatty acids and render them nontoxic. The unbound fatty acids may then cause increased cytotoxicity. Another frequent cause, trauma, disrupts the integrity of the ductal system and allows extravasation of digestive enzymes into the pancreatic parenchyma and adjacent fat. A common but often underappreciated cause of acute pancreatitis, low blood flow as in shock, produces pancreatic inflammation and necrosis through an ischemia or reperfusion injury.

Acinar Cell Events. Evidence from animal studies suggests that one of the earliest events in acute pancreatitis is the colocalization of digestive zymogens and lysosomal hydrolases in acinar cells.[77,79] Normally, the digestive zymogens and lysosomal hydrolases are packaged separately, but a disruption of normal secretion, as induced in experimental pancreatitis, leads to a defect in intracellular transport and sorting of enzymes. Consequently, digestive zymogens and lysosomal hydrolases colocalize within the same cytoplasmic vacuoles. The lysosomal hydrolases, in particular cathepsin B, activate trypsinogen. During this process, the vacuoles disintegrate and release their contents into the cytoplasm and injure the acinar cells. Multiple lines of evidence support the colocalization theory, including the observations that formation of cytoplasmic vacuoles containing digestive and lysosomal enzymes occurs before evidence of cell injury, that cathepsin B can activate trypsinogen in vitro, that trypsin activation peptide first appears in these cytoplasmic vacuoles, that specific cathepsin B inhibitors can prevent the activation of trypsin in isolated acinar cells after hyperstimulation with cerulein, and that cathepsin B–deficient mice have a defect in trypsinogen activation after cerulein hyperstimulation. On the release of the vacuole contents, trypsin can activate other zymogens, freeing them to damage the acinar cells.

Although autodigestion of the acinar cell by digestive enzymes plays a central role in most theories of acute pan-

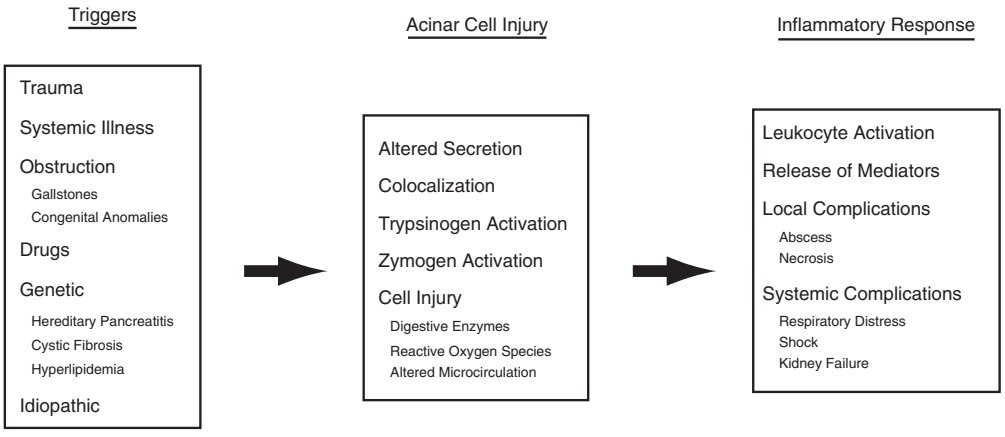


FIGURE 6-4 A model for the pathophysiology of pancreatitis.

creatitis, other processes may also contribute to acinar cell damage in early pancreatitis. Several authors have touted the role of reactive oxygen species in acute pancreatitis.[80,81] They base their arguments on data showing an increase in lipid peroxides during experimental pancreatitis, an alteration in cytoskeleton function by lipid peroxidation leading to abnormalities in transport of digestive enzymes and to their premature activation in acinar cells, and an increase in cell permeability correlated with the high production of both free oxygen radicals and activated zymogens. Additionally, abnormalities of the blood supply probably contribute to early injury. Vascular injuries can clearly induce acute pancreatitis, but alterations in the microcirculation have been documented in other forms of pancreatitis. In experimental pancreatitis, hypoperfusion occurs in severely injured regions, whereas less injured regions maintain good perfusion.[82] Finally, activation of resident macrophages in the pancreas and the migration of activated leukocytes into the pancreas contribute to the severity of gland inflammation in acute pancreatitis.[83–85] Nude mice lacking lymphocytes have decreased severity of pancreatitis in experimental models. The transfer of T lymphocytes into nude mice increases the severity of cerulein-induced acute pancreatitis.

Late Events. Acinar cell damage produces pancreatic edema and a local inflammatory response associated with the release of inflammatory mediators into the systemic circulation.[86,87] These cytokines and chemokines mediate a systemic inflammatory response, a common pathway in many forms of injury. The clinical severity of pancreatitis depends in part on the magnitude of this systemic response, and the balance between proinflammatory and anti-inflammatory mediators ultimately influences the clinical course. In response to a brisk systemic inflammatory response, activated leukocytes migrate into other organs, particularly the lungs, kidneys, and liver, and cause tissue edema and damage. Thus, according to current data, the activated immune response plays the major role in the systemic complications of acute pancreatitis.[86,87] Most likely, the damage of distant organs by circulating pancreatic digestive enzymes is minimal.

Chronic Pancreatitis

Early in the course, chronic pancreatitis may be difficult to distinguish from acute pancreatitis on clinical grounds.[45] In chronic pancreatitis, continued inflammation of the pancreas produces irreversible morphologic changes in the gland. When available, histology shows irregular fibrosis, acinar cell loss, islet cell loss, and infiltration by inflammatory cells. The clinical diagnosis depends on identifying decreased function and chronic changes by imaging studies, which represent late changes in the disease. The difficulties in diagnosing chronic pancreatitis early in its development hinder clinical studies of natural history and of potential therapies to halt the disease process. Consequently, this uncertainty has fostered the proposal of many different theories to explain the pathophysiologic mechanisms in chronic pancreatitis.

Theories of Pathogenesis. Over the past 50 to 60 years, various investigators have suggested theories to connect the development of chronic pancreatitis with environmental factors, many concentrating on alcohol consumption.[88] The dominant view throughout the latter half of the twentieth century held that recurrent acute pancreatitis progressed to chronic pancreatitis. Some authors looked at clinical data and concluded that acute and chronic pancreatitis were separate diseases and developed various theories that did not include acute pancreatitis in the development of chronic pancreatitis. Sarles and colleagues introduced the concept of a primary defect in the pancreatic ducts leading to intraductal obstruction and tissue damage by increased intraductal pressure.[89] Others suggested that chronic pancreatitis results from the toxic and metabolic effects of the inciting agent, in particular alcohol, or from repeated oxidative stress.[90,91] In the early 1990s, Kloppel and Maillet refined the recurrent acute pancreatitis theory and proposed an important role for necrosis and subsequent fibrosis in the development of chronic pancreatitis.[92,93] Accordingly, they believed that repeated episodes of acute pancreatitis would induce areas of focal necrosis and healing with fibrosis. Although some skepticism over the necrosis-fibrosis hypothesis remains, the model recently gained strong support from the descriptions of the molecular defect in hereditary pancreatitis and the understanding that acute pancreatitis leads to typical chronic pancreatitis in these patients. A new proposal, the sentinel acute pancreatitis event or SAPE hypothesis, incorporates elements from each of these theories.[94] An additional feature of this model is the requirement for an initiating event to trigger the first episode of acute pancreatitis, the beginning of chronic pancreatitis, on the background of a continuous stress such as alcohol consumption (Figure 6-5).

Etiology and Risk Factors for Chronic Pancreatitis. Often after the diagnosis of chronic pancreatitis, the etiology remains unclear (Table 6-2). Even the presence of established risk factors does not provide a clear etiology in most cases because the majority of people, for instance those who consume alcohol, with these risk factors do not develop chronic pancreatitis. Other factors must be present for disease to occur, and, with this concept in mind, recent studies have focused on the importance of genetic predisposition to chronic pancreatitis.[45]

Hereditary Pancreatitis. First described in 1952, hereditary pancreatitis causes recurrent episodes of acute pancreatitis and, in about 75% of patients, chronic pancreatitis.[88] Multiple pedigrees have been well described, and it is clear that hereditary pancreatitis has an autosomal dominant inheritance pattern, with about 80% penetrance and a variable clinical course even among family members.[88]

The inheritance pattern of hereditary pancreatitis suggests that a single gene defect produced the disease. In 1996, a single point mutation in the third exon of the gene encoding cationic trypsinogen was shown to segregate with the disease.[95] The point mutation causes an arginine to histidine substitution at position 122. Subsequently,

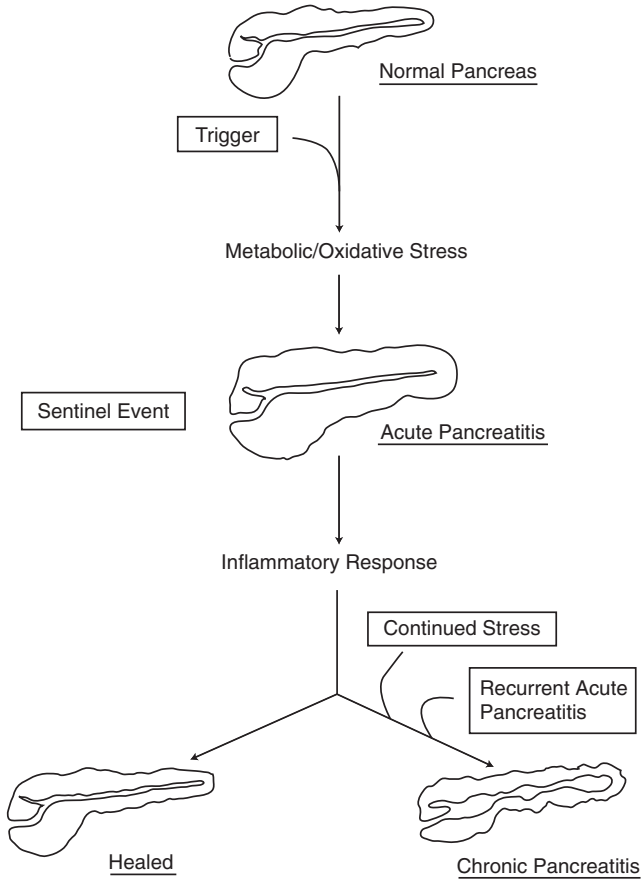


FIGURE 6-5 A hypothesis for the pathophysiology of chronic pancreatitis, the sentinel acute pancreatitis event hypothesis proposed by Whitcomb.[94] In this model, the normal pancreas is exposed frequently to metabolic or oxidative stresses, either drugs or toxins. In some patients, this exposure produces the initial episode of acute pancreatitis, the sentinel event. During the event, activated lymphocytes, macrophages, and stellate cells increase in number within the gland and produce cytokines and small amounts of collagen. With time, the number of cells diminishes, and the gland returns to normal. In the presence of continued stress or recurrent pancreatitis, the tissue macrophages and stellate cells remain active and continue to release cytokine and deposit collagen, a process that eventually causes the fibrosis characteristic of chronic pancreatitis.

other studies of pedigrees with hereditary pancreatitis revealed additional mutations in the gene encoding cationic trypsinogen, including N29I, A16V, D22G, K23R, and R122C. Three of these mutations, R122H, R122C, and N29I, account for the majority of patients.[88] The other mutations are extremely rare and are only weakly associated with pancreatitis.

Increased resistance of the R122H mutant trypsin to hydrolysis has been proposed as a model for the defect in hereditary pancreatitis.[45] Normally, the pancreas synthesizes trypsin as an inactive precursor, trypsinogen, along with pancreatic secretory trypsin inhibitor, SPINK1, at a ratio of 5 to 1. SPINK1 provides a first line of defense against premature trypsinogen activation inside the acinar cell (Figure 6-6). The second line of defense depends on the degradation of trypsin by autolysis and, perhaps, by other proteases. Degradation begins with hydrolysis after Arg122. In most cases of hereditary pancreatitis, the substitution of histidine at position 122 prevents autolysis. In vitro studies confirm the resistance of R122H to autolysis and also demonstrate that the mutation increases autoactivation of the mutant trypsinogen.[96] Similar studies on the N29I human cationic trypsinogen reveal that the mutation results in faster autoactivation and increased trypsin stability.[97] Consequently, both N29I and R122H trypsin mutants are more likely to accumulate in acinar cells and cause increased activation of other zymogens, and people with one of these mutations develop pancreatitis more readily than people who have normal trypsinogen.

Cystic Fibrosis Transmembrane Regulator. Cystic fibrosis is the most common cause of pancreatic insufficiency in pediatrics.[98] The autosomal recessive disorder results from mutations in the cystic fibrosis transmembrane regulator (CFTR), a membrane protein found on the apical membrane of ductal cells, where it regulates chloride conductance and water flow. Over 900 different mutations in CFTR cause disease. These mutations fall into five major classes (Figure 6-7). Classes I, II, and III result in complete loss of CFTR function because of defective protein production, abnormal protein processing, and abnormal regulation of chloride conductance, respectively. In general, these mutations cause more severe disease manifestations. Class IV and V mutations produce milder symptoms because these mutations result in mutant CFTR with decreased conductance properties or in decreased synthesis of normally active CFTR. Individuals with cystic fibrosis may be homozygous for one genetic mutation or heterozygous for two different mutations. About 85% of patients with cystic fibrosis have pancreatic insufficiency. Patients homozygous for severe mutations, classes I, II, and III, are affected. Those with at least one less severe mutation are usually pancreatic sufficient.

TABLE 6-2 ETIOLOGIES OF CHRONIC PANCREATITIS

TOXIC-METABOLIC
Medications
Hyperlipidemia
Hypercalcemia
Toxins
GENETIC
Cystic fibrosis
Hereditary pancreatitis
SPINK1 mutations
OBSTRUCTIVE
Pancreas divisum
Anomalous insertion of duct
Tumor
Crohn disease
Post-traumatic duct strictures
IDIOPATHIC
AUTOIMMUNE
Collagen vascular disease
Isolated autoimmune pancreatitis

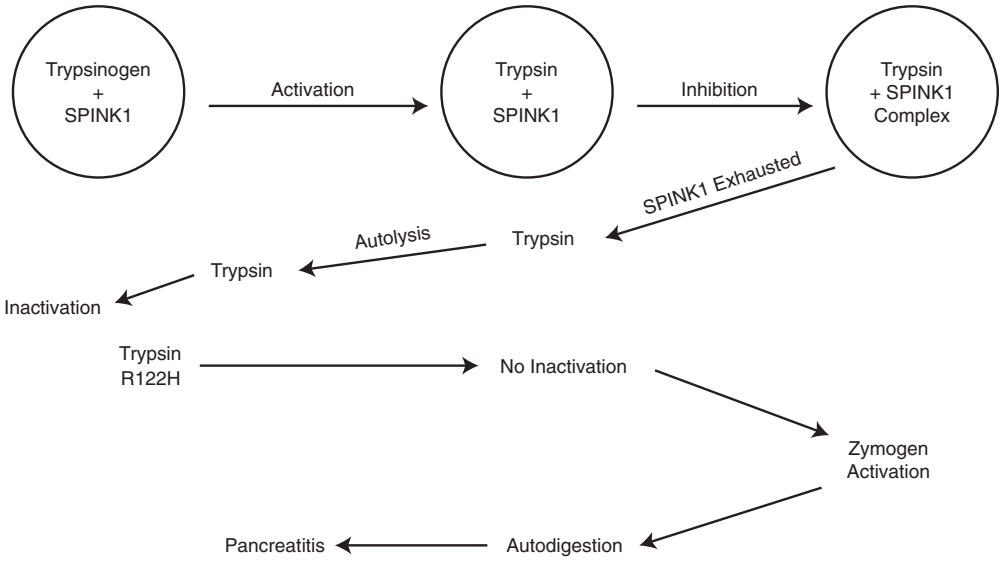


FIGURE 6-6 Model of hereditary pancreatitis. Trypsinogen and SPINK1 are packaged in zymogen granules at a 5:1 molar ratio. When trypsinogen is activated within the acinar cell, inhibition by SPINK1 represents the first line of defense against activation of the zymogen cascade. In the presence of robust trypsinogen activation, SPINK1 concentrations are overwhelmed, and protection depends on the inactivation of trypsin by autolysis. Trypsin hydrolysis begins with cleavage after Arg122, the mutated residue in hereditary pancreatitis. Consequently, this protective mechanism fails in patients with hereditary pancreatitis, and free trypsin levels accumulate, thereby favoring the activation of other zymogens and the development of acute pancreatitis.

The histopathology of the pancreas in cystic fibrosis varies considerably. Patients with mild disease may have a normal pancreas. Severely affected patients have a shrunken, cystic, and fibrotic pancreas with fatty changes.[99] Pancreatic damage results from obstruction of small ducts by precipitated proteins and cellular debris, changes that can be found in the prenatal pancreas. Involvement of large ducts, usually stenosis, is uncommon. Cystic spaces filled with eosinophilic, calcium-containing concretions develop secondary to duct blockage and acinar cell damage (Figure 6-8). Mild inflammatory changes and fibrosis develop around damaged acini. Even as fibrosis progresses, the islets of Langerhans remain intact until later in life.

Decreased chloride and bicarbonate secretion into the duct produces the pancreatic pathology observed in cystic fibrosis.[76,98] Although the mechanism remains debatable, the secretion of chloride by CFTR facilitates the secretion of bicarbonate by ductal cells. The increased concentration of these anions draws water into the duct, thereby producing the alkaline fluid needed to keep the highly concentrated digestive enzymes soluble in pancreatic juice. When CFTR is not present, pancreatic duct cells have greatly impaired secretion of chloride, bicarbonate, and water. The net effect diminishes flow and increases protein concentration in the ducts, a situation that promotes protein precipitation and the formation of inspissated secretions. These secretions obstruct the ducts, which causes acinar cell injury. In addition, the low pH of the duct lumen may inhibit membrane trafficking at the apical surface of the acinar cells, further disrupt secretion, and provide more protein for plug formation.[100]

CFTR and Idiopathic Pancreatitis. Recently, evidence has accumulated to implicate abnormal CFTR alleles in chronic pancreatitis in patients without other clinical features of cystic fibrosis.[101,102] In 1998, two groups reported an association between mutations in the gene encoding CFTR and patients with idiopathic chronic pancreatitis.[103,104] Although the initial reports found a correlation between mutations in a single CFTR allele and chronic pancreatitis, a later study with more detailed analysis of the CFTR alleles correlated risk with having two CFTR mutations.[101] These patients had compound heterozygous genotypes consisting of a severe CFTR mutation and a mild-variable mutation resulting in residual CFTR function. Their risk for developing chronic pancreatitis was increased 40-fold over the general population. The mechanism is probably similar to that described above for patients with cystic fibrosis.

Pancreatic Secretory Trypsin Inhibitor. The association of cationic trypsinogen with hereditary pancreatitis (see above) led to the search for families with mutations in SPINK1. SPINK1 is synthesized in acinar cells and packaged with trypsinogen, where it acts as the first line of defense against premature trypsinogen activation. Loss of SPINK1 function would presumably allow the accumulation of trypsin in the acinar cell and permit activation of other zymogens to cause acute pancreatitis and, eventually, chronic pancreatitis. In 2000, mutations in the gene encoding SPINK1 were correlated with idiopathic chronic pancreatitis.[105] Later, mutations in the SPINK1 gene were associated with tropical pancreatitis in Bangladesh.[106] Interestingly, the mutations N34S and P55S, implicated in

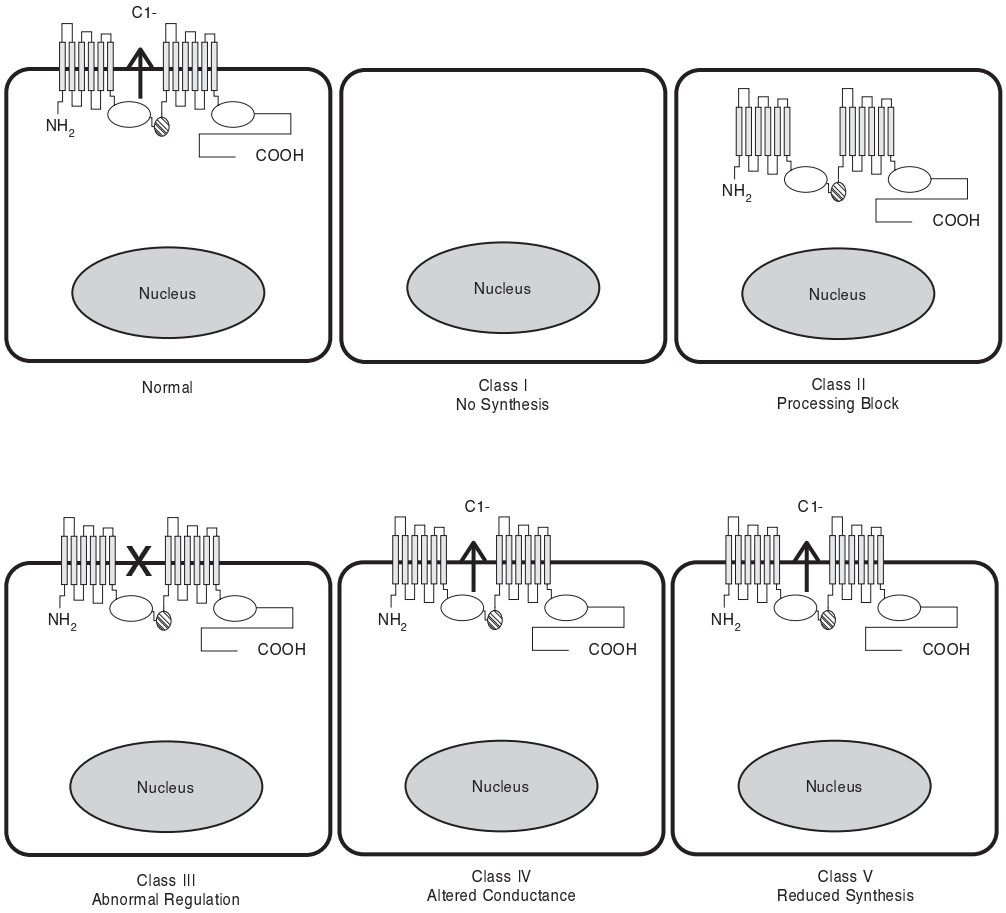


FIGURE 6-7 Mechanisms of defective cystic fibrosis transmembrane regulator (CFTR) function for the various classes of CFTR mutations. Class I: nonsense or frameshift mutations prevent the translation of CFTR protein. Class II: missense or deletion mutants do not block translation of CFTR protein, but the resultant protein does not fold properly and is degraded within the cell without reaching the cell surface. Class III: missense mutations that alter the properties of the regulatory units in CFTR, producing an inactive or poorly active transporter. Class IV: missense mutations that reduce the chloride flux through CFTR. Class V: missense mutations that decrease the synthesis of active CFTR.

chronic pancreatitis, represent 1% and 2%, respectively, of the alleles in the general population. Because of the high prevalence of these alleles and the low incidence of chronic pancreatitis, the risk of a SPINK1 mutation carrier developing chronic pancreatitis is only about 1%. Thus, the disease mechanism cannot simply be autosomal recessive inheritance. It must be more complex. Currently, SPINK1 mutations are considered disease modifiers, as illustrated by studies that analyzed patients with idiopathic chronic pancreatitis for both CFTR and SPINK1 mutations.[45] The risk for developing chronic pancreatitis was increased 900-fold in patients with mutations in both genes.[101]

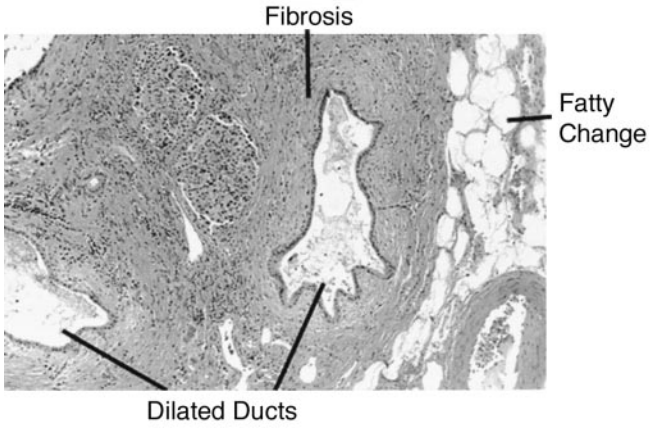


FIGURE 6-8 Histology of the pancreas in cystic fibrosis.

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CHAPTER 7

MITOCHONDRIAL FUNCTION AND DYSFUNCTION

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The study of the structure and function of mitochondria represents one of the most fascinating chapters in the history of cellular and molecular biology.[1] First described by Altman in 1890, mitochondria were among the first organelles to be studied with the electron microscope by Sjostrand and Palade.[1] An important development in the understanding of mitochondrial physiology was the discovery by Lehninger and others that the process of oxidative phosphorylation (OXPHOS) occurs in mitochondria. Later, the link between the electron transfer of OXPHOS and the synthesis of adenosine triphosphate (ATP) was established by Mitchell, for which he was awarded the Nobel Prize. Major breakthroughs in genetics were the discovery of mitochondrial deoxyribonucleic acid (mtDNA) and the sequencing of human mtDNA. More recently, in 1996, another Nobel Prize was awarded to Walker and Boyer for their studies of the structure and function of mitochondrial adenosine triphosphatase (ATPase).

Mitochondria have been considered the powerhouse of cells because they supply most of the cellular energy needs by synthesizing ATP, following the oxidation of pyruvate via the Krebs cycle and of fatty acids.[2] Notwithstanding, other important mitochondrial functions are the synthesis of heme, the disposal of ammonia via the urea cycle, and the supply of intermediate products for gluconeogenesis and lipogenesis. More recently, the central role of mitochondria in the generation of reactive oxygen species (ROS) and in the regulation of programmed cell death or apoptosis has been recognized.[3]

Following the increasing knowledge of mitochondrial biology, a variety of disorders related to abnormalities of mitochondrial function have been described. The first mitochondrial disease was described by Luft and colleagues in 1962 in a patient who had a hypermetabolic state associated with uncoupled OXPHOS and changes in mitochondrial structure.[4] Subsequently, more than 200 different defects in mtDNA and in nuclear DNA (nDNA) encoding for mitochondrial proteins have been described and associated with a variety of neurologic, muscular, metabolic, cardiac, renal, and gastrointestinal diseases.[5,6] More recently, mitochondrial abnormalities have been implicated in the pathophysiology of such diverse conditions as aging, cancer, and neurodegenerative diseases.[7]

This chapter provides an overview of mitochondrial structure, genetics, and function, as well as of pathologic conditions associated with primary or secondary dysfunction of mitochondria. The reader can find in-depth discussions on these areas in several books and reviews provided as references in this chapter and on specific gastrointestinal metabolic disorders in Chapter 55, "Genetic and Metabolic Disorders."

MITOCHONDRIAL STRUCTURE

Mitochondria are cytosolic organelles varying in size between 0.2 and 5 µm in diameter, with a length up to 20 µm.[8] The number of mitochondria in eukaryotic cells varies among species and tissues. In humans, the number of mitochondria may range from a few in the spermatozoa to about 800 in hepatocytes and approximately 100,000 in the oocyte. The total volume of mitochondria may account for as much as 25% of the cytosol. Within the cytosol, mitochondria may cluster in different areas of the cell. For example, in the spermatozoa, mitochondria are close to the tail, whereas in muscular cells, mitochondria are distributed more regularly along the length of the myofibrils. In other cells, the mitochondria may have a more random distribution.

Mitochondria are mobile.[9] They closely associate with the cytoskeleton and particularly with microtubules, actin and intermediate filaments that serve as cellular tracks. The motion of the mitochondria involves active hydrolysis of ATP and the participation of different motor proteins of the kinase superfamily (eg, KIF1B, KLP67A, KIF5B) and dynamin-related proteins.[10] Mitochondria may constitute an interconnected reticulum that extends throughout the cytosol.[11] The reticular conformation of the mitochondria seems to be necessary for delivering energy to distant areas of the cell and involves fusion and fission mechanisms redistributing the enzymatic respiratory activity of the organelle.[9,12] Fusion and fission are also important in mitochondrial biogenesis. In yeast, the fusion process also allows segregation of the mtDNA during budding.[12] The study of different mutants in yeast has permitted the identification of different proteins belonging to the dynamin family playing a role in mitochondrial dynamics.[10] Some of these proteins (mdm1p, mdm20p, mdm14p, and Rsp5p)

localize in the cytoplasm, whereas others are expressed on the mitochondrial surface (mdm10p, mmm1p, mdp12p). In infertile males of *Drosophila* with defective spermatogenesis, abnormal aggregation of mitochondria was observed, forming "fuzzy onion"–like structures.[10] The identified gene (*fzo*) causing the abnormality encodes a protein belonging to a new family of mitochondrial guanosine triphosphatases. *Fzo* homologues have also been reported in yeast, and *fzo* mutants have shown mitochondrial fragmentation and blocking of fusion.[13,14] Recently, two *fzo* family members, mitofusins 1 (mfn1) and 2 (mfn2), have been identified in humans.[15]

Mitochondria have two membranes: an outer membrane and an inner membrane separated by the intermembrane space.[8,16,17] The matrix is the space within the boundary of the inner membrane. The inner membrane has multiple infolds projecting into the matrix, the cristae. The cristae membrane is connected to the inner membrane by defined tubular structures called cristae junctions. The outer and inner membranes are separated but in close juxtaposition, except in several areas in which the two membranes fuse. It is believed that these contact sites serve as places for direct import of proteins from the cytosol into the matrix. The size of the intermembrane space and the matrix changes according to the metabolic state of the mitochondrion.

The inner and outer membranes have different lipid and protein composition.[18] The outer membrane has a similar composition to the cell membrane with roughly equal proportions of protein and lipid. It contains, among other proteins, a transmembrane porin or voltage-dependent anion channel (VDAC) permitting the passage of small molecules (< 5,000 D) and carnitine palmitoyltransferase I (CPT I), a protein involved in the transport of fatty acids from the cytosol into the mitochondria. Compared with the outer membrane, the inner membrane has less cholesterol and more cardiolipin, a phospholipid containing four fatty acid esters that decreases the ionic permeability of the membrane. The inner membrane has a higher protein-to-lipid ratio (3:1) and contains, among other proteins, the proteins involved in electron transport and OXPHOS.

The intermembranous space contains only a few proteins; some of them are involved in apoptosis. In contrast, the matrix is rich in proteins, such as the enzymes of the Krebs cycle, fatty acid oxidation, urea metabolism, and synthesis of heme. The matrix also contains chaperone proteins (heat shock protein [Hsp]60 and Hsp70) participating in the folding of proteins imported into the mitochondria, enzymes involved in the synthesis of mitochondrial proteins (ribonucleic acid [RNA] and DNA polymerases), and mtDNA.

GENETICS OF MITOCHONDRIA

The sequencing of human mtDNA[19] and the study of mtDNA in a variety of organisms have shed light on the functional and evolutionary aspects of mitochondria.

According to the endosymbiotic hypothesis, mitochondria are derived from an ancestral protobacteria (eubacteria) related to the current *Rickettsia* group, which became incorporated into a host archaebacterium or primitive eukaryotic cell.[20] During the course of evolution, variable transfer of genetic material involving protein synthesis codons and RNA genes from the protomitochondria to the nuclear genome occurred.[21] This reduction in the protomitochondria genome may explain the diverse size and genetic information present in the mtDNA of current living organisms.The size of mtDNA among different organisms ranges from 6 kb in *Plasmodium falciparum*, 14 to 20 kb in metazoan, 40 to 60 kb in fungi, to 200 to 400 kb in plants. Most organisms have circular mtDNA, but others (eg, *Saccharomyces cerevisiae*) may have linear mtDNA. The mitochondria genome encodes for different proteins of the respiratory chain and for a repertoire of ribosomal ribonucleic acid (rRNA) and transfer ribonucleic acid (tRNA). Differences in the expression of specific peptides and RNA exist between organisms.

Human mtDNA is a double-stranded circular DNA composed of 16,569 base pairs (Figure 7-1).[19,22] Based on their separation in alkaline cesium chloride, heavy (H) and light (L) strands have been described. Human mtDNA encodes for two rRNAs (12S and 16S), 22 tRNAs, and 13 peptides of the multimeric respiratory chain proteins. Specifically, mtDNA encodes for 7 (ND1, -2, -3, -4, -4 L, -5, and -6) of 42 subunits of complex I (reduced nicotinamide adenine dinucleotide [NADH]–coenzyme Q [CoQ] oxidoreductase), 1 (cytochrome-*b*) of 11 subunits of complex III (CoQ–cytochrome-*c* oxidoreductase), 3 (subunits I, II, and III) of 13 subunits of complex IV (cytochrome-*c* oxidase [COX]), and 2 (subunits A6 and A8) of 16 peptides of complex V (ATP synthase). The 4 subunits of complex II (succinate-CoA reductase), the remaining subunits of the respiratory chain complexes, and the proteins necessary for the transcription, translation, and replication of mtDNA (such as mtDNA and RNA polymerases) are encoded by the nDNA.

There are between 1,000 and 10,000 copies of mtDNA in somatic cells, whereas in germ cells, the number varies between 100 in sperm and as many as 100,000 in the human egg. Each mitochondrion may have 2 to 10 copies of mtDNA. After fertilization, the paternal mtDNA is eliminated by mechanisms not well understood. As a result, mitochondria and their DNA are maternally derived.

The replication of mtDNA is initiated by the synthesis of an RNA primer by mitochondrial RNA polymerase and of the mitochondrial transcription factor (mtTFA), both encoded by nDNA.[23,24] It is followed by the transcription initially of the heavy chain (clockwise) and subsequently of the light chain (counterclockwise) directed by two promoters, heavy and light strand promoters, respectively. The synthesis of the heavy strand starts at the origin of heavy strand replication (Oh close to position 200) and that of the light strand begins at the origin of light strand replication (Ol near position 5750). The replication requires the presence of DNA polymerase γ, a mitochondrial RNA polymerase, and the mitochondrial single-stranded DNA binding protein (mtSSB). The transcription of both strands is complete and involves the entire sequences, each producing a large polycistron. The translation of the light strand includes the messenger ribonucleic acid (mRNA) for sub-

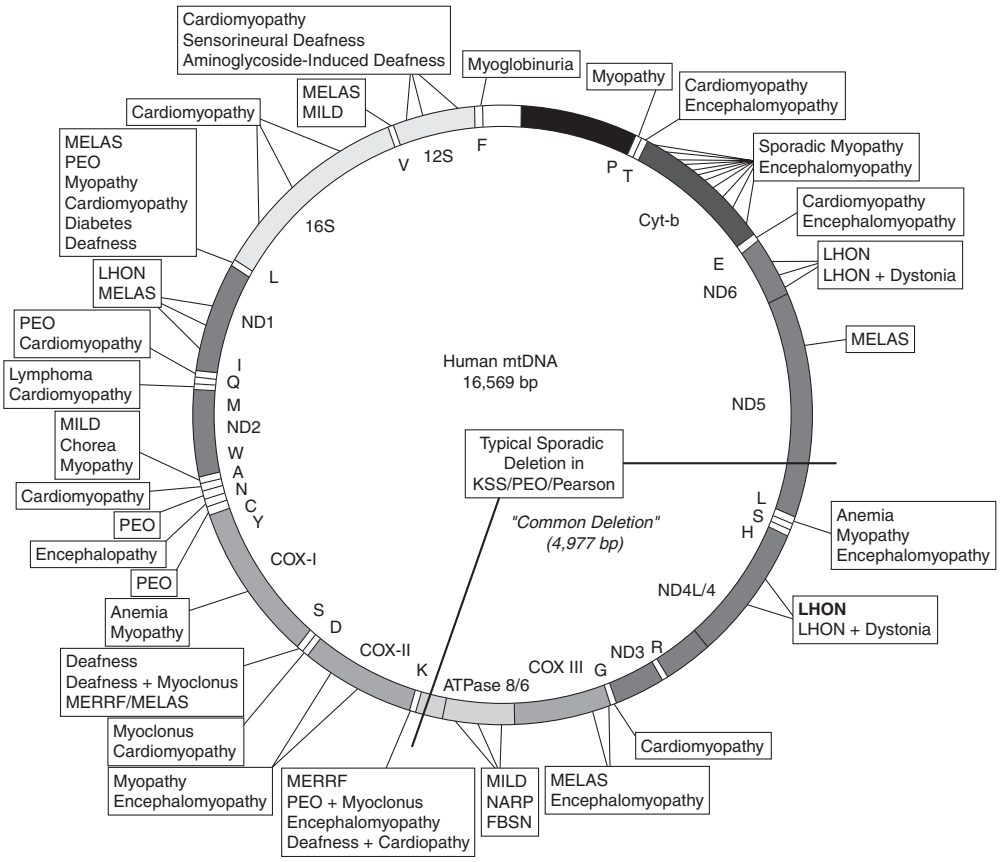

FIGURE 7-1 Mitochondrial deoxyribonucleic acid (mtDNA) and morbidity map of mitochondrial genome. The map of the 16.6 kb mtDNA shows differently shaded areas representing the protein-coding genes for the seven subunits of complex I (ND), the three subunits of cytochrome-*c* oxidase (COX), cytochrome-*b* (Cyt-*b*), and the two subunits of adenosine triphosphatase (ATPase) synthase (ATPases 6 and 8); the 12S and 16S ribosomal ribonucleic acid (rRNA); and the 22 transfer ribonucleic acids (tRNA) identified by one-letter codes for the corresponding amino acids. FBSN = familial bilateral striatal necrosis; KSS = Kearns-Sayre syndrome; LHON = Leber hereditary optic neuropathy; MELAS = mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes; MERRF = myoclonic epilepsy with ragged red fibers; MILD = maternally inherited Leigh disease; NARP = neuropathy, ataxia, retinitis pigmentosa; PEO = progressive external ophthalmoplegia. Adapted with permission from DiMauro S.[453]

unit ND6 of complex I and eight tRNAs, whereas the translation of the heavy strand includes the sequences for other peptides of the respiratory chain and the remainder tRNA. The termination of transcription requires the presence of a factor called mitochondrial transcription termination factor. The transcripted polycistron is then cleaved to produce rRNA, tRNA, and mRNA sequences. The genetic code of mtDNA differs from nDNA in that in mitochondrial translation, AGA and AGG sequences act as stop codons instead of coding for arginine. Also, sequence UGA codes for tryptophan instead of being a stop codon, and AUU sequence codes for methionine instead of isoleucine.

The translation of mitochondrial mRNA requires the presence of peptides derived from both nuclear and mitochondrial genomes. The replication of mtDNA within the cell does not correlate with that of nDNA.[25] The exact mechanisms participating in the coordination of the two genomes are not understood. Recently, several factors, including NRF1 and NRF2, and other nuclear coactivators (PGC and PRC) have been described as nuclear transcriptional regulators of mitochondrial biogenesis.[26] There is evidence that the two genomes mtDNA and nDNA have coevolved. In experiments in which mtDNA was paired with nDNA of different species, only genomes being phylogenetically very close were able to sustain functional OXPHOS.[27]

Mitochondrial proteins synthesized from nDNA need to be imported into the mitochondria by a complex mechanism. The process requires different proteins that translocate peptides from the cytosol through the outer and inner mitochondrial membranes (Figure 7-2).[28,29] For this purpose, many proteins to be imported into the mitochondria have an N-terminal leader signaling sequence. In hydrophobic membrane proteins, the signaling peptide may reside inside the protein. Proteins located in the mitochondrial intermembrane space may have both N-terminal and internal signal sequences, whereas other proteins may have multiple targeting signals. The proteins bind initially to the chaperone molecules, Hsp70, or the mitochondrion import stimulating factor to keep the peptides unfolded.

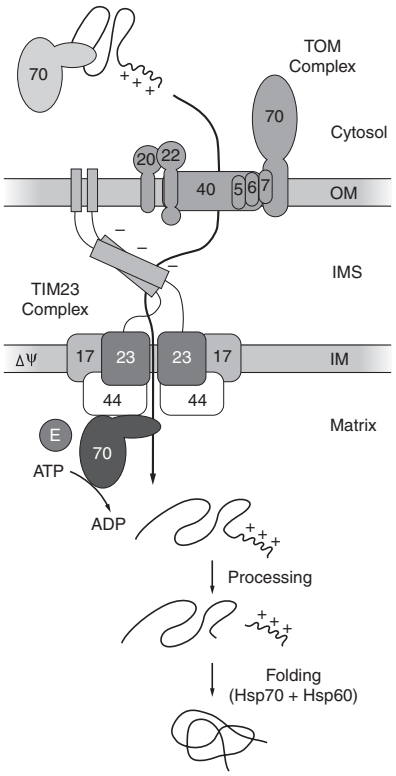

FIGURE 7-2 Mitochondrial import pathway for matrix-targeted preproteins. The nuclear encoded preproteins are synthesized in the cytosol with N-terminal positively charged matrix targeting signals and are bound by cytosolic heat shock protein (Hsp)70 chaperones. The Tom20/Tom22 receptors recognize the mitochondrial preproteins on the surface of the mitochondria and transfer them to the protein-conducting channel of the TOM complex. For translocation across the inner membrane, the TOM complex cooperates with the TIM23 translocase, which initiates the translocation of the preproteins across the inner membrane in a $\Delta\Psi$-dependent manner. In the matrix, the preproteins are bound by mitochondrial Hsp70, which, together with its cochaperone Mge1p, drives the adenosine triphosphate (ATP)-dependent translocation further. The matrix-targeting signals are processed by the mitochondrial processing peptidase, and the preproteins fold into their native conformation with the help of chaperones. Numbers refer to the molecular masses of the respective component in kD. ADP = adenosine diphosphate; E = Mge1p; IM = inner membrane; IMS = intermembrane space; OM = outer membrane; $\Delta\Psi$ = membrane potential across the inner membrane. Reproduced with permission from Paschen SA, Neupert W, Taylor and Francis Ltd (<http://www.tandP.co.uk/journals>).[28]

Import of proteins in the mitochondria is mediated by a translocase in the outer membrane, the TOM complex, and two translocase complexes in the inner membrane, TIM23 and TIM22. Each of these complexes is made of different proteins encoded by nuclear genes. TOM is constituted by the receptor proteins Tom20, Tom22, and Tom70; the channel protein Tom40; and three other associated proteins, Tom5, Tom6, and Tom7. In the mitochondrial outer membrane, proteins to be imported interact with receptor proteins Tom20 and Tom22, which mainly bind proteins with an N-terminal leader sequence, and with Tom70, which recognizes internal sequences. The traveling peptide then passes through the general import (GIP) complex, which includes Tom40, Tom5, Tom6, and Tom7. Of these proteins, Tom40 serves as the protein-conducting channel, and the others have a supportive role. Tom5 facilitates the transfer of proteins from Tom22 to Tom40. Tom6 contributes to the stability of the GIP complex, and Tom7 intervenes in the release of proteins in the outer membrane. Proteins to be integrated into the inner membrane, after passage through the outer membrane, can interact with other TIM molecules in the intramembranous space (Tim8, Tim9, Tim10, Tim12, and Tim13), avoiding aggregation and facilitating the interaction with the TIM22 complex. TIM22 is constituted by three integral membrane proteins, Tim22, Tim54, and Tim18, and by three peripheral proteins, Tim12, Tim10, and Tim9. TIM22 pore activity is dependent on the mitochondrial membrane potential $\Delta\Psi$ and inserts imported multispanning membrane proteins (eg, several metabolic carriers) into the lipid phase of the inner membrane.[30] In the case of molecules to be integrated into either mitochondrial membrane, the leader signal is commonly accompanied by hydrophobic sorting sequences recognizable by TOM and TIM22 complexes, stopping the proteins in the outer or inner membranes.

For proteins with the matrix as the final destination, the passage through the inner membrane occurs after the importing sequence interacts with TIM23, another translocase complex in the inner membrane (see Figure 7-2). The TIM23 complex consists of two transmembrane proteins, Tim23 and Tim17, the former being the actual pore through which the protein is transferred in a process dependent on the membrane potential $\Delta\Psi$. The proteins then associate with a complex of Tim 44, a translocase on the matrix side of the inner membrane, Hsp70, and a cochaperone protein, Mge 1p. The signaling sequence is cleaved by an ATP-dependent mitochondrial processing peptidase. The protein is then refolded with the assistance of other chaperone molecules, such as Hsp70, Hsp60, or peptidyl-prolyl *cis-/trans*-isomerases.

The mechanisms of transport of proteins, such as the components of the respiratory chain from the matrix into the inner membrane, are not well defined. Recently, two proteins, Oxa 1 and Pnt 1, have been proposed as playing a role in this process.[31,32]

MITOCHONDRIAL FUNCTION

Energy Production

The production and use of energy are the fundamental metabolic processes of a living organism. Mitochondria play a pivotal role in the production and storage of energy through the oxidation of nutritional substrates coupled to the synthesis of ATP. The main substrate for the production of energy in the mitochondria is acetyl coenzyme A (CoA) derived from the oxidation of pyruvate via the Krebs cycle or from the β-oxidation of fatty acids (Figure 7-3).[2]

In the cytosol, the initial degradation of glucose produces two molecules of pyruvate and four molecules of ATP, of which two molecules are reused in the glycolytic pathway. Pyruvate is imported into the mitochondria by a pyruvate carrier and is oxidized in the matrix to acetyl CoA

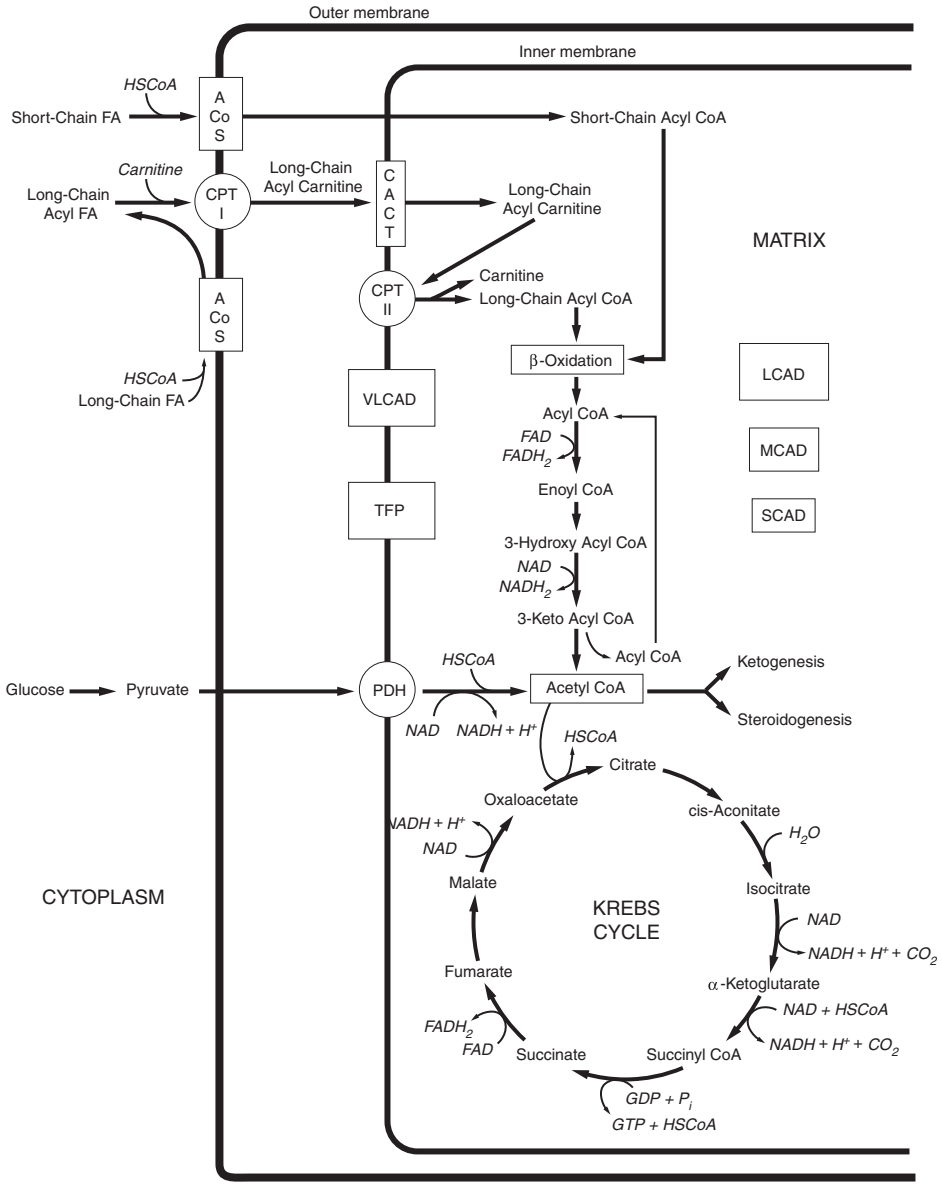

FIGURE 7-3 Oxidation of metabolic substrates pyruvate and fatty acids (FA) by mitochondria. Pyruvate produced by glycolysis in the cytosol is imported into the mitochondria and is oxidized by pyruvate dehydrogenase (PDH), producing acetyl CoA, which undergoes further metabolism in the Krebs cycle. Short- and long-chain FAs are acylated by acyl-CoA synthases (ACoAS) in the outer membrane. Long-chain acyl FAs are transported into the mitochondria after forming acyl carnitine ester by carnitine palmitoyltransferase I (CPTI), which is passed through the inner mitochondrial membrane by a carnitine acylcarnitine translocase (CACT) into the matrix. The acyl carnitine ester is hydrolyzed by carnitine palmitoyltransferase II (CPTII), producing long-chain acyl CoA and carnitine. Acyl-CoA FA then undergoes β-oxidation mediated by the activity of different acyl-CoA dehydrogenases. Reduced nicotinamide adenine dinucleotide (NADH) and flavine adenine dinucleotide ($FADH_2$) can be oxidized in the inner mitochondrial membrane by the electron transport chain. FAD = flavin adenine dinucleotide; GDP = guanosine diphosphate; GTP = guanosine triphosphate; LCAD = long-chain acyl dehydrogenase; MCAD = medium-chain acyl dehydrogenase; NAD = nicotinamide adenine dinucleotide; SCAD = short-chain acyl dehydrogenase; TFP = trifunctional protein; VLCAD = very-long-chain acyl dehydrogenase.

in the presence of CoA and nicotinamide adenine dinucleotide (NAD) by pyruvate dehydrogenase:

$$\text{Pyruvate} + \text{CoA} + \text{NAD}^+ \rightarrow \text{NADH} + \text{acetyl CoA} + \text{CO}_2$$

Pyruvate dehydrogenase is a multimeric protein composed of three units (each with multiple subunits): E1 or pyruvate decarboxylase, E2 or lipoamide transacetylase, and E3 or dihydrolipoyl dehydrogenase. The enzymatic activity requires the presence of five coenzymes: oxidized nicotinamide adenine dinucleotide (NAD^+), flavin adenine dinucleotide (FAD), CoA, lipoamide, and thiamine pyrophosphate (TP).

Krebs Cycle. The 2-carbon acetyl CoA enters the Krebs or citric acid cycle by combining with oxaloacetate to form a 6-carbon molecule, citric acid (see Figure 7-3). This reaction is mediated by citrate synthase. Further metabolism by this pathway involves the formation of isocitrate by aconitase, an enzyme containing a nonheme iron sulfur cluster, which also participates in the cell handling of iron. The synthesis of α-ketoglutarate from isocitrate requires the activity of isocitrate dehydrogenase, NAD^+, and adenosine diphosphate (ADP). α-Ketoglutarate is metabolized to succinyl CoA by α-ketoglutarate dehydrogenase. The subsequent formation of succinate is mediated by succinyl-CoA synthase and requires guanosine diphosphate as a P_i acceptor. The oxidation of succinate to fumarate is catalyzed by succinate dehydrogenase, with the release of two electrons and two protons, with FAD becoming the proton acceptor. Fumarate is next hydrated to form malate by a fumarase. Further dehydrogenation by malate dehydrogenase in the presence of NAD^+ yields oxaloacetate and NADH.

The complete aerobic metabolism of one molecule of glucose results in the production of 6 molecules of CO_2, 10 molecules of NADH, 2 molecules of reduced flavin adenine dinucleotide ($FADH_2$), and 36 molecules of ATP.

Fatty Acid Oxidation. Fat oxidation represents an important source of energy during periods of metabolic

need such as starvation, exercise, or stress. In several tissues, such as the myocardium and skeletal muscle, energy is obtained mainly from fatty acid oxidation.[33] Fatty acids are derived from triglycerides stored in adipose tissue or from triglyceride-rich lipoproteins, such as chylomicrons and very-low-density lipoproteins, after hydrolysis by lipases. Free fatty acids are then bound to albumin and transported into the cell by diffusion or by several fatty acid transporters.[34] Once in the cytosol, fatty acids bind to fatty acid binding proteins.[35]

Most fatty acid β-oxidation occurs in the mitochondria by sequential removal of two carbon acetyl-CoA units.[36,37] Additional α- and β-oxidation of fatty acids may occur in the peroxisomes.[38] Peroxisomes are particularly important in the oxidation of branched- and very-long-chain fatty acids and may initiate the oxidation of long-chain fatty acids. Fatty acid oxidation in peroxisomes may proceed only until the formation of octanoyl CoA and is not accompanied by synthesis of ATP. Compared with mitochondria, the peroxisomes contribute less to the oxidation of fatty acids below 20-carbon chain length.[39]

Long-chain fatty acids enter the mitochondria after esterification with CoA in the outer membrane. The reaction is mediated by a variety of ATP-dependent acyl-CoA synthases, including members of the fatty acid binding proteins, followed by binding of the ester to acyl-CoA binding protein.[37] The entry of the activated fatty acids into the mitochondria proceeds initially by the formation of an acyl carnitine ester mediated by a tissue-specific enzyme, CPTI. The fatty acid–CoA ester is then transferred across the inner membrane into the matrix by carnitine acylcarnitine translocase (CACT) and is reconstituted as acyl CoA and free carnitine in the inner membrane by CPTII. The oxidation of fatty acid occurs in the matrix by different acyl-CoA dehydrogenases (ACADs) for very-long (VLCAD), long-chain (LCAD), medium-chain (MCAD), and short-chain (SCAD) fatty acids. VLCADs localize in the inner membrane and the trifunctional protein (TFP) complex. TFP is an octamer consisting of two tetramers, α and β, which contain the enzymatic activity of long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD), 2-enoyl-CoA hydratase, and 3-ketoacyl-CoA thiolase.[40] The other acyl hydrolases, LCAD, MCAD, and SCAD, are present in the matrix. According to the length of the fatty acids, the substrate activity of ACADs has been reported to be C24 to C12 for VLCAD, C20 to C6 for LCAD, C16 to C4 for MCAD, and C6 to C4 for SCAD.[41]

In the first step, a specific length acyl CoA is hydrolyzed by an ACAD specific for a given fatty acid chain length in the presence of FAD as a cofactor, yielding *trans*-Δ2-enoyl CoA and reduced $FADH_2$. The electrons present in $FADH_2$ are then transferred to the electron transfer flavoproteins (ETFs). Subsequently, the electrons present in reduced ETF can be transferred to ubiquinone by another inner mitochondrial membrane, the ETF-ubiquinone oxidoreductase, entering the electron transport chain. Enoyl CoA is then hydrated by 2,3-enoyl-CoA hydratase, producing 3-hydroacyl CoA, which, in the presence of NAD^+ and 3-hydroacyl-CoA dehydrogenase, results in the formation of 3-ketoacyl CoA and NADH. The last step consists of the cleavage of 3-ketoacyl CoA producing acetyl CoA and acyl CoA (shortened by 2 carbons and continuing a similar cycle). In addition, depending on the location of the double bond (even or odd numbered carbons), the oxidation of polyunsaturated fatty acids requires the activity of auxiliary enzymes, including enoyl CoA, dienoyl CoA isomerases, and 2,4-dienoyl CoA reductase.[41]

Acetyl CoA released during fatty acid oxidation may enter the Krebs cycle, be used in ketogenesis, or serve as a substrate in the synthesis of mevalonate, a precursor of cholesterol. The production of the ketone bodies acetoacetate and hydroxybutyrate occurs mainly in the liver,[42,43] but other tissues, such as kidney, intestine, and adipose tissue, also have ketogenic ability. Initially, acetyl CoA reacts with acetoacetyl CoA in the presence of 3-hydroxy-3-methylglutaryl CoA synthase, forming 3-hydroxy-7-methylglutaryl CoA, which is subsequently metabolized into acetoacetate by a specific lyase. The reduction of acetoacetate to hydroxybutyrate is carried by the 3-hydroxybutyrate dehydrogenase. Ketones are used as a source of energy in peripheral tissues, particularly in the brain during glucose deficiency circumstances.[44]

Electron Transport and OXPHOS. Chemical reactions involve the transfer of electrons between reagent molecules. Oxidation refers to the loss of electrons from individual atoms or molecules, whereas reduction refers to the gain of electrons. These processes are coupled in oxidation-reduction reactions. The reduction potential, E, of a molecule describes the facility to gain electrons. The oxidative potential describes the change in charge when there is loss of an electron. In an oxidoreduction reaction, the sum of these potentials, ΔE, defines the direction of the reaction.

In mitochondria, most of the energy produced during the oxidation of sugars and fatty acids is retained in the reduced coenzymes NADH and $FADH_2$ and is released during the oxidation to NAD^+ and FAD.[2] The oxidation of NADH and $FADH_2$ involves the removal of a hydride ion yielding one proton and two electrons ($H^- \rightarrow H^+ + 2e^-$).

The sequential transfer of electrons occurs via the oxidoreduction of several prosthetic groups present in the protein complexes of the electron transport chain and is coupled with the transfer of protons from the matrix to the intermembranous space (Figure 7-4). Because the inner mitochondrial membrane is impermeable to protons, the transfer of protons across the inner membrane creates a proton concentration gradient and therefore a pH gradient of about 1 unit between the matrix and the intermembranous space. Because the matrix side of the inner membrane becomes relatively electronegative owing to the loss of protons, a voltage gradient of about –160 mV is also generated across the membrane: the membrane potential, usually designed as ΔΨ. The sum of the proton concentration gradient and ΔΨ is expressed as the proton motive force. This proton electrochemical gradient drives the synthesis of ATP by the ATP synthase (complex V) present in the mitochondrial inner membrane. The oxidation of one molecule

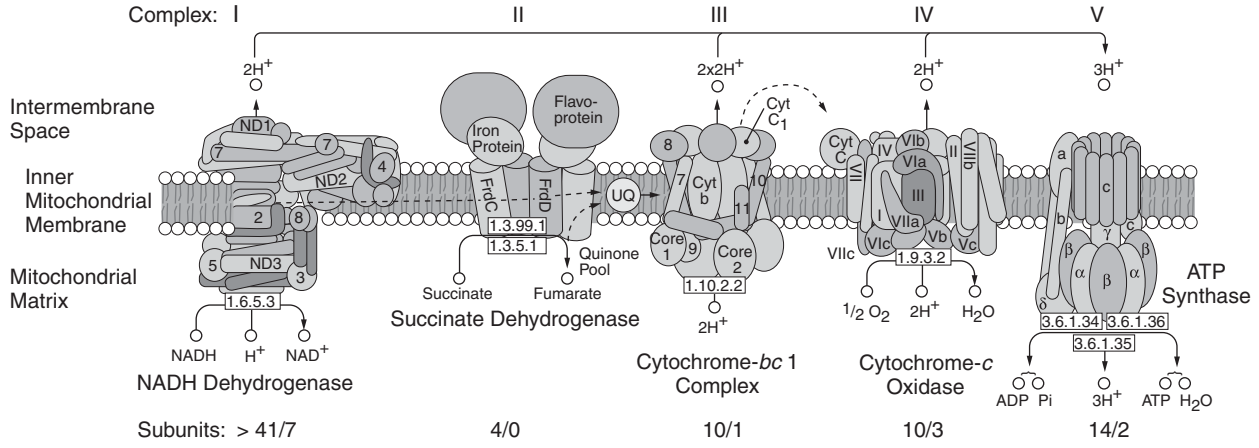

FIGURE 7-4 Electron transport chain. Schematic representation of mammalian electron transport complexes (I–IV). Electrons flow from reduced nicotinamide adenine dinucleotide (NADH) or succinate to complex I or II, respectively, and subsequently to ubiquinone (UQ). Electrons then flow from ubiquinone through complexes III and IV to the final acceptor, molecular oxygen. Electron flow is coupled to proton movement across the inner membrane in complexes I, III, and IV. The resulting proton gradient is harvested by complex V to generate adenosine triphosphate (ATP). The number of subunits encoded by the nuclear/mitochondrial genomes is at the bottom of the figure. ADP = adenosine diphosphate; NAD = nicotinamide adenine dinucleotide. Adapted from Mandavilli BS et al.[170]

of NADH produces three molecules of ATP, whereas that of $FADH_2$ produces two molecules of ATP because the oxidation of succinate is initiated at the level of the second complex in the respiratory chain.

The electron transport chain includes four electron transport proteins (complexes I, II, III, and IV), the ATP synthase (complex V), and two electron shuttles: the CoQ, also called quinone (Q) or ubiquinone (UQ), and cytochrome-*c*. The five complexes are multimeric proteins residing in the mitochondria inner membrane (see Figure 7-4).[45–47] Except for complex II (succinate-CoA reductase), which is encoded by nDNA, the subunits of the other complexes are derived from both the nuclear and mitochondrial genomes and assembled by poorly understood mechanisms. CoQ is a lipophilic molecule containing 10 isoprenoid units. The quinone moiety can be reduced to reduced coenzyme Q ($CoQH_2$) or quinol by collecting the protons entering the respiratory chain and oxidized to CoQ by passing the electrons to the electron transfer chain complexes I and II. Cytochrome-*c* is one of four different cytochromes in the respiratory chain that contains iron inside a porphyrin ring. The iron then may be oxidized or reduced in the coupled reactions involved in the electron transport.

***Complex* I (NADH-Q*uinone* O*xidoreductase*).** In mammalian mitochondria, complex I is the largest of the electron transport proteins, with a molecular weight close to 1,000 kD.[48,49] The enzyme is L shaped, and the primary structures of the 42 units in humans have recently been reported.[49] The complex is made of three different fractions: a flavoprotein, an iron sulfur, and a hydrophobic protein fraction. The latter contains seven subunits: ND1 to 6 and subunit 4L. The complex binds NADH and CoQ and uses flavin mononucleotide, eight to nine iron sulfur clusters, and CoQ as prosthetic groups in the redox reactions. Complex I catalyzes the reaction by which CoQ is reduced, and four protons are transported from the matrix across the inner membrane:

$$NADH + CoQ + 5\ H^+ \text{ (from the matrix)} \rightarrow NAD^+ + CoQH_2 + 4\ H^+$$

The details of the electron transfer and proton translocation within the complex are not completely established, but it is believed that the initial transfer of electrons starts by the transfer of a hydride ion to flavin mononucleotide, and then the electrons are passed to different iron sulfur clusters to reach the ubiquinone.[48]

***Complex* II (*Succinate-Ubiquinone* Reductase).** Succinate-ubiquinone reductase is composed of four subunits: A (70 kD), B (27 kD), C (15 kD), and D (13 kD).[45–47,50] Units A and B constitute a water-soluble peripheral domain with succinate dehydrogenase activity. Unit A, also referred to as flavoprotein, contains a molecule of FAD as a prosthetic group, covalently bound to a histidyl residue. The prosthetic groups of unit B, also known as iron sulfur protein, are three iron sulfur clusters designated as S1, S2, and S3. Units C and D are hydrophobic and constitute the membrane integral domain of the molecule. This domain carries containing another cytochrome-*b*–like redox component that has heme as a prosthetic group. Complex II catalyzes the two electrons oxidation of fumarate and the reduction of ubiquinone to ubiquinol:

$$\text{Succinate} \rightarrow \text{fumarate} + 2H^+ + 2e^-$$
$$CoQ + 2H^+ + 2e^- \rightarrow CoQH_2$$

The reaction, although thermodynamically favorable, does not provide enough energy to pump protons across the inner membrane. The nuclear genes encoding for the four subunits SDHA, SDHB, SDHC, and SDHD have been identified.[51]

***Complex* III (U*biquinone–Cytochrome-c* O*xidoreductase*).** Complex III is a dimeric protein with a monomer mass of 240 kD made of 11 peptides.[45–47] It contains two cytochrome-*b* molecules in subunit III: one with high reduction potential (280 mV), *bh*, and the other with lower potential (30 mV), *bl*. Subunit IV contains another heme-containing cytochrome (cytochrome-*c*1). Two other iron-sulfur clusters are located in subunit V, also know as the Rieske protein. Complex III has two binding sites for the oxidation of ubiquinol: one, Qo, localizes on the cytosolic side of the inner membrane, whereas the other, Qi, locates on the matrix side of the protein. The enzyme catalyzes the reaction:

$$CoQH_2 + 2\ \text{cyt-}c^{3+} + 2H^+\ \text{(from the matrix)} \rightarrow CoQ + 2\ \text{cyt-}c^{2+} + 4H^+$$

The reaction occurs sequentially via the Q cycle.[52] Reduced ubiquinone ($CoQH_2$) originating in complex I diffuses into the membrane and binds to the Qo site of complex III, where oxidation is initiated, freeing two protons and one electron (of two electrons present in the ubiquinone molecule). The first electron is then transferred to the Rieske iron-containing subunit, passed on to cytochrome-*c*1 and subsequently to cytochrome-*c*. The ubisemiquinone ($CoQH^{\bullet}$) passes the other electron, called cyclic electron, sequentially to the cytochrome molecules *bl* and *bh* and to the Qi site of the molecule. After two cycles (two ubisemiquinones passing one electron each), the ubiquinone is reconstituted in its reduced form, and a total of four protons are passed across the inner membrane.

***Complex* IV (*Cytochrome* O*xidase*).** Bovine heart complex IV is a dimeric protein constituted by 13 subunits with a molecular weight of 204 kD.[53] The three larger subunits, I, II, and III, encoded by mtDNA are the core of the protein, which is surrounded by 10 other peptides (IV, Va, Vb, VIa, VIb, VIc, VIIa, VIIb, VIIc, VIII). The molecule contains two heme groups, a and a_3, and two copper centers, Cu_A and Cu_B. Subunit I contains the heme groups a and a_3 and the Cu_B center, whereas subunit II binds the Cu_A center. The molecule catalyzes the reaction:

$$4\ \text{cyt-}c^{2+} + 8\ H^+\ \text{(from the matrix)} + O_2 \rightarrow 4\ \text{cyt-}c^{3+} + 4\ H^+ + 2\ H_2O$$

The transfer of electrons occurs sequentially from cytochrome-*c* to redox centers Cu_A, to heme a, and to the Cu_B-a3 active site, where oxygen is reduced to water with a coupled oxidation of iron (present in heme) and copper. In the process, eight protons from the matrix are used, and four protons are transported to the intermembranous space.

***Complex* V (ATP S*ynthase*).** The ATP synthase is constituted by 12 to 14 subunits arranged into two multimeric complexes: one is embedded in the inner mitochondrial membrane (Fo), whereas the other protrudes into the matrix (F1) (see Figure 7-4).[54–56] In mammals, depending on the species, Fo contains several subunits, including subunits A6, A8, a, and b; several subunits of c; and subunits, e, f, and g. A proton channel is present between the a and c subunits. The F1 section is also multimeric. The protruding stalk contains subunits γ and ε, connected to the hydrophilic segment in the matrix, a hexamer composed of three α subunits intercalated with three β subunits. Another δ subunit associated with the hexamer connects with the b subunit of Fo. According to the current model, the protein has a rotor constituted by the ensemble of hydrophobic c subunits making part of the Fo portion of the molecule, rotating around an axle composed of the δ and ε subunits of F1. The hexamer made of subunits α and β is stabilized by the subunits δ and b (the stator), avoiding rotation around the shaft or stalk when the complex is not active. The protein ensemble may be seen as a turbine or rotary motor transmitting the motion initiated in Fo to F1 through a cam (the γ and ε subunits).

ATP synthase catalyzes the reaction ADP + $P_i \rightarrow$ ATP + H_2O. Under standard conditions, it is an endergonic reaction with a Δ G of 7.3 kcal/mol, requiring input of energy.[2] That energy is provided by the passage of protons through the enzyme, flowing down from the intermembrane space into the matrix. This proton transport is driven by the proton motive force (the sum of the membrane potential, ΔΨ, and the H^+ concentration gradient, Δ pH). The electrostatic interaction between the positive charge of the protons and negatively charged amino acids residues located in the c unit (Glu 58 in the mammalian enzyme) induces the complete rotation of the c unit, releasing the proton into the matrix. The revolving movement of the rotor and the asymmetric shaft inside the matrix portion of the molecule changes the conformation of the catalytic sites located in the β subunits of the αβ hexamer. According to the model of rotational catalysis proposed by Boyer, the catalytic sites may assume three different configurations.[54,55] In the loose configuration (L), ADP and P_i bind to the β subunit. The catalytic synthesis of ATP occurs when the subunit assumes a tight configuration (T). The energy for the reaction comes from the binding of the substrates ADP and P_i to the subunit. The rotational movement of the hexamer induced by the proton translocation then changes the conformation of the β subunit to an open (O) configuration, overcoming the strong affinity of ATP for the subunit, allowing then the release of ATP. By continuous rotation of the hexamer, the open configuration is reverted to the loose configuration. The synthesis of one molecule of ATP appears to require the flow of four protons through complex V, and the enzyme may have a rotation rate close to 8,000 rpm.[57] Depending on the energy requirement of the cell and the relative availability of substrates (ATP, ADP, P_i), the enzyme can have reverse rotation and ATPase activity, binding ATP and yielding ADP and P_i. The rotatory process is under control of the ε subunit, which, depending on its contact with the αβ hexamer, may act as a unidirectional ratchet.[58] The energy derived from ATP hydrolysis may then be used to pump protons from the matrix across the inner mitochondrial membrane.

Mitochondrial Transport Systems

The performance of many of the biologic functions and reactions occurring within the cell necessitates the trans-

port of different molecules among the mitochondria, the cytosol, and other cell organelles.

One of these essential processes is the transfer of newly synthesized ATP from the mitochondrial matrix to the cytosol and the import into the matrix of ADP, produced after cytosolic hydrolysis of ATP. This coupled exchange is mediated by the ADP-ATP translocase or adenine nucleotide translocator (ANT).[59] The ANT is an integral dimeric protein member of the mitochondrial carrier family, with a molecular weight of about 32 kD that can bind the nucleotides ATP and ADP on both sides of the inner mitochondrial membrane. A nuclear gene encodes for three isoforms, with variable tissue expression and possibly different kinetic properties.[60] The ANT molecule exchanges ADP and P_i from the intermembrane space with ATP from the matrix. The cotransport depends on the proton motive force and is energetically expensive, using a quarter of the energy produced by the electron chain transport. The activity of the transporter maintains a high ATP-to-ADP ratio within the cell. In addition to the interchange of ATP and ADP, ANT may function as a nonspecific pore leading to increased permeability of the inner membrane to solutes up to 1,500 D. Along with other proteins, ANT makes part of the permeability transition pore complex (PTPC), a structure important in the process of apoptosis (see below).[61]

The outer mitochondrial membrane is highly permeable to small molecules (< 5,000 D) owing to the presence of pores formed by the porin protein, whereas the inner membrane is impermeable to most molecules. Therefore, intermediate products necessary in different metabolic pathways require specific transport mechanisms between the matrix and the cytosolic compartments.[2] The transport of major fuel substrates (fatty acid and pyruvate) across the mitochondrial membranes has already been described. Once formed in the mitochondria, acetyl CoA necessary for the synthesis of fatty acid in the cytosol cannot cross the inner membrane and needs to convert to citrate to exit the matrix. In the cytosol, citrate is converted to oxaloacetate and acetyl CoA. Oxaloacetate is then reduced to malate, which enters into the mitochondrial matrix using two different transporters. One, the tricarboxylate transporter, couples the ingress of malate into the mitochondria to the exit of citrate, isocitrate, or *cis*-aconitate from the matrix. The second, the α-ketoglutarate transporter, is a dicarboxylic acid translocase that transports α-ketoglutarate from the matrix to the cytosol in exchange for malate.

Although cytosolic coenzymes produced in the cytosol, such as NADH, cannot enter the matrix, the electrons from cytosolic NADH can be transferred inside the mitochondria to be used in OXPHOS by two shuttle systems: the malate-aspartate shuttle and the α-glycerol phosphate shuttle. In the malate-aspartate shuttle, the NADH electrons are used in the synthesis of maleate from oxaloacetate in the presence of a cytosolic malate dehydrogenase. Malate can then be imported into the mitochondrial matrix, where a mitochondrial maleate dehydrogenase in the presence of NAD^+ regenerates NADH, yielding oxaloacetate. Because the mitochondrial membrane is not permeable to oxaloacetate, to leave the matrix, oxaloacetate needs to combine with glutamate to form aspartate. Aspartate can exit the matrix via a specific transporter that also imports glutamate from the cytosol into the matrix. In the α-glycerol phosphate shuttle, the oxidation of NADH to NAD^+ in the cytosol is coupled to the transfer of two electrons to dihydroxyacetone phosphate, a glycolytic intermediate yielding glycerol-3-phosphate. In the mitochondrial membrane, glycerol-3-phosphate dehydrogenase reverts the reaction, oxidizing glycerol-3-phosphate, regenerating dihydroacetone phosphate. The reaction is accompanied by the transfer of the two electrons, reducing the FAD prosthetic moiety of the mitochondrial glycerol-3-phosphate dehydrogenase. Then the two electrons present in $FADH_2$ may enter the respiratory chain by reducing CoQ.

Ions are also transported in and out of the mitochondrial membrane. The phosphate transporter is an HPO_4^{2-}/OH^- carrier that imports HPO_4^{2-} into the matrix coupled to the transfer of OH^- from the matrix to the intermembrane space. Calcium is an important modulator of the production of energy by the mitochondria by activating pyruvate dehydrogenase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase, increasing the NADH-to-NAD^+ ratio possibly by regulation of cytochrome-*c* and ATPase.[62] In addition, compartmentalization of calcium in the mitochondria may regulate different calcium-dependent cellular processes by affecting the availability of calcium in the cytosol and organelles (such as the endoplasmic reticulum).[63] Calcium influx into the mitochondria is facilitated by $\Delta\Psi$ and is mediated by a uniporter that also transports other cations.[64] The efflux of calcium from the matrix is an active transport that occurs via Na^+-dependent and Na^+-independent calcium transporters. A recent report suggests that the VDAC or mitochondrial porin located in the outer membrane also controls the influx and efflux into and from the mitochondria.[65] Na^+ may be transported by an Na^+-H^+ antiporter, and K^+, which regulates mitochondrial volume, is transported by a channel activated by ATP and also by a K^+-H^+ antiporter.

Control of OXPHOS

OXPHOS requires the availability of electron donor intermediates NADH and $FADH_2$ derived from fuel catabolism of oxygen and a supply of ADP and P_i as substrates for the synthesis of ATP by complex V. In vitro, OXPHOS is mainly regulated by the availability of ADP and P_i.[66,67] The ratio between the amount of phosphorylated ADP and oxygen consumption is designed as the P-to-O ratio. In the presence of ADP, there is maximal consumption of O_2 by the mitochondria (stage 3), which decreases once ADP is transformed into ATP (stage 4). The ratio between stage 3 and stage 4 of respiration is called the respiratory control ratio and represents a measure of the coupling between electron transport and ATP synthesis. At the cellular level, the short-term control of OXPHOS may also depend on the consumption of ATP and the intramitochondrial concentration of ADP. The ATP-to-ADP cellular ratio may have an effect on OXPHOS by regulating the activity of enzymes such as pyruvate dehydrogenase, increasing the availability

of NADH.[47] In addition, the ATP-to-ADP ratio could regulate OXPHOS via an allosteric effect on cytochrome-*c* activity.[68] However, other levels of control may exist because, in vivo, no significant changes in ADP occur under maximum respiration,[69] and the increase of ATP synthesis and respiration in muscle appears to occur faster than predicted by the availability of ADP.[70] Based on the observation that mitochondrial O_2 consumption inversely relates to the nitric oxide (NO)-to-O_2 ratio in mitochondria, it has been proposed that NO may have a physiologic role in the control of OXPHOS.[71,72]

In uncoupled respiration, respiration by the mitochondria and the generation of $\Delta\Psi$ can occur independently of the synthesis of ATP. In uncoupled respiration, in the absence of ADP (stage 4), proton import from the intermembranous space into matrix through the complex V is limited, and the consumption of oxygen depends on leakage of protons through the inner membrane by other pathways.[73] Under these conditions, the energy generated by the transfer of electron is then not conserved as ATP but rapidly dissipates as heat. The uncoupling of mitochondrial respiration is important in thermoregulation and in the regulation of activity of certain enzymes involved in mitochondrial steroid synthesis in the adrenal cortex.

Recently, three uncoupling proteins (UCP1, UCP2, and UCP3) belonging to the mitochondrial supercarrier family have been described in mammals.[74,75] UCP1 consists of 306 amino acids, is expressed exclusively in brown adipocyte tissue, requires fatty acid to induce uncoupling, and is up-regulated by cold, stress, and leptin. UCP2 is expressed in most tissues, and UCP3 is expressed predominantly in skeletal muscle. Both proteins are about 300–amino acid monomers and require fatty acid for their activity. In rats, fasting increased the expression of UCP3 and, to a lesser degree, of UCP2.[76] Refeeding rats with an isocaloric low-fat diet decreased the expression of both proteins.[77] This effect, however, was not seen with a high-fat diet. In humans, polymorphisms of the three genes have been reported, and the effects of these polymorphisms on body mass index, fat oxidation, and energy expenditure have been reported for UCP2 and UCP3.[74,75]

Other Mitochondrial Functions

In addition to its role in the oxidation of carbohydrates and fatty acids, mitochondria contribute to gluconeogenesis and protein metabolism by the interconversion of aspartate and oxaloacetate by the aspartate aminotransferase and of alanine and pyruvate by the activity of alanine aminotransferases. Also, in mitochondria, pyruvate carboxylase may convert excessive pyruvate to oxaloacetate. Oxaloacetate may be decarboxylated, yielding phosphoenoylpyruvate, a glucose precursor in a reaction catalyzed by phosphoenoylpyruvate carboxykinase. Mitochondria intervene in lipogenesis by providing acetyl CoA for the synthesis of fatty acid and cholesterol. One of the ways in which mitochondria participate in protein metabolism is via the urea cycle, in which excess nitrogen is converted into excretable urea (see Chapter 55, "Genetic and Metabolic Disorders").[78] In the mitochondria, carbamoyl phosphate synthetase forms carbamoyl phosphate from bicarbonate and from ammonia residues derived from amino acid catabolism. In another reaction, carbamoyl phosphate condenses with ornithine to form citrulline by ornithine transcarbamoylase, another mitochondrial matrix enzyme. Citrulline is then transferred to the cytosol, where, in the presence of aspartate, it forms arginine. The latter, after hydrolysis in the liver, yields urea and ornithine.

Heme is an important component of proteins such as hemoglobin and cytochromes such as cytochrome P-450, the prosthetic group of the electron transport chain and of several enzymes, such as catalase and peroxidase.[79] Mitochondria participate in the synthesis of heme initially by synthesizing 5-aminolevulinic acid (ALA) from glycine and succinyl CoA by the mitochondrial enzyme ALA synthase. ALA is transferred to the cytosol, where, by a series of enzymatic steps, it is converted into porphobilinogen and coproporphyrinogen. The latter returns to the mitochondria, where heme is synthesized by a series of reactions involving the coproporphyrinogen oxidase, yielding protoporphyrinogen IX, which is oxidized by protoporphyrinogen oxidase to protophorphyrin. The final reaction mediated by ferrochelatase introduces ferrous iron into the protophorphyrin ring, synthesizing heme.

Production of ROS

Mitochondria use about 90% of the total oxygen consumption by the cell.[80] As described above, the oxygen is reduced to form water in the electron transport chain. It has been calculated, however, that an electron leak of 0.25 to 2% occurs during mitochondrial respiration.[80,81] ROS are by-products of metabolized oxygen in the mitochondria.[3,82,83] The reduction of oxygen to water involves the transfer of four electrons to the molecule of oxygen. Molecular oxygen has two unpaired electrons with a similar spin in different orbitals. Because this electron configuration hampers the insertion of new electrons, oxygen reduction to form water proceeds by the sequential addition of one electron at a time. In the first reaction, oxygen may form superoxide O_2^- by accepting one electron from ubisemiquinone ($CoQH^\bullet$) produced during the electron transport:

$$CoQH^\bullet + O_2 \rightarrow O_2^- + CoQ$$

Most superoxide is produced in complexes I and III.[82] Recent evidence also suggests a role for complex II in the production of ROS.[83,84] Although the majority of oxygen reduction occurs in complex IV (COX), the generation of superoxide is minimal in this reaction, probably because of the high affinity of the molecule for partially reduced oxygen intermediates. Superoxide does not diffuse through the cell and may react with iron sulfur groups present in NADH-ubiquinone oxidoreductase, in succinate dehydrogenase, and as other enzymes such as aconitase, releasing ferrous ions that deactivate the enzymes.[85] Superoxide is rapidly reduced to form hydrogen peroxide:

$$O_2^- + e^- + 2H^+ \rightarrow H_2O_2$$

This reaction is catalyzed by several dismutases,[86] including manganese superoxide dismutase (SOD2) in the mitochondria or in the cytosol by copper zinc superoxide dismutase (SOD1). Another copper zinc superoxide dismutase (SOD3) is present extracellularly.

Recently, the importance of dismutases in avoiding oxidative damage has been demonstrated in knockout animals for these enzymes.[87–93] Mice defective in SOD3 were healthy until 14 months but, when submitted to oxidative stress, had severe pulmonary edema and short survival.[87] Animals with SOD1 deficiency were apparently normal but had significant motor loss after axonal injury.[88] Knockout mice for SOD2 presented with dilated cardiomyopathy, metabolic acidosis, liver steatosis, and fat deposition in muscle and died within the first 10 days. They also had a significant reduction in the activity of complexes I and II; inactivation of aconitase, especially in the heart; organic-aciduria; and DNA damage.[89,90] In a different strain of SOD2 knockout mice, the animals had severe anemia, progressive weakness, and motor abnormalities.[91] Neuronal degeneration in the brain was also observed, and marked mitochondrial injury was seen in neurons and cardiac myocytes. Compared with normal mice, liver mitochondria of animals heterozygous for SOD2 deficiency (SOD +/–) had a 30% reduction in glutathione levels. Oxidative damage of mtDNA but not of nDNA was reported. There was oxidative damage of mitochondrial proteins, including complex I and aconitase, along with an increase in the permeability of the mitochondrial membrane.[92] More recently, abnormalities in mitochondrial function characterized by respiratory inhibition, oxidative damage, proton leak, and enhanced permeability of the mitochondrial membrane were seen in SOD2-deficient animals. The changes were more significant and appeared earlier in SOD2 (–/–) than in SOD2 (+/–) mice.[93]

Hydrogen peroxide does not possess free electrons for oxidation but diffuses freely and may react with ferric or cupric ions, forming a hydroxyl radical, $HO^{\bullet}$. This reaction may be increased by Ca^{2+}, probably by mobilization of intracellular pools of iron.[83] The hydroxyl radical has a short life, causing peroxidation of proteins, lipid, and DNA. Hydrogen peroxide generated by dismutases is metabolized by mitochondrial and cytosolic glutathione peroxidase (GSHPx) in the presence of reduced glutathione to form water and oxidized glutathione.[94] Mutant mice lacking GSHPx produced fourfold more hydrogen peroxide and were 20% leaner than the wild type, probably owing to chronic oxidative stress.[95] Liver mitochondria from the knockout animals also had a lower mitochondria respiratory control ratio. In addition to GSHPx, other enzymes involved in the clearance of peroxide include peroxisomal and mithochondrial catalases[96] and thioredoxin peroxidases.[97] Recently, it was reported that mitochondrial isocitrate dehydrogenase may also be an important enzymatic defense against ROS by supplying the reduced nicotinamide adenine dinucleotide phosphate necessary to regenerate reduced glutathione in mitochondria.[98]

Another potential source for ROS is NO, a highly diffusible signaling molecule involved in many biologic processes. NO is more soluble in membranes than oxygen[99] and binds reversibly to the ferrous heme group in proteins.[100] NO is synthesized from arginine by two constitutional (one neuronal and another endothelial) and one inducible NO synthase.[101] Recently, the existence of a mitochondrial NO synthase has been reported.[102] NO may form NO_2 and NO_3 in the presence of oxygen and peroxynitrite ($ONOO^-$) by reacting with superoxide.[103,104] These NO derivatives or reactive nitrogen species (RNS) may oxidize or nitrate other molecules. Peroxynitrite may nitrite tyrosines and oxidize tryptophan, damaging a variety of enzymes, including aconitase, creatine kinase, ATP synthase, and SODs, as well as producing lipid peroxidation. In addition, NO and RNS may directly affect OXPHOS by inhibiting complexes I,[105] II,[106] and IV.[107] The inhibition of the activity of complex IV is due to reversible competition of NO for the binding site of O_2 located in the Cu_B-cytochrome a_3 center. It seems that in the presence of NO, the initial inhibition occurs at the level of complex IV, but with prolonged exposure, inactivation of complex I owing to *S*-nitrosylation of the protein occurs.[105] These NO effects on mitochondrial respiration may result in enhanced production of ROS, collapse of $\Delta\Psi$, and inhibition of ATP synthesis, leading the cell to necrosis or apoptosis.[108–110]

The generation of ROS depends on the redox status of the mitochondria. The production of ROS decreases during stage 3 of mitochondrial respiration, when the electron chain transport is more oxidized,[111] and is enhanced when the electron transfer decreases, and the system is reduced (stage 4 of respiration).[112] It seems that these different effects relate to the magnitude of $\Delta\Psi$, defining a threshold for ROS production. The importance of cell energy balance in the control of ROS production is illustrated by studies performed in knockout mice for the adenine translocator ANT-1.[113,114] In these animals, the defect has expressed phenotypically with cardiac hypertrophy, exercise intolerance, lacticacidemia, and the presence of ragged red fibers. The muscle showed ragged red fibers, proliferation of mitochondria, and a severe defect in coupled respiration.[113] Mitochondria from muscle, heart, and brain (tissues expressing the ANT-1 isoform) produced large amounts of hydrogen peroxide. Of interest, the increase in ROS production was associated with enhanced production of SOD2 in muscle tissue and muscle mitochondria. The heart, however, had a minimal compensatory increase in SOD2. A moderate increase in GSHPx was seen in both muscle and heart. Noteworthy, mtDNA rearrangements were more significant in the heart than in muscle, likely owing to the lesser ability of the heart to scavenge ROS.[114]

The production of ROS also increases in the presence of mitochondrial calcium loading, hyperoxia, and the use of specific inhibitors of the respiratory chain, such as actinomycin A, and is decreased by uncouplers of the mitochondrial respiration.[83] Recently, a role for UCPs in mitochondrial ROS production has been suggested.[115] In one study, in vitro inhibition of UCP2 induced hydrogen peroxide production.[116] Further support for a role of UCP2 in ROS production came from studies on the UCP2 (–/–) mice. In these animals, no abnormalities in terms of obe-

sity or thermoregulation were seen, but they were resistant to *Toxoplasma gondii* infection owing to increased cell ROS production.[117] Also, in UCP3 knockout mice, no defects in thermogenesis or obesity were observed, but superoxide anion production in the skeletal muscle was present.[118]

The ability of the mitochondria and the cell to limit the deleterious effect of RNS and ROS depends on the balance between the production of these radicals, the enzymatic capability of the mitochondria, and the capacity of the cell to neutralize them, as well as on the presence of lipid-soluble antioxidants such as α-tocopherol, glutathione, and $CoQH_2$.[119] ROS have been long considered to be toxic by-products of oxygen metabolism with only deleterious effects on cells. However, there is growing evidence that both ROS and RNS are participants in multiple physiologic responses. For instance, as described before, NO may regulate mitochondrial respiration by competing reversibly with O_2 for binding to COX.[71,72] In addition, as reviewed, both ROS and RNS are involved in a variety of signaling pathways regulating the expression of numerous genes and serving as messengers for several growth factors.[120,121]

Role of Mitochondria in Apoptosis

Life depends on maintaining the homeostasis of the different biologic processes in the presence of variable conditions in the environment. The integrity of the organism relies on adequate mechanisms of cell replication, differentiation, and proliferation. As part of these regulated processes, the cell possesses an intrinsic, programmed mechanism for cell death, or apoptosis, which removes damaged, aged, or excessive cells. Apoptosis was described in 1972 by Kerr and colleagues[122] and was differentiated from necrosis based on morphologic changes observed in the cell. In necrosis, swelling of the cell and organelles is followed by disintegration (karyorrhexis) and dissolution of the nucleus (karyolysis), as well as rupture of organelles and cell membrane. In contrast, apoptotic changes typically include condensation of the cell and nuclei with aggregation of chromatin, DNA fragmentation, and membrane blebbing. Abnormalities in the regulation of programmed cell death characterized by enhancement of apoptotic mechanisms may be associated with neurodegerative diseases and reperfusion injury, whereas down-regulation of apoptosis may be involved in autoimmunity and cancer.[123–125] Apoptosis is an intricate mechanism involving signals to initiate cell death, followed by the complex interaction of numerous proteins that may promote or inhibit the process. Among cell organelles, mitochondria play a central role in apoptosis.[123,126–130]

A cell may be induced to apoptosis by different normal or abnormal factors. Examples of such stimuli are the binding of specific ligands to receptors of the tumor necrosis factor (TNF) superfamily at the cell surface; the deprivation of growth factors, nutrients, or oxygen; the exposure to xenobiotics or biologic toxins; the presence of oxidative stress; signaling from second messengers (eg, calcium, ceramide); or cellular damage induced by radiation. Once the cell has been induced to apoptosis, an execution phase follows, activating a cascade of interactions between different proteins and cell products, committing the cell to death. The final phase is cell degradation by the action of several catabolic enzymes.

Proteins Involved in Apoptosis. Studies in *Caenorhabditis elegans* by Horvitz identified three different genes inducing cell death: *ced-3*, *ced-4*,and *eg1-1*.[131] Another gene, *ced-9*, was found to inhibit the process. Cd-4, an adaptor protein, likely activates *cd-3*. The activation of *cd-3* by *cd-4* may be inhibited by *cd-9,* and this inhibitory effect can be removed by *egl-1*. Homologous gene products have been identified in mammals, including caspases, proteins of the Bcl-2 family, and the apoptotic protease activating factor 1 (Apaf-1).

Caspases (cysteine aspartate–specific proteases) are a family of 14 proteins homologous to *ced-3*, classified into three different subgroups according to their substrate activity.[132] Caspases are proenzymes with three domains that can be catalytically activated, forming multimeric complexes. The substrate of their activity includes several proteins, including poly-ADP ribose, lamin, actin, DNA-dependent protein kinase, and other caspases themselves, as well as other regulators of apoptosis, such as Bcl-2 proteins. Caspases 2, 8, 9, and 10 participate in the induction and amplification of the cell death program, whereas caspases 3, 6, and 7 are the effector executioners. Depending on the signal and pathway of apoptosis, different caspases are activated (see below). Notwithstanding the important effects of caspases in apoptosis, in several experimental models, inhibition of caspases has not prevented cell death, suggesting that apoptosis may also occur by caspase-independent mechanisms.[133]

Caspases may be inhibited by several members (X-linked inhibitor of apoptosis [XIAP], cellular inhibitor of apopotis protein [c-IAP] 1, c-IAP2, and survivin) of the inhibitor of apoptosis proteins (IAPs) family, a group of proteins initially identified in baculovirus.[126,134] The functional unit of these proteins is an 80–amino acid baculovirus IAP repeat, and multiple repeats are present in most of them. The inhibition of caspase activity by these proteins may be suppressed by a mitochondrial protein called Smac (second mitochondria–derived activator of caspases) or Diablo (direct IAP binding protein with low pH), increasing the number of active caspases available for apoptosis.[135,136] The Smac protein is synthesized as a 239–amino acid precursor with an N-terminal signal of 55 amino acids that targets the protein to the mitochondria and is cleaved on entering the organelle. Another inhibitor of caspases is the Htr A2/Omi protein, a serine protease also imported into the mitochondria.[127]

The Bcl-2 family of proteins is a group of proteins homologous to the ced-9 protein in *C. elegans*.[137] These proteins contain the Bcl-2 homology (BH) domains 1, 2, 3, and 4. The family includes proapoptotic and antiapoptotic proteins. Most of the antiapoptotic proteins (Bcl-2, Bcl-X_L, Bcl-w, Mcl-1, A1, Bfl-1, Brag-1) contain the BH4 domain. The subgroup of proapoptotic proteins is subdivided in those containing the BH3 motif, being homologous with the egl-1 protein described in *C. elegans* (Bid, Bad, Bim, Noxa, Puma) and the Bax subfamily (Bax, Bak, Bok).

The mammalian protein homologous to *C. elegans ced-4* is Apaf-1.[138] Apaf-1 appears to bind to caspases via a caspase recruitment domain, which is also present in *ced-3* and *ced-4*.[139]

Pathways of Apoptosis. Two pathways for apoptosis have been defined. The first, or extrinsic, pathway is initiated by the binding of specific ligands to death receptors of the TNF receptor superfamily, including TNF-R1, Fas (CD95), tumor necrosis factor receptor apoptosis inducing ligand (TRAIL)-R1, TRAIL-R2, death receptor (DR)-3, and DR-6.[129,130,140] These receptor proteins have an extracellular cysteine-rich domain that binds specific ligands, as well as a cytoplasmic death domain capable of binding several adaptor proteins initiating the activation of caspase 8.[141] For example, binding of Fas ligand to the Fas receptor or TNF-α to the TNF-R1 receptor induces trimerization of the receptors and the recruitment of adaptor proteins, FADD (Fas-associated protein with death domain)[142] and TRADD (TNFR-1–associated death domain).[143] The association of TNF-R1/TRADD and FADD constitutes the so-called death-inducing activation complex that activates caspase 8. Activated caspase 8 may then trigger the cascade activation of effector or executioner downstream caspases 3, 6, and 7 with the subsequent cleavage of structural cytoskeleton proteins and the induction of nuclear degradation. In addition to this proapoptotic pathway, TRADD may also induce cell survival by recruiting receptor interacting protein and TRAF-2 (TNFR-associated factor 2), activating the signal transduction pathways for nuclear factor-κB (NF-κB) and JNK/c-Jun. NF-κB is a transcription regulator of several cell survival genes, including the inducible NO synthase,[144] members of the Bcl-2 family (Bcl-2 and Bcl-X_L),[145] c-IAP,[146] and SOD2.[147] Another regulatory mechanism of protection against apoptosis via the extrinsic pathway is provided by FLIPs (FADD-like interleukin-1β converting enzyme such as protease [FLICE/caspase 8]), which binds to the receptor-FADD complex, inhibiting caspase 8 activation.[148]

A second, or intrinsic, pathway for apoptosis is mediated by mitochondria (Figure 7-5).[123,126–128] As described above, a wide range of stimuli, other than the specific ligands that trigger the extrinsic pathway, may induce this intrinsic mechanism of apoptosis. Central events in this pathway are the increase in mitochondrial membrane permeability (MMP), the loss of $\Delta\Psi$, and the release of several mitochondrial proteins from the intermembranous space, including cytochrome-*c*, followed by caspase activation. The increase in MMP, also called mitochondrial permeability transition, appears to be regulated by the PTPC.[149] The nature of the complex is not completely understood but is believed to be composed of several proteins from both mitochondrial membranes and from the intermembrane and matrix spaces. The PTPC includes a cytosolic hexoquinase; two proteins of the outer membrane, the peripheral benzodiazepine receptor and the VDAC or porin; an intermembrane protein, creatine kinase; the ANT localized in the inner membrane; and cyclophilin D, located in the matrix. The pore may assume an open or closed configuration depending on several physiologic or pathologic signals. For example, MMP is induced by increased levels of calcium in the matrix, oxidative stress, fatty acid, bilirubin and bile acids, bacterial and viral proteins, β-amyloid, and respiratory chain inhibitors.[128] In contrast, several other factors may be inhibitors of MMP. For example, divalent cations (other than calcium) such as Mg^{2+} and Sr^{2+} are inhibitory, probably by competing with calcium for binding sites to the complex.[129] Also, an acidic pH in the matrix inhibits the pore activity, as well as adenine nucleotides (ADP, ATP, adenosine monophosphate).[149] The activation of MMP decreases $\Delta\Psi$, uncouples the respiratory chain, increases the formation of ROS, and results in breakdown of energy metabolism.[123,149]

As already stated, proteins of the Bcl-2 family are important regulators of MMP and apoptosis. Antiapoptotic members of the family possessing a BH4 domain (Bcl-2 and Bcl-X_L) are present in the outer mitochondrial membrane and apparently associate with the PTPC,[123,150,151] although the details of that interaction are not clear. Both Bcl-2 and Bcl-X_L prevent the drop in $\Delta\Psi$ induced by different apoptotic stimuli.[123,152] It has been suggested that the antiapoptotic effect of these proteins could be due to modulation of mitochondrial calcium traffic,[153] by regulation of the cellular redox potential,[154] or by interference with the pore-forming ability of other proapoptotic membranes of the family, such as Bax and Bak.[155] The mechanisms of interaction between the pro- and the antiapoptotic molecules of the Bcl-2 family are not completely understood.

There is controversy about the mechanisms involved in the release of mitochondrial soluble intermembrane proteins such as cytochrome-*c* into the cytosol.[123,156] In one model, on receiving an apoptotic signal, proapoptotic proteins of the BH3 family (eg, Bim, Bid, or Bid truncated by caspase 8) interact with Bax or Bak, changing their conformation.[157] The change in conformation of Bax or Bak promotes the formation of oligomers that form pores in the outer membrane, allowing the leakage of proteins from the intermembrane space. In this model, the antiapoptotic proteins Bcl-2 and Bcl-X_L interfere with the conformational change of Bax and Bad, preventing apoptosis. The second model proposes that MMP induced by a variety of stimuli causes massive swelling of the mitochondria, leading to the rupture of the outer membrane.[123,158,159] According to the model, proapoptotic Bcl-2 proteins activate MMP by changing the permeability of VDAC or ANT, affecting the flux of ADP or ATP, water, and ions between the cytosol and the matrix. Antiapoptotic proteins could then block the activation of MMP by mechanisms that have not been elucidated.

The disruption of the outer membrane permeability induces the release of mitochondrial intermembrane proteins, including cytochrome-*c*, caspase inhibitors (Smac/Diablo and Htr A2/Omi), several procaspases (2, 3, 9), the apoptosis-inducing factor (AIF), and endonuclease G into the cytosol.[127] AIF is a protein imported into the mitochondria that has oxidoreductase activity similar to that of flavoproteins. It produces apoptotic changes, including disruption of MMP, condensation of chromatin, and DNA fragmentation.

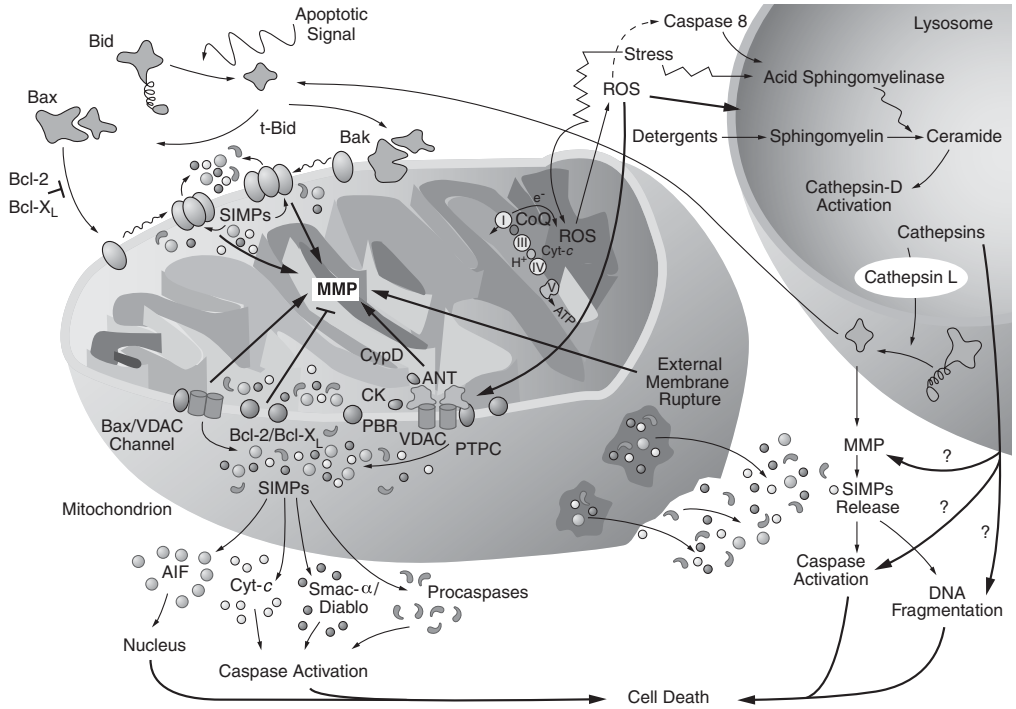

FIGURE 7-5 Organelle-specific permeabilization reaction in apoptosis. Mitochondria undergo membrane permeabilization (MMP) in response to molecules activating Bid by proteolytic cleavage (eg, caspase 8 and cathepsin L), stimulating the insertion or oligomerization of Bax or Bak in the outer membrane or agents acting on the permeability transition pore complex (PTPC). Membrane permeabilization then causes the release of soluble intermembrane proteins (SIMPs), including apoptosis-inducing factor (AIF), cytochrome-*c*, Smac/Diablo, and procaspases. Simultaneously, mitochondria generate reactive oxygen species (ROS) as a consequence of uncoupling of inhibition of the respiratory chain. In response to stress, lysosomes undergo membrane permeabilization and/or local activation and release of cathepsins. Cathepsins then can cause the proteolytic activation of Bid and might have direct effects on caspases and nuclear deoxyribonucleic acid (DNA). Antiapoptotic proteins Bcl-2 and BCL-X_L can inhibit the proapoptotic effect of Bax and Bak. ANT = adenine nucleotide translocator; CK = creatine kinase; CoQ = coenzyme Q; CYP D = cyclophilin D; Cyt-*c* = cytochrome-*c*; PBR = peripheral benzodiazepine receptor; t-Bid = truncated Bid; VDAC = voltage-dependent anion channel. Adapted with permission from Ferri KF and Kroemer G.[128]

Endonuclease G, a mitochondrial nuclease also imported into the mitochondria, translocates to the nucleus, where it produces DNA cleavage.

Once released in the cytosol, cytochrome-*c* binds to Apaf-1, inducing a conformational change that facilitates the binding of deoxyadenosine triphosphate (dATP) or ATP, the unmasking of caspase recruitment domains, and the oligomerization of the complex. This complex, known as the apoptosome, initiates the catalytic activation of procaspase 9.[160] On binding and activation of executer caspases 3 and 7 by caspase 9, downstream activation of caspases 2, 6, 8, and 10 proceeds. These activated caspases, acting on different substrates mentioned previously, initiate the damage of structural elements of the cell and the nucleus. The effects may be amplified by the proteolytic cleavage of apoptosis inhibitors such as XIAP, a member of the IAP family, or of ICAD, an inhibitor of caspase-activated deoxyribonuclease (CAD).[161]

Other organelles, besides mitochondria, also participate in apoptosis, not only as targets for the caspases but also as places where apoptotic signals may be originated.[128] For instance, nDNA damage or oncogene expression may result in activation of the tumor suppressor protein p53, which may activate proapoptotic proteins and suppress the expression of antiapoptotic members of the Bcl-2 family, as well as promote ROS production.[128,162] Under stressful signals, the endoplasmic reticulum (ER) retains unfolded proteins, activates several mediators, and releases calcium, which, in turn, can induce MMP. ER can also influence the traffic and activation of Bcl-2 proteins. For instance, antiapoptotic protein Bcl-2 targeted to the ER protected against mitochondrial apoptosis activation by interacting with an active form of Bad, a BH3 protein, preventing the activation of Bax, another proapoptotic protein.[163] Recently, data have been presented demonstrating that Bax and Bad, both proapoptotic proteins, as well as Bcl-2, localize in the ER, modulating the ER calcium concentration and influencing apoptotic mechanisms in the mitochondria.[164] There is evidence that cathepsins and probably other lysosomal products participate in the apoptotic process. Lysosomal extracts have the ability to cleave Bid, which, in turn, may induce cytochrome-*c* release in the mitochondria.[165] In addition, lysosomes may degrade damaged mitochondria by autophagy, a process in which the membrane of the ER

wraps defective mitochondria, forming a vacuole that fuses with lysosomes to form autophagosomes.

Mitochondria not only play a pivotal role in apoptosis but also probably define the ultimate outcome of the cell. Lemasters and colleagues have proposed a model in which the final fate of the cell depends on the number of mitochondria affected and the availability of energy in the cell. According to their model, when a few mitochondria undergo dysfunction, autophagy is stimulated. If the number of dysfunctional mitochondria increases, but there is still enough ATP available, the cell proceeds with apoptosis. When the mitochondrial failure is extensive, and the cell is unable to generate ATP, the hydrolysis of existent ATP is accelerated and the cell undergoes necrosis.[166]

MITOCHONDRIAL DYSFUNCTION

The metabolic machinery of the mitochondrion results from the coordinated synthesis and assembly of proteins encoded by both nuclear and mitochondrial genomes, and a variety of clinical conditions have been associated with abnormalities in mitochondrial genetics and function.[5–7] Mutations in mtDNA may be maternally inherited or occur sporadically during embryogenesis. Somatic mutations of mtDNA occur later in life and are likely due to exposure to ROS. An exemption to these mechanisms of mtDNA mutation was recently reported in a man who inherited a novel mtDNA mutation in the *ND2* gene from his father, probably owing to a defective mechanism in the exclusion of paternally derived mtDNA during early embryogenesis.[167] Although the majority of mitochondrial products are encoded by nDNA, only a minority of nDNA mutations have been identified. In contrast to mtDNA, which is maternally derived, nDNA mutations affecting mitochondrial function or the replication and integrity of mtDNA follow a mendelian pattern of inheritance (autosomal recessive, autosomal dominant, and X-linked).[5,7]

Compared with nDNA, mtDNA has a high mutation rate.[168] This difference has been attributed to an increased risk of mtDNA damage by ROS generated by the mitochondria[169] and by inefficient repair of mtDNA.[170] When a mtDNA mutation occurs, both mutated mtDNA and normal mtDNA are present in the same cell, a state known as heteroplasmy, whereas normal cells carry only normal mtDNA (homoplasmy).[5,171] Replication of mtDNA occurs independently of cellular division, and different templates of mtDNA may replicate several times during a cell cycle.[172] During cell division, mitochondria partition randomly among daughter cells, and the proportion of abnormal mtDNA may vary in different cells. Hence, during periods of high cell division such as embryogenesis, replicative segregation occurs. This segregation determines differences in the distribution and proportion of mutant mtDNA among cell lineages, producing homoplasmic and heteroplasmic cell populations with variable content of mutant mtDNA.

Depending on the proportion of abnormal mtDNA and the energy requirements of a particular tissue, functional abnormalities manifest after reaching a phenotypic threshold.[5,7,173] The phenotypic threshold may be evident not only depending on the ratio between mutant and normal mtDNA but also according to the ability of the cell and tissue to compensate for the defect at translational, transcriptional, enzymatic, and respiratory levels. Given different energy needs, the threshold varies among tissues, with the brain being the organ most susceptible to mitochondrial dysfunction, followed by the heart, skeletal muscle, endocrine system, kidney, and liver. The impaired mitochondrial function may result in decreased energy production, increased ROS production, and cellular damage. Depending on the magnitude of the defect, a decrease in functional cell mass within a specific organ may occur, presenting with variable degrees of dysfunction or failure.

Clinical Manifestations of Mitochondrial Diseases

Because the process of OXPHOS is vital in all cells and tissues, manifestations of abnormalities in mitochondrial function may be evident in one or several organs.[5,7,174] Given the high energy requirements of brain and muscle, mitochondrial diseases frequently present with neuromuscular abnormalities. Common symptoms are seizures, ataxia, delay or regression of developmental milestones, cortical blindness, muscular weakness, exercise intolerance, and hypotonia. Cardiac manifestations vary from conduction abnormalities to cardiomyopathy. Kidney involvement may present with renal tubular abnormalities or renal failure. Endocrine manifestations such as diabetes, thyroid or parathyroid diseases, adrenal insufficiency, and growth hormone deficiency can also be seen in patients afflicted with mitochondrial disorders. Deafness and retinopathy may be manifestations of the involvement of sensory organs. Defects in the hematopoietic system may result in sideroblastic anemia, neutropenia, and thrombocytopenia and, occasionally, myelodysplasia. Children affected with mitochondriopathies may have poor feeding and malnutrition as a consequence of the neuromuscular or cardiac manifestations of the disease. Other gastrointestinal manifestations include diarrhea, failure to thrive, intestinal dysmotility, pancreatic insufficiency, and liver abnormalities, including fulminant liver failure. Onset of symptoms may occur early or later in life, and multisystemic manifestations may present acutely or insidiously and progressively.

Patients with mitochondrial diseases may have abnormalities in biochemical tests, imaging studies, or tissue biopsy, particularly of muscle.[174,175] Commonly, but not always, patients with mitochondrial diseases have lactic acidosis with an increased lactate-to-pyruvate ratio (usually higher than 20). Accumulation of lactate in the brain may be found in the cerebrospinal fluid or may be demonstrated by proton magnetic spectroscopy. In patients with OXPHOS disorders, a ratio of 3-hydroxybutyrate to butyrate in plasma higher than 2 can be observed. Low levels of free carnitine with a relative increase in plasma acyl carnitines may also suggest a mitochondrial disorder. Analysis of organic acids in the urine may reveal increased amounts of 3-methylglutaconic acid and excretion of lactate and citric acid intermediates. Plasma amino acid analysis may demonstrate relatively high levels of alanine owing to transamina-

tion of accumulated pyruvate and low levels of citrulline. Muscle biopsies may show ragged red fibers in which there are irregular patches of abnormal mitochondria under the sarcolemma. The presence of ragged red fibers, however, is not pathognomonic, nor it is present in all types of mitochondrial diseases.[5] Electron microscopy of biopsies may demonstrate abnormalities in the morphology of mitochondria, including alterations of the cristae, formation of megamitochondria, and the presence of osmiophilic or paracrystalline inclusions (Figure 7-6). Biochemical analysis of the activity of the respiratory complexes in lymphocytes, fibroblasts, or tissue, as well as immunocytochemistry studies, may also give diagnostic clues in patients with clinical symptoms suggestive of mitochondrial disease. Specific molecular genetic studies available at specialized research centers may also be helpful in characterizing the mitochondrial abnormality if present.

Classification of Mitochondrial Diseases

Mitochondrial disorders have been classified based on biochemical and genetic abnormalities.[5,176,177] The following integrated classification proposed by DiMauro and colleagues describes the mitochondrial defects according to the abnormalities in mtDNA and nDNA, including the biochemical defects.[176] Table 7-1 illustrates the phenotype and the associated genetic defect of several mitochondrial diseases. The reader will notice that different genotypes may be responsible for similar phenotypes and that a given genotype may have diverse phenotypic expression.

Mitochondrial DNA Defects. mtDNA-related diseases may be due to (1) point mutations in mitochondrial tRNA or in rRNA genes affecting protein synthesis and in protein coding genes for different subunits of the respiratory chain and (2) rearrangements of mtDNA with deletion of one or more tRNAs, producing protein synthesis defects (see Figure 7-1). In the majority of cases, individuals with mtDNA disorders have mutations in tRNAs.

Point Mutations. More than 100 point mutations have been described. The majority of them occur in tRNA. Examples of these mutations include MELAS (mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes),[178] which is commonly associated with a tRNA leucine mutation (A3243G), and MERRF (myoclonic epilepsy and ragged red fibers), which is frequently related to tRNA lysine (A 8344G).[179] Several cases of isolated progressive external ophthalmoplegia, myopathy without ophthalmoplegia, and other disorders have also been described in patients with a variety of tRNA mutations.[176] Less common are rRNA mutations that have been described in subjects with deafness[180] or cardiomyopathy.[181]

Point mutations for coding genes of several subunits of the respiratory chain complexes have also been reported.[15,177] Point mutations in several subunits of complex I (ND1, -2, -4, -5, and -6) have been described in patients with Leber hereditary optic neuropathy,[7] MELAS,[182] and exercise intolerance.[183] Patients with exercise intolerance have been found to harbor mutations in the cytochrome-*b* of complex III.[184] Mutations encoding for subunit I of COX-I have been described in patients with sideroblastic anemia[185] and of subunit III (COX-III) in MELAS.[186] Recently, a heteroplasmic G9379A mutation creating a stop signal for the COX-III subunit was reported in a patient with lactic acidosis, exercise intolerance, and poor growth.[187] Mutations in subunit 6 of ATPase have been associated with NARP (neuropathy, ataxia, retinitis pigmentosa) and maternally inherited Leigh disease.[188–190]

***Rearrangements of mt*DNA.** A variety of multisystemic phenotypes are seen in cases of rearrangements of mtDNA, probably owing to differences among insertions and deletions and the variable proportion of abnormal mtDNA in different tissues.[191] Single deletions, including

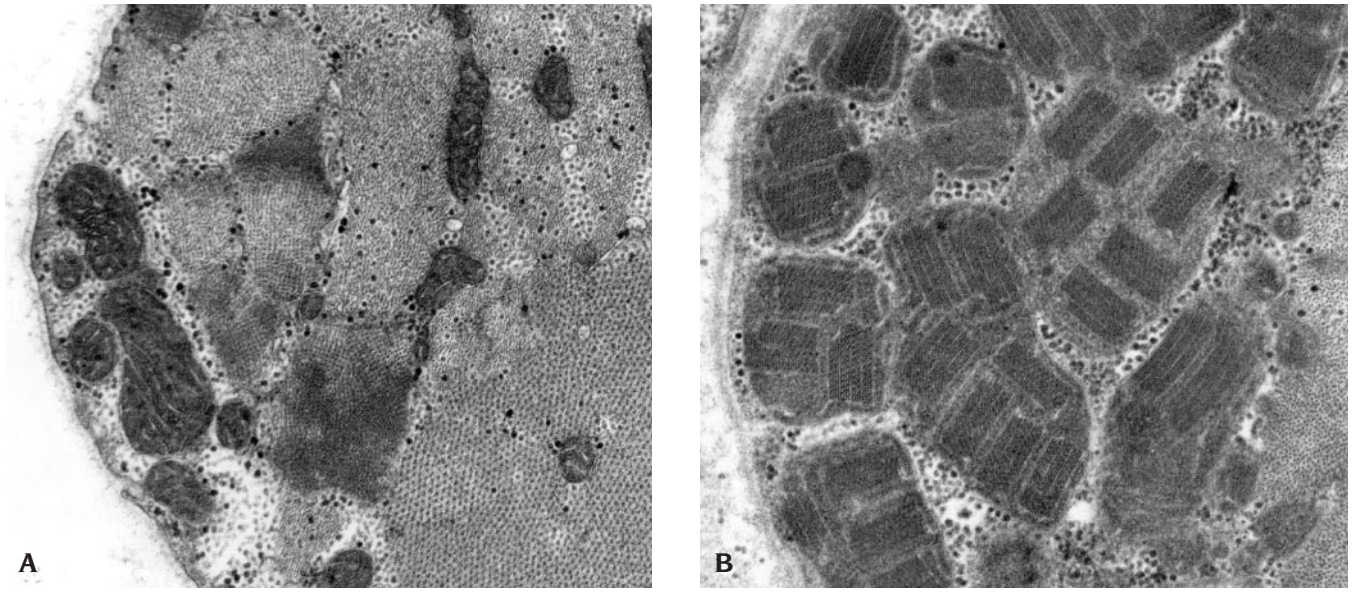

FIGURE 7-6 Muscle electron microscopy. *A* illustrates normal mitochondria. *B* shows proliferation of subsarcolemmal mitochondria containing crystalline inclusions (×40,000 original magnification). Courtesy of Drs. Eduardo Bonilla and Salvatore DiMauro, Columbia University, NY.

TABLE 7-1 PHENOTYPIC EXPRESSION AND ASSOCIATED GENETIC ABNORMALITIES IN SEVERAL MITOCHONDRIAL DISEASES

DEFECT	PHENOTYPE	GENETIC ABNORMALITY
MELAS	Strokes, seizures, lactic acidosis, headaches, ataxia, dementia, ophthalmoplegia, diabetes mellitus, hearing loss, limb weakness, exercise intolerance, basal ganglia calcifications, muscular ragged red fibers; onset frequently occurs before 15 years old	Point mutations in $tRNA^{leu\ (UUR)}$, $tRNA^{phe}$, $tRNA^{val}$, $tRNA^{cys}$, complex I (ND1, ND5), complex IV (COX-III)
MERRF	Myoclonus, ataxia, seizures, myopathy, multiple lipomas, hearing loss, retinopathy, ophthalmoparesis, short stature; onset in childhood or adulthood	Point mutations in $tRNA^{lys}$, $tRNA^{ser}$, $tRNA^{leu\ (UUR)}$; multiple deletions
Progressive external ophthalmoplegia	Ptosis, ophthalmoplegia, and limb weakness; onset in adolescence or adulthood	Point mutations in $tRNA^{leu\ (UUR)}$, $tRNA^{leu\ (UCN)}$, $tRNA^{ile}$, $tRNA^{asn}$, $tRNA^{tyr}$, ANT1; single deletions and/or duplications
AD-PEO	Hearing loss, tremor, ataxia, nystagmus, mental retardation; onset in childhood or adolescence	Multiple mtDNA deletions, mutations in *ANT1*, *POLG*, *Twinkle* genes
Diabetes (maternally inherited diabetes mellitus; diabetes and deafness)	Diabetes	Point mutations in $tRNA^{leu\ (UUR)}$, $tRNA^{lys}$
Cardiomyopathy	Hypertrophic or multisystemic cardiomyopathy	Point mutations in $tRNA^{ile}$, $tRNA^{lys}$, $tRNA^{leu}$, $tRNA^{gly}$, 12S rRNA,complex III (cyt-*b*)
	Dilated cardiomyopathy	Point mutations in 12S rRNA, 16S rRNA, $tRNA^{ala}$, $tRNA^{asp}$, $tRNA^{glu}$, $tRNA^{thr}$, complex I (ND2)
Myopathy	Myopathy	Point mutations in $tRNA^{leu\ (UUR)}$, $tRNA^{met}$, $tRNA^{trp}$, $tRNA^{leu\ (CUN)}$, $tRNA^{phe}$, complex III (cyt-*b*), $tRNA^{pro}$; multiple deletions
LHON	Progressive loss of vision; onset in second or third decade of life	Point mutations in complex I (subunits ND1, ND2, ND4, ND5, ND6), complex III (cyt-*b*), complex IV (COX-I, COX-III), complex V (ATPase 6)
NARP	Sensory neuropathy, neurotaxia, seizures, developmental delay, dementia, retinitis pigmentosa	Point mutation in ATPase 6
Kearns-Sayre syndrome	PEO, retinopathy, cardiac conduction block, ataxia, peripheral neuropathy, hearing loss, endocrinopathy	Multiple mtDNA deletions
Leigh disease	Infantile onset, ataxia, developmental regression, hypotonia, optic atrophy, nystagmus, occasional liver involvement and cardiomyopathy, bilateral degeneration of basal ganglia	Point mutations in PDH-E_1, NDUFS 7, NDUFS 8, *SURF1*, $tRNA^{trp}$, $tRNA^{val}$, ATPase 6, SDHA

Adapted from Sevidei S,[6] Rossignol R et al,[173] and Shon EA and DiMauro S.[177]
AD-PEO = autosomal dominant progressive external ophthalmoplegia; ANT = adenine nucleotide translocase; ATPase = adenosine triphosphatase; COX = cytochrome oxidase; cyt-*b*, cytochrome-*b*; LHON = Leber hereditary optic neuropathy; MELAS = mitochondrial encephalomyopathy with lactic acidosis and strokelike episodes; MERFF = myoclonus epilepsy with ragged red fibers; mtDNA = mitochondrial deoxyribonucleic acid; NARP = neuropathy, ataxia, retinitis, pigmentosa; NDUFS = reduced nicotinamide adenine dinucleotide–ubiquinone oxidoreductase Fe-S protein; PDH-E_1 = pyruvate dehydrogenase $E_{1\alpha}$; PEO = progressive external ophthalmoplegia; POLG = mitochondrial deoxyribonucleic acid polymerase gamma; rRNA = ribosomal ribonucleic acid; SDHA = succinate dehydrogenase 2 flavoprotein subunit; *SURF1* = surfeit-1 gene; tRNA = transfer ribonucleic acid.

multiple tRNA genes, are usually heteroplasmic and may occur spontaneously. These abnormalities have been described in patients with Kearns-Sayre and Pearson syndromes and progressive external ophthalmoplegia, as well as in children with non-neuromuscular multisystemic disorders.[192–195] About one-third of patients share the same "common deletion" between ATPase 8 and the *ND5* gene (see Figure 7-1).[177]

Nuclear DNA Defects. The vast majority of the more than 1,000 mitochondrial proteins are encoded by nDNA.[22] Mutations in nDNA may affect the transport and metabolism of substrates, OXPHOS, the import of mitochondrial proteins, the function of mitochondrial transporters, and the intergenomic communication between nDNA and mtDNA.[177,196]

Defects in Substrate Transport. Defects in substrate transport include deficiencies in the transport of fatty acid by CPTI, CPTII, and CACT, producing hypoketotic hypoglycemia and cardiomyopathy in infants.[197]

Defects in Substrate Metabolism. Defects in substrate use involve abnormalities in the metabolism of fatty acid and pyruvate. Deficiencies in several enzymes participating in fatty acid oxidation frequently manifest with hypoglycemia, hepatic dysfunction, including liver failure, and neuromuscular and cardiac abnormalities (see also Chapter 55).[36,197]

In the majority of cases, disorders in the function of the pyruvate dehydrogenase complex are due to X-linked point mutations or, less frequently, to insertions or deletions in the E1α subunit gene.[198, 199] Patients with these defects may present in the neonatal period or early in infancy with lactic acidosis, lethargy, hypotonia, and seizures. The course may be fatal or less severe, with mild lactic acidosis and ataxia or exercise intolerance.[5,199]

Defects in pyruvate carboxylase, the enzyme that transforms pyruvate into oxaloacetate, have variable presenta-

tion, from mild lactic acidosis and delayed development to a severe neonatal presentation with metabolic acidosis, hepatomegaly, hyperammonemia, and death. The deficiency is autosomal recessive, and two missense mutations in the gene have been reported.[200]

Krebs cycle disorders are rare.[201] Patients with α-ketoglutarate dehydrogenase deficiency present early in life with lactic acidosis, encephalopathy, and hypotonia. As of 2000, 13 cases of fumarase deficiency have been described in infants presenting with encephalopathy, hypotonia, seizures, brain magnetic resonance imaging abnormalities, dysmorphic features, and fumaric aciduria.[201,202] Aconitase deficiency has been described in patients with exercise intolerance and myoglobinuria who also had succinate dehydrogenase deficiency.[203]

Nuclear Gene Defects in OXPHOS. nDNA encodes for 73 of 82 structural subunits of the five complexes involved in OXPHOS and for the proteins assembling them. It has been estimated that nDNA defects could be responsible for 80 to 90% of the respiratory chain disorders.[196]

Of the 42 subunits of complex I, mutations in NADH-ubiquinone oxidoreductase flavoprotein 1 have been identified in patients with macrocephaly, leukodystrophy, and myoclonic epilepsy.[204] Mutations in the NADH-ubiquinone oxidoreductase Fe-S proteins 4, 7, and 8 have been seen in Leigh disease and Leigh disease–like syndromes.[205–207] Mutations in the four subunits of complex II have also been reported.[51] Of them, mutation in the succinate dehydrogenase 2 flavoprotein subunit was associated with Leigh disease[208] and optic atrophy with myopathy and ataxia.[209] Interestingly, mutations in units SDHB, SDHC, and SDHD have not manifested with neuromuscular or multisystemic syndromes but with the presence of hereditary paragangliomas, a group of slow-growing neuroectodermal tumors. No nDNA mutations in the structural genes of complex III, IV, or V have as yet been described.

Abnormalities in the function of OXPHOS complexes owing to defects in their assembly have been described. Missense mutations in BCS1L, a protein involved in the assembly of the Rieske Fe-S subunit of complex III, were found in children with encephalopathy, liver failure, and tubulopathy.[210] Mutations in the surfeit 1 gene (*SURF1*), a homologue of a yeast gene, *SHY1*, that contributes to the assembly of COX, were reported in patients with low COX activity and Leigh disease.[211,212] Subsequently, mutations in two mitochondrial copper chaperone genes involved in COX assembly, *SCO2* and *SCO1*, were also described. Defects in the *SCO2* gene have been seen in patients with cardiomyopathy and lactic acidosis[213,214] and of *SCO1* in neonates with liver failure and encephalopathy.[215] Another missense mutation in the *COX10* gene encoding for the heme A farnesyltransferase was found in a patient with proximal tubulopathy and leukodystrophy who also had reduced activity in the COX-II subunit.[216] Because differences exist in tissue expression of COX deficiency, it has been suggested that pathways of COX assembly and regulation may be tissue specific.[217]

***Defects in* Mitochondrial Protein Import, Translocases, and Transporters.** Mutations in TIMM8a, a gene homologue to yeast TIM8p protein of the mitochondrial protein import machinery, was reported in families with deafness-dystonia syndrome. [218] Methylmalonicacidemia, isovaleryl-acidemia, and gyrate atrophy result from mutations in the mitochondrial import leader sequences of methylmalonyl-CoA mutase,[219] isovaleryl-CoA dehydrogenase,[220] and ornithine aminotransferase,[221] respectively. Defects in the ornithine transcarbamoylase gene reduce mRNA levels of the enzyme, causing abnormal synthesis of urea and defective removal of endogenous ammonia.[222] Missense mutations affecting the heart and muscle isoforms of ANT1 have been described in patients with autosomal dominant progressive external ophthalmoplegia.[223,224] Interestingly, consistent with the possible role of ANT in apoptosis, patients with autosomal dominant progressive external ophthalmoplegia and ANT1 mutations have shown no evidence of apoptosis in muscle biopsies.[225]

More recently, mutations in *SLC25A13*, a gene encoding for citrin, an aspartate-glutamate transporter and component of the malate aspartate NADH shuttle, were found in patients with late-onset citrullinemia.[226] These patients present with hyperammonemia in the second decade of life or in adulthood, and the disorder is fatal. A neonatal presentation has recently been described in Japanese children with cholestasis.[227–229] Liver dysfunction is self-limited after a few months, but one case of liver failure necessitating liver transplant has been reported.[229]

Mutations in an ATP binding cassette transporter, the *ABC7* gene, were described in X-linked sideroblastic anemia.[230] Another disorder related to mitochondrial iron metabolism is Friedreich ataxia, which has been found to be due to mutations in the frataxine gene, an orthologue of the yeast *YFHi* gene.[231]

A model for abnormalities in mitochondrial fusion and fission processes necessary in mitochondrial biogenesis is dominant optic atrophy. The defect is caused by a mutation in the *OPA1* gene that encodes for a mitochondrial importing motif similar to some yeast dynamin–related guanosine triphosphatases (eg, mitochondrial genome maintenance proteins in *S. cerevisiae*).[232,233] An example of nuclear mutations related to processes of mitochondrial protein assembly and turnover is spastic paraplegia, which has been associated with mutations in the paraplegin gene, a homologue of yeast AFG3/RCA1 coding for metalloproteases of the Atpases associated with diverse cellular activities (AAA) family.[234]

***Defects in* Intergenomic Signaling/Coordination.** nDNA is responsible for most of the tasks required in the functional expression of mtDNA and its repair. Clinical disorders associated with abnormalities in these processes include a decrease in the number of copies of mtDNA (mtDNA depletion) or defects in the integrity of mtDNA (mtDNA deletions).[235]

The mtDNA depletion syndrome is the first autosomal recessive quantitative disorder of mtDNA described.[235–237] In this syndrome, the affected tissues are almost devoid of mtDNA. Characteristically, they have a significant decrease in the respiratory activity of the mtDNA-encoded complexes I, III, and IV, with normal activity of complex II, which is encoded exclusively by nDNA. Significant proliferation of

mitochondria and the presence of ragged red fibers may be seen in muscle biopsies. One mode of presentation is fatal myopathy with onset in the neonatal period (congenital myopathy) or around the first year of life (infantile myopathy). Another variant is seen in infants with liver failure occurring in the first weeks of life (see below). Mutations in the mitochondrial thymidine kinase (*TK2*) gene were recently reported in some cases of the myopathic form[238] and in the deoxyguanosine kinase (*dGK*) gene in some cases of hepatocerebral mtDNA depletion.[239–241] These findings suggest that imbalances in the cellular pool of deoxyribonucleotide could affect the integrity of mtDNA.

Another example of potential alteration in mitochondrial nucleotide pools affecting mtDNA maintenance includes mutations in the TP gene, found in patients with mitochondrial neurogastrointestinal encephalomyopathy (MNGIE), an autosomal recessive condition with multiple mtDNA deletions (see below).[242] Three different gene mutations associated with multiple mtDNA deletions have been reported in patients with autosomal dominant progressive external ophthalmoplegia. One occurs in the *ANT1* gene, as already described. Another localizes in the *C10orf2* gene encoding for a protein called Twinkle (because of star-like staining in mitochondria), with homology to a T7 bacteriophage helicase.[243,244] A third mutation occurs in the gene for mtDNA polymerase γ (*POLG*).[245,246]

Primary Mitochondriopathies of the Digestive System

Primary Mitochondrial Hepatopathies. Liver manifestations of mitochondrial diseases may be due to defects in OXPHOS, in fatty acid transport and oxidation, in the electron transfer flavoproteins, or in the metabolism of pyruvate.[247] This chapter focuses mainly on defects in OXPHOS associated with liver dysfunction. The reader is invited to review other possible metabolic causes of hepatopathy in Chapter 55.

Among inborn errors of metabolism, OXPHOS disorders are a relatively common cause of liver failure in infancy. In a study of 80 infants with liver failure, respiratory chain disorders accounted for 21% of cases, and inborn errors of metabolism were found in 42%.[248] In another series of 157 children with respiratory chain disorders, 20% developed liver failure.[249] Cormier-Daire and colleagues studied 22 infants with isolated respiratory chain defects and described two modes of presentation.[250] In the first group, which included 40% of patients, a severe neonatal form was found in children who presented with progressive liver failure, hypotonia, and myoclonic seizures in the first weeks of life, followed by death early in infancy. In the second group, initial symptoms of hepatomegaly and jaundice occurred between 2 and 18 months, followed by liver failure. About two-thirds of patients had neurologic impairment such as myoclonic seizures or psychomotor retardation, and 40% of them had a fatal course.

In several reports, children with OXPHOS-related liver failure had isolated deficiencies in complex I or IV,[248,250–252] but children with complex III deficiency presenting with neonatal onset of liver failure and encephalopathy have also been reported.[253] Selective defects of several complexes have also been observed.[248,253]

Patients with mtDNA depletion syndrome may have multiple deficiencies in the activity of mtDNA-encoded respiratory chain complexes I, III, and IV, with normal activity of complex II in different tissues.[236,237,254–257] Hepatic depletion of mtDNA can be observed in as many as 30% of these patients,[235] and symptoms of liver dysfunction, including liver failure, occur more frequently in the immediate neonatal period or during the first months of life.[241,255,257–260] The liver may be the only organ affected. Recently, 21 children with severe liver mtDNA depletion (70 to 80% decrease of mtDNA) were studied.[235] All patients had liver failure, 2 had cardiomyopathy, and 14 (67%) had neurologic symptoms, including myoclonus, hypotonia, developmental delay, and myopathy. The course was fatal in 70% of children in the first 2 years of life. In only three of these patients, mutations in the *dGK* gene were found[241]—hence the possibility of other gene defects causing hepatic mtDNA depletion. An abnormal mosaic pattern of expression of mtTFA and of mtSSB was seen in a child with liver failure and severe mtDNA depletion.[260] The significance of these abnormalities regarding mtDNA replication remains unclear and probably represents a secondary phenomenon.

Children with hepatic mitochondriopathies may present with feeding difficulties preceding other symptoms. They frequently have lactic acidosis with elevated lactate-to-pyruvate ratios, as well as hypoglycemia. Liver function tests most commonly show mild or moderate elevation of transaminases, hyperbilirubinemia, and coagulation abnormalities in cases of liver failure. Liver biopsy findings include cholestasis and steatosis, with variable degrees of fibrosis and iron load (Figure 7-7).[257,261] Electron microscopy reveals abnormalities in the distribution of mitochondria, with matrix changes and disarray of the cristae. The clinical course can be fatal or may stabilize. Occasionally, recovery from liver failure may happen, as reported in a child with cytochrome-*c* oxidase deficiency[252] and in another with mtDNA depletion.[258] Liver transplant is controversial and has been performed in patients with liver failure secondary to OXPHOS disorders.[262–264] Of 18 patients described in these publications, 10 patients died, and the remainder survived after a follow-up of 5 months to 8 years.

Delayed hepatic failure is characteristic of Alpers-Huttenlocher syndrome, also known as progressive infantile polydystrophy.[265,266] Alpers-Huttenlocher syndrome is a neurodegenerative process characterized by microcystic cerebral degeneration with gliosis, spongiosis, and neuronal loss. The onset of symptoms frequently occurs in the first 2 years of life. Common manifestations are feeding difficulties, hypotonia, developmental delay, and ataxia. Patients may subsequently develop seizures, followed by liver failure a few months later. Several of these patients have had liver failure shortly after the use of valproic acid (VPA) for treatment of seizures, and hepatotoxicity owing to the medication was initially considered to be the cause

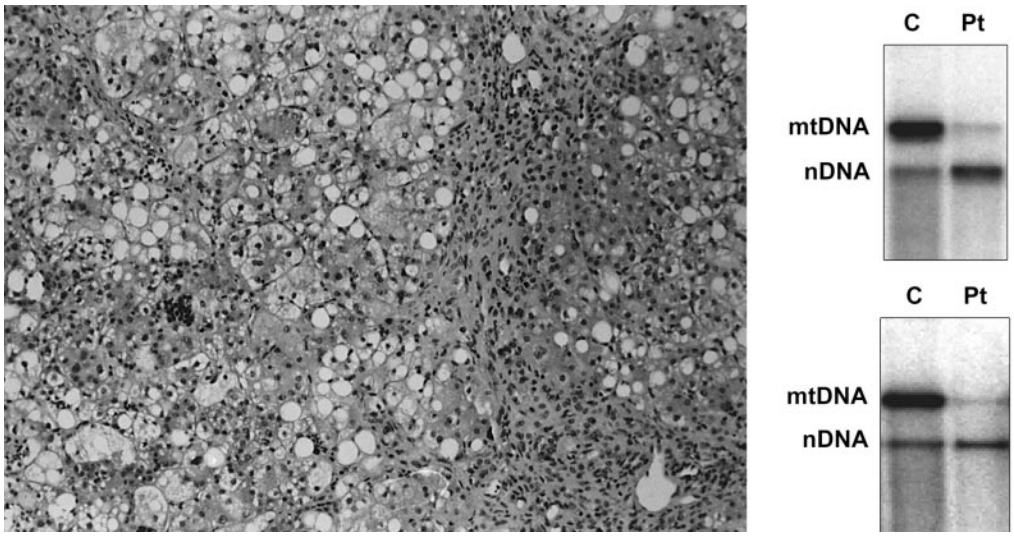

FIGURE 7-7 Liver histology and Southern blots of mitochondrial deoxyribonucleic acid (mtDNA) from an infant with mtDNA depletion. The left panel illustrates the liver biopsy showing steatosis, fibrosis, portal inflammation, and giant transformation of hepatocytes (hematoxylin and eosin stain; ×400 original magnification). Courtesy of Dr. Cyril D'Cruz, Newark Beth Israel Medical Center, NJ. The panels on the right illustrate the Southern blots of mtDNA and nuclear deoxyribonucleic acid (nDNA) from the liver (*top*) and muscle (*bottom*) from a control subject (C) and from the patient (Pt) with mtDNA depletion. Courtesy of Drs. Tuan H. Vu and Salvatore DiMauro, Columbia University, NY.

of the hepatic dysfunction.[267–270] In most cases, death occurs before the fourth year. Patients who underwent liver transplant succumbed to neurologic complications within the first year after transplant.[268,270] Although the cause of the condition has not been established, several mitochondrial defects have been described, including defects in complexes I[271,272] and IV,[273, 274] mtDNA depletion owing to deficiency in mtDNA polymerase,[275] and alterations in pyruvate metabolism and the citric cycle,[271,276] as well as the presence of muscular ragged fibers with cytochrome-*c* oxidase–negative fibers.[277]

Navajo neurohepatopathy is a candidate primary mitochondrial hepatopathy. The condition has been described in Navajo infants and children with sensory neuromotor neuropathy, corneal ulcerations, acral mutilations, failure to thrive, and hepatopathy.[278] Liver disease may be evident during the first months of life or later and progress to liver failure.[279] Histology of the liver shows cholestasis, steatosis, portal fibrosis, cirrhosis, and giant cell transformation. Electron microscopy has demonstrated pleomorphism of the mitochondria with abnormalities in the cristae. A preliminary report suggests that the syndrome might result from a deficiency in the multidrug resistance 3 gene (*MDR3*), based on a significant reduction of *MDR3* mRNA found in the liver of several children affected by the condition.[280] However, no *MDR3* mRNA mutations have been identified to date, and the correlation of the proposed defect with the neuropathy has not been established.[281] Evidence linking Navajo neurohepatopathy to mitochondrial depletion has been recently reported by Vu and colleagues in two infants who developed liver failure in the first 6 months of life.[282] In these infants, compared with controls, the activity of COX was decreased in liver, and one of them also had less activity of complexes II and III. Quantitative Southern blot showed about 85% depletion of mtDNA in the liver. No mutations were found in mtDNA or in the mtTFA gene.

Pearson Syndrome. Pearson syndrome was first described in children with sideroblastic anemia, thrombocytopenia, vacuolization of erythroid and myeloid precursors in the bone marrow, and pancreatic insufficiency.[283] The majority of patients present during the first year of life with hematologic abnormalities,[283–285] although a case without hematologic involvement has been reported.[286] Patients may have gastrointestinal symptoms, including vomiting, diarrhea, and failure to thrive, without evidence of pancreatic insufficiency.[285,287] Others develop renal tubulopathy,[285,288] hepatic dysfunction, including liver failure,[285, 289] neuromuscular symptoms,[285,290] diabetes,[291,292] or heart failure.[293] Frequently, affected patients have lactic-acidemia with high lactate-to-pyruvate ratios, and the analysis of organic acids in urine may demonstrate 3-methylglutaconicaciduria.[294] Pearson syndrome is caused by sporadic mtDNA rearrangements, including deletions and duplications.[285,295] The disorder has also been described in children of mothers with similar deletions.[296,297] The majority of patients have a 4,977 bp deletion in mtDNA, spanning a region between the sequences encoding for the ND5 subunit of complex I and the ATPase 8 subunit (see Figure 7-1). This deletion also occurs in patients with Kearns-Sayre syndrome,[298] a multisystemic disorder presenting with progressive external ophthalmoplegia, retinopathy, and cardiac, renal, and neuromuscular abnormalities. Patients suffering from Pearson syndrome frequently die in the first year of life. Survivors of Pearson syndrome can develop Kearns-Sayre syndrome.[285,299] Some patients improve hematologic abnormalities, presumably

by segregation of normal mtDNA in the hematogenic precursors during cell division.

Mitochondrial Neurogastrointestinal Encephalomyelopathy

This syndrome is characterized by the association of several gastrointestinal, neurologic, and muscular symptoms. Patients with MNGIE have multiple mtDNA deletions and depletion in muscle, associated with various mutations in the TP gene.[242,300] The enzyme catalyzes the phosphorolysis of thymidine to thymine. It is believed that the defect can produce an imbalance in the nucleotide pool available for mtDNA synthesis.[300] As reviewed by Nishino and colleagues[300] and Teitelbaum and colleagues,[301] onset of symptoms most commonly occurs late in the second decade of life. Initial gastrointestinal symptoms are cachexia, vomiting, diarrhea, and abdominal pain secondary to gastroparesis and partial obstruction. Small bowel diverticula may be found in as many as two-thirds of affected subjects. Sensory and motor symptoms owing to peripheral neuropathy are present and are usually mild. Ocular findings, including ptosis and ophthalmoplegia, have been reported in the majority of cases. Hearing loss can also be part of the syndrome in about half of cases. Patients may have lactic acidosis, and muscle biopsy shows ragged fibers, with COX-deficient fibers in most subjects. Other pathologic findings include atrophy of the intestinal muscularis propia with the presence of abnormal mitochondria in ganglion and smooth muscle cells, as well as microvesicular steatosis in liver, skeletal and intestinal muscle, and Schwann cells.[302] The activity of TP as measured in peripheral leukocytes by the conversion of thymidine to thymine is almost undetectable. Brain magnetic resonance imaging (MRI) shows leukoencephalopathy in all patients, and electromyography consistently demonstrates neurogenic changes in all patients. Forty percent of patients may also have myopathic findings.[300] Death commonly occurs in the fourth decade.

Other Mitochondrial Enteropathies. There are several reports of patients in whom clinical symptoms are similar to MNGIE but in whom no evidence of leukoencephalopathy or mutations in the TP gene have been found.[300,303] Mitochondrial enteropathies other than MNGIE, manifesting in infancy and childhood, have been reported. Cormier-Daire and colleagues described two children who presented with severe vomiting, diarrhea, and malnutrition at 6 and 15 months, respectively.[304] During the course of their disease, they were found to have partial villous atrophy and lactic acidosis while receiving a high-carbohydrate diet. Liver and renal abnormalities were present without evidence of pancreatic insufficiency or hematologic disorders. Temporary improvement was noted, and they were able to receive continuous enteral nutrition. Both patients subsequently developed cerebellar ataxia and sensorineural deafness. One of them had retinitis pigmentosa and proximal muscle weakness and the other had diabetes and renal failure. Both children died at 12 years of age. mtDNA deletions (3,380 bp and 4,191 bp) encoding for several tRNAs and subunits of complex I were found.

Verma and colleagues reported a child who, at 3 years of age, had persistent intermittent abdominal pain, episodic vomiting, and diarrhea, resulting in growth failure.[305] He was found to have a borderline gastric emptying time. Gastric and intestinal biopsies were normal. At age 7 years, he developed seizures and, years later, lactic acidosis, cerebellar ataxia, sensorineural deafness, peripheral neuropathy, and retinitis pigmentosa. He did not have ophthalmoplegia or limb myopathy. MRI demonstrated abnormalities in the white matter, thalamus, and basal ganglia. He died when he was 15 years old. A novel mitochondrial tRNA lysine (G8313A) mutation was found in muscle and fibroblasts. The authors suggest that intestinal dysmotility in patients with mithochondrial disease can be secondary to abnormalities in the intestinal muscle or in the neuronal network.

More recently, Chitkara and colleagues reported six children who presented with poor suck, food refusal, vomiting, and diarrhea or constipation in the first days of life.[306] Owing to feeding difficulties, gastric tube feedings were necessary, and in three children, additional parenteral nutrition was required. Neurologic symptoms began after the second year of life and included seizures, muscular hypotonia or hypertonia, and cortical blindness. Brain MRI was normal in four children. Mild dilatation of ventricles and brain atrophy were seen in one patient, and periventricular leukomalacia was reported in another. All patients had abnormal antroduodenal manometry. Several abnormalities in OXPHOS were reported in all patients, but no evidence of mtDNA mutations was seen in multiple mtDNA genes screened. At the time of the publication, patients had been followed for several years, with no fatalities reported.

Secondary Dysfunction of Mitochondria

Abnormalities in mitochondrial structure and function have been observed in a variety of clinical conditions related to apoptosis, to increased production of ROS, or to direct mitochondrial damage produced by different toxins and drugs.

Cholestasis. Patients with liver disease frequently present with cholestasis, which can further compromise liver function by affecting mitochondria. In animal models, bile duct ligation produced swelling of the mitochondria, shortening of the cristae, and changes in the shape of the organelle.[307] Several factors may be responsible for these bile acid–induced abnormalities. Bile acids may derange mitochondrial respiration and electron transfer,[308,309] increase production of ROS,[310,311] and produce cellular ATP depletion.[312] Bile acids may also activate the Fas receptor–mediated pathway of apoptosis.[313,314] The final outcome of these abnormalities may be changes in MMP with release of cytochrome-*c* and initiation of apoptosis.[315,316] Interestingly, ursodeoxycholate, a hydrophilic bile salt, confers cytoprotection against apoptosis induced by other bile salts.[315,316] Noteworthy, in rat neuron and astrocyte cultures, ursodeoxycholate and tauroursodeoxycholate were protective against the apoptotic effects of unconjugated bilirubin

and β-amyloid peptide, two neurotoxins implicated in kernicterus and Alzheimer disease, respectively.[317]

Infections. Several bacterial and viral products may interact with mitochondria, affecting their function.[318,319] For instance, *Neisseria* species possess porin proteins in the outer membrane that have structural similarities to VDAC and localize in the host mitochondria, affecting apoptosis. *Neisseria gonorrhea* has a porin B protein (PorBg) that induces MMP and apoptosis.[320] Interestingly, porin B of *Neisseria meningiditis* (PorBm), which has a minor structural difference with PorBg, has the opposite effect, inhibiting MMP and apoptosis.[321] The N-terminal of the vacuolating cytotoxin A from *Helicobacter pylori* also interacts with VDAC, causing apoptosis.[322] Similarly, *Clostridium difficile* toxin A localizes in host cell mitochondria, causing apoptosis and generation of ROS.[323]

Sepsis is the systemic reaction of the host to infection and can progress to septic shock and multiorgan failure. Evidence in animals and humans has shown mitochondrial respiration abnormalities during sepsis.[324–326] In a recent study, compared with controls, septic patients had decreased OXPHOS, and decreased ATP production, depletion of glutathione, and overproduction of NO. Of clinical relevance, the magnitude of the changes seen in these patients related to their survival.[324]

Several viruses, including Epstein-Barr virus,[327,328] Kaposi sarcoma–associated herpesvirus 8,[329,330] and adenovirus,[331] produce apoptosis inhibitor homologues to Bcl-2 proteins. Others, such as cytomegalovirus, encode an apoptosis inhibitor, viral mitochondrial inhibitor of apoptosis, which interacts with the ANT.[332] Virus can also produce proapoptotic mediators. For example, viral protein R of human immunodeficiency virus (HIV) interacts with ANT, promoting apoptosis and caspase activation.[333,334] Another HIV protein, the transactivating protein (Tat),[335] induced caspase activation, mitochondrial calcium uptake, ROS accumulation, and mitochondrial membrane depolarization. Interestingly, Tat may induce calcium-dependent ion secretion in Caco cells and human colonic mucosa, suggesting a role for this protein in HIV-1 enterophathy.[336]

Factors producing cellular damage in viral hepatitis are complex. There is evidence that in chronic hepatitis B and C, apoptosis may result from activation of Fas-mediated mechanisms.[337–339] In addition, the X gene product of the hepatitis B virus (HBV-X) interacts with mitochondrial VDAC,[340,341] decreasing $\Delta\Psi$,[340] inducing oxidative stress and activating transcription factors NF-κB and signal transducer and activator of transcription-3.[342] There is also evidence of increased oxidation in patients with hepatitis C.[343,344] Experimental cell overexpression of the hepatitis C core protein demonstrated association of the protein with mitochondria, increased ROS production, and release of cytochrome-*c*.[345]

Drugs. The liver plays a central role in the metabolism of potential toxins, including drugs, which can affect mitochondria by several mechanisms.[346,347] For example, amiodarone, an antiarrhythmic medication, may produce liver dysfunction and affect mitochondria at different levels, including inhibition of the electron transport chain, uncoupling of OXPHOS, and inhibition of mitochondrial fatty acid metabolism.[348,349] Several enzymes of fatty acid β-oxidation can be inhibited by tetracyclines, glucocorticoids, nonsteroidal anti-inflammatory medications such as ibuprofen, and several tricyclic antidepressants.[347] Buprenorphine, an analgesic used as a substitute drug in heroine addicts, when used intravenously, produces severe hepatitis secondary to inhibition of β-oxidation, uncoupling and inhibition of mitochondrial respiration, and collapse of $\Delta\Psi$.[350]

VPA is an 8-carbon branched carboxylic acid widely used for the treatment of seizures. Two forms of hepatotoxicity associated with the use of VPA have been described.[351] One is dose dependent and is characterized by mild elevation of transaminases and a minimal, if any, increase in bilirubin. Patients have minimal symptoms. Abnormalities in liver tests and symptoms subside after adjusting the dose or discontinuing the medication. The second form of hepatotoxicity is idiosyncratic and dose independent. It typically presents during the first months of treatment and is heralded by nausea, vomiting, and anorexia, followed by lethargy and seizures. The elevation of hepatic enzymes may be significant but most commonly is mild or moderate and is accompanied by an abnormal coagulation profile indicative of liver failure. The greatest risk occurs in children younger than 2 years, in those receiving multiple medications, or in patients affected by neurodevelopmental delay or metabolic disorders.[352–354] Underlying mitochondrial disease appears also to be a risk factor for VPA-induced liver failure.[355,356] Although reversal of liver failure has been reported,[354,357] the clinical course is usually fatal. Except for one reported case,[358] liver transplant has been unsuccessful.[353,359] The toxicity of VPA has been attributed to abnormalities in the β-oxidation of fatty acid owing to formation of valproyl CoA decreasing the formation of acyl CoA or to the toxic effects of VPA metabolites.[351] In rat liver, VPA[360,361] and its metabolites[361] have been shown to decrease mitochondrial respiration. In a similar animal model, mitochondrial respiratory abnormalities were associated with a decrease in the content of cytochrome aa_3 in complex IV.[362] In vitro studies on rat liver mitochondria have demonstrated the ability of VPA to induce MMP without significant effects on mitochondrial $\Delta\Psi$.[363] The potential apoptotic effects of VPA have also been seen in human leukemic cells. In these cells, treatment with VPA produced apoptotic changes, including cytochrome-*c* release, caspase activation, and DNA fragmentation.[364] Liver and muscle steatosis associated with morphologic abnormalities in mitochondria have been reported as a toxic manifestation of VPA treatment.[365,366]

Epidemiologic evidence in children has correlated the use of aspirin during viral illness with the development of Reye syndrome.[367,368] The incidence of the syndrome has decreased dramatically after the decline in the use of aspirin in children.[369] Reye syndrome usually presents during a viral illness with vomiting and lethargy, followed by variable degrees of encephalopathy, brain edema, and hepatopathy.[370] The encephalopathy may be accompanied

by seizures, and the initial lethargy can progress to severe coma, with decortication and decerebrate posturing and, eventually, brainstem herniation. Hepatomegaly has been found in 50% of cases, and transaminases are elevated, but bilirubin levels are usually normal or minimally increased. Variable degrees of coagulopathy may be present. Analysis of serum fatty acid has demonstrated increased concentration of dicarboxylic acids, suggestive of a fatty acid oxidation defect, which correlates with the clinical status.[371] Other biochemical abnormalities include hypoglycemia, hyperammonemia, aminoaciduria, and lactic acidosis. Liver biopsies characteristically show microvesicular steatosis without necrosis. Steatosis may also be present in renal tubular cells, muscle, myocardium, lungs, and pancreas. Electron microscopy has shown swelling of mitochondria with cristae abnormalities.[372] It has been suggested that salicylates, in conjunction with abnormalities in cell calcium regulation produced by viral illness, might uncouple OXPHOS, inducing MMP and apoptosis.[373]

Nucleoside analogues such as zidovudine, stavudine, and zalcitabine used in antiretroviral treatment may interact with DNA polymerase γ, causing termination of mtDNA replication.[374–376] Significant structural and functional mitochondrial damage, including mtDNA depletion in patients treated with these medications, has been reported.[375,377–380] HIV-infected patients developing mitochondriopathy after the use of these medications have had lactic acidosis, as well as neuromuscular, hematologic, and hepatic symptoms. Fialuridine, another antiviral agent and an inhibitor of mtDNA replication,[381] was tried in patients with chronic hepatitis B but was withdrawn from the market because it caused hepatopathy and lactic acidosis in several patients.[375] Of interest, interferon-α used to treat chronic hepatitis B and C affected mtDNA transcription and reduced mitochondrial mRNA in vitro.[382,383] A recent publication suggests that down-regulation of mRNA in cells treated with interferon could be due in part to mRNA degradation by a mitochondrial ribonuclease.[384] In both, intact cells and isolated mitochondria interferon treatment decreased electron transport.[385]

Alcohol Liver Disease. The pathophysiology of alcohol liver disease is multifactorial and complex.[386] Mitochondria are affected early in the course of the disease and can be the source or the target of ROS.[387,388] Mitochondrial abnormalities, characterized by swelling of the organelle with disorganization of the cristae and formation of megamitochondria, have been correlated with alcohol consumption.[389,390] Alcohol impairs OXPHOS, producing a decline in active respiration of the mitochondria (stage 3).[391,392] Chronic ethanol ingestion can decrease the synthesis of the 13 OXPHOS peptides encoded by mtDNA[393] and reduce the number of mitochondrial ribosomes.[394] The most significant quantitative effect occurs in the activity of COX, which can be reduced by at least 50%.[395,396] Also, the activity of ATPase synthase is impaired[397] as a result of defective synthesis of subunits 6 and 8 of the enzyme.[398] Lesser impairment in the activity of cytochrome-*b* and of the iron sulfur center of complex I has also been reported.[395,399] Isolated fetal hepatic mitochondria exposed to ethanol showed a decrease in the activity of complexes I and IV accompanied by lowered ATP synthesis.[400]

Alcohol may lower the cellular threshold for oxidative damage owing to alterations in the membrane fluidity, decreasing the import of glutathione from the cytosol into the mitochondria.[401,402] In addition, alcohol enhances susceptibility to oxidative damage by reducing the activity of GSHPx.[403] Acute administration of ethanol enhances production of ROS, lowers $\Delta\Psi$, increases MMP, and causes apoptosis.[404] As expected, ethanol-induced ROS damage proteins and DNA and produce lipid peroxidation.[405] Marked loss of hepatic mtDNA in rats after an acute dose of ethanol was reported, suggesting that ethanol-induced ROS affect the integrity of mtDNA.[406] This is of relevance because mtDNA deletions have been observed in alcoholics.[407]

Nonalcoholic Fatty Liver Disease. Nonalcoholic fatty liver disease is the most common hepatic disease in America and is characterized by fatty infiltration of the liver and hepatomegaly.[408] The condition is frequently seen in patients with obesity, non–insulin-dependent diabetes, and hypertriglyceridemia.[409,410] Fat liver accumulation may cause cell necrosis and inflammation with elevation of transaminases, an association known as nonalcoholic steatohepatitis (NASH). The condition may progress to fibrosis and cirrhosis.[411,412] Recently, the role of mitochondria in NASH has been examined. Ultrastructural abnormalities of mitochondria have been described in NASH, including swelling, the appearance of multilamellar membranes and crystalline inclusions, loss of cristae, and formation of megamitochondria.[412,413] A decrease in the synthesis of ATP has been found in patients with NASH,[414] and preliminary reports have described lower activity of the respiratory chain,[415] as well as abnormalities in mtDNA.[416] Similar to alcohol-induced steatohepatitis, in NASH, enhanced production of ROS and lipid peroxidation appear to be central in inducing cellular damage and cytokine production by hepatocytes and Kupffer cells.[409] Recently Sanyal and colleagues showed that compared with individuals with nonalcoholic fatty liver disease, subjects with NASH had higher fasting levels of insulin and free fatty acids, as well as enhanced hepatic fatty oxidation.[413] The authors suggest that patients with NASH may constitute a different subpopulation of individuals who may carry a silent mitochondriopathy, which is then unmasked when patients develop insulin resistance.

Wilson Disease and α1-Antitrypsin Deficiency. Mitochondrial abnormalities have been described in patients with Wilson disease and α_1-antitrypsin (α_1-AT) deficiency. Wilson disease is characterized by cirrhosis and degeneration of the basal ganglia owing to abnormalities in copper metabolism produced by mutations in a copper-transporting ATPase (ATP7B).[417] The ATP7B product is a copper-transporting P-type ATPase present in the cell in two forms: one isoform of 160 kD[418,419] localizes in the *trans*-Golgi, whereas another of 140 kD apparently localizes in the mitochondria.[418] The molecule is necessary for the incorpora-

tion of copper into ceruloplasmin and the excretion of copper into the bile.[420] Polymorphic changes of mitochondria, accompanied by crystalline inclusions, abnormalities in the cristae, and the presence of vacuoles in the matrix, have been seen in liver biopsies from patients with Wilson disease.[421] Using an animal model, Sokol and colleagues demonstrated that copper load decreases COX activity, enhances ROS production, and produces oxidation of protein and lipids.[422] Similar findings were reported in patients with Wilson disease.[423] Deletion of mtDNA secondary to oxidative stress was reported in 50% of Wilson disease patients younger than 30 years of age.[424] A more recent study described significant decrease in the activities of aconitase and in the activity of respiratory complexes I, II, III, and IV in three Wilson disease patients, supporting the notion that abnormalities in mitochondrial OXPHOS may contribute to the pathogenesis of the disease.[425]

α_1-AT deficiency is the most common genetic liver disorder in children.[426] The condition is due to abnormalities in the PiM allele for the gene. Individuals with cirrhosis secondary to α_1-AT deficiency have a gene base substitution (342 lysine), and the mutated allele is known as PiZZ. The mutated gene product has impaired secretion and is retained in the ER, triggering an autophagic response. Recent studies in patients with α_1-AT deficiency showed marked autophagia of the mitochondria in the liver of α_1-AT patients.[427] Noteworthy, many mitochondria not associated with the phagosome vacuoles showed variable degrees of damage, which included condensation of the cristae and matrix, formation of multilamellae, and loss of matrix structures. Similar mitochondrial changes associated with activation of caspase 3 were seen in the liver of PiZ transgenic mice, a model for α_1-AT deficiency. Of interest, these abnormalities improved in animals receiving cyclosporine, an inhibitor of MMP. The cause and possible consequences of these mitochondrial abnormalities in α_1-AT deficiency await further investigation.

Ischemia-Reperfusion. Ischemia and hypoxemia occur in a variety of clinical circumstances and affect the metabolism, energy production, and function of tissues and organs. The re-establishment of appropriate blood flow with adequate supply of nutritional substrates and oxygen paradoxically may further impair cell and organ function. Several pathophysiologic events seen in ischemia-reperfusion injury, such as increased ROS production, mitochondrial dysfunction, failure in energy production, induction of inflammatory response, and production of cytokines acting in concert, may lead the cells to apoptosis or necrosis.[428–430] Given the different metabolic rates of specific tissues, the ability to recover depends on the magnitude of the ischemic-hypoxia insult and the intrinsic adaptive mechanisms of the tissue. Because mitochondria are central in the consumption of oxygen and production of energy, the important role of mitochondria in the pathophysiology of ischemia-reperfusion is not surprising. For example, in the liver, morphologic alterations of mitochondria have been observed early under hypoxemic conditions, which are reversible after reoxygenation.[428] Hypoxia and ischemic injury affect OXPHOS and the production of ATP. In vitro studies on mitochondria have shown that after reoxygenation, mitochondria uncoupled respiration and decreased phosphorylation activity.[431] In liver cells, without suitable energy substrates, respiratory inhibition produced by ischemia-reperfusion increased ATPase activity, leading to cellular ATP depletion.[429] Mitochondria can also be the source of ROS under hyperoxia or hypoxia-hyperoxia, and there is experimental evidence of ROS production during ischemia-reperfusion[432,433] Therefore, ROS generation may contribute to cellular and tissue damage occurring in ischemia-reperfusion. Alterations in oxygen and nutrient cell supply during ischemia, along with the above-mentioned mitochondrial abnormalities, are triggering signals for apoptosis.[434–437]

Aging, Cancer, and Degenerative Disorders. Aging is a complex biologic process involving genetic and environmental factors resulting in functional decline of cells and organs. Aging is frequently associated with the development of degenerative disorders and increased incidence of malignancy. Since Harman proposed that the life span of an organism relates to the rate of oxygen use and accumulation of oxidative damage in the mitochondria,[438] the relationship between aging and mitochondrial function has been the focus of extensive research.[439,440]

The mitochondrial free radical theory of aging considers that aging is accompanied by a progressive dysfunction of OXPHOS and energy production, proton leakage, and enhanced production of free radicals, causing further mitochondrial and cellular damage.[441] Miguel suggested that during aging, oxidative damage of mtDNA in postmitotic cells could produce defects in replication and mutation of mtDNA, resulting in lower cell energy production.[442] According to this model, a vicious circle is established in which the abnormalities in mtDNA amplify the process by further affecting OXPHOS and ATP synthesis, increasing the production of ROS. The role of primary mtDNA mutations in aging, however, has not been completely established.[443]

Mitochondrial dysfunction and oxidative stress have also been considered to have a common link with cancer[7,444,445] and degenerative diseases.[446,447] Early in the past century, Warburg and later Szent-Gyorgyi proposed that abnormalities in mitochondria could be related to malignant transformation.[446] Mitochondria could play a role in oncogenesis by different mechanisms. For example, it is hypothesized that in addition to inducing mtDNA damage, oxidative stress could damage nDNA, activating oncogenes or deleting tumor suppressor genes, resulting in activation of mitogenic pathways. An altered cellular redox state and enhanced ROS can affect cell differentiation and transformation via the regulation of expression of numerous genes, of which the MAP kinase and the NF-κB signal transduction pathways have been more investigated.[448] Another potential role for mitochondria in oncogenesis could be through its role in apoptosis because abnormalities in apoptosis have been associated with cancer.[125] Other possible roles for mitochondria in the development of malignancy have been considered, including incorporation of fragments of mtDNA into nDNA, the transmission of onco-

genic viral DNA, the mitochondrial activation of chemical carcinogens, and the regulation of calcium homeostasis.[444]

Several mitochondrial genetic abnormalities have been identified in various types of cancer, as recently reviewed.[449,450] Somatic mtDNA changes include point mutations, insertions, transitions, and deletions. In several tumors (eg, breast, colon, hepatic, gastric), mtDNA mutations frequently occur in the D-loop region. Abnormalities in mitochondria have also been described in Alzheimer[451] and Parkinson disease, the two most common neurodegenerative disorders.[452]

SUMMARY

The presence of metabolic functions is central to life. The most important aspect of metabolism is the generation of energy, without which no life phenomena can be sustained.

From a thermodynamic perspective, living organisms may be considered as open, nonequilibrium systems exchanging matter and energy with their environment. Mitochondria in synthesizing ATP are pivotal in this process by harvesting the energy contained in electrons present in $NADH^+$ and $FADH_2$ produced during the oxidation of fat and carbohydrates. Mitochondria and specifically the electron transport chain may be considered the interface at which the transformation of energy contained in substrates from endogenous or exogenous sources is made available for the biologic functions of aerobic organisms. Conversely, impairments in the generation of ATP by the mitochondria may lead to cellular depletion of energy, failure of vital systems, and, ultimately, death.

Living organisms maintain their integrity by adapting to changes in the inner and outer environments. The presence of an atmosphere rich in oxygen determined adaptation in the evolution of life, providing aerobic organisms with the potential to couple the generation of energy with the reduction of oxygen in the mitochondria. In these reactions, however, potentially cell-damaging ROS may be produced, and mechanisms to neutralize them may emerge. Cell differentiation, development, and growth are constitutive processes in the life of multicellular organisms. As part of these processes, metazoans developed mechanisms of cell death, eliminating damaged, abnormal, or excessive cells to maintain tissue homeostasis. As described in this chapter, mitochondria play a central role in apoptosis, a cell death process involving the complex, concerted action of several proteins, resulting in disruption and collapse of energy production. Mitochondria are therefore at the crossroad of life and death and, not surprisingly, mitochondrial dysfunction and failure frequently associated with ROS production may be the ultimate consequence of diverse pathologic states.

Advances in the understanding of the biology and genetics of mitochondria have permitted the recognition of diseases owing to abnormalities in mitochondrial function. Primary mitochondrial diseases can occur as a consequence of abnormalities in mtDNA or in nDNA affecting mitochondrial proteins or the integrity of mtDNA. These disorders are frequently, but not always, multisystemic and involve organs and tissues of high energy demand, such as brain, heart, and muscle, although other systems may also be compromised. Manifestations in the digestive system include liver dysfunction and, frequently, liver failure, but pancreatic insufficiency, intestinal dysmotility, and failure to thrive may also occur. Secondary dysfunction of mitochondria may accompany a broad group of conditions, including aging, cancer, neurodegenerative diseases, ischemia-reperfusion injury, certain infections, and exposure to endogenous or exogenous toxic substances. Clinicians should consider the possibility of a mitochondrial disorder in the differential diagnosis of patients affected with multisystemic and progressive disorders, without forgetting that mitochondrial diseases may also manifest in one system.

A better understanding of the synthesis, transport, assembly, and function of known mitochondrial proteins, as well as the perspective of new discoveries in mitochondrial biology and function, will certainly have an impact on the diagnosis and the treatment of mitochondrial disorders.

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CHAPTER 8

GASTROINTESTINAL INJURY

1. *Drug-Induced Bowel Injury*

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Pharmacotherapy is often associated with adverse effects in the gastrointestinal tract. Oral administration of drugs exposes gastrointestinal mucosa to relatively high concentrations of the drug. Even parenteral use of drugs may cause adverse reactions, which are specific to the gastrointestinal system.

The classification system of adverse drug reactions (ADRs) proposed by Patterson and colleagues categorizes them as predictable or unpredictable, providing a practical framework to deal with the phenomenon that often leads to significant morbidity and mortality.[1] Predictable ADRs are further divided into side effects (commonly seen unwanted effects stemming from the drug's pharmacology and mechanisms of action), secondary effects (relatively rare side effects), drug interactions, and toxicity (a consequence of overdose). Unpredictable ADRs include intolerance (exaggerated effects at usual drug doses), idiosyncratic effects (severe unwanted effects that may not be fully explained by the drug's mechanisms of action), and allergic reactions mediated by immunologic mechanisms.

ADRs that target the gastrointestinal tract are numerous, ranging from nausea and vomiting without significant pathology to severe colitis. This chapter describes drug-induced bowel injury defined as gastrointestinal ADRs with pathologic changes. Although nausea and vomiting without gastrointestinal pathology are commonly encountered, they are not discussed. Similarly, overdoses and poisonings with corrosive nonmedicinal agents are beyond the scope of the chapter. The ADRs are discussed in a section of each major target anatomic site, but it is important to note that multiple sites may be involved. Because the field of pediatrics encompasses premature infants to young adults, the chapter covers both pediatric-specific ADRs and those commonly reported in adults.

ESOPHAGUS

Pill Esophagitis

Tablets and capsules are common drug formulations for older children, adolescents, and adults. These drug formulations are designed for optimal dissolution in an appropriate gastrointestinal environment for absorption. As a conduit for ingested substances, the esophagus is not directly involved in drug absorption. However, a swallowed tablet or capsule may lodge itself in the esophagus, releasing its contents onto the esophageal mucosa. If a pill is dissolved in such a confined small area, concentrations of active ingredients in the local milieu may become extremely high. If exposed to the drug at a high concentration for a prolonged period of time, the esophageal mucosa may be damaged. Hence, lodged and immobilized pills become a cause of esophageal mucosal injury. This condition is called *pill esophagitis* or *pill-induced esophageal injury*.[2,3] Although the risks of developing pill esophagitis may be small, it is estimated that 10,000 cases occur per year in the United States.[2]

Case reports indicate that patients' ages vary, ranging from 3 to 98 years.[2] Surprisingly, associations with specific underlying conditions, including esophageal disease, are not known, and the majority of pill esophagitis cases are reported in patients with no apparent predisposing conditions.[2] However, swallowing without water, supine positioning, and certain surface characteristics of pills (eg, larger pills, gelatinous capsules, certain sustained-release formulations) are considered risk factors for developing the condition.[4,5] Despite the lack of apparent association, patients with preexisting esophageal functional and/or anatomic pathology that delays esophageal emptying should be considered at risk until proven otherwise.

Drugs reported to cause pill esophagitis are numerous (Table 8.1-1),[2,3] but the most frequently quoted medications include antibiotics, potassium chloride, nonsteroidal anti-inflammatory drugs (NSAIDs), and quinidine, comprising nearly 90% of all reported cases.[2] However, the drug-specific risks of developing the condition are unknown. Despite the recent introduction into clinical practice, case reports of esophageal injury by bisphosphonates such as alendronate and pamidronate are relatively common and severe in nature.[2] The exact mechanisms of caustic effects probably differ among these offending drugs and are not fully understood.

The symptoms include acute-onset retrosternal pain that is continuous or worsened by swallowing (ie, odynophagia).

TABLE 8.1-1 DRUGS FREQUENTLY IMPLICATED FOR ESOPHAGEAL MUCOSAL INJURY

ANTIMICROBIALS
Doxycycline
Tetracyclines
Oxytetracycline
Minocycline
Penicillin
Ampicillin
Zidovudine
NSAIDS AND SALICYLATES
Naproxen
Aspirin
Ibuprofen
Indomethacin
Piroxicam
OTHERS
Bisphosphonates (alendronate, pamidronate)
Potassium chloride
Ferrous sulfate/succinate
Quinidine
Theophylline
Corticosteroids

Adapted from Kikendall JW.[2]
NSAID = nonsteroidal anti-inflammatory drug.

Dysphagia may also be associated. A long-standing injury may lead to a stricture. Esophageal hemorrhage (especially in those receiving aspirin and NSAIDs), perforation, penetration, strictures, and mediastinitis have been reported in those receiving drugs such as NSAIDs,[6,7] potassium chloride,[8] alendronate,[9] sustained-release ferrous sulfate, and sustained-release valproate.[10] Endoscopic examinations usually confirm the diagnosis. The symptoms usually subside in a few days to weeks on discontinuation of the drug therapy, although cases with complications may require intensive therapy, including total parenteral nutrition and surgery.

ORAL AND ESOPHAGEAL MUCOSITIS INDUCED BY CANCER CHEMOTHERAPY

Anticancer drugs affect tissue turnover and remodeling maintained by rapidly proliferating epithelial cells in the gastrointestinal tract. Consequently, ulceration and inflammation may occur following systemic administration of cancer chemotherapy agents throughout the gastrointestinal system, including oral cavity mucosa, the esophagus, and other parts of the gastrointestinal tract. Usually, oral lesions accompany the esophageal changes. Methotrexate, vinca alkaloids (such as vincristine and vinblastine), dactinomycin, doxorubicin, bleomycin, cytosine arabinoside, and 5-fluorouracil are often implicated as causative agents.[11] Treatment is supportive, and uncomplicated lesions usually heal in 2 weeks. A well-known example of effective prevention of toxicities caused by high-dose methotrexate regimens is folinic acid rescue (leucovorin).

STOMACH AND DUODENUM

NSAID-INDUCED GASTRODUODENAL ULCER

NSAIDs (in this chapter, NSAIDs indicate nonselective cyclooxygenase [COX]-1 inhibitors and do not include selective COX-2 inhibitors, unless otherwise stated), including aspirin, are by far the most common causes of drug-induced mucosal injury of the stomach and duodenum in children and adults.[12] The mechanisms are multifactorial but most likely involve inhibition of COX in the mucosa of the gastrointestinal tract, thereby reducing mucosa-protective prostaglandins.[12–14] This pathologic process develops not only through topical mucosal contact with the offending agents but, more importantly, via the systemic effects of the drugs.[12–16]

Overall, about 15 to 30% of patients receiving NSAIDs on a regular basis have either dyspeptic symptoms without overt ulcer, endoscopically proven mucosal damages without symptoms, or both, each comprising roughly one-third of the cases.[12] Risk factors for developing NSAID-induced gastroduodenal ulcers include advanced age, past history of ulcer, use of concurrent corticosteroids, higher NSAID doses, multiple NSAID use, anticoagulant use, and serious systemic disorder.[12] In adults, the risk of NSAID-induced upper gastrointestinal mucosal damage is highest within the first month of its use, although the risk seems to accumulate during the first 6 months.[17]

In a large prospective study of more than 8,000 adult patients with rheumatoid arthritis, the rate of serious gastrointestinal complications, such as perforation, gastric outlet obstruction, and bleeding, was found to be about 0.8% per 6 months,[17] approaching an annual incidence of 2%. In patients without risk factors such as old age (75 years or older) and previous peptic ulcer and bleeding, they reported that the logistic regression model predicted a risk for developing gastrointestinal complications such as bleeding and endoscopically proven mucosal lesions of 0.4%.[17] The risk in pediatric patients is probably close to this estimate, although there are no explicit data. In a retrospective study of 702 pediatric patients with juvenile rheumatoid arthritis, 5 children had a total of 10 events of symptomatic "gastropathy," defined as esophagitis, gastritis, or peptic ulcer disease, which were associated with NSAIDs, including tolmetin (seven episodes), diclofenac, aspirin, and indomethacin.[18] In premature infants receiving corticosteroids or NSAIDs for lung maturation or closure of patent ductus arteriosus, severe gastrointestinal complications such as perforation have been well recognized and are discussed in this chapter (see dexamethasone-induced gastrointestinal perforation and NSAID-induced intestinal injury in the section on the small intestine and colon).

Although there is an apparent rank order of ulcer bleeding and perforation risks of NSAIDs (eg, low risk: ibuprofen and diclofenac; medium risk: naproxen, indomethacin, and piroxicam; high risk: ketoprofen and azapropazone),[19] caution should be exercised in interpreting it because the data are not available for risks standardized by equivalent doses. Given the dose-response relationship between NSAID use and the risk of gastrointestinal complications, this aspect cannot be ignored.

Children with the acute phase of Kawasaki disease are treated with immunoglobulin and high-dose aspirin, followed by low-dose aspirin therapy during the convalescent phase. Surprisingly, there has been no systematic study on

aspirin-induced gastrointestinal damages in children with Kawasaki disease. A 1996 report described two children with overt gastrointestinal hemorrhage during aspirin therapy for Kawasaki disease in the convalescent phase.[20]

A new subclass of NSAIDs (eg, celecoxib and rofecoxib) has recently been introduced, which consists of relatively selective inhibitors for COX-2, one of the two isoforms of the COX enzyme. They are called coxibs, COX-2 inhibitors, or COX-1–sparing NSAIDs. The gastrointestinal tract constitutively expresses COX-1, which plays a key role in protecting and maintaining the integrity of the gastrointestinal mucosa by producing mucosa-protective prostaglandins.[16] Therefore, by sparing COX-1, these COX-2 inhibitors appear to be beneficial in minimizing the risk of the mucosal damages.

Clinical trials in adults suggest that the COX-2 inhibitors are better tolerated than nonselective NSAIDs such as ibuprofen, diclofenac, or naproxen.[21–25] In patients receiving no aspirin, the annualized occurrence rate of gastroduodenal ulcers and their complications was 0.44% for celecoxib and 1.27% for the nonselective NSAIDs.[21] Similarly, in adults with rheumatoid arthritis, rofecoxib was associated with fewer gastrointestinal ulcers and complications (2.1% per year) than naproxen (4.5% per year); the difference was most pronounced in the frequency of gastric ulcer.[22] Together with other studies,[23–25] these data suggest an improved adverse-effect profile of the COX-2 inhibitors, especially for complicated ulcer. However, the difference may not be so distinct for alleviating dyspeptic symptoms. Although more data are clearly needed, COX-2–selective inhibitors seem a reasonable alternative for nonselective NSAIDs in adult patients with risk factors such as a history of gastroduodenal ulcers.[26] However, given the relatively low risk of gastroduodenal ulcers and complications in children and young, otherwise healthy adults, the cost-effectiveness of the relatively costly COX-2–selective inhibitors over cheaper nonselective NSAIDs with or without antiulcerogenic therapy in pediatric populations remains to be clearly demonstrated.

Pharmacologic approaches to counteract ulcerogenic effects of NSAIDs include the use of misoprostol,[17,27–29] proton pump inhibitors,[30–32] histamine$_2$ (H_2) receptor antagonists,[33–38] and nitric oxide.[39,40] Misoprostol, a prostaglandin analogue, is expected to replace cytoprotective prostaglandins depleted by NSAIDs. Although misoprostol 100 to 200 μg four times a day in adults was shown to prevent NSAID-induced gastroduodenal ulcers, dyspeptic symptoms were not improved.[28] H_2 receptor antagonists such as ranitidine were shown to be effective in reducing dyspeptic symptoms and preventing duodenal ulcers in patients receiving NSAIDs,[33–37] although they may mask ulcer-associated symptoms.[12,38] Proton pump inhibitors such as omeprazole, pantoprazole, and lansoprazole have recently been advocated as an effective modality for NSAID-induced peptic ulcer diseases. In adult patients receiving NSAIDs regularly, omeprazole is more effective than ranitidine in healing and preventing gastroduodenal erosions and ulcers.[30] Omeprazole is also as effective as misoprostol but more tolerable owing to a lack of diarrhea,[31] a major side effect of misoprostol. In comparing proton pump inhibitors and H_2 receptor antagonists, a prevailing view[41] is that the level of acid suppression, rather than a drug class–specific mechanism, is the key to successful therapy and prevention for NSAID-induced gastroduodenal ulcers, although high-dose H_2 receptor antagonists are usually more costly than equipotent proton pump inhibitors. In children, a retrospective study suggested that misoprostol alleviates gastrointestinal symptoms associated with NSAIDs.[27] In that series, only 1 of the 25 children receiving an NSAID and misoprostol had diarrhea, which is in sharp contrast to the adult study.[28] Table 8.1-2 summarizes the major drugs and drug groups for NSAID-induced gastroduodenal damages.

Nitric oxide shares multiple antiulcerogenic effects with mucosa-protective prostaglandins. A case-control study showed that the use of nitric oxide–releasing nitrovasodilators is associated with a reduced risk of upper gastrointestinal bleeding in adults receiving NSAIDs or low-dose aspirin.[39] Nitric oxide–releasing NSAIDs are currently under investigation for clinical use.

In summary (see Table 8.1-2), a proton pump inhibitor or an H_2 receptor antagonist may be used for NSAID-induced dyspepsia symptoms. Misoprostol or a proton pump inhibitor is for prophylactic therapy of gastrointestinal ulcers associated with nonselective NSAIDs; alternatively, a COX-2 inhibitor may replace an NSAID in patients with significant risk factors for developing gastroduodenal ulcers. Patients with active ulcer, who need to continue NSAIDs, should be treated with a proton pump inhibitor.[12]

Helicobacter pylori infection and NSAID use are major and independent risk factors for gastroduodenal mucosal injury. Although the prevalence of *H. pylori* infection is lower in the pediatric population than in adults, it remains an etiologically important risk factor for gastroduodenal lesions. When endoscopically confirmed mucosal damages caused by NSAIDs are used as an end point, *H. pylori* infection does not seem to substantially increase the frequency and severity of the NSAID-induced mucosal damages for both short- and long-term (more than 4 weeks) use of the drug.[42] However, it is recommended that *H. pylori* be eradicated in those NSAID-receiving patients with *H. pylori*–associated ulcers because the two conditions are indistinguishable.[41,42]

TABLE 8.1-2 MAIN PHARMACOLOGIC APPROACHES FOR NSAID-INDUCED GASTRODUODENAL INJURY

DRUG/DRUG GROUP	INDICATIONS
Misoprostol	Ulcer prophylaxis
Proton pump inhibitors	Ulcer prophylaxis Dyspepsia treatment Ulcer treatment*
H_2 receptor antagonists	Dyspepsia treatment Ulcer treatment†

*In patients who continue nonsteroidal anti-inflammatory drug (NSAID) treatment.
†For those who discontinued NSAIDs.

Currently, no evidence exists to clearly support or refute *H. pylori* eradication before NSAID use in adult patients with no preexisting mucosal lesions.

Prostaglandin E_1–Induced Antral Hyperplasia

Antral mucosal hyperplasia, with no evidence of pyloric stenosis, causing gastric outlet obstruction has been reported in neonates with congenital heart disease such as transposition of great arteries and hypoplastic left heart syndrome, who were receiving prostaglandin E_1 infusion to sustain circulation dependent on the ductus arteriosus.[43] The condition is distinct from infantile hypertrophic pyloric stenosis because there is significant hyperplasia of the antral mucosal glands with no muscular thickening at the pyloric sphincter. Diarrhea is recognized as an adverse effect of prostaglandin E_1 infusion, which responds to dose reduction. One study showed that 5 of 74 neonates evaluated had clinical, radiologic, and pathologic evidence of the gastric outlet obstruction with no evidence of hypertrophic pyloric stenosis.[43] These 5 patients received the drug for a significantly longer period (mean 569 hours) at significantly higher cumulative doses than the remaining neonates. On withdrawal of the drug in 2 patients, the condition improved clinically and ultrasonographically. The authors recommended close monitoring of the gastric outlet functioning in neonates receiving prostaglandin E_1 for more than 120 hours.

Erythromycin-Induced Infantile Hypertrophic Pyloric Stenosis

The association between perinatal and neonatal exposures to erythromycin and development of infantile hypertrophic pyloric stenosis has been reported since 1976.[44] In particular, data indicating a close epidemiologic link of early neonatal exposure have been accumulating. Systemic use of erythromycin within the first 2 weeks of life (eg, for pertussis prophylaxis) appears to pose a 7- to 10-fold increased risk of developing pyloric stenosis over the incidence in the general population of about 1.5 to 4 patients per 1,000 live births.[45–47] Data are scarce to define the exact relationship between the dose and duration of the therapy and the development of the condition.

Erythromycin is a potent gastrointestinal kinetic agent through its action on the receptor for motilin, a peptide stimulating gastrointestinal contraction. Although the exact mechanism is not fully understood, breakdown products generated in the stomach from acid-labile erythromycin may play an important role in its prokinetic actions.[48] Because of its prokinetic action, erythromycin has been investigated for the treatment of feeding intolerance for premature infants. So far, however, no report exists linking the drug to hypertrophic pyloric stenosis in this population.[49,50]

The association between maternal use of erythromycin late in the pregnancy or early in the lactation period and the development of infantile hypertrophic pyloric stenosis is less clear and still controversial.[46,51–53] There has been no report linking other macrolides, such as clarithromycin and azithromycin, to pyloric stenosis.[48]

SMALL INTESTINE AND COLON

Neutropenic Enterocolitis (Typhlitis, Necrotizing Enterocolitis, Ileocecal Syndrome)

Severe inflammation leading to bowel necrosis may be seen in the cecum, ascending colon, and terminal ileum of leukemic patients receiving chemotherapy.[54] The condition is also seen in noncancer patients,[55–57] such as those with drug-induced neutropenia, acquired immune deficiency syndrome (AIDS), or organ transplants. The patient usually has severe granulocytopenia (< 500–1,000 cells/mm^3), and mortality may be as high as 50%.[58] Although the etiology is likely to be multiple and the pathophysiology poorly understood, cytotoxic drugs apparently play a key role in some patients. Vinca alkaloid–induced myenteric nerve damages may contribute to adynamic ileus and cecal distention that further enhances intestinal ischemia.

Pain in the right lower quadrant of the abdomen, fever, diarrhea with or without blood, and nausea and vomiting are usually seen. As the disease progresses, clinical signs of intestinal perforation, peritonitis, and sepsis may become apparent. Abdominal computed tomography, ultrasonography, and radiography provide diagnostic clues, including a fluid-filled dilated lumen, pneumatosis, and cecal wall thickening.

Treatment for the drug-induced neutropenic enterocolitis is no different from treatments for other forms of necrotizing enterocolitis. Namely, the therapy includes antibiotics covering intestinal bacteria, antifungal agents, supportive measures, and surgical resection.

Intestinal Mucositis Induced by Cancer Chemotherapy

Cancer chemotherapeutic agents affect intestinal cells with rapid cell turnover, disrupting the integrity of the epithelia (see "Esophagus"). Methotrexate is one of the most commonly cited offending agents. The affected patients have abdominal pain, diarrhea, vomiting, and, occasionally, melena. Protein-losing enteropathy has also been reported.

Drug-Induced Intestinal Hypomotility

Intestinal motility is reduced by drugs with anticholinergic properties (eg, anticholinergics, tricyclic antidepressants, and opioids). These are consequences of their pharmacologic actions of functional cholinergic inhibition. In contrast, vincristine damages nerve tissues, including the myenteric plexus, which may lead to adynamic ileus.[11,59,60] The condition develops within 2 to 3 days of the therapy.[11] Conservative treatment usually brings complete recovery in 2 weeks unless other complications, such as neutropenic enterocolitis (above), develop. If patients concomitantly receive itraconazole, vincristine neurotoxicity, including adynamic ileus, may become more severe.[61] This vincristine-itraconazole interaction may be due to itraconazole inhibition of a drug-metabolizing enzyme (eg, cytochrome P-450 3A4) and a drug transporter (eg, P-glycoprotein), which handle vincristine as a substrate.

Dexamethasone-Induced Intestinal Perforation in Premature Infants

A clinical trial of early use of high-dose dexamethasone in extremely low birth weight infants to prevent chronic lung disease was terminated before completion owing to a high incidence of spontaneous gastrointestinal perforation.[62] The rate of gastrointestinal perforation without necrotizing enterocolitis during the first 2 weeks of life was 13% (14 of 111) in the dexamethasone group that received a 10-day tapered course of the drug starting at 0.15 mg/kg/d within 24 hours after birth. The incidence in the placebo group was 4% (4 of 109). The sites of perforation were the small bowel (13 infants), the stomach (1), and unknown (4). A concurrent use of indomethacin appeared to contribute to development of the adverse event in both groups. Trends toward higher rates of spontaneous perforation associated with dexamethasone or indomethacin were also reported in other studies.[63–67] Given the established inhibitory effects of corticosteroids and NSAIDs on gastrointestinal prostaglandin synthesis, it is not surprising to see an increased incidence of spontaneous gastrointestinal perforation in this vulnerable patient population.

Antibiotic-Associated Diarrhea

Antibiotics are responsible for about one-fourth of the cases of drug-induced diarrhea.[68] About 70 to 80% of the cases of diarrhea associated with antibiotics use are nonspecific, self-limited, and unrelated to *Clostridium difficile*.[69,70] Some of these patients may develop the condition as a result of altered carbohydrate metabolism induced by changed bacterial flora in the large intestine. Erythromycin may cause diarrhea owing to its prokinetic property. The nonspecific diarrhea subsides on discontinuation of the antibiotics.

C. difficile infection is a nosocomial illness, comprising about 20% of antibiotic-associated diarrhea.[69] *C. difficile* produces toxins, causing intestinal damage with a wide spectrum of severity. *C. difficile* disease occurs 4 to 18 days after a first dose of the offending agent[71] and usually requires a cascade of events: changes in normal gut flora, acquisition and colonization of the bacteria, and toxin production.[69] Clearly, loss of colonization resistance as a result of disruption of the normal intestinal microflora by antibiotics is an important etiologic process, although *C. difficile* disease in pediatric patients may be less dependent on prior exposures to antibiotics than in adults.[70] The most commonly implicated antibiotics are ampicillin, amoxicillin, cephalosporins (second and third generations), lincomycin, and clindamycin, but use of virtually any antibiotic can be a predisposing factor.[72–74] Notably, as many as 60% of neonates and infants are asymptomatically colonized by *C. difficile*, the mechanism of which is not fully understood.[69,75–77] Compared with neonates, the isolation rates of the bacteria decrease to 0 to 3% in older asymptomatic children, similar to those seen in asymptomatic healthy adults.[70] Clinical pictures of *C. difficile* infections range from asymptomatic carriers, mild diarrhea, and uncomplicated colitis to pseudomembranous colitis and fulminant colitis.

Pseudomembranous enterocolitis affects mainly the large intestine and rarely the small intestine. *C. difficile* has been the most common cause, often induced by preceding antibiotic therapy. Other causes of pseudomembranous colitis include ischemia, verotoxin-producing *Escherichia coli*, and drugs such as chlorpropamide, gold, and NSAIDs.[69]

C. difficile infections usually present with profuse watery or mucoid diarrhea with or without blood, abdominal pain, and fever. Supportive care and discontinuation of the offending antibiotics, if any, may be sufficient for those with mild symptoms. Symptomatic therapy with antidiarrheal agents should be avoided. Patients with severe symptoms, for whom supportive therapy has failed, may be treated with oral metronidazole or vancomycin for 1 to 2 weeks. Of those who underwent the therapy for the first time, as high as 40 to 60% may relapse.[78–81] Currently, a new strategy to neutralize the toxin by a synthetic oligosaccharide mimicking toxin receptors is being tested for treating recurrent *C. difficile* infections.[70]

NSAID-Induced Intestinal Injury

Intestinal damages inflicted by NSAIDs manifest themselves in several distinct pathologic forms. Some of the lesions share the same pathologic processes as NSAID-induced gastric damages.

Ulceration. Whereas gastroduodenal damages caused by NSAIDs have been long known and relatively well characterized, NSAID-induced small intestinal and colonic lesions are a recent addition to its adverse-effect profile. An autopsy study involving 249 adult patients on NSAIDs found the prevalence of small intestinal ulcers to be 8.4% compared with 0.6% in the control group.[82] Although gastric and duodenal ulcers were also seen in the NSAID users, no correlation was found between the gastric or duodenal lesions and the small intestinal ulcers,[82] implying that prediction of the small intestinal ulcers from the upper gastrointestinal damages may not be valid. Small intestinal perforation associated with slow-release NSAIDs is well documented in adults,[83,84] suggesting that slow-release formulations simply shift a target site of NSAID-induced mucosal injury from the stomach to the more distal intestinal tract.

In children, data are scarce, but preterm neonates appear to be more susceptible to the adverse effects of NSAIDs. For example, 1 in 10 premature infants with a patent ductus arteriosus, who received indomethacin, were found to have intestinal perforation compared with none in surgically treated babies.[85] Overall, perforation has been reported to occur throughout the gastrointestinal tract in premature neonates receiving NSAIDs.[63,65–67,85] Common mechanisms of local prostaglandin depletion seem to underlie gastric and intestinal perforation associated with NSAID therapy.

Strictures. NSAIDs cause strictures in the small intestine and colon in adults after prolonged use, which is not necessarily a consequence of ulceration. Pathologic characteristics range from nonspecific strictures to multiple, thin, and web-like diaphragms that are considered patho-

gnomonic of NSAID use.[86] An apparently low incidence of the condition may be an underestimate as a result of difficulty in diagnosis. There is no report of the NSAID-induced web-like strictures in pediatric patients.

NSAID Enteropathy. NSAID enteropathy may be defined as a disturbance of the small intestinal function associated with NSAID use in the absence of macroscopic pathology such as macroscopic ulceration and bleeding. The underlying alterations are probably diffuse intestinal inflammation and increased mucosal permeability.[87] However, how NSAIDs cause these changes is unknown. Clinical pictures include iron deficiency anemia owing to chronic blood loss, a protein-losing enteropathy, and mild lipid malabsorption. Although as many as 70% of NSAID users are estimated to be at least partially affected by the condition, overt manifestations are uncommon.[87] Recently, measurement of fecal calprotectin, a neutrophil protein, has been suggested as a simple method to diagnose the condition.[88]

Colitis. Chronic use of NSAIDs, usually for 6 months or longer, may cause various forms of colitis, whose symptoms include watery and/or bloody diarrhea. Case reports in adults indicate that eosinophilic, collagenous, pseudomembranous, and nonspecific colitis are associated with NSAID use. Mefenamic and fulfenamic acids are most often implicated as causative NSAIDs, but epidemiologic data are scarce to define drug-specific risks.

In a recent study with a case-crossover design (a median patient age of 35 years old, ranging from 4 months to 89 years),[89] NSAID use within 6 days prior was shown to increase the risk of acute diarrhea by about threefold, although information on concurrent medications, if any, was not given, and the diversity of NSAIDs used made it impossible to analyze drug-specific risks. Interestingly, however, none of their study patients received meclofenamate,[88] which was reported to induce diarrhea as much as 30% in clinical trials.[90]

The mechanism of NSAID-induced colitis is largely unknown, although inhibition of prostaglandin synthesis coupled with relative overproduction of leukotrienes may be a contributing factor. It is clinically characterized by acute diarrhea with or without mucus or blood.

In addition to the de novo colitis, NSAIDs may activate inflammatory bowel disease, especially ulcerative colitis, within a few days of the start of the therapy in some patients.[87,91] The mechanism is not fully understood, but inhibition of the COX by NSAIDs may shift the arachidonic acid metabolism pathway sideways to produce the proinflammatory leukotrienes.

NSAID-induced pathology in the colon is similar to those in the small intestine but less commonly reported. This may be due to a progressively small amount of orally ingested NSAIDs reaching the colon in most patients as a result of nearly complete absorption in the proximal intestinal tract. Indeed, ulcers and strictures (broad based or diaphragms) are seen, if at all, mostly in the right side of the colon.[87,92,93]

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2. *Radiation Enteritis*

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In children, the use of radiation therapy for abdominal malignancies has diminished in recent years, but some specific applications remain.

Two decades ago, radiotherapy to the abdomen was commonly given for lymphoma, neuroblastoma, rhabdomyosarcoma, teratoma, and Wilms tumor.[1] In the last decade, external radiotherapy has been used postoperatively for Wilms tumor, rarely for Hodgkin disease of the abdomen and Ewing sarcoma of the pelvic bones,[2] and for some complicated malignancies with poor prognosis. In children, irradiation for neuroblastoma is provided by intravenous ^{131}I metaiodobenzylguanidine, which emits β-radiation locally without penetration to surrounding tissues. For rhabdomyosarcoma in the pelvic region,[1] brachytherapy is a treatment in which needles are inserted in the pelvic region for postoperative local irradiation without damaging radiation effects on the surrounding tissues.

Presently, doses of irradiation to the abdomen rarely exceed 20 to 30 Gy and are usually lower, so they do not exceed tolerance of the kidneys, liver, intestine, or other organs. However, radiation to the pelvis for Ewing sarcoma may require a dose up to 60 Gy.[2]

INCIDENCE OF RADIATION ENTERITIS IN CHILDREN

The incidence of radiation enteritis during and after abdominal pelvic radiation therapy varies in adults between 2.5 and 25% and is characterized by reversible abnormalities in small intestinal or anorectal mucosa. Acute damaging effects are mostly reversible, but months to years after irradiation, mucosal changes may develop, and sometimes they are irreversible. In 69 children aged 6 months to 8 years, treated by total or partial irradiation (20–35 Gy) with chemotherapy for Wilms tumor, neuroblastoma, or rhabdomyosarcoma, gastrointestinal symptoms occurred in 74% and diarrhea in 42%. In 55%, a 10% body weight loss was observed in the acute phase. Long-term persistent gastrointestinal symptoms occurred in 36% of children treated in the same institution.[3,4] The majority have some loose stools, which do not interfere with their daily activities.[5]

PATHOPHYSIOLOGY

Gastrointestinal damage depends on the physical characteristics of radiation exposure. The modern megavoltage (MV) linear accelerators use a wave guide to accelerate electrons, bombarding a target at high energy to produce x-rays in the range of 4 to 25 MV. Radioactive atoms have unstable nuclei, which release energy in the process of spontaneous disintegration. Ionizing irradiation with protons (x-rays or γ-rays), electrons, neutrons, or pions interacts with tissues and produces radicals. They damage nuclear deoxyribonucleic acid (DNA), leading to cell death.

The accepted radiation dose unit is 1 Gy or 100 rad (1 rad = 1 cGy).

Today radiation treatment is usually administered in 2 Gy treatment fractions; these regimens were empirically developed, and they do not clearly separate the responses of normal tissues and malignant tumors. For most pediatric tumors, external beam irradiation is used. An exception is pelvic rhabdomyosarcoma, for which brachytherapy uses per operatively placed ^{192}I needles in sheets.[6]

As cells are irradiated with increasing doses, one observes a shoulder in their survival curve; this shoulder reflects the cells' capicity to accumulate sublethal damage, which becomes effective at higher doses, leading to rapid cell death. Between fractions of radiation treatment, however, this accumulated damage may dissipate.[7]

A significant factor determining the response of a cell to radiation is the phase of its division cycle. There is a brief period after mitosis when there is no DNA synthesis (G_1); then there is a DNA synthetic phase (S), followed by a short second phase without DNA synthesis (G_2), followed by cell division (mitosis). Sensitivity to irradiation is most pronounced in the mitotic and G_2 phases, and resistance is most pronounced in the synthesis phase. These findings may explain how chemotherapeutic drugs can alter the sensitivity of cells to irradiation because they act mainly on the synthetic phase of the cell cycle. Chemotherapeutic drugs, at times used in concert with radiation, such as doxorubicin, 5-fluorouracil, and actinomycin D, are also capable of injuring intestinal cells.[8] The tissue perfusion of tumor cells modifies their sensitivity to irradiation. Hypoxic cells are particularly radiation resistant. Usually, but not always, radiation injury is most severe in rapidly dividing cells, which are less differentiated and have a high mitotic activity, like malignant cells. In the intestine, enterocytes are comparable to hematopoietic and tumor cells in the rapidity of their mitotic activity. Therefore, radiation treatment directed at injuring tumor cells is likely to damage enterocytes within the radiation field. The earliest impact of radiation on the gut epithelium occurs in the crypts, where epithelial cells are dividing most rapidly.[9] These cells mature during migration out of the crypts and move to the

villous tips, where they are extruded or go into apoptosis. The whole replacement procedure requires 4 to 6 days in humans for the small intestine, colon, and rectum. After radiation exposure, crypt intestinal epithelial cells go into apoptosis and stop dividing, migrating, and maturing. Repopulation of enterocytes of the villous epithelium is impaired, and villous atrophy is inevitable. Usually, epithelial integrity is maintained, but ulceration may occur. With fractionated radiation dosages, these changes rarely cause symptoms, but for some reason, possibly a variability on individual radiosensitivity, the intestinal epithelial barrier may be destroyed in some patients who develop severe diarrhea, mucosal bleeding, and, rarely, septicemia from bacterial translocation. After discontinuation of radiation treatment, crypts regenerate, and the epithelium repopulates with enterocytes on the muscosal surface; abnormalities in mucosal morphology may persist for longer periods.

It is interesting to note that with exclusion of pancreatic juices from the intestinal lumen, epithelial radiation injury does not develop, but exclusion of biliary secretions has no influence on radiation injury.[10] In the acute phase, pancreatic juices seem to be involved in radiation effects, but their role has not been investigated in the chronic phase.[11,12]

Subepithelial structures in the gut mucosa are less rapidly dividing than epithelial cells, but irradiation of vascular endothelial cells and connective tissue can cause late and permanent changes in the vascular supply to the musosa.

Radiation leads initially in the acute phase to capillary endothelial swelling, capillary leakage, lymphatic leakage, and edema.[13] This damage is followed in the recovery phase by vascular and connective tissue changes, which can progress to obliterative endarteritis and endophlebitis, causing intestinal ischemia, ulcerations, and necrosis.[14] Strictures are caused by progressive fibrosis owing to this vasculopathy.[15] Mucosal surface destruction is secondary to these vascular changes, as are possible motility disorders (Table 8.2-1).[9,16]

This scenario has been turned on its head. The radiation administered to the mouse intestine preferentially damages the endothelial cells of the gut microvasculature. The death of epithelial stem cells might be a secondary event to the demise of endothelial cells. Microvascular endothelial cells express the receptor for basic fibroblast growth factor (bFGF), whereas epithelial stem cells in the crypt do not; systemic administration of bFGF enhances epithelial stem cell survival after irradiation. Irradiation of microvascular endothelial cells generates ceramide, a proapoptotic lipid that facilitates endothelial cell death. bFGF, an endothelial cell mitogen, overrides the ceramide signal. This was supported by a compelling experiment in which mice lacking the gene for acid sphingomyelase, which is required for ceramide production and is highly expressed in endothelium, were protected from radiation-induced destruction of the gut mucosa. This two-compartment model is well known for tumor growth, but gut epithelial cells are highly hypoxia resistant; therefore, this hypothesis has been challenged.[17–19]

Tissue injury at a molecular level has been studied for leukocyte–endothelial cell interactions after radiation-induced tissue injury, in which E (endothelial)-selectin and intercellular adhesion molecule 1 (ICAM-1) are involved in mediating leukocyte sequestration in irradiated normal tissues. In inflammation, leukocyte–endothelial cell interactions are important for the trapping of inflammatory cells into the inflammatory infiltrate.[20] It has been shown that leukocytes roll on irradiated endothelial cells via E-selectin and then are arrested by ICAM-1. Leukocytes have a very slow rolling velocity on E-selectin substrate, suggesting the involvement of additional rolling receptors on the irradiated vasculature. Other potential receptors are P-selectin and L-selectin. Also, the observed radiation-mediated effects on CD31 expression imply a role for CD31 in mediation of leukocyte transmigration into irradiated tissues.[21] In this process, up-regulation of ICAM-1 might also be involved.[22]

This elucidation of specific molecules involved in radiation-induced leukocyte extravasation permits speculation on possible specific targeted therapeutic strategies for patients. The ICAM-1 knockout mice do not exhibit an increase in leukocyte infiltration of the lung after irradiation. This observation identifies ICAM-1 as a possible therapeutic target for the amelioration of radiation-induced leukocyte–endothelial cell interactions.[23] Inhibiting leukocyte ligand binding by inhibiting ICAM-1 on the endothelial surface might reduce leukocyte influx in normal tissues after irradiation. Injection of monoclonal ICAM-1 antibodies into irradiated lungs of mice attenuated infiltration of leukocytes into lung tissue. Theoretically, these antibodies might inhibit endothelial cell surface ICAM-1 and diminish inflammation in normal human tissues after irradiation. Anti-CD31 antibodies might be of therapeutic benefit to block transmigration of leukocytes into irradiated tissues. Glucocorticoids might be effective by inhibiting nuclear factor-κB (NFκB) activation. NFκB is implicated in inducing transcription of E-selectin and ICAM-1 in irradiated endothelium. Leukocyte infiltration of normal tissues after irradiation might be an important factor in the pathogenesis of late radiation damage.

Another important determinant of radiation-induced tissue injury is platelet adherence to the vascular wall and abnormal endothelial cell proliferation, leading to vascu-

TABLE 8.2-1 CLINICAL FINDINGS: RADIATION ENTERITIS IN CHILDREN

Acute enteritis
Vomiting
Diarrhea
Weight loss
Intestinal hemorrhage (rare)
Chronic enteritis
Bowel obstruction (complete or partial)
Vomiting
Diarrhea
Abdominal pain
Abdominal distention
Late effects of enteritis
Bowel obstruction (complete or partial)
Esophageal obstruction
Dysphagia
Vomiting
Substernal pain

lar occlusion. A dose of 2 Gy increases von Willebrand factor release from endothelial cells with increased platelet adhesion to the extracellular matrix; endothelial cells detach from the underlying matrix, leading to platelet adhesion, thrombus formation, and, ultimately, vascular occlusion of the lumen.[24]

A transmembrane glycoprotein thrombomodulin (TM) is located on the luminal surface of endothelial cells and is pivotal in maintaining thrombohemorrhagic balance. TM forms a complex with thrombin and prevents fibrin formation but activates protein C, a major anticoagulant. Radiation causes local TM deficiency, insufficient activation of protein C, and reduced scavenging of thrombin, thus enhancing activation of protease-activated receptor 1, which increases twofold in vascular and intestinal smooth muscle cells in irradiated intestine. This up-regulation has proinflammatory, profibrogenic, and promitogenic effects and might be implicated in radiation-induced vascular sclerosis and in radiation fibrosis of the intestinal wall.[25]

Matrix metalloproteinases (MMP-2, MMP-9 [gelatinases]) are present in the mucosa of the gut, and it has been shown that these metalloproteinases are increased after irradiation in human rectal mucosa.[26] The mucosal extracellular matrix (ECM) is a functionally active scaffolding meshwork that, in addition to maintaining normal tissue stricture, is pivotal in the integration of cellular interactions. MMPs play a central role in regulating the equilibrium between the ECM, synthesis, and breakdown. Up-regulation might signify damage and atrophy of the ECM, although publications are conflicting in their results so far, and protective effects during irradiation might not be excluded.[26]

Immediately after irradiation, the bowel is hyporesponsive to secretagogues, with less inducible nitric oxide synthase derived. Nitric oxide goes parallel with decreased tissue resistance, which makes the epithelium more vulnerable to epithelial translocation. The same first 3-day period, the tissue is less responsive to exogenous 5-hydroxytryptamine (5-HT); simultaneously, the sensitivity of 5-HT_3 and 5-HT_4 receptors was attenuated. The subsequent day, the decreased 5-HT tissue content was normalized, with a higher sensitivity of the tissue to 5-HT owing to up-regulation of 5-HT_3 and 5-HT_4 receptors. The 5-HT–mediated pathways are neural and non-neural, respectively, and in the first days after irradiation diminished colonic water and electrolyte transport was related to hyporesponsiveness of the epithelium and at recovery of diarrhea increased responsiveness the days after. With respect to the above, it might be apparent that a paradox exists. The irradiated epithelial cells secrete less chloride and water, although diarrhea is one of the side effects of radiotherapy. However, secretion, compared to absorption, in the colon is a minor event. It is just sufficient to reserve the microenvironment. It might be conceived that the low-volume secretory processes are surpassed by phenomena of diminished absorption in the colon. It is shown that the Na^+/K^+–adenosine triphosphatase activity is diminished and is related to malabsorptive electrolyte diarrhea.[27,28]

In animals, granisetron (5-HT_3 receptor antagonist) prevented diarrhea and improved colonic motility.[29]

The cell death attributable to radiation of the gut goes parallel with apoptosis. In radiation-induced apoptosis, expression of the tumor suppressor gene *TP53* in the stem cell region is increased. In knockout mice, no apoptosis increase was observed after irradiation of small and large intestine, so *TP53* seems to be mandatory for this apoptosis increase. Also, at the same time, the expression of apoptosis-protecting gene *BCL2* is increased. Animals lacking *BCL2* have increased apoptosis after mucosal irradiation. Probably, a balance of these proapoptotic and protecting genes is involved in radiation damage.[30]

BCL2 is involved in protection of the large intestine, whereas *BCLW*, another physiologic regulator, is an important determinant in protecting the small intestine during irradiation.[31]

Induction of apoptosis measured in human rectal specimens showed increased permeability in a size-dependent fashion for marker molecules in Ussing chambers.[32]

After ionizing radiation, transforming growth factor-β (TGF-β) is increasingly expressed. It is a potent fibrogenic and immunomodulatory cytokine leading to hyperplasia of connective tissue mast cells and leukocyte migration into the intestinal wall. Interestingly, 26 weeks after radiation, only the isoform TGF-β1 remains elevated in vascular endothelial cells, fibroblasts, and smooth muscle cells. It is increased strongly only in areas of persisting radiation-induced injury and is probably related to the development of vascular sclerosis and fibrosis of the serosa and muscularis propria.[33]

Fibroblast growth factors (FGF-2) enhance survival of epithelial cells after injury induced by ionizing radiation. The biologic effects of FGFs are mediated by binding to an activating cell-surface receptor tyrosine kinase. FGF-2 is elevated in small intestine after radiation injury, it is localized to mesenchymal cells surrounding regenerating crypts, and it enhances crypt stem cell survival when it is administered before but not after radiation. With other cytokines or intracellular regulatory molecules in animal models, it has been shown that only when administered before irradiation do they protect epithelial stem cells and enhance recovery, including TGF, tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-11, prostaglandin E_2 (PGE_2) and misoprostol. Clinical studies are lacking so far, but it is conceivable that some effects might lead to clinical application.[34]

The most abundant gastrointestinal prostaglandin, PGE_2, is probably involved in radiation injury; the biologic activities are mediated through interaction with plasma membrane G protein–coupled receptors, the E-prostanoid (EP) receptors, which are up-regulated in the intestinal mucosa after irradiation.[35]

In rats, growth hormone in low- but not high-dose radiation maintained body weight and growth.[36]

In animals, by gene transfer through a herpes virus as vector, human manganese superoxide dismutase in the small intestine delivered by the luminal route was overexpressed and prevented radiation damage, suggesting a role for oxygen free radicals to initiate cytokine cascades, leading to fibrosis after chronic radiation injury.[37]

EFFECTS OF RADIATION ON MOTILITY

Radiation of the gut does not significantly affect normal migrating motor complexes. It was observed that giant migrating contractions are generated after the first dose of irradiation and continue for the duration of the irradiation period. Afterward, motility returns to normal patterns. These changes in motility pattern occurred in small and large intestine and resulted in rapid propulsion of luminal contents. Although lowered contractile activity in the proximal small intestine was observed, these increased giant migrating contractions overwhelm and increase transit. In animal models, during irradiation in gastrointestinal tissue, changes in tissue and serum levels of various neuroendocrine products were shown in a region-specific manner, including acetylcholinesterase, vasoactive intestinal polypeptide (VIP), substance P (SP), and peptide YY, but no relationship to altered motility was provided. Also, the increased sensitivity to cholinergic stimuli up-regulation of 5-HT_3 and 5-HT_4 receptors suggests a role for serotonin, but relationships are unclear so far.[38]

In human colonic specimens in nerve fibers, SP and VIP were increased in mucosal and circular muscle fibers but were decreased after 6 weeks postirradiation, which might have an influence on inflammation and motility.[39] In animals, increased SP directly after irradiation of the intestine was related to increased contractility and release of proinflammatory mediators such as TNF.[40]

In animal morphometric studies on the intestine after irradiation, the storage function of the irradiated gut is and stays decreased, further supported by persistent increased stress in the proximal part to the affected site.[41] This is supported by observations in the rectum in humans after radiation therapy for prostate cancer in which anorectal function is preserved, although with a decreased storage capacity and rectal compliance.[42–44]

Long-term effects are seen in 11% of patients.[45] Radiation doses in the 1960s and 1970s were up to 40 Gy, but now 20 Gy is rarely exceeded. In pelvic irradiation, long-term effects are more likely to occur in children owing to the application of higher doses.[45] Up-regulation of CD31 expression on endothelial cells, which plays an important role in platelet adherence, disrupting regulation of endothelial cell proliferation, is a focal event, resulting in protrusions into the lumen. Strategies to prevent late radiation reactions should be targeted to radiation-mediated CD31 expression and attempts to prevent leukocyte influx, thrombus formation, and abnormal endothelial cell proliferation. Blocking ICAM-1 could reduce leukocyte influx in irradiated tissues. Because chronic inflammatory processes are important for late radiation effects, prevention and amelioration of inflammatory processes are realistic treatment prospects for childhood malignancies.[23]

MICROSCOPIC ABNORMALITIES IN RADIATION-DAMAGED BOWEL

At the onset of damage to the bowel, reduced mitotic activity is observed under light microscopic examination. The migration of normal mature enterocytes continues, and for a few days, the epithelial villous structure remains intact. Gradually, a lack of migrating enterocytes stretches them out, failing to cover the surface with enterocytes, and a gradual reduction in villus size follows. In the crypt, apoptotic cells are present within 24 hours. Abnormalities increase gradually because radiation is usually administered in 2 Gy/d until the total dose is achieved.

At the electron microscopic level, radiation can cause reduced numbers of microvilli, disrupted tight junctions,[46] dilated endoplasmatic reticulum, and sometimes mitochondrial swelling or fragmentation of internal crystae. Chemotherapy can enhance these effects because methotrexate,[47] actinomycin D, vincristine,[48] and doxorubicin induce comparable effects on the small intestinal epithelium. In addition, hyperemia, edema, and inflammatory infiltration occur, and, occasionally, crypt abscesses are visible. Vascular abnormalities are difficult to discern; they are predominantly responsible for the late effects.[15]

Late effects after radiation to the abdomen are due mainly to vascular injury. The typical lesions are subintimal fibrosis with degeneration of the full thickness of the walls of different-sized vessels in the submucosa; these lesions are distributed irregularly. With the vascular changes, there is ischemic bowel and consequent infarction, atrophy, and fibrosis. The obvious result is fibrosis of the submucosa and muscular layers of the gut, leading, in some cases, to luminal narrowing of the intestine and obstruction.[15]

Intestinal resections within 2 years of irradiation show serosal adhesions and ulcers, but after 8 years, ulcerative strictures are seen in 82%, with damaged vessel walls in 73%, enteritis cystica profunda in 73%, atypical epithelia in 64%, and fistula in 18%. These sequelae suggest a need for careful long-term follow-up of all of these patients.[49]

ACUTE RADIATION ENTERITIS

Early Clinical Symptoms in Children

Symptoms of acute radiation enteritis can develop within hours after the first exposure, but usually they begin during the first to second week, or even after completion of abdominal or pelvic radiotherapy. Some diarrhea is usually noticed; even bloody diarrhea can occur during radiation therapy. Occasionally, nausea, vomiting, abdominal cramps, and abdominal pain may occur. Irradiation to the abdomen in children usually involves the small bowel; pelvic irradiation, which can damage the distal large bowel and ileal loops, is administered relatively infrequently to young patients. When it does occur, proctosigmoiditis produces mucoid discharge, tenesmus, and rectal bleeding, suggesting mucosal ulceration (Table 8.2-2). The clinical picture here resembles acute ulcerative colitis.[50] In a retrospective study, 70.5% of children undergoing abdominal radiation therapy had early symptoms, consisting of severe vomiting or diarrhea that was severe, requiring intravenous fluids, in 30%.[4]

Thoracic radiation can cause acute radiation esophagitis. Symptoms are highly variable, from mild substernal burning to dysphagia and swallowing-induced, angina-like chest pain.[5]

TABLE 8.2-2 MECHANISMS OF RADIATION ENTERITIS

ACUTE RADIATION ENTERITIS
Interruption of enterocyte replacement
Villous atrophy
Disaccharidase deficiency
Occasional mucosal ulceration
CHRONIC RADIATION ENTERITIS
Mainly vascular obliteration
Obliterative endoarteritis
Endophlebitis
Intestinal ischemia
Diffuse chronic mucosal inflammation
Villous atrophy
Progressive fibrosis
LATE EFFECTS OF RADIATION ENTERITIS
Degeneration of vessel walls
Subintimal fibrosis
Ischemic bowel with infarction
Submucosal and muscular atrophy and fibrosis
Ongoing perivascular inflammation
Stricture formation

Mechanisms of Early Clinical Symptoms

In the affected loops, malabsorption is initially due to edema, followed by villous atrophy and osmotic diarrhea, which is the result of a secondary deficiency of brush border enzymes with dietary disaccharide intolerance.[51,52] The affected surface area predicts the extent of clinical symptomatology. Chemotherapy might cause the same disaccharidase deficiency and might enhance the symptomatic impact of radiation. In about 60% of cases, steatorrhea develops during radiation therapy mainly in those receiving concurrent chemotherapy; probably the basis for this fat malabsorption is villous atrophy.[53,54] Chemotherapy alone can cause steatorrhea, and radiation therapy seems to be a potentiating factor. 5-HT_3 receptors might play an important role in radiation-induced nausea and vomiting by direct stimulation of specific receptors on peripheral afferent nerves by free radicals or endotoxins or stimulation of central nervous centers by circulating substances. In fact, 5-HT_3 receptor antagonists are effective in controlling radiation-induced vomiting and nausea.

Diagnosis

Minor symptomatology in the early treatment phase does not justify intensive investigations unless a reduction in therapeutic fraction size or an increase in fraction interval is being considered. Duodenoscopy with mucosal biopsy will identify villous atrophy and an inflammatory lesion. On endoscopic visualization, hyperemia, friability, erosions, and ulcerations may be apparent. When the pelvic region is within the radiation field, sigmoidoscopy might show edema, hyperemia, friability, and ulceration of the rectal mucosa. Isotopic or barium imaging studies are not warranted. Hydrogen breath testing can be used to detect disaccharide intolerance, but many of these children are receiving antibiotics, which negates the usefulness of the test.[12,52]

Treatment

In children, symptoms are usually mild and rarely dictate adjustment of the radiation dose. On occasion, for severe symptoms, radiation treatment must be stopped, the dose decreased, or the interval between doses increased. Symptoms usually abate in a fortnight after cessation of radiation therapy. Treatment should be instituted according to symptomatology and the probable site of injury.[55] Mild diarrhea owing to lactose intolerance diminishes on a lactose-free diet.[53] Nausea and vomiting are sensitive to treatment with 5-HT_3 antagonists.[56] These drugs might also inhibit serotonin-mediated small intestinal dysmotility. A combination of nausea, vomiting, and diarrhea seems to be sensitive to nonsteroidal anti-inflammatory drugs (NSAIDs). Aspirin, ibuprofen,[57] and indomethacin[58] have been helpful in reducing painful cramps, diarrhea, and nausea in adults; they have also been useful in treating radiation-induced esophagitis.[59–61] Cholestyramine, which can improve diarrhea in adults after pelvic irradiation, might be used in children with radiation treatment to the same site.[62,63] Some relief of symptoms during radiation can occur with elemental feedings or total parenteral nutrition,[64–66] but these techniques are usually reserved for children with severe symptoms. Children who develop significant malnutrition should receive aggressive nutritional support given enterally or parenterally.[50] In animals, neither oral arginine nor glutamine had any effect on radiation-induced intestinal injury.[67]

Severe rectal bleeding has been successfully treated with laser therapy,[68] and rectal sucralfate enemas may mitigate radiation proctitis.[59]

For erosive blood loss, 4% formalin solutions applied rectally might have some effect clinically when laser coagulation is not available.

Argon plasma coagulation is suitable for treating hemorrhagic gastrointestinal tract lesions. Ulcers might develop locally, but these are asymptomatic.[69]

Prophylaxis

Radiation delivery to an abdominal tumor sometimes can be modified to diminish gastrointestinal side effects.[70–72] For example, in adults undergoing pelvic irradiation, the volume of small intestine in a pelvic radiation field can be determined by barium studies. If indicated by these studies, the patient can be placed in the prone position for pelvic irradiation to decrease the exposure of normal small intestine.[73] Children requiring pelvic radiation therapy, especially those with previous abdominal surgery and the risk of fixation of the small bowel in the pelvis, should have a similar assessment, and then their radiation fields should be adjusted to a hyperfractionated regimen to minimize late side effects. Also, in adults, use of an elemental diet or parenteral nutrition showed diminished diarrhea and improved nitrogen balance during the acute phase of abdominal radiation treatment.[74–76] NSAIDs given prophylactically may ameliorate acute radiation enteritis.[57]

Topical sucralfate and hydrocortisone, but not mesalazine (5-acetylsalicylic acid), have some preventive effect in radiation proctitis, but this cannot be extrapolated to radiation effects on other usually affected parts of the intestine in children.[77] Amifostine is cytoprotective in adults against radiation mucositis.[78]

Indomethacin reduces the endoscopic manifestations of radiation esophagitis but not the microscopic abnormalities.[58] Exclusion of pancreatic juices in animals can reduce intestinal radiation damage, but antiproteases, such as trypsin inhibitors from soy beans or *Ascaris*, have not been studied in a clinical setting.[11,79]

In children, the appearance of late symptoms can be preceded by gastrointestinal complaints during the early radiation period, but 10 children presenting with ileus 10 years after abdominal radiation had not experienced acute-phase symptoms.[4]

CHRONIC RADIATION ENTERITIS

Eleven percent of children develop a delayed intestinal syndrome consisting of vomiting and diarrhea with a distended abdomen after completion of radiotherapy.[5] Delayed symptoms first appeared within 2 months after completion of radiotherapy. In the French experience, no child developed delayed radiation enteritis without previously having had an early reaction during radiotherapy, but in our experience, only 41% had been asymptomatic during the original radiation treatment. These intestinal lesions are characterized by adhesions and fibrosis, leading in some cases to intestinal obstruction and enteroenteric fistulae steatorrhea, which have been observed in children up to 6 months after completion of radiotherapy. Late and very late symptoms are rare in children; they may be reported at any time after the well-defined period of delayed reactions within 2 months after cessation of radiotherapy.[4,58]

In cases of delayed radiation damage, the extent of bowel involvement is almost always more extended than suspected. In most cases, both small bowel and large bowel are involved. If Ewing sarcoma or rhabdomyosarcoma has been treated with pelvic irradiation, the terminal ileum may suffer from late effects, but, usually, other regions of small bowel and, occasionally, the colon are involved. Increasing pain or a change in pain pattern requires early investigation for obstruction, perforation, abscess formation, or infarction. A recurrent malignancy always must be considered. Minor symptoms should not be overlooked.[80–83]

Pathophysiology of Delayed Reactions

In some children and many adults, mucosal abnormalities such as villous atrophy, abnormal crypts, venous obliteration, and lymphatic ectasias may persist for years because of a combination of epithelial, arteriovenous, and lymphatic obliterative lesions, resulting in vascular and structural dysregulation.[84–86] Subtotal villous atrophy can certainly persist for up to 6 months after radiation therapy. Persisting villous atrophy related to submucosal vascular abnormalities is likely to cause malabsorption if enough irradiated bowel surface area is involved.[87–89] Secondary disaccharidase deficiency with osmotic diarrhea can be a functional sequela of this lesion. Protein-losing enteropathy can occur, with edema and hypoproteinemia owing to permanently dilated lymphatic vessels from obliterative lymphatic lesions.[90–92] Also, bacterial overgrowth with bacterial deconjugation of bile acids can contribute to this malabsorption because the vascular lesions, fibrosis, and stricture formation may cause intestinal stasis with bacterial overgrowth.[93,94]

Deficiency of vitamin B_{12} occurs with long-standing ileal involvement in radiation enteritis, when it might be secondary to bowel stasis with bacterial overgrowth. Rarely, hypocalcemia and vitamin D deficiency occur during chronic mucosal malabsorption.[95] It has to be emphasized that vascular abnormalities without stricturing might cause diarrhea, steatorrhea, nausea, or (sub)clinical signs of deficiencies without obvious alarming symptomatology. Small bowel intestinal obstruction caused by vascular abnormalities after radiation exposure is usually symptomatic owing to strictures and adhesions, with abdominal discomfort, distention of the abdomen in combination with abdominal pain, and sometimes vomiting. Chronic blood loss or massive bleeding owing to radiation ulcers or telangiectasia occurs but very rarely in children. Abdominal abscesses after bowel necrosis and fistulization after pelvic irradiation have been observed in children, but only at laparotomy. Also, septicemia and peritonitis are very rare in children.

Diagnosis

Routine imaging can be helpful, but there are many pitfalls. Strictures and dilatations of the intestine may be detected, but the regions of intestinal wall involved in chronic lesions usually cannot be localized radiologically. Isotope labeling of leukocytes can be used to detect inflammation and abscesses[96,97]; intensive concentrations of isotope suggest an ongoing inflammatory process preceding stricture formation and fibrosis. Also, abnormalities in the vessels of the submucosa are detectable with these scans. Endoscopy is rarely helpful because the involved areas are usually out of reach of the endoscope in children, but small bowel biopsy may show chronic villous atrophy. Malabsorption should be evaluated with fecal fat balance studies. Labeled triglyceride breath tests might be helpful, and in cases of bile acid malabsorption, bile acid breath tests have been used. The Schilling test to assess vitamin B_{12} absorption and the ^{13}C-xylose breath test are helpful in detecting bacterial overgrowth owing to strictures and dilated areas.[98] Occasionally, arteriography may be applied to show arterial stenosis after radiation.

Treatment

Conventional treatment of mild cases of radiation enteritis is supportive. Surgical intervention may be attempted if there is intestinal obstruction, but in children, adhesions, fibrosis, and inflamed bowel loops usually make operative dissection unacceptably hazardous. For reasons that are not yet clear, a diet free of gluten and cow's milk (lactose) and low in fiber and fat content can be very beneficial to symptomatic children.[99] Five children with progressive intestinal dysfunction improved dramatically on such a diet. For undernourished patients not responding to the measures described above, some approaches, used mainly in adults to date, can be attempted.[100] These strategies include a low-fat diet with supplemented medium-chain triglycerides and essential fatty acids and pancreatic enzyme supplements.[101]

If there is fat malabsorption, it is prudent to give supplements of fat-soluble vitamins: A, D, E, and K.

In those rare childhood cases in which terminal ileum has been included in the radiation field and there is watery diarrhea, cholestyramine treatment can be tried. Bacterial overgrowth, which might be anticipated where there is stasis of contents, can be treated with antibiotic regimens.

The management of small intestinal inflammation and proctocolitis has been largely empiric, but a combination of sulfasalazine with prednisone has been effective in a few patients.[102] For proctitis and colitis after pelvic irradiation, anti-inflammatory agents have been of some benefit,[103] and short-chain fatty acid enemas have been used to improve symptomatic radiation proctitis.[104]

Severe bleeding or moderate severe bleeding should be traced by endoscopy and treated locally.

VERY LATE EFFECTS OF RADIATION THERAPY

Gastrointestinal problems can develop in long-term survivors of childhood malignancies exposed to therapeutic abdominal radiation. These complications are very rarely reported, perhaps because, previously, long-term survival was not possible. These late gastrointestinal lesions caused by irradiation may be related to new treatments and new combinations of treatment. Supportive therapies such as blood transfusions can influence the immune apparatus of the gut and induce chronic infections, such as *Candida* esophagitis and cytomegalovirus gastritis and colitis during treatment.[105] New drugs are always appearing for use in childhood cancer, but, so far, no long-term direct gastrointestinal sequelae have been reported in children. Most delayed effects in adults are seen between 6 months and 5 years after irradiation.[106] Later events are called "late event" and beyond 20 years (up to 29 years[107,108]) are designated "very late." In children, these time periods are more difficult to define because of small numbers of cases.[109]

In pediatric cancer patients, benign esophageal stricture formation has been reported in five children directly or 2 years after cessation of radiotherapy.[110] Investigations showed esophageal dysmotility with absent peristalsis in these children.

Symptoms were progressive dysphagia. One child with a total radiation dose of 1,320 cGy responded well to esophageal dilatations, three children with a total dose of 5,400 to 6,800 cGy remained symptomatic after 7 to 50 esophageal dilatations, and one child finally required an esophageal replacement. Some of the children with esophageal strictures after radiotherapy had experienced several disease recurrences of rhabdomyosarcoma or Hodgkin disease.[111–114] Another 21-year-old young man had an esophageal stricture 14 years after radiation therapy for Hodgkin disease.[115] Investigations showed esophageal dysmotility with absent peristalsis in all six children. Fibrosis, dense collagen depositions, and ulcerations without an inflammatory infiltrate are seen in pathologic specimens.[116] Gastric lesions have been reported in adults after a dose of 5,500 cGy or more; atrophic gastritis and ulcers are extremely rare. In children, no gastric lesions have been reported.

Very late effects on the small intestine have presenting symptoms of vomiting, abdominal pain (localized or generalized), constipation,[117] diarrhea, bleeding with or without anemia, anorexia, fatigue, wasting, and rarely chronic hypercholereic metabolic acidosis. These symptoms are mainly the expression of subacute intestinal obstruction; they are intermittent and treatable by operation in most cases. A few patients have a continuum of symptoms after acute radiation enteritis advancing into delayed radiation enteropathy without a latent period. The incidence of clinically significant problems is enhanced by abdominal surgery and possibly radiomimetic chemotherapy. Frequency of late effects depends on the dosage delivered. Overall, there is a 5% incidence of fibrosis after 4,000 to 5,000 cGy and an incidence as high as 36% after 6,000 cGy or more. The late effects are anatomically related to fixed parts of the gut, duodenum, or terminal ileum or loops fixed by adhesions after surgery.[118,119]

More mobile parts of the small intestine seem to recover better from acute radiation enteritis. In the affected regions, diffuse fibrosis of the muscularis propria and fibrosis of the submucosa are detected, along with stenosis and ulceration. Vascular lesions in the submucosa and in the mesentery are characterized by variable myointimal proliferations and media fibrosis. These changes involve small arteries and arterioles and small veins. Some small arteries show an acute vasculitis with heavy infiltration of lymphocytes and neutrophils in the media and adventitia, and variable necrosis of the vessel wall. In late radiation, enteritis fibrosis without an inflammatory infiltrate is common, but focal acute vasculitis distant from ulcerations signifies ongoing fibrosis. Villous atrophy is noticed in all resected specimens, suggesting long-term permanent villous alterations in patients treated with radiation therapy to the abdomen.

The incidence of proctosigmoiditis on long-term follow-up is probably 2 to 5% and dose dependent; above 5,000 cGy, the incidence increases at 1-year follow-up to 18 to 37% and above 6,000 cGy to 37%.[4,5]

Secondary malignancies are extremely rare. One child with Wilms tumor developed colon cancer years after chemotherapy, surgery, and radiation.[120]

Another with Wilms tumor developed hepatocellular carcinoma 16 years after chemotherapy, surgery, and radiation.[120]

From the atom bomb survivors in Japan, a slight increase in carcinomas of the esophagus and colon but not the stomach, small intestine, or rectum was reported 25 years after observation. In adults assessed 30 years after irradiation for benign uterine bleeding with a dose range between 695 and 1,050 cGy, the increased risk of secondary malignancy appears to be related to low- and medium-range dosages of radiation instead of a high dosage.[121]

It seems clear that children who have been treated with abdominal radiation for malignant disease must be observed over their lifetimes for possible complications, including secondary malignancy.[122,123]

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CHAPTER 9

ACUTE DIARRHEA

Stefano Guandalini, MD

DEFINITION AND EPIDEMIOLOGY

Acute diarrhea is the abrupt onset of increased fluid content of the stool above the normal value of approximately 10 mL/kg/d. Usually, this situation implies an increased frequency of bowel movements, which can range from 4 to 5 to more than 20 times per day. The augmented water content of the stools is the result of an imbalance in the function of the small and large intestinal processes involved in the absorption and secretion of electrolytes, organic substrates, and thus water.

Acute-onset diarrheal episodes, most often the result of infections of the gastrointestinal tract, continue to be a major problem for worldwide child health. Although in developed countries, its prevalence and severity have declined, acute diarrhea remains an extremely common and often a severe problem. In developing countries, an average of three episodes per child per year in children below 5 years of age is reported, but there are areas with 6 to 8 episodes per year per child. In these settings, malnutrition is an important additional risk factor for acute and prolonged diarrhea.[1] Childhood mortality associated with diarrhea has constantly but slowly declined during the past two decades, mostly because of the widespread use of oral rehydration solutions (ORSs).[2] A recent review of studies published during the previous 10 years found that global diarrheal disease mortality fell to 2.5 million per year.[3] However, despite this progressive reduction in global diarrheal disease mortality, diarrhea morbidity in published reports from 1990 through 2000 has slightly increased worldwide compared with previous reports.[4] Furthermore, we should not ignore that in countries where the toll of diarrhea is highest, poverty also adds an enormous additional burden, and long-term consequences of the vicious cycle of enteric infections, diarrhea, and malnutrition are devastating.[5]

In the United States, there are 1 to 2 episodes per child per year in children below 5 years of age, with 220,000 hospital admissions (or about 10% of all admissions for children in this age range) and about 400 deaths per year.[6,7] Furthermore, acute diarrhea causes 20% of physician referrals for children below the age of 2 years[8] and 10% for those below the age of 3 years.[9] Throughout the world, the most underprivileged populations are most severely affected. In the United States, the mortality rate among blacks is four times that for whites (32.2 vs 8.2 deaths per 100,000 live births).[10,11] Even though gastrointestinal infections are by far the most common cause of acute diarrhea, the sudden onset of increased stool fluid output can indeed be caused by many different disorders. Table 9-1 lists these potential causes in the approximate order of frequency. Many different pathogens can be responsible for infectious diarrhea. Table 9-2 lists the main agents known to infect the intestine and cause acute diarrhea in children. In a multicenter investigation conducted over a period of 1 year in several European countries, my colleagues and I identified a pathogen in 65.6% of 287 children, most commonly *Rotavirus* (35.1%).[12] The frequency with which a particular pathogen is isolated varies widely between different geographic areas and different age

TABLE 9-1 KNOWN CAUSES OF ACUTE DIARRHEA

INFECTIONS
Enteric infections (including food poisoning)
Extraintestinal infections
DRUG INDUCED
Antibiotic associated
Other drugs
FOOD ALLERGIES
Cow's milk protein allergy
Soy protein allergy
Multiple food allergies
DISORDERS OF DIGESTIVE/ABSORPTIVE PROCESSES
Sucrase-isomaltase deficiency
Late-onset (or "adult type") hypolactasia
CHEMOTHERAPY OR RADIATION-INDUCED ENTERITIS
"Surgical" conditions
Acute appendicitis
Intussusception
VITAMIN DEFICIENCIES
Niacin deficiency
INGESTION OF HEAVY METALS
Copper, tin, zinc

TABLE 9-2 MAIN CAUSES OF ACUTE INFECTIOUS DIARRHEA

PATHOGEN IN DEVELOPED COUNTRIES	APPROXIMATE FREQUENCIES IN CASES OF SPORADIC DIARRHEA (%)
VIRUSES	
Rotavirus	25–40
Calicivirus	1–20
Norwalk-like virus	10
Astrovirus	4–9
Enteric-type adenovirus	2–4
BACTERIA	
Campylobacter jejuni	6–8
Salmonella	3–7
Escherichia coli	3–5
Enterotoxigenic	
Enteropathogenic	
Enteroaggregative	
Enteroinvasive	
Enterohemorragic	
Diffusely adherent	
Shigella	0–3
Yersinia enterocolitica	1–2
Clostridium difficile	0–2
Vibrio parahaemolyticus	0–1
Vibrio cholerae 01	Unknown
Vibrio cholerae non-01	Unknown
Aeromonas hydrophila	0–2
PARASITES	
Cryptosporidium	1–3
Giardia lamblia	1–3

groups. For instance, bacteria are generally more common in the first few months of life and then again in school-age children. *Rotavirus*, the single most pervasive cause of infectious diarrhea worldwide, peaks between the ages of 6 and 24 months. In developed countries, intestinal infections are usually sporadic, but outbreaks of foodborne or waterborne infections are well described and continue to occur. Recently, a growing epidemiologic role for Norwalk-like virus has been reported. Norwalk-like virus is now considered to cause an impressive 10% of sporadic cases in developed countries.[13] It should be mentioned that all of the above applies to the general population: in patients with immune disorders, specifically acquired immune deficiency syndrome (AIDS), a much wider array of pathogens is seen. Etiologic diagnosis, not considered necessary in most sporadic cases occurring in immunocompetent children, in subjects with human immunodeficiency virus (HIV) infection is very important for treatment, and it is currently thought that endoscopy is necessary.[14] See Chapter 38, "Infections," for a detailed presentation of individual enteric infections.

It is well accepted that extraintestinal infections (eg, middle ear, lung, and urinary tract infections) can result in acute diarrhea, which is usually mild and self-limited, but the mechanisms for such a relationship are not understood.

Many drugs can induce acute diarrhea as a side effect. Among them, antibiotics have a special place because they frequently cause diarrhea. *Clostridium difficile*[15] may be responsible for many cases of antibiotic-associated diarrhea, but this is not always the case. Indeed, it is thought that in children, the majority of episodes of diarrhea secondary to antibiotic use are not related to *C. difficile*.

The incidence of food allergies has risen during the past decade. Presently, it is assumed that about 3% of all infants are affected by food allergies, of which by far the most common is cow's milk protein allergy (CMPA). A vast array of signs and symptoms are linked to CMPA, but acute diarrhea (typically accompanied by vomiting) is a very common modality of onset. Thus, CMPA should be taken into consideration in the differential diagnosis of acute-onset diarrhea, particularly when the diarrhea fails to resolve within 10 to 14 days.

Although disorders of digestive and absorptive processes are more commonly considered causes of chronic diarrhea or malabsorption syndromes, it is worth remembering that sucrase-isomaltase deficiency may be mistaken for an acute diarrheal illness if the relationship with the intake of sucrose is not detected by accurate history taking. Likewise, lactose intolerance of the older child may not be distinguishable from other forms of diarrhea with an acute onset. In general, the child presenting with acute diarrhea may be showing the early symptoms of a chronic malabsorption syndrome.

In patients treated for cancer by chemotherapy or radiation therapy, acute diarrhea may also ensue as a result of the damage to the intestinal absorptive area. See Chapter 8, "Gastrointestinal Injury," for details.

Several "surgical" conditions (eg, appendicitis[16]) can also present with acute diarrhea as the most obvious clinical sign. This should always be kept in mind when approaching a child with acute-onset diarrhea.

Much rarer disorders resulting in acute diarrhea are niacin deficiency and ingestion of heavy metals.

Finally, it should be noted that despite an aggressive search for the cause of acute diarrhea in children, only in 60 to 70% of cases is it possible to make a diagnosis (usually an intestinal infection). Whatever the cause, typically acute diarrhea in developed countries runs a mild course and resolves, by definition, in less than 14 days. In the previously mentioned study, my colleagues and I found that acute-onset diarrhea lasted a mean of 5.0 ± 2.2 days.[12] Overall, only about 10% of all children had a course more prolonged than 7 days (Figure 9-1).

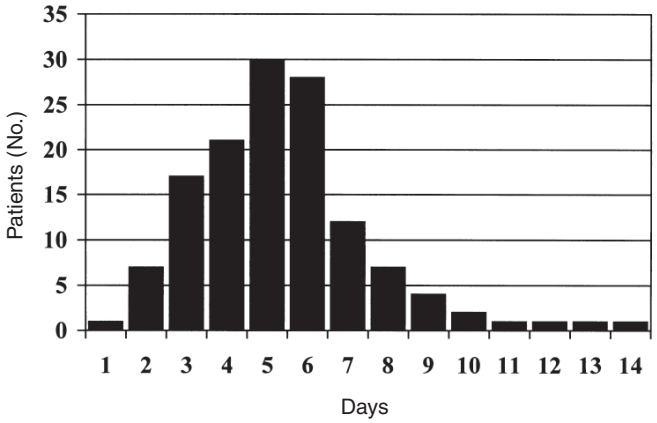


FIGURE 9-1 Bar graph with duration of diarrhea in 456 Italian patients.

PATHOGENESIS

As previously stated, diarrhea results from an imbalance in the intestinal handling of water and electrolytes. Under normal circumstances, the small intestine absorbs large quantities of sodium, chloride, and bicarbonate. It also secretes H^+ ions and, to a lesser extent, bicarbonate and chloride. Water then passively follows the net transport of solutes. The overall absorption of water, sodium, and chloride can therefore be viewed as the result of two opposing unidirectional fluxes of ions, one absorptive and the other secretory. Even though these two processes were once thought to be completely anatomically separated (absorption taking place in the mature epithelial cells lining the villi, whereas secretion is predominantly a crypt process), we now know that they are not, with both absorption and secretion occurring in villi and crypts.[17] Still, the majority of absorption takes place in the villous region, and the bulk of secretion does originate from the crypts. Because the absorptive capacity of the enterocytes quantitatively far exceeds secretory activity, the net result in health is absorption of water and electrolytes.

The basic cellular mechanisms that determine electrolyte absorption and secretion and therefore, when altered, the presence of diarrhea are schematically illustrated in Figure 9-2. The most important ion in drawing net water and nutrient absorption in the gut is sodium. Three different processes of sodium absorption have been described, all driven by Na, K adenosine triphosphatase (ATPase), a basolateral membrane enzyme with a key role in intestinal absorption of ions and nutrients. In fact, Na, K ATPase generates and maintains a Na electrochemical gradient between the gut lumen and the interior of the intestinal epithelial cell, which allows Na to enter the cell downhill along one of the following three paths.

Sodium Absorption Coupled to Nutrients

The entry of glucose and of several groups of amino acids is coupled with high affinity to that of Na throughout the small intestine. A specific carrier called sodium-glucose transporter 1 (SGLT-1) [18] is involved in coupling the entry of glucose (and galactose) across the brush border to that of Na. Furthermore, several, but not all, carriers for different categories of amino acids also couple their entry into the enterocyte with the downhill transport of Na.[19,20] Dipeptide absorption, on the other hand, is not directly coupled to Na absorption but is nevertheless an electrogenic, active process, occurring coupled to the entry of a proton ion (H^+) across the brush border.[21] The existence of the Na-coupled glucose absorption, and its substantial integrity during most acute diarrheal disorders (see below), is considered the pathophysiologic basis for the use of orally administered hydration solutions in children with diarrhea.

Electrogenic, Amyloride-Sensitive Na Absorption

This process allows sodium to enter the cell down its electrochemical gradient, through selective channels, uncoupled to other substrates in the ileum and throughout the colon, where it is preponderant. In the large intestine, electrogenic Na^+ absorption via the epithelial Na^+ channel takes place in the surface epithelium and upper crypts of the distal colon. The cystic fibrosis transmembrane conductance regulator (CFTR) is expressed throughout the colonic epithelium and dominates in the crypts.

It should also be noted that Cl is absorbed throughout the gastrointestinal tract whenever a potential difference is created (serosal side positive) as a result of electrogenic Na absorption through either of the two pathways described above.

Neutral NaCl Absorption

By far the most important process involved in vectorial absorption of Na (and Cl) is the Na-Cl cotransport. This transport process operates throughout the gastrointestinal tract, but it predominates in the small intestine. Transport of the ionic pair NaCl is actually mediated by two coupled antiports; one exchanges Na^+/H^+ (cation

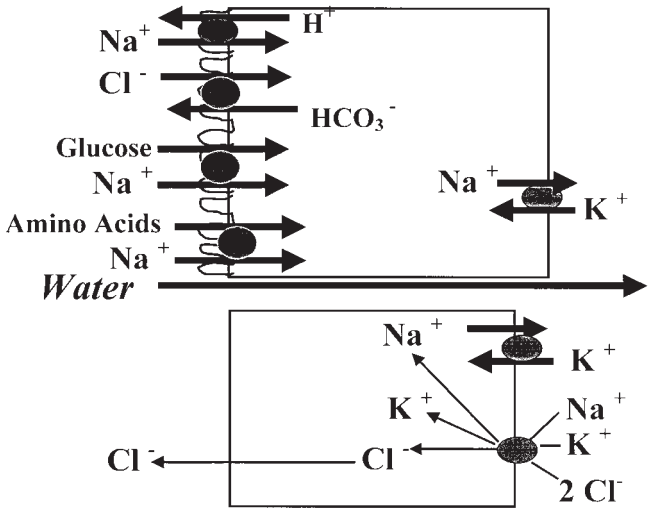


FIGURE 9-2 Main intestinal absorptive/secretory processes for electrolytes. In the villous cell (*top panel*), Na, K adenosine triphosphatase (ATPase) actively extrudes Na in exchange for K, thus maintaining the low intracellular Na concentration, which allows the "downhill" entry of the ionic pair Na-Cl and of the Na-coupled nutrients such as glucose and amino acids. It can also be seen that the entry of the ionic pair Na-Cl is in reality, across most of the intestinal tract, the result of a double antiport, Na being exchanged with H and Cl with HCO_3. In the crypt cell (*bottom panel*), the low Na cell concentration maintained by Na, K ATPase builds a Na gradient between the extracellular compartment and the cell. Energized by this gradient, a carrier in the basolateral membrane (lower part of the figure) couples the flow of one Na, two Cl, and one K from the serosal compartment into the crypt cell. As a result, Cl accumulates above its electrochemical equilibrium and under physiologic circumstances leaks into the lumen across a semipermeable apical membrane. Because absorptive activity in the villous cell quantitatively far exceeds the minor secretion from the crypts (as suggested in the figure by the arrows' sizes), the net result is absorption of electrolytes and nutrients. Water absorption then passively follows, mainly through the intercellular tight junctions.

exchanger), and the other exchanges Cl^-/HCO_3^- (anion exchanger). The Na^+/H^+ antiport operates via specific carriers that have been identified in the past several years and are named the Na-hydrogen exchangers (NHEs). There are six isoforms of NHE (1 to 6)[22]: NHE3 is located in the brush border of the enterocytes and is thought to be the main isoform involved in the transepithelial absorption of Na. The isoform NHE1 is mostly involved in maintaining intracellular pH.[23]

Anion Secretion

Chloride and HCO_3 are the major anions being actively secreted into the gut lumen. Such secretion takes place mainly in the crypts and is electrogenic. As a result, passive diffusion of a cation (usually Na) and water follow.

Overall, the intestinal transport of water and electrolytes is a finely tuned process resulting from the complex interplay of regulatory mechanisms involving the enteric nervous system, cells in the lamina propria, and the epithelial cells. The regulatory agents released and ultimately responsible for the maintenance of this homeostasis include hormone peptides, active amines, arachidonic acid metabolites, and nitric oxide (Table 9-3). Under normal circumstances, such complex interactions eventually act on the intestinal epithelial cells, affecting the described ion transport processes and ultimately resulting in net water and electrolyte absorption. The bulk of absorbed water crosses the intestinal epithelium between the cells (across the tight junctions), following the osmotic gradient generated by the transport of nutrients and electrolytes.

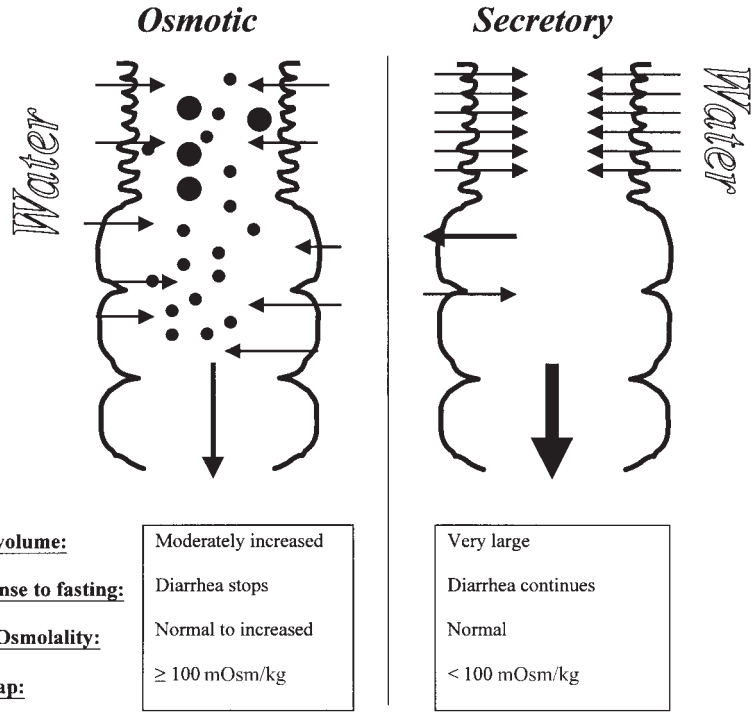
Diarrhea, therefore, is the reversal of this normal net absorptive status to secretion. Such a derangement can be the result of either an osmotic force that acts in the lumen to drive water into the gut or the result of an active secretory state induced in the enterocytes. In the former case, diarrhea is osmolar in nature, as observed after the ingestion of nonabsorbable sugars such as lactulose or of lactose in lactose malabsorbers. In the typical active secretory state, there is enhanced anion secretion mostly by the crypt cell compartment, best exemplified by enterotoxin-induced diarrhea.

These two basic pathogenetic mechanisms are summarized in Figure 9-3. In osmotic diarrhea, the intestinal mucosa cannot digest and/or absorb one or more nutrients. As a consequence, these solutes exert an osmotic force, proportional to their concentration, which drives water, mainly across the leaky tight junctions, into the lumen. Furthermore, as the unabsorbed nutrient is often a carbohydrate, when it reaches the colon it undergoes further digestion by the resident microflora, generating smaller particles and, hence, further contributing to the osmotic drive of water into the lumen. The main features of osmotic diarrhea are described in Figure 9-3. Stool output is proportional to the intake of the unabsorbable substrate and is usually not massive; diarrheal stools promptly regress with discontinuation of the offending nutrient, and the stool ion gap is high, exceeding 100 mOsm/kg. In fact, the fecal osmolality in this circumstance is accounted for not only by the electrolytes but also by the unabsorbed nutrient(s) and their degradation products. The ion gap is obtained by subtracting the concentration of the electrolytes from total osmolality, according to the formula: ion gap = osmolality – [(Na + K) × 2].

In secretory diarrhea, the epithelial cells' ion transport processes are turned into a state of active secretion. This situation can occur as a result of many processes. When dealing with *acute* secretory diarrhea, the most common cause is a bacterial infection of the gut. Several mechanisms may be at work (see Chapter 38).[24] After colonization, enteric pathogens may adhere to or invade the epithelium; they may produce enterotoxins (exotoxins that elicit secretion by increasing an intracellular second messenger) or cyto-

TABLE 9-3 ENDOGENOUS REGULATORS OF INTESTINAL WATER AND ELECTROLYTE TRANSPORT

SOURCE	STIMULATE ABSORPTION	STIMULATE SECRETION
Mucosal epithelial cells	Somatostatin	Serotonin Gastrin cholecystokinin Neurotensin Guanylin Nitric oxide
Lamina propria cells	?	Arachidonic acid metabolites Nitric oxide Several cytokines Bradykinin
Enteric neurons	Norepinephrine Neuropeptide Y	Acetylcholine Serotonin Vasoactive intestinal polypeptide Nitric oxide Substance P Purinergic agonists
Blood	Epinephrine Corticosteroids Mineralocorticosteroids	Vasoactive intestinal polypeptide Calcitonin Prostaglandins Atrial natriuretic peptide



	Osmotic	Secretory
Stool volume:	Moderately increased	Very large
Response to fasting:	Diarrhea stops	Diarrhea continues
Stool Osmolality:	Normal to increased	Normal
Ion Gap:	≥ 100 mOsm/kg	< 100 mOsm/kg

FIGURE 9-3 Scheme of osmotic and secretory diarrhea. The left panel shows the situation in osmotically induced diarrhea. Undigested or unabsorbed substrates remain in the gut lumen and exert an osmotic force proportional to their concentration that drives fluid into the lumen. In the case of unabsorbed carbohydrates, colonic flora enzymes may partially digest them, thus further contributing to the osmotic load and water flow into the lumen. Secretory diarrhea (*right panel*) is characterized by a state of active secretion of anions by the enterocytes. In vivo, equivalent amounts of cations also passively follow, and the result is net secretion of water and electrolytes. The role of the colon varies according to the cause of the secretion.

toxins. They also may trigger release of cytokines attracting inflammatory cells, which, in turn, contribute to the activated secretion by inducing the release of agents such as prostaglandins or platelet-activating factor. Features of secretory diarrhea, reported in Figure 9-3, are a high purging rate, a lack of response to fasting, and a normal stool ion gap (≤ 100 mOsm/kg), indicating that nutrient absorption is intact. The role of the colon in secretory diarrhea varies with the causes.[25–27] Generally speaking, the colonic mucosa's absorptive capacity is maximized; thus, the large intestine partially compensates for the increased small intestinal water loss. However, there are instances in which the colon is either directly involved in the stimulated secretion (ie, when the secretion is the result of a bacterial infection involving the colon) or, even if not challenged topically by a pathogen, may be put in a secretory state via the enteric nervous system as a result of enterotoxin-stimulated secretion going on in the small intestine.[24]

Three intracellular second messengers (cyclic adenosine monophosphate [cAMP], cyclic guanosine monophosphate [cGMP], and Ca^{++}/protein kinase C) have long been recognized as key mediators of secretion (see Table 9-3). They are activated by physiologic events and by various secretagogues, notably bacterial enterotoxins. Increases in any of these second messengers result in a series of biochemical events that activate protein kinases, which act directly on ion channels, inhibiting NaCl-coupled influx and increasing Cl efflux (Figure 9-4). Enterotoxins elaborated by bacterial pathogens selectively and specifically increase either cAMP (eg, cholera toxin and *Escherichia coli* heat-labile toxin) or cGMP (eg, enterotoxigenic *E. coli* [ETEC], enteroaggregative *E. coli* [EAEC], or *Klebsiella* heat-stable enterotoxin—STa). In the mature villous cells, cAMP and cGMP appear to be equally powerful inhibitors of NaCl entry, whereas cAMP is more potent than cGMP in stimulating anion secretion. In the crypts, several components are involved in the cyclic nucleotide and Ca-dependent electrogenic anion secretion. Na, K ATPase in the basolateral membrane maintains a low intracellular Na concentration, thereby allowing a gradient favorable to Na entry from the extracellular environment. Because of this gradient, one Na, two Cl, and one K flow via a carrier in the basolateral membrane from the serosal fluid into the cell (see Figure 9-4). Whereas Na and K may recycle out of the cell, Cl accumulates in the cell above its electrochemical equilibrium. Protein kinases, activated by these cyclic nucleotides and by Ca, then open Cl channels, allowing anions to leave the cell down a favorable electrochemical gradient. The major Cl channel, sensitive to all of the described second messengers, is the CFTR protein,[28] but other anion channels are also present.

More recently, another mechanism for fluid secretion has been elucidated by studies of the zonula occludens toxin from *Vibrio cholerae*.[29] This toxin loosens tight junctions between small intestinal enterocytes,[30] leading to fluid secretion into the lumen.[31] Although examples of purely osmolar and purely secretory diarrheas do occur, in most acute-onset diarrhea, both mechanisms coexist. For example, in rotaviral enteritis, a serious disruption of absorptive functions occurs as a result of the selective invasion of the mature enterocytes by the invading organisms. In this circumstance, osmolar diarrhea ensues. However, the reduction of absorptive cells in the gut lining also unmasks the secretion in the crypts, and a secretory component is superimposed. Furthermore, the secretory nature of rotavirus diarrhea is also augmented by an enterotoxin, the nonstructural protein NSP4, which acts as a viral enterotoxin to induce diarrhea, causing Ca^{++}-dependent transepithelial Cl

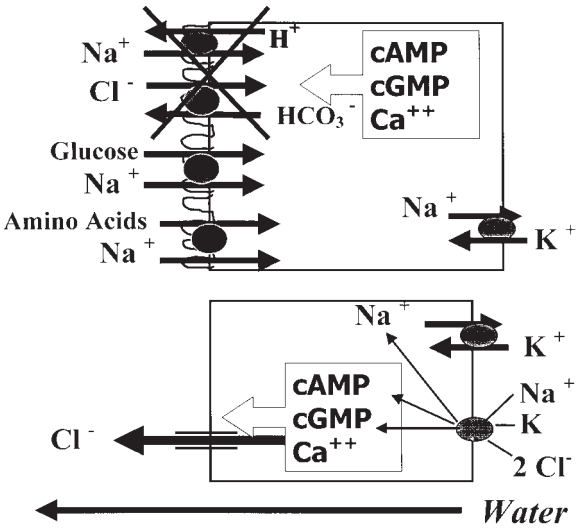


FIGURE 9-4 Secretory changes induced by second messengers. Cyclic adenosine monophosphate (cAMP), cyclic guanosine monophosphate (cGMP), and Ca^{++}/protein kinase C have similar effects. In the mature villous cell (*top panel*), they inhibit the electrically neutral, coupled influx of Na and Cl (which results from the double antiport of Na/H and Cl/HCO_3). In the undifferentiated crypt cell, cAMP, cGMP, and Ca^{++}/protein kinase C act by opening Cl channels (mainly the cystic fibrosis transmembrane conductance regulator) in the luminal membrane. As a consequence, Cl leaves the cell moving down its electrochemical gradient. Because the epithelium cannot secrete only anions, cations (Na) flow across the paracellular pathway, driven by the electrical gradient created by the secretory transport of Cl. Thus, antiabsorptive (mostly but not exclusively in the villous cell) and prosecretory (mostly but not exclusively in the crypt cell) forces combine to shift ions, and with them water, from absorption to secretion.

secretion.[32] Of interest, recently a second example of an enterotoxin-like action by a viral protein has been found, the HIV-1 Tat protein, which acts on ion secretion and on cell proliferation in human intestinal epithelial cells.[33]

Table 9-4 summarizes the main pathogenic mechanisms displayed by the most common agents of infectious diarrhea, along with their predominant site of action within the gut. Chapter 38 contains additional information on the pathogenesis of viral, bacterial, parasitic, and fungal infections.

CLINICAL FEATURES

Acute diarrhea in developed countries is almost invariably a benign, self-limited condition, subsiding within a few days. The clinical presentation and course of illness are dependent on the host and on the infecting organism.

As for the host, age and nutritional status appear to be the most important elements. In fact, the younger the child, the higher is the risk for severe, life-threatening dehydration as a result of the high body water turnover and limited renal compensatory capacity of very young children. Whether younger age also means a risk of running a prolonged course is an unsettled issue. In a series of 453 children, no correlation was found (r = .01, p = not significant) between age at onset and diarrhea duration, but in developing countries, it has been reported that persistent postenteritis diarrhea (PPD) shows a strong inverse correlation with age.[34,35] Nutrition plays an essential role in determining the severity of the diarrheal episode, an effect mediated by several factors, including an altered small intestinal mucosal permeability.[36]

As for the infecting organism, the different pathogenic mechanisms deployed by different infectious agents result in a variable pattern of clinical features (see Table 9-4). Table 9-5 reports clinical features at admission in the previously quoted series of 287 patients.[12] It can be seen that this group of well-nourished children with acute diarrhea from 10 European centers presented mainly with watery or loose stools, often with vomiting and fever. They were mostly in good or fair condition and with either mild or no dehydration in almost 80%. By looking at the clinical features of children grouped according to the pathogen identified, it is evident that these features do vary according to etiology, as one would expect based on the varying pathophysiology. Table 9-6 reports data pooled from our recent series[12] and from a previous series of 154 children,[37] 3 months to 5 years of age. Only patients in whom a single pathogen was identified are reported. When compared with children with either invasive etiology or enterotoxigenic bacteria, it is obvious that patients with rotaviral diarrhea tend to have severe dehydration, vomiting, and watery stools. Fever, crampy abdominal pain, and blood mixed with stools are more common in patients with invasive pathogens such as *Salmonella*, *Shigella*, *Yersinia*, *Campylobacter*, and *Entamoeba histolytica*. The smaller group of children affected by STa-producing ETEC had milder illness, with fecal electrolyte content consistent with a secretory pathogenesis.[38]

In developed countries, diarrhea is basically a self-limited condition. If the proper replenishment of water and electrolytes lost with the stools is provided on an ongoing basis, hydration will be maintained, and the condition will fade within a few days. Sometimes diarrhea fails to subside and undergoes a prolonged course. See Chapter 10, "Persistent Diarrhea," for a detailed presentation of persistent diarrhea. The World Health Organization (WHO) has defined PPD as an episode beginning acutely and lasting for at least 14 days.[39] Between 0 and 1% (in developed countries) and up to 20% (in developing countries) of acute-onset diarrheal episodes run a prolonged course. Table 9-7 lists the main causes leading to the syndrome of PPD. As can be seen, most of the same pathogens that cause acute diarrhea are involved in PPD. Predisposing factors have been described, but by far the most important is a weakened underlying immune status and malnutrition. In fact, PPD, which is frequently seen in association with malnutrition, causes 30 to 40% of all diarrheal deaths in underdeveloped countries.[40,41] In this setting, PPD, in turn, undermines nutritional status, leading to a vicious cycle of diarrhea, malnutrition, and diarrhea.[42] An episode of acute infectious enteritis might evolve into a prolonged course by a variety of mechanisms,[43] including many different morphologic and biochemical abnormalities in the small intestine. In

TABLE 9-4 PATHOGENIC MECHANISMS AND LOCALIZATION OF THE MAIN INTESTINAL PATHOGENS

PREDOMINANT PATHOGENESIS*	SITE OF INFECTION	AGENT	CLINICAL FEATURES
Direct cytopathic effect	Proximal small intestine	*Rotavirus* Enteric-type adenovirus Calicivirus Norwalk-like virus EPEC *Giardia*	Copious watery diarrhea, vomiting, mild to severe dehydration; frequent lactose malabsorption, no hematochezia Course may be severe
Enterotoxigenicity	Small intestine	*Vibrio cholerae* Enterotoxigenic *Escherichia coli* Enteroaggregative *E. coli* *Klebsiella pneumoniae* *Citrobacter freundii* *Cryptosporidium*	Watery diarrhea (can be copious in cholera or ETEC), but usually mild course; no hematochezia
Invasiveness	Distal ileum and colon	*Salmonella* *Shigella* *Yersinia* *Campylobacter* Enteroinvasive *E. coli* *Amoeba*	Dysentery: very frequent stools, cramps, pain, fever, and often hematochezia with white blood cells in stools Variable dehydration Course may be protracted
Cytotoxicity	Colon	*Clostridium difficile* Enterohemorrhagic *E. coli* *Shigella*	Dysentery, abdominal cramps, fever, hematochezia EHEC or *Shigella* may be followed by hemolytic uremic syndrome

EHEC = enterohemorragic *Escherichia coli*; EPEC = enteropathogenic *Escherichia coli*; ETEC = enterotoxigenic *Escherichia coli*.
*Elaboration of various types of enterotoxins affecting ion transport has been demonstrated as an additional virulence factor for almost all of the bacterial pathogens.

some cases, the mucosal villous architecture is almost normal, and in others, continuous or patchy atrophy is seen. Abnormalities of brush border and/or intraluminal digestive enzymes, gut permeability, and/or small bowel intraluminal bacterial flora can be found. In developed countries, the rare occurrence of PPD in otherwise healthy children is most often due to a food protein sensitization. In infants, cow's milk proteins are the most common offending proteins.[44] If acquired during the acute phase of the intestinal infection, this sensitized state might be provoked by increased antigen penetration, secondary to damaged mucosa and increased permeability.

PRINCIPLES OF MANAGEMENT

REHYDRATION

The obvious major risk in acute diarrhea is the loss of water and electrolytes with consequent dehydration and possibly even loss of Na homeostasis. Rehydration or maintenance of hydration is therefore the cornerstone of treatment. Until the mid-1960s, this was accomplished almost exclusively via the intravenous route. Subsequently, the expanded understanding of the pathophysiologic events in intestinal transport processes allowed a dramatic change of approach. In fact, it became apparent that enterotoxigenic bacteria such as *V. cholerae* or ETEC leave intact small intestinal mucosal morphology and absorptive functions. In particular, the glucose-coupled Na influx was found to be fully functional in cholera toxin and other cAMP-induced secretory diarrheas, studied in vitro and in vivo.[45–47] This was later confirmed for the other cyclic nucleotide, cGMP, and its related enterotoxins.[48] Thus, the ongoing absorption of Na and glucose during secretion promotes fluid absorption and allows rehydration to take place in spite of the ongoing fluid loss seen in enterotoxigenic diarrheas. It must be noted that ORSs have been found to be effective even in situations such as rotavirus

TABLE 9-5 CLINICAL FEATURES AT PRESENTATION OF ACUTE DIARRHEA IN 287 PATIENTS

FEATURE	MEAN ± SD OR %
Age (mo)	12.3 ± 4.1
Sex (% females)	39
Weight (kg)	8.8 ± 1.6
Height (cm)	73.7 ± 8.4
Weight/height (percentile)	32 ± 18
Partially breastfed (%)	22.5
Stool characteristics (%)	
Watery	71
Loose	20.8
Mucousy	28.5
Bloody	8.3
Vomiting	60
Fever	59.3
Condition (%)	
Good	50.2
Fair	7.7
Poor	41.3
Dehydration (%)	
Absent	29
Mild	48.5
Moderate	21.8
Severe	0.7

Adapted from Guandalini S et al.[12]

TABLE 9–6 COMPARISON OF CLINICAL FEATURES ASSOCIATED WITH SPECIFIC PATHOGENS: 306 EUROPEAN CHILDREN WITH ACUTE INFECTIOUS DIARRHEA

SYMPTOM/SIGN	ROTAVIRUS (*N* = 180) (%)	INVASIVE PATHOGENS* (*N* = 104) (%)	ENTEROTOXIGENIC *ESCHERICHIA COLI* (*N* = 22)
Shock	2	—	—
Dehydration (moderate to severe)	62	29	18
Vomiting	71	35	14
Fever	26	68	23
Abdominal crampy pain	25	51	32
Watery stools	81	47	64
Hematochezia	5	47	4

Adapted from Guandalini S et al.[12,37]
*Invasive pathogens combine *Salmonella* (*n* = 41), *Shigella* (*n* = 6), *Campylobacter* (*n* = 38), *Yersinia enterocolitica* (*n* = 8), *Entamoeba* (*n* = 11).

enteritis, despite a diffuse damage to the epithelium, which in vitro results in the inhibition of nutrient transport.

These concepts provided the pathophysiologic basis for the WHO-UNICEF–supported and highly successful global program for oral rehydration therapy (ORT). Oral rehydration solutions have proved both safe and effective worldwide in hospital settings and also in the home to prevent dehydration. For more than two decades, the WHO has recommended a standard formulation of glucose-based ORS with 90 mmol/L of sodium and 111 mmol/L of glucose, with a total osmolarity of 311 mmol/L. However, many in vitro and in vivo studies during the 1980s and 1990s had consistently shown that lower concentrations of sodium and glucose enhance solute-induced water absorption and might therefore be superior to the solution with a higher osmolarity, as reviewed in several articles.[49–51]

The ORS originally proposed by the WHO, and so successfully employed over the years in huge numbers of adults and children, might still be preferable for use in areas where the prevalence of cholera is high.[52] In fact, cholera, still a major cause of morbidity and mortality in some parts of the world,[53] is known to induce the highest rates of Na purging, around 90 mmol/L of stools. It should be noticed, however, that when investigated in a controlled trial that compared the two solutions even in children with cholera, they performed equally well.[54] Reduced-osmolarity solutions have concentrations of glucose and Na inferior to those in the WHO solution: glucose ranges between 75 and 100 and Na between 60 and 75 mmol/L, so osmolarity is maintained at 225 to 260 mOsm/L. The use of such ORSs in children of developed countries was originally proposed in 1992 by an ad hoc committee of the European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN).[55] The composition recommended is reported in Table 9-8. Hypo-osmolar ORSs appear to have the additional advantage of allowing a reduced stool output while being just as effective in obtaining and maintaining rehydration and can be safely given throughout the duration of diarrhea, as shown in both developed countries[56] and, more recently, by a large multicenter trial, in developing countries.[57] Indeed, a recent large meta-analysis of all published controlled trials comparing low-osmolarity solutions with standard WHO formulas appearing in the Cochrane Library concluded that "in children admitted to hospital with diarrhoea, reduced osmolarity ORS when compared to WHO standard ORS is associated with fewer unscheduled intravenous fluid infusions, lower stool volume post randomization, and less vomiting. No additional risk of developing hyponatraemia when compared with WHO standard ORS was detected."[58] Thus, the time is probably right for the WHO approach to recommend a global ORS based on a reduced-osmolarity composition.[59]

Studies comparing glucose- versus rice-based ORSs generally show that, although the rice-based ORS tends to reduce stool output during the first 12- to 24-hour periods, both solutions are well accepted and equally efficacious.[60,61]

In a large meta-analysis, ORT was found to provide effective treatment for over 96% of children of developed countries,[62] without need to resort to intravenous rehydration.

In summary, ORT with a glucose-based ORS must be viewed as by far the safest, most physiologic, and most effective way to provide rehydration and maintain hydration in children with acute diarrhea worldwide, as recommended by the WHO, by the ad hoc committee of ESPGHAN,[55,63] and by the American Academy of Pediatrics.[64] Overall, according

TABLE 9-7 MAIN CAUSES OF PERSISTENT POSTENTERITIS DIARRHEA

INTESTINAL INFECTIONS
Enteritis and/or colitis
Rotavirus
Adenovirus
Enteropathogenic *Escherichia coli*
Enteroaggregative *E. coli*
Salmonella
Shigella
Clostridium difficile
Giardia
Cryptosporidium
Small bowel
Bacterial overgrowth
FOOD ALLERGY
Cow's milk protein intolerance
Soy protein intolerance
Multiple food intolerances

TABLE 9–8 COMPOSITION OF THE ORAL REHYDRATION SOLUTIONS RECOMMENDED BY ESPGHAN AND BY WHO

INGREDIENT	ESPGHAN CONCENTRATION (MMOL/L)	WHO CONCENTRATION (MMOL/L)
Glucose	74–111	111
Na	60	90
K	20	20
Base	10 (citrate)	30 (bicarbonate)
Cl	60	80
Osmolality (mOsm/kg)	225–260	331

ESPGHAN = European Society of Pediatric Gastroenterology, Hepatology and Nutrition; WHO = World Health Organization.

to the WHO, the use of ORT for patients with diarrhea rose during the years 1980 to 1993 from less than 5% to about 50% of all episodes of diarrhea worldwide.[65] Indeed, developed countries remain behind in fully using such effective means of treatment, as shown in both Europe[63] and the United States,[66] where rates of use are disappointingly low.

Refeeding

It has been clear for many years that, when affected by gastroenteritis, breastfed infants should be continued on breast milk without any need for interruption. In fact, breastfeeding not only has a well-known protective effect against the development of enteritis,[67] it also promotes faster recovery and provides improved nutrition.[68] This is even more important in developing countries, where withdrawal of breastfeeding during diarrhea has been shown to have a deleterious effect on the development of dehydration in infants with acute watery diarrhea.[69]

In artificially fed infants, however, the issue is much more controversial. For years, the popular remedy for acute diarrhea has been that of fasting, on the intuitive basis that "gut rest" would be beneficial. This long-held view has been challenged during the past several years to the point that today the evidence in favor of rapid refeeding is overwhelming. In fact, many well-conducted studies have provided evidence that in weaned children not severely dehydrated or acidotic, a rapid return to full feeding after having completed oral rehydration in the first 4 to 6 hours is well tolerated.[70–72] Indeed, ORT itself has a beneficial effect on nutrition by stimulating the child's appetite as a result of the improved water and potassium balance. Furthermore, rapid refeeding after adequate ORT has been shown to allow a faster recovery from the abnormally increased intestinal permeability owing to induced enteritis.[73]

Furthermore, evidence has been provided that even in the United States, delaying reintroduction of normal feeds in diarrheic children may have devastating nutritional effects.[74] The Working Group on Acute Diarrhea of ESPGHAN evaluated in a multicenter study the effect of early versus delayed resumption of full feedings in European children with acute diarrhea.[75] The children were first orally rehydrated with a reduced-osmolality solution, formulated according to the previous recommendations,[55] and were then assigned to either the "gradual" or the "early" refeeding group. Two hundred thirty patients (mean age 14 months) were examined; their profile on entry into study showed no statistically significant difference between the two groups. After 4 hours of ORT, the patients who were "early" fed resumed their normal diet, including lactose-containing formulas, whereas the "late feeders" continued to receive only ORS for 24 hours before returning to normal foods. Weight gain proved significantly greater in patients refed early, not only during the first 1 or 2 days after rehydration but also throughout hospitalization, and persisted as late as at day 14 postenrolment. Most important, the two groups did not differ in the incidence of emesis and watery stools. Of note, by day 5, no patient in either group had lactose intolerance.

Lactose intolerance was once thought to be a major problem in children with diarrhea and a reason to delay refeeding milk-based formulas. In fact, human milk is particularly rich in lactose. In spite of the presence of reducing substances in the stools of infants with diarrhea, it is believed that lactose intolerance is not a concern for the vast majority of patients in developed countries.[76] In the ESPGHAN study, only 3% of the children at admission had signs of lactose malabsorption and none at day 5 postenrolment after receiving a normal, lactose-containing formula.[75] The occurrence of lactose intolerance must not be completely disregarded. Occasionally, and with increased frequency in malnourished children, diarrhea may worsen on reintroduction of milk or "normal" formulas. If fecal pH decreases and more than 0.5 to 1% reducing substances are found in the stools, lactose intolerance should be assumed and a lactose-free formula employed at least temporarily to prevent PPD.[77,78]

There is overwhelming evidence that rapid refeeding for most infants beyond the first 3 months of age is safe and effective in acute diarrhea.[42,68,70–72,79–81] Based on this evidence, including the large meta-analysis by Brown and colleagues of the published trials comparing different refeeding regimens,[82] the ESPGHAN Working Group recommended that "the optimal management of mild-to-moderately dehydrated children in Europe should consist of (A) oral rehydration with ORS over 3 to 4 hours, and (B) rapid reintroduction of normal feeding thereafter."[83,84] These principles are equally applicable to the developing world.

Other Treatments: Antibiotics, Micronutrients, Immunoglobulins, Drugs, and Probiotics

Appropriate management of dehydration, electrolyte status, and nutrition, as outlined above, remain the corner-

stones of therapy. In bacterial enteritis, the decision to treat a child with antibiotics is not easy. Pediatric data on newer antimicrobials, such as the fluoroquinolones, are scanty. Furthermore, enteric bacterial pathogens show increased resistance to standard therapy, antibiotics are variably effective, and their use may prolong the carrier status.

In terms of recommended antimicrobial treatment in the nonimmunocompromised host, enteric bacterial and protozoal pathogens can be grouped as follows: (1) agents for whom antimicrobial therapy is always indicated; the consensus includes in this category only *V. cholerae*, *Shigella*, and *Giardia lamblia*; (2) agents for whom antimicrobial therapy is indicated only in selected circumstance; (3) infections by enteropathogenic *E. coli*, when running a prolonged course; enteroinvasive *E. coli*, based on the serologic, genetic, and pathogenic similarities with *Shigella*; (4) *Yersinia* infections in subjects with sickle cell disease; and (5) *Salmonella* infections in the very young infants, if febrile, or with positive blood culture.[85]

Micronutrient deficiencies found in malnourished children with diarrhea include zinc deficiency. In the past few years, a great deal of interest has been generated by the possible role of zinc supplementation in either the prevention or the treatment of acute diarrhea in developing countries, particularly in India and Bangladesh. In a double-blind, controlled study in Bangladesh, Roy and colleagues showed that the supplementation of 20 mg/d of elemental zinc to malnourished children with acute diarrhea resulted in shorter duration of diarrhea, lesser stool output, better weight gain, and improved zinc serum status.[86] All of these changes were again more evident in initially zinc-deficient subjects. Further evidence was subsequently provided in support of the role of zinc supplementation to prevent and treat acute diarrheal diseases in children of developing countries.[87–90] A meta-analysis of trials of adjunctive zinc supplementation in children with diarrhea showed that zinc reduced the duration of the illness by 24%.[91]

In light of these latest acquisitions, it is conceivable that supplementation of zinc should be implemented for children with acute diarrhea or even more generally to malnourished children living in areas with a high risk of developing diarrhea.[92]

Several articles have documented, either in case series or in small uncontrolled trials, the efficacy of oral or enteral immunoglobulin in the treatment of rotavirus diarrhea.[93–96] Although these data are interesting and may apply to certain patients with severe rotavirus diarrhea (immunocompromised or immunocompetent), it does not appear that the cost-benefit ratio would justify this approach on a widespread basis.

The search for the ideal drug to treat acute diarrhea is certainly not new. Considering the burden of morbidity of this condition, it is obvious that having a safe and effective drug would be beneficial. This quest has, however, been largely unsuccessful, and all current guidelines warn against the use of drugs that may be more harmful than useful. Even the impressive search that has taken place in the past two decades as a result of the increased knowledge on the pathophysiology of intestinal secretion has been disappointing because most of the "newer" antisecretory agents (such as chlorpromazine, loperamide, and octreotide) have a limited effect and/or are not devoid of potentially hazardous side effects, making them not an option for acute diarrhea. Recently, however, a new investigational drug has appeared that does show some promise: the enkephalinase inhibitor Acetorphan (racecadotril).[97–100] This molecule is supposed to maintain higher levels of enkephalins by preventing their breakdown: enkephalins would then act by reducing intracellular levels of the cyclic nucleotides cAMP and cGMP, thus ultimately reducing their role in elicited ion secretion (see the previous section on pathophysiology). The drug, which in vivo has been effective in reducing jejunal secretion stimulated by cholera toxin,[101] has been experimented initially in adults with acute-onset diarrhea (reviewed in Lecomte[100]) and later also in children with acute diarrhea of various etiologies, but mostly owing to *Rotavirus*.[102,103] In both pediatric studies, the drug given at 1.5 mg three times a day significantly reduced the stool output and the duration of diarrhea compared with placebo.

One of the most rapidly expanding areas in the treatment and prevention of diarrheal disease has been the use of "probiotics," a term meant to stress the derivation of these bacteria from healthy, live microflora and their beneficial effect on the host.[104] See Chapter 76.2B, "Probiotics," for more details. The use of several strains of lactic acid bacteria to treat human diseases is not new; indeed, lactobacilli are among the most commonly employed bacterial species used to promote health and counteract intestinal infections. Table 9-9 lists the most thoroughly investigated probiotics. Among them, *Lactobacillus rhamnosus* strain GG (ATCC 53103) is by far the most widely investigated. The capacity of this strain to

TABLE 9-9 PROBIOTIC MICROORGANISMS

LACTIC ACID BACTERIA
Lactobacilli
Lactobacillus acidophilus
Lactobacillus rhamnosus
Lactobacillus gasseri
Lactobacillus casei
Lactobacillus reuteri
Lactobacillus plantarum
Lactobacillus bulgaricus
Lactobacillus johnsoni
Lactobacillus lactis
Bifidobacteria
Bifidobacterium bifidum
Bifidobacterium longum
Bifidobacterium breve
Bifidobacterium infantis
Bifidobacterium adolescentis
OTHERS
Bacteria
Escherichia coli
Enterococcus fecalis
Streptococcus thermophilus
Yeasts
Saccharomyces boulardii

transiently colonize the human gut, unlike the strains employed for the production of commonly marketed yogurts, is well established.[105] *Lactobacillus* GG has a number of diverse, potentially beneficial, biologic effects, and in several well-conducted clinical trials, it proved effective in the prevention[106,107] and/or treatment of acute diarrheal disease in children[108,109] and in adults.[110] The effect is definitely most pronounced in rotavirus diarrhea, where a shortening of the duration of the illness,[12,111–113] prevention of PPD,[12] and reduced duration of viral shedding[114] have been documented. Indeed, after many double-blind, placebo-controlled, and randomized investigations have been reported on the efficacy of some strains of *Lactobacillus* in children with acute diarrhea, three recent rigorous meta-analyses have confirmed their safety and effectiveness.[115–117] Some evidence is also starting to mount that probiotics, among them particularly *Lactobacillus* GG, are more beneficial if administered early in the course of the disease.[12,118] Additionally, a meta-analysis found that there could well be a dose-effect pattern (Figure 9-5), with the effect on the duration of diarrhea depending on the dose of probiotic administered.[117] These data are of importance as they point to our need to learn more about the pharmacokinetics of these agents in order to employ them effectively. All of these effects are undoubtedly important in the management of children with acute diarrhea; this modality of treatment therefore shows exciting promise.

Probiotics also appear to be useful in the prevention of nosocomial-acquired infectious diarrhea[119] and of antibiotic-associated diarrhea. A recent meta-analysis of nine published randomized, double-blind, placebo-controlled trials of probiotics suggested that they can be used to prevent antibiotic-associated diarrhea and that both lactobacilli and the yeast *Saccharomyces boulardii* have the potential to be used in this situation.[120]

As more and more candidate probiotics are proposed, it is evident that each one must be studied individually and extensively to prove its efficacy and safety before use in acute diarrhea is recommended.

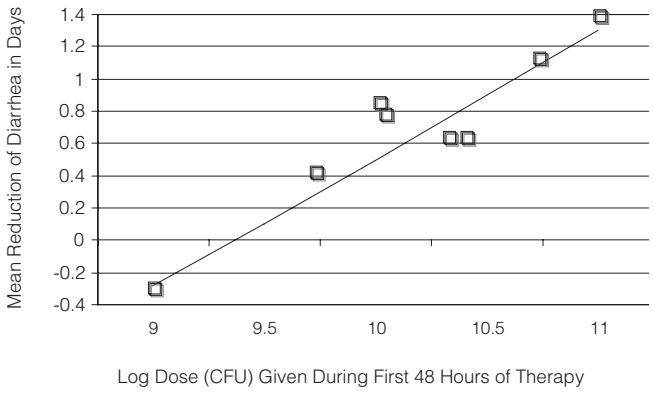


FIGURE 9-5 Dose-effect response between *Lactobacillus* and reduction in duration of diarrhea. A clear dose-effect relationship is apparent from the eight controlled studies that reported diarrhea duration as an outcome. CFU = colony-forming units. Reproduced with permission from Van Niel CW et al.[117]

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CHAPTER 10

PERSISTENT DIARRHEA

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Diarrheal disorders are a major health problem in children worldwide. Studies published in the last 10 years indicate that the global incidence remains unchanged at about 3.2 episodes per child per year, despite a reduction in mortality.[1] Most diarrheal diseases resolve in 1 week, but a small, although consistent, number of cases persist beyond this time. Especially in developing countries, these cases are associated with a high risk of mortality, accounting for most diarrhea-associated fatalities.[2]

A number of definitions have been used for persistent diarrhea, leading to discrepancies in either mortality rates and responsible etiologies in published studies.[3] The World Health Organization (WHO) defines persistent diarrhea as an episode of diarrhea that begins acutely and lasts for 14 days or more.[4] This definition was intended to exclude specific causes of chronic diarrhea such as celiac disease or inflammatory bowel diseases. The difficulties in defining and differentiating diarrhea at its onset and the possibility that a chronic diarrhea may begin acutely support the concept that persistent diarrhea is mainly defined by its duration. In this respect, persistent diarrhea is not different from chronic diarrhea, the conventional duration of which is at least 14 days. However, the term persistent diarrhea is more related to an acute-onset diarrhea that continues behind the expected duration of an infectious diarrhea. Narrowing the spectrum, persistent diarrhea may be defined as protracted and severe diarrhea, that is, diarrhea that persists for more than 2 weeks, leading to nutritional impairment, which may require clinical nutrition.

The definition of persistent diarrhea thus encompasses a wide spectrum of conditions ranging from long-lasting infectious diarrhea to intractable diarrhea, which implies, in its classic definition, a high risk of death.

EPIDEMIOLOGY

The incidence and prevalence of persistent diarrhea show a distinct pattern in developing and industrialized countries. Studies from Asia, Latin America, and Africa support an average incidence of persistent diarrhea as high as 10% of all cases of diarrhea, ranging from 5 to 25% in different settings.[5–7] In light of the burden of infectious diarrhea, persistent diarrhea has a major impact in developing countries. In developed countries, the incidence of chronic diarrhea is inadequately documented. A report from the United Kingdom indicates an incidence of 3 to 5%,[8] but it is likely that this incidence has been reduced in recent years. More importantly, mortality rates associated with persistent diarrhea are dramatically high in developing countries (23–62%) and are responsible for most diarrhea-associated deaths. No data on the general incidence of persistent diarrhea-related fatal outcomes are available in Western countries, but indirect observations suggest a very low mortality rate, with the exception of the so-called intractable diarrhea syndrome. These epidemiologic differences clearly indicate that persistent diarrhea is a distinct disease in developing countries in comparison with developed countries. The concept of two substantially different clinical conditions is supported by studies on primary etiologies and on the risk factors responsible for persistent diarrhea in the two socioenvironmental settings.

PATHOPHYSIOLOGY

The pathophysiologic mechanisms of persistent diarrhea are generally divided into secretory and osmotic, but, in several cases, diarrhea is the result of both mechanisms. Secretory diarrhea is usually associated with large volumes of watery stools and persists even when oral food is withdrawn. In contrast, osmotic diarrhea is dependent on oral feeding, and stool volumes are usually not as massive as in secretory diarrhea.

Secretory Diarrhea

It is characterized by increased electrolyte and water fluxes toward the intestinal lumen, resulting from either the inhibition in neutral NaCl absorption by villous enterocytes or the increase in electrogenic chloride secretion by secretory crypt cells. The classic example of secretory diarrhea is that induced by *Vibrio cholerae* and enterotoxigenic *Escherichia coli*. Cholera toxin produced by *V. cholerae* binds to specific enterocyte membrane receptors activating the adenyl cyclase through the stimulation of an enterocyte G protein. The resulting increase in intracellular cyclic adenosine monophosphate (cAMP), in turn, activates specific signalling proteins, inducing the opening of chloride channels. Intestinal fluid secretion predominantly results from electrogenic chloride secretion through the activation of the cystic fibrosis transmembrane regulator chloride channel, located on the apical membrane of the enterocyte. The

other components of the enterocyte ion secretory machinery are (1) the Na:K:2Cl cotransporter for the electroneutral chloride entrance into the enterocyte; (2) the Na:K pump, which decreases the intracellular Na^+ concentration, determining the driving gradient for further Na^+ inlet; (3) the K^+ selective channel, which enables intracellular K^+, once entered because of coupled Na^+ movement, to return to the extracellular fluid (Figure 10-1).

The stimulation of chloride secretion, in response to cholera or cholera-like toxins, is mediated by the up-regulation of intracellular concentration of mediator cAMP. Other enterotoxins induce intestinal secretion through the activation of cyclic guanosine monophosphate or a rise in intracellular calcium concentration. Recently, another intracellular mediator, nitric oxide, was proposed as a key factor controlling chloride secretion.[9] The classic concept that only bacteria induce secretory diarrhea has now been challenged by the evidence of similar ion secretion pathways induced by viruses and protozoan agents. *Rotavirus*, the most frequent agent of infectious diarrhea, produces a nonstructural protein (NSP4) that stimulates calcium-mediated chloride secretion.[10] Also, human immunodeficiency virus (HIV) induces secretory diarrhea: a protein expressed by HIV, the *trans*-activating transfer factor Tat, acts as an enterotoxin via up-regulation of calcium intracellular concentration.[11] Thus, viruses may induce secretory diarrhea. The protozoon *Cryptosporidium parvum*, a major agent of severe and protracted diarrhea in immunocompromised children,[12] also induces secretory diarrhea through an enterotoxic activity that has been detected in stools and is able to induce chloride secretion through a calcium-mediated pathway.[13] Secretory diarrhea may also be of a noninfectious nature. Several hormones and neurotransmitters have been implicated in intestinal secretion as part of a complex neuroendocrine network that integrates the intestinal response to external stimuli.[14] Electrolyte secretion can be activated by microbial enterotoxins or by other secretagogues of endocrine or nonendocrine origin (Table 10-1). Also, inflammatory cytokines, such as interleukin-1, may exert a direct secretory effect on the enterocyte (see Table 10-1).[15]

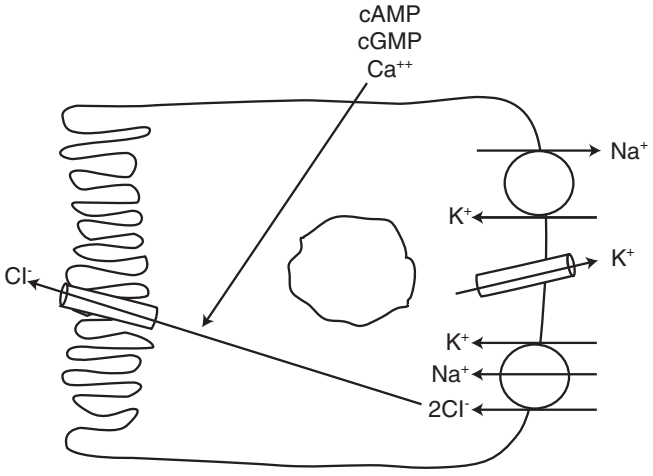

FIGURE 10-1 Components of the secretory machinery in the intestinal epithelial cell. The intestinal crypt cells maintain a secretory tone by a balanced movement of anions and cations through the epithelial monolayer. The Na:K:2Cl cotransporter is responsible for the elecroneutral chloride entrance, from the basolateral membrane, into the enterocyte. The Na:K pump decreases the intracellular Na^+ concentration, indirectly determining a driving gradient for chloride entrance, and the K^+ selective channel enables intracellular K^+ to return to the extracellular fluid. The up-regulation of cyclic adenosine monophosphate (cAMP), cyclic guanosine monophophate (cGMP), or Ca^{2+} induces active chloride secretion of the enterocyte through chloride channels, such as the cystic fibrosis transmembrane regulator, located onto the apical membrane of the enterocyte.

A different mechanism of secretory diarrhea is the inhibition of the electroneutral NaCl-coupled pathway that involves the Na^+-H^+ and the Cl^--HCO_3^- antiporters. Defects in the genes of the Na^+-H^+ and the Cl^--HCO_3^- exchangers are responsible for congenital Na^+ and Cl^- diarrhea, respectively.[16]

Osmotic Diarrhea

Osmotic diarrhea is caused by the presence of nonabsorbed nutrients in the gastrointestinal tract and is generally associated with intestinal damage. The osmotic force driving water into the lumen is provided by nonabsorbed solutes either deriving from food or from injured mucosa. A classic example of osmotic diarrhea is lactose intolerance associated with congenital or acquired lactase deficiency. Lactose, being not absorbed in the small intestine, reaches the colon in its intact form. The colonic microflora ferment the sugar to short-chain organic acids, generating an osmotic load that drives water into the lumen. The ingestion of sugar-containing carbonated fluids exceeding the transport capacity, as well as the ingestion of magnesium salts and sorbitol, both not absorbed, also results in an osmotic load.

In general, osmotic diarrhea occurs whenever digestion and/or absorption are impaired. Reduction or absence of pancreatic enzymes and bile acid disorders are responsible for impaired digestion. Intestinal villi are blunted in overt celiac disease because of antigen-driven immune response. In congenital microvillous atrophy, the functioning absorptive surface is reduced because of a genetic developmental disorder involving the brush border (Figure 10-2). In short-bowel

TABLE 10-1 MAIN FACTORS DETERMINING ELECTROLYTE AND WATER SECRETION

Cyclic adenosine monophosphate dependent
Bacterial enterotoxins: *Vibrio cholerae*, *Escherichia coli* (heat labile), *Shigella*, *Salmonella*, *Campylobacter*, *Pseudomonas*
Hormones: vasoactive intestinal peptide, gastrin, secretin
Anion surfactants: bile acids, ricinoleic acid
Cyclic guanosine monophosphate dependent
Bacterial enterotoxins: *E. coli* (heat stable) enterotoxin, *Klebsiella pneumoniae*, *Citrobacter freundii*, *Yersinia enterocolitica* enterotoxin
Hormones: guanylin
Calcium dependent
Bacterial enterotoxins: *Clostridium*, *Cryptosporidium*
Viral enterotoxins: *Rotavirus* nonstructural protein 4, HIV Tat
Endogenous factors: histamine, interleukin-1β and -8, bradykinin, cholecystokinin
Neurohormones: acetylcholine, serotonin, galanin

HIV Tat = human immunodeficiency virus *trans*-activating transfer factor.

syndrome, surgical removal of a large portion of the intestine does not leave enough of the absorbing intestine. Intestinal absorption depends on an intact epithelium but also on an adequate time for digestion and contact between the nutrients and the absorptive surface. Thus, alterations in intestinal transit times, particularly reductions in small intestinal and whole-gut transit times, may result in impaired nutrient, electrolyte, and water intestinal absorption. Finally, increased gut permeability, secondary to inflammation or cytotoxic agents, is responsible for excessive protein loss, as in protein-losing enteropathies. Infectious agents induce diarrhea with an osmotic mechanism when they are responsible for direct epithelial or mucosal damage, as in the case of enteroadherent or enteropathogenic *E. coli* (Figure 10-3).

However, various pathways generally contribute to persistent diarrhea, interacting with each other and producing a synergic vicious circle. A paradigm of the complex pathophysiology of persistent diarrhea is provided by HIV infection. Chronic diarrhea is considered an acquired immune deficiency syndrome (AIDS)-defining condition in the classification scheme for pediatric HIV infection.[17] Malnutrition can be an early manifestation of HIV infection and is associated with a rapid decrease in the CD4+ cell number and an increased rate of opportunistic infections.[18] A long list of combined dysfunctions of the digestive-absorptive processes is observed in 60 to 80% of children with HIV infection naive to antiretroviral therapy and may involve the intestine, the liver, and the pancreas.[19] Clinical manifestations of the so-called HIV enteropathy may be limited, but iron and lactose malabsorption is very common.[20] Overall, nutrient malabsorption certainly contributes to malnutrition and eventually to wasting, the terminal feature of HIV infection. The pathophysiology of intestinal dysfunction is complex and involves multiple factors. There is little evidence of a role of specific enteric pathogens in HIV-associated intestinal dysfunction, even though *Cryptosporidium* is recognized as specifically responsible for intestinal inflammation and secondary digestive abnormalities in these children.[21] A role of HIV itself has been hypothesized, and recent data showed that Tat protein released by the virus functions both as a viral cytotoxin and an enterotoxin, directly interacting with the enterocyte to impair cell growth and proliferation, as well as ion transport.[11] The role of HIV in intestinal dysfunction is supported by the finding that children shifted to highly active antiretroviral therapy showed a rapid normalization of intestinal function tests, in parallel with a decrease in viral load and an increase in CD4+ cell number.[22] Interestingly, it was also shown that nutritional rehabilitation is effective in the improvement of the immune status, thus supporting the cause-and-effect relationship between malnutrition, intestinal dysfunction, and immune derangement.[23] Thus, HIV-associated intestinal dysfunction, malnutrition, and immune impairment produce a vicious circle and represent the paradigm of the high risk for persistent diarrhea and its high mortality in poor countries, following intestinal infections (Figure 10-4).

RISK FACTORS

Most studies on risk factors for persistent diarrhea have been performed in developing countries. Caloric and proteic malnutrition appears to be the most relevant risk factor for an intestinal infection to evolve into persistent diarrhea. The coexistence of malnutrition in children with infectious diarrhea consistently increases the probability of a protracted duration, with an inverse correlation between nutritional status and the severity of the diarrhea.[24,25] Also, specific micronutrient deficiencies are related to persistent diarrhea. Vitamin A and zinc deficiencies are significantly associated with persistent diarrhea, and clinical trials assessing the efficacy of their supplementation support these observations.[26–31] Infants who are not breastfed are at increased risk of persistent diarrhea, whereas prolonged breastfeeding is a

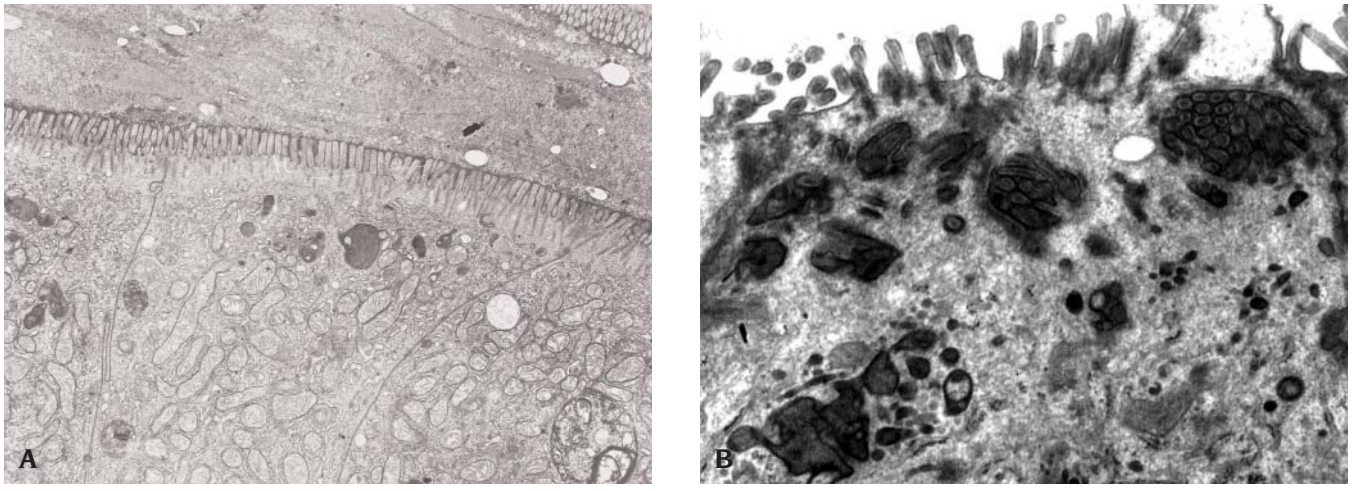

FIGURE 10-2 Ultrastructural evaluation of enterocytes. *A*, Enterocytes show signs of mild, nonspecific damage; microvilli look shorter than normal; cytoplasm is normal with the exception of an increased vacuolization. Courtesy of M. Morroni, Ancona, Italy (transmission electron micrograph, stained with lead citrate). *B*, Microvillous inclusion disease is characterized by loss and disorganization of microvilli of the brush border. In the cytoplasm, secretory granules and inclusion bodies with microvilli are evident. Courtesy of A. Phillips, London, UK (transmission electron micrograph, double stained with lead citrate and uranyl acetate).

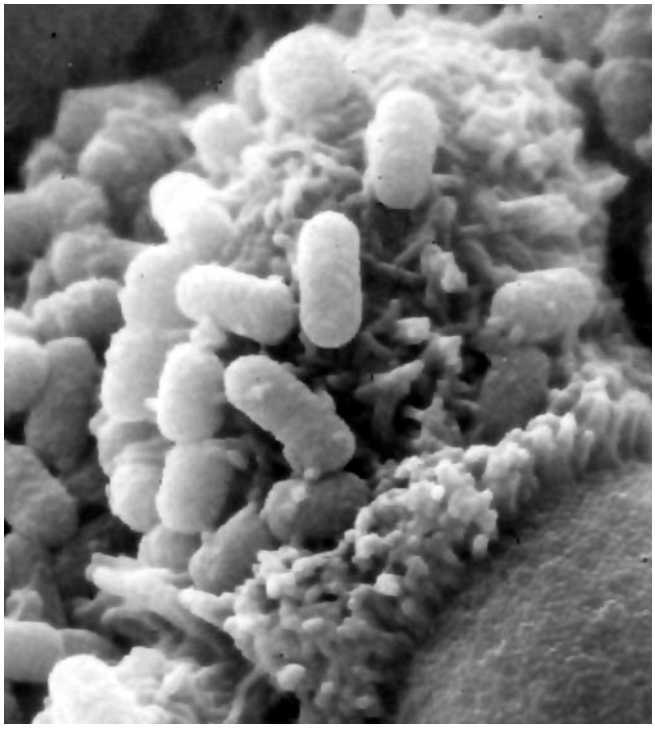

FIGURE 10-3 Enteropathogenic *Escherichia coli* adhesion to intestinal epithelial cells. Adherence is the first stage of enteropathogenic *E. coli*–induced diarrhea. Following that, a number of bacterial proteins are translocated by a type III secretion system, driving the enterocyte effacement, pedestal formation, and intimate bacterial attachment to the host cell. Courtesy of A. Phillips, London, UK (scanning electron micrograph, critically point dried in liquid carbon dioxide and coated with gold-palladium).

protective factor.[25,32] Because malnutrition is also associated with extraintestinal infections, persistent diarrhea is significantly associated with a broad range of health problems, such as pneumonia, urinary tract infections, and anemia.[33] Prior illnesses have also been implicated in triggering protracted diarrhea, and among them, measles carries an increased risk for diarrhea, which may persist as long as 6 months because of its immunosuppressive effect.[34] The major etiology of acquired immunosuppression, the HIV infection, is the leading underlying condition of persistent diarrhea in developing countries, where access to effective antiretroviral therapies is limited or not available.[35] Selected intestinal infections, induced by classic enteropathogenic agents, including *Shigella* and enterotoxigenic *E. coli*, have been implicated as determinants of persistent diarrhea in developing countries.[36,37]

A diarrhea presenting with increased severity at its onset may be associated with an increased risk of long duration, especially when malnutrition is present.[38] Persistent diarrhea is generally more frequent in males, with a male-to-female ratio of 1.2 to 2.6:1 and in the age range of 6 to 24 months.[6,25,36] The age of the mother may also be a determinant of persistent diarrhea, with an increased risk found in children born to younger mothers.[39,40] The use of antibiotics for acute gastroenteritis is not generally recommended and has recently been reported as a specific risk factor for persistent diarrhea.[25]

On the other side, risk factors for persistent diarrhea also exist in developed countries and depend on the specific etiology of persistent diarrhea. For selected genetic diseases such as congenital Na^+ or Cl^- diarrhea, a history of intractable diarrhea in the family may be reported. A family history of fatal diarrhea was identified as a risk factor for the intractable diarrhea syndrome in a population of 32 consecutive children admitted to receive parenteral nutrition.[41] In the same population, the lack of breastfeeding, parental atopy, and early (less than 3 months) onset of symptoms had an increased incidence compared with controls.[41]

ETIOLOGY AND SPECIFIC CLINICAL FEATURES

The overall prevalent etiologic pattern of persistent or chronic diarrhea is substantially different in the two distinct socioenvironmental settings considered. Persistent diarrhea in developing countries has its own epidemiology, risk factors, and, more importantly, a peculiar etiologic pattern, different from that seen in children living in industrialized countries. These differences can be mainly ascribed to the overwhelming rate of intestinal infections observed in developing and transitional countries, where other causes of persistent diarrhea become almost negligible. In addition, diagnostic facilities are not easily accessible in developing countries, raising problems in diagnosing the rare diseases that are responsible for persistent diarrhea in industrialized countries. A list of etiologies of persistent diarrhea is reported in Table 10-2.

Enteric infections are by far the most frequent cause of persistent diarrhea in developing countries. Extensive efforts have been made to identify specific pathogens responsible for persistent diarrhea.[42] The results are scattered through different geographic regions. Consecutive cultures during episodes of persistent diarrhea have shown that the same organism is not always found during prolonged illness, suggesting that sequential infections with the same or a different pathogen may be responsible for prolonged symptoms.[43] Enteroadherent *E. coli* have been specifically implicated in persistent diarrhea (see Figure

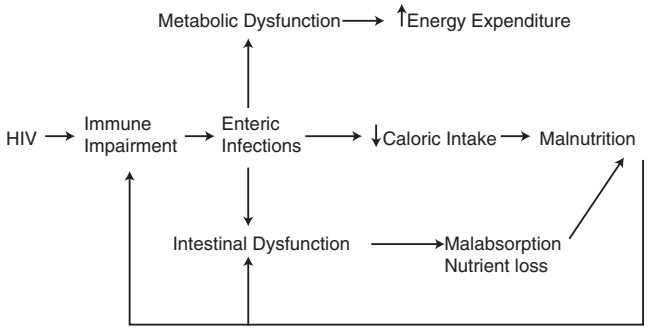

FIGURE 10-4 Pathways of malnutrition in human immunodeficiency virus (HIV)-infected children. A complex interplay exists among several conditions, leading to malnutrition in HIV-infected children. Enteric infections and intestinal dysfunction are major determinants of wasting, an acquired immune deficiency syndrome (AIDS)-defining condition.

TABLE 10-2 CAUSES OF PERSISTENT DIARRHEA

Infections
Bacterial: *Shigella*, *Salmonella*, *Yersinia enterocolitica*, *Escherichia coli*, *Clostridium difficile*, *Campylobacter jejuni*, *Vibrio cholerae*, *Mycobacterium avium* complex
Viral: rotavirus, adenovirus, astrovirus, torovirus, cytomegalovirus, HIV
Parasitic: *Cryptosporidium*, *Giardia*, *Entamoeba histolytica*, *Isospora*, *Strongyloides*
Postenteritis syndrome
Small bowel overgrowth
Tropical sprue
Diarrhea associated with exogenous substances: excessive intake of carbonated fluid, dietetic foods containing sorbitol, mannitol, or xylitol; excessive intake of antacids or laxatives containing lactulose or $Mg(OH)_2$; excessive intake of methylxanthine-containing drinks (cola, tea, coffee)
Abnormal digestive processes: cystic fibrosis, Shwachman-Diamond syndrome, isolated pancreatic enzyme pancreatitis, chronic pancreatitis, Pearson syndrome; trypsin/chymotrypsin, enterokinase deficiency
Disorders of bile acids: chronic cholestasis, use of bile acid sequestrants, primary bile acid malabsorption, terminal ileum resection
Carbohydrate malabsorption: congenital or acquired sucrase-isomaltase deficiency, congenital or acquired lactase deficiency, glucose-galactose malabsorption, fructose malabsorption
Immune-based disorders: food allergy, celiac disease, eosinophilic gastroenteritis, inflammatory bowel disease, autoimmune enteropathy, primary immunodeficiencies
Structural defects: microvillous inclusion disease, tufting enteropathy, phenotypic diarrhea, heparan-sulfate deficiency, $\alpha_2\beta_1$ and $\alpha_6\beta_4$ integrin deficiency, lymphangiectasia
Defects in electrolyte and metabolite transport: congenital chloride diarrhea, congenital sodium diarrhea, acrodermatitis enteropathica, selective folate deficiency, abetalipoproteinemia
Motility disorders: Hirschsprung disease, intestinal pseudo-obstruction (neurogenic and myophatic), thyrotoxicosis
Surgical causes: congenital or acquired short bowel (secondary to stenosis, segmental atresia, malrotation)
Neoplastic diseases: neuroendocrine hormone-producing tumors: VIPoma, APUDomas, mastocytosis

HIV = human immunodeficiency virus.

10-3).[44–46] Less compelling evidence suggests a role for *Shigella*, enterotoxigenic *E. coli*, and *Campylobacter*.[36,37] *Cryptosporidium* was often found in persistent episodes in Bangladesh but not in Peru.[43,47] Fatal diarrhea induced by *Cryptosporidium* was associated with a specific deficiency in interferon-γ production.[48] *Giardia lamblia* shows similar incidence rates in acute and persistent diarrhea. Among viruses, *Rotavirus* has been associated with severe and protracted diarrhea[43,49] and implicated in life-threatening intractable diarrhea syndrome in developed countries.[50] Cytomegalovirus is a possible cause of intractable diarrhea, also in the immunocompetent child.[51] Torovirus has been found in children with persistent diarrhea but may be associated with enteroadherent *E. coli*.[52] Also, astrovirus has recently been associated with persistent diarrhea.[53]

In children with AIDS, opportunistic agents are a major cause of persistent diarrhea. Opportunistic agents are defined as microorganisms that induce diarrhea exclusively or, in unusually severe forms, in target populations, such as immunocompromised children. Enteric cryptosporidiosis is the most frequent cause of severe and protracted diarrhea in HIV-infected children.[12] Infections with *Blastocystis hominis*, *Coccidia*, *Mycobacterium avium*, *Isospora belli*, and *Candida albicans* should be specifically considered in children with AIDS and persistent diarrhea.[54,55] Finally, HIV may be directly responsible for diarrhea and the so-called HIV enteropathy.[11]

In rich countries, persistent infections are directly responsible for a relative minority of persistent diarrhea cases.[41,56,57] However, indirect consequences of enteric infections do play a major role. Postenteritis syndrome is a clinicopathologic condition in which small intestinal mucosal damage persists following acute gastroenteritis. Sensitization to food antigens and secondary disaccharidase deficiency were classically considered to be responsible for postenteritis syndrome. More recent studies have demonstrated that the incidence of disaccharidase deficiency is very low following acute diarrhea.[58,59] Similarly, the role of sensitization to food antigens has a relatively lower incidence than previously thought, and international guidelines discourage the use of hypoallergenic or diluted milk formulas during acute gastroenteritis.[60,61] A third mechanism of postenteritis syndrome is believed to be an infection or reinfection with an enteric pathogen. However, the mechanisms of postenteritis diarrhea remain to be fully clarified.

Small bowel bacterial overgrowth induces persistent diarrhea through multiple mechanisms. In normal conditions, bacterial load in the proximal jejunal fluid does not exceed 10^4 colony forming units (CFU)/mL of aerobic bacteria. An increase over 10^5 CFU/mL in duodenal fluid, or the presence of anaerobic bacterial species that are normally detected only in more distal intestinal segments, is believed to be responsible for severe impairment of digestive and absorptive processes. Diarrhea may be the result of either a direct interaction between a microorganism and the enterocyte or the consequence of deconjugation and dehydroxylation of bile salts and hydroxylation of fatty acids operated by enteric bacteria.

Persistent diarrhea may be the result of maldigestion owing to pancreatic disorders. In most patients with cystic fibrosis, pancreatic insufficiency results in fat and protein malabsorption. In Shwachman syndrome, exocrine pancreatic hypoplasia can be associated with neutropenia, bone changes, and intestinal protein loss. Specific isolated pancreatic enzyme defects result in fat or protein malabsorption. Familial pancreatitis, associated with a mutation in the trypsinogen gene, is associated with chronic pancreatitis, pancreatic insufficiency, and persistent diarrhea.

Liver disorders, such as cholestasis, may lead to a reduction in the bile acid pool with fat malabsorption. Bile acid

loss may be associated with terminal ileum diseases, such as Crohn disease, or with terminal ileum resection. A rare disease is primary bile acid malabsorption attributable to mutations of the ileal bile acid transporter,[62] and neonates and young infants present with fat malabsorption and chronic diarrhea. Long-term use of bile acid binders such as cholestyramine may be responsible for continued bile acid loss in the stools, leading to decreased bile acid pool size.

Increased intraluminal osmolarity and subsequent diarrhea are the end points of excessive sugar-containing carbonated fluid or fruit juice intake, which exceeds the transport capacities of the small intestine in younger infants. Excessive intake of sorbitol, magnesium hydroxide, and lactulose is also responsible for persistent diarrhea. Persistent diarrhea may also be the manifestation of carbohydrate malabsorption because of specific molecular defects. These include lactose intolerance, sucrase-isomaltase deficiency, and congenital glucose-galactose malabsorption. Lactose intolerance is rarely associated with congenital lactase deficiency but is more frequently a consequence of lactase deficiency owing to mucosal damage. A progressive loss of lactase activity, which affects about 80% of the nonwhite population, may be responsible for persistent diarrhea in older children receiving cow's milk.[63]

The extreme severity spectrum of persistent diarrhea includes a number of heterogeneous conditions leading to the so-called intractable diarrhea syndrome. This was originally described as diarrhea lasting more than 2 weeks, with no detectable infectious etiology, starting in the first 3 months of age and loaded with a high mortality rate.[64] More recent definitions reflect the concept that the syndrome, in its typical setting, is the result of a permanent defect in the structure or function of intestine, leading to progressive intestinal failure with the need for parenteral nutrition for survival.[56] The intractable diarrhea syndrome has an evolving etiologic spectrum in which enteric infections and food intolerances have been replaced by more rare congenital conditions (Table 10-3).[41,56,57] The main groups of diseases causing severe and protracted diarrhea include structural enterocyte defects, immune-based disorders, multiple food intolerance, motility disorders, and short gut.[57] This classification is based on the main disorder responsible for diarrhea and has implications for the outcome of the disease.[57] However, the etiology of persistent diarrhea includes a larger number of specific diseases that are herein briefly described.

An increasing number of molecular defects are responsible for a wide variety of electrolyte transport defects. In congenital chloride diarrhea, a mutation in the solute carrier family 26 member 3 gene (*SLC26A3*) leads to severe intestinal Cl^- malabsorption owing to the defect or absence of the Cl^-/ HCO_3^- exchanger.[65] The consequent defect in HCO_3^- secretion leads to metabolic alkalosis and acidification of the intestinal content, with further inhibition of Na^+/H^+ exchanger-dependent Na^+ absorption. Patients with congenital sodium diarrhea show parallel clinical features because of a defective Na^+/H^+ exchanger in all segments of the small and large intestine, the presence of extremely high Na^+ fecal concentration, and severe acidosis.[66]

Structural enterocyte defects, based on specific, yet largely unknown, molecular defects, are responsible for early-onset severe diarrhea.[67] In microvillous inclusion disease, there is a net reduction of the absorptive surface area, associated with massive active secretion of electrolytes in the stools. The ultrastructural hallmark of the disease is the lack of microvilli on the apical enterocyte surface and the presence of secretory granules and membrane-bound inclusions lined by microvilli (see Figure 10-2).[68] Evidence has been obtained that inclusion bodies originate from autophagocytosis of the apical membrane of enterocytes, with engulfing of microvilli.[69] Intestinal epithelial dysplasia (or tufting enteropathy) is relatively more common than microvillous inclusion disease and is characterized by various degrees of morphologic abnormalities mainly localized in the epithelial layer, including disorganization of surface enterocytes with focal crowding and formation of tufts (Figure 10-5).[70] Abnormal laminin and heparan sulfate proteoglycan deposition on the basement membrane have been detected in intestinal epithelia from infants with tufting enteropathy.[71] Also, a defect in the distribution of integrins has been reported.[71] These ubiquitous proteins are involved in cell-cell and cell-matrix interactions and play a crucial role in cell differentiation and tissue development. An abnormal intestinal distribution of $\alpha_2\beta_1$ and $\alpha_6\beta_4$ integrins has been implicated in tufting enteropathy.[72] Congenital heparan sulfate deficiency is an extremely rare disorder,

TABLE 10-3 EVOLVING ETIOLOGIES OF SEVERE PROTRACTED DIARRHEA IN CHILDREN IN ITALY

	1977–1993 (*N* = 38)	1993–1996 (*N* = 32)	1997–2001 (*N* = 61)
ETIOLOGY	***n*** **(%)**	***n*** **(%)**	***n*** **(%)**
Enteric infection	18 (48)	4 (12)	2 (3)
Food intolerance	8 (22)	3 (10)	10 (17)
Autoimmune enteropathy	2 (5)	8 (25)	7 (12)
Structural enterocyte defects	2 (5)	7 (22)	16 (26)
Celiac disease	1 (2.5)	0 (0)	0 (0)
Eosinophilic enteropathy	1 (2.5)	1 (3)	0 (0)
Lymphangiectasia	1 (2.5)	1 (3)	2 (3)
Motility disorders	2 (5)	3 (9)	16 (26)
Munchausen syndrome by proxy	0 (0)	0 (0)	1 (1.5)
Unknown	3 (7.5)	5 (16)	7 (11.5)

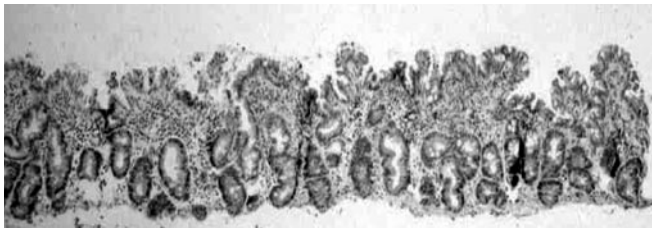

FIGURE 10-5 Small intestinal mucosal histology of a child with tufting enteropathy. The epithelial layer appears partially detached by the basal membrane. On tips of villi, enterocytes are focally crowded with formation of typical tufts. Courtesy of A. Barabino and C. Marino, G. Gaslini Hospital, Genoa, Italy (hematoxylin and eosin; ×10 original magnification).

with severe enteric albumin loss presenting within the first weeks of life.[73] Heparan sulfate is a glucosaminoglycan component of the basement membrane with multiple roles in the intestine, including restriction of charged macromolecules within the vascular lumen.

Phenotypic diarrhea is characterized by immunodeficiency and facial abnormalities, woolly hair, and intractable diarrhea with a typical familiar pattern (Figure 10-6).[74]

Persistent diarrhea may have an immune or allergic pathogenesis. Cow's milk protein allergy, as well as other food allergies, may determine abnormalities of the small intestinal mucosa. Multiple food intolerance is included in most series of intractable diarrhea syndrome. However, this is usually an exclusion diagnosis based on a relationship between any ingested food and diarrhea. In most cases, multiple food intolerance is not eventually confirmed by oral challenge.

The intestine may be the target of specific autoimmune processes that are responsible for persistent diarrhea and, in more severe cases, for intestinal failure. Autoimmune enteropathy is characterized by the production of antienterocyte antibodies, primarily immunoglobulin G, directed against components of enterocyte brush border or cytoplasm. In association, a cell-mediated autoimmune response is detected with a mucosal T-cell activation.[75] Abnormal immune function, as seen in patients with agammaglobulinemia, isolated immunoglobulin A deficiency, and combined immunodeficiency disorders, can result in persistent diarrhea induced by a wide spectrum of microorganisms, as in AIDS.

Disorders of intestinal motility are an emerging group of intestinal diseases associated with persistent diarrhea. Motility disorders include alterations of the enteric nervous system development and function, such as in Hirschsprung disease, aganglionosis, and chronic intestinal pseudo-obstruction (which encompasses both neurogenic and myogenic forms).[76,77] Recently, alterations of the connective-tissue plexus layer, which roots circular and longitudinal intestinal muscles, have been identified in children with chronic intestinal pseudo-obstruction.[78] Other motility disorders may be secondary to extraintestinal disorders, such as in hyperthyroidism. Motility disorders are associated with either constipation or diarrhea, or both, with the former usually dominating the clinical picture.

Short-gut syndrome is associated with persistent diarrhea. All intestinal abnormalities, such as stenosis, segmental atresia, and malrotation, may require surgical resection. In these conditions, the residual intestine may be insufficient to carry on its normal digestive-absorptive functions. Alternatively, small bowel bacterial overgrowth may be the main mechanism involved in diarrhea, such as in blind loop syndrome.

In rare cases of severe persistent diarrhea, the gastrointestinal symptoms may be the initial manifestation of a mitochondrial disease.[79] Finally, in cases in which a cause of diarrhea is not detected and the clinical course is inconsistent, Munchausen syndrome by proxy should be considered.

FIGURE 10-6 Intractable diarrhea associated with phenotypic abnormalities. *A*, In rare cases, intractable diarrhea may be associated with facial dysmorphism, hypertelorism, and woolly, easily removable hair with trichorexis nodosa. *B*, Jejunal biopsy specimens show total or partial villous atrophy with crypt necrosis and inconstant T-cell activation. All of the patients have defective antibody responses despite normal serum immunoglobulin levels. Courtesy of A. Barabino and C. Marino, G. Gaslini Hospital, Genoa, Italy (hematoxylin and eosin; ×10 original magnification).

The natural history of intractable diarrhea is related to the primary intestinal disease.[57] Food intolerances generally resolve in a few weeks or months, as does autoimmune enteropathy, when appropriate immunosuppression is started. Children with motility disorders show more severe, long-lasting symptoms but a less severe course, whereas those with structural enterocyte defects never recover, undergo a more severe course, generally needing parenteral nutrition, and often become candidates for intestinal transplant.[57] The natural history of short gut encompasses all the outcomes, depending on the length of remaining gut, its function, child age, and management.

APPROACH TO THE CHILD WITH PERSISTENT DIARRHEA

Because of the wide etiologic spectrum of persistent diarrhea in children, medical decisions should be based on diagnostic algorithms that begin with the age of the child, then consider clinical and epidemiologic factors, and always take into account the results of microbiologic investigations.

Specific clues in the family and personal history may provide useful indications. A history of chronic or intractable diarrhea in a relative suggests a genetic disease, particularly if presentation occurred in the first months of life. A family history positive for immune or atopic diseases points toward allergy or autoimmunity.

The presence of polyhydramnios is consistent with congenital chloride or sodium diarrhea. A previous episode of acute gastroenteritis is suggestive of postenteritis syndrome, whereas a history of recent intestinal resection strongly points to short-bowel syndrome or blind loop syndrome. Finally, the association of diarrhea with ingestion of specific foods should always be considered for possible intolerance to one or more food antigens.

Initial clinical examination should include the evaluation of general and nutritional status. It is not rare to face a critical clinical condition with dehydration, marasmus, or kwashiorkor, requiring prompt supportive interventions to stabilize the patient. The presence of eczema or asthma is associated with an allergic disorder, and specific extraintestinal manifestations (eg, arthritis, diabetes, thrombocytopenia) may suggest an autoimmune disease. Specific skin lesions may be suggestive of enteropathic acrodermatitis. Typical facial abnormalities and woolly hair are associated with phenotypic diarrhea (see Figure 10-6).

If the child with persistent diarrhea lives in a developing country or comes from a poor social setting in which the typical risk factors for persistent diarrhea, including malnutrition, are common, an intestinal infection should be suspected. However, in all cases of persistent diarrhea, irrespective of risk factors, the diagnostic approach should include stool cultures and a search for parasites, *Rotavirus*, and other enteric viruses.

The evaluation of nutritional status is crucial to establish the need for rapid intervention. It should start with the evaluation of the weight and height curves and of the weight-for-height index to determine the impact of diarrhea on growth failure. Malnutrition may precede the onset of diarrhea, thus contributing to its long duration, or it could be the consequence of the disease, suggesting the presence of malabsorption. Weight gain is generally impaired before height growth, but, with time, linear growth is also affected, and both parameters may be equally abnormal in the long term. Assessment of nutritional status includes dietary history and biochemical and nutritional investigations. Caloric intake should be quantitatively determined. Biochemical markers include albumin (half-life 20 days), prealbumin (half-life 2 days), retinol binding protein (half-life 12 hours), transferrin (half-life 8 days), serum iron, iron binding capacity, and micronutrient concentrations. The half-life of serum proteins may help distinguishing short- and long-term malnutrition. Assessment of body composition may be performed by measuring midarm circumference and triceps skinfold thickness or, more accurately, by bioelectrical impedance analysis or dual-emission x-ray absorptiometry scans.

The relationship between weight modifications and energy intake should be carefully considered. In infants and children who are apparently thriving or overweight while suffering from chronic diarrhea, a 1-week dietary record may be used to explore the hypothesis that the youngster is being overfed or is drinking excessive amounts of juices or beverages with a high sucrose content. Conversely, a child with persistent diarrhea and suspected malabsorption may be receiving diluted hypocaloric formula or even clear liquids in an effort to reduce diarrhea, and persistent diarrhea may be an indirect consequence of ongoing malnutrition.

Search for etiology may be based on the pathophysiology of diarrhea. Electrolyte concentrations in fecal samples discriminate between secretory and osmotic diarrhea and may provide important information for guiding the subsequent diagnostic approach (Table 10-4). In parallel, assessment of intestinal function has a key role in the diagnostic approach and should be performed in a noninvasive manner (Table 10-5).

Microbiologic investigation of stool samples should include a thorough list of agents and may provide valuable information. A search for proximal intestinal bacterial overgrowth is part of a microbiologic investigation. The breath hydrogen test, after glucose oral load, may identify an abnormal bacterial proliferation in the small bowel. The breath test can also be used for detecting carbohydrate malabsorption.

Diagnostic workup of persistent diarrhea is largely based on the specific diagnostic tools available; however, endoscopy and histology provide essential information. Small intestinal biopsy was effective in detecting a primary intestinal etiology in more than 90% of cases of chronic

TABLE 10-4 DIFFERENTIAL DIAGNOSIS BETWEEN SECRETIVE AND OSMOTIC DIARRHEA

	SECRETORY	OSMOTIC
Osmotic gap	< 50 mOsm/kg	> 135 mOsm/kg
Cl^- concentration	> 40 mEq/L	< 35 mEq/L
pH	> 6.0	<5.6
Na^+ concentration	> 70 mEq/L	< 70 mEq/L

TABLE 10-5 NONINVASIVE INTESTINAL FUNCTION TESTS

TEST	NORMAL VALUES	IMPLICATION	REFERENCE
α_1-Antitrypsin concentration	< 0.9 mg/g	Increased intestinal permeability/protein loss	Catassi C, Cardinali E, D'Angelo G, et al. Reliability of random fecal alpha 1-antitrypsin determination on nondried stools. J Pediatr 1986;109:500–2.
Steatocrit	< 2.5% (older than 2 yr); fold increase over age-related values (below 2 years)	Fat malabsorption	Guarino A, Tarallo L, Greco L, et al. Reference values of the steatocrit and its modification in diarrheal diseases. J Pediatr Gastroenterol Nutr1992;14:268–74.
Feces-reducing substances	Absent	Carbohydrate malabsorption	Lindquist BL, Wranne L. Problems in anlysis of faecal sugar. Arch Dis Child 1976;51:319–21.
Calprotectin concentration	< 50 µg/g (older than 4 yr)	Intestinal inflammation	Fagerberg UL, Loof L, Mersoug RD, et al. Fecal calprotectin levels in healthy children studied with an improved assay. J Pediatr Gastroenterol Nutr 2003;37:468–72.
Fecal leukocytes	< 5/microscopic field	Colonic inflammation	Harris JC, Dupont HL, Hornick RB.Fecal leukocytes in diarrheal illness. Ann Intern Med 1972;76:697–703.
Nitric oxide in rectal dialysate	< 5 µM of NO_2^-/NO_3^-	Rectal inflammation	Canani RB, Cirillo P, Bruzzese E, et al. Nitric oxide production in rectal dialysate is a marker of disease activity and location in children with inflammatory bowel disease. Am J Gastroenterol 2002;97:1574–6.
Elastase concentration	> 200 µg/g	Pancreas function	Carroccio A, Fontana M, Spagnuolo MI, et al. Pancreatic dysfunction and its association with fat malabsorption in HIV infected children. Gut 1998;43:558–63.
Chymotrypsin concentration	> 7.5 U/g; > 375 U/24 h	Pancreas function	Carroccio A, Iacono G, Lerro P, et al. Role of pancreatic impairment in growth recovery during gluten-free diet in childhood celiac disease. Gastroenterology 1997;112:1839–44.
Fecal occult blood	Absent	Fecal blood loss	Fine KD. The prevalence of occult gastrointestinal bleeding in celiac sprue. N Engl J Med 1996;334:1163–7.

Adapted from Eherer AJ, Fordtran JS. Fecal osmotic gap and pH in experimental diarrhea of various causes. Gastroenterology 1992;103:545–51; and Fine KD, Schiller LR. AGA technical review on the evaluation and management of chronic diarrhea. Gastroenterology 1999;116:1464–86.

diarrhea.[80] Colonoscopy should be performed in all cases of chronic diarrhea in which gross or occult blood is detected in the stools or when an increased frequency of mucoid stools and abdominal pain suggests colonic involvement. Biopsies should be performed at multiple sites, even in a normal-appearing intestine, because at least 5% of apparently normal colons will yield specimens positive for colitis, when a disease characterized by patchy lesions is responsible for the observed symptoms. Histology is important to establish the degree of mucosal involvement, through grading of intestinal damage and the presence of associated abnormalities, such as inflammatory infiltration of the lamina propria. Morphometry provides additional quantitative information on epithelial changes. In selected cases, light microscopy may help identifying specific intracellular agents, such as cytomegalovirus from the presence of large inclusion bodies in infected cells[51] or intracellular parasites. Electron microscopy is indicated in all cases of intractable diarrhea of unknown etiology and is essential to detect microvillous inclusion disease or other cellular ultrastructural abnormalities. Immunohistochemistry allows the study of mucosal immune activation and of other cell types (smooth muscle and neuronal cells and enterocytes), as well as components of the basal membrane. An immunohistologic classification of intractable diarrhea, with prognostic implications, was originally proposed by Cuenod and colleagues.[81] The authors recognized a group of children with severe immune activation and epithelial damage and another group with no mucosal damage and likely affected by inborn defects of enterocyte differentiation.

Imaging has a major role in the diagnostic approach to persistent diarrhea. Preliminary abdominal radiography is useful for detecting gaseous distention, suggestive of a gastrointestinal obstruction. Intramural or biliary gas may be seen in necrotizing enterocolitis or intestinal invagination. Structural abnormalities such as diverticula, malrotation, stenosis, blind loop syndrome, and motility disorders may be appreciated after a barium meal and an entire bowel follow-through examination.

Specific investigations should be carried out when a specific diagnostic suspect is posed. A valuable tool in the diagnosis of bile acid malabsorption is the measure of retention, in the enterohepatic circulation, of the bile acid analogue $^{(75)}$Se-homocholic acid taurine as an index of ileal bile acid absorption.[82] A scintigraphic examination, with labeled octreotide, is indicated in cases of suspected APUD (amine precursor uptake decarboxylation) cell neoplastic diseases.[83] In other diseases, other specific imaging techniques, such as computed tomography or nuclear magnetic resonance imaging, may have an important diagnostic value.

Once infections have been excluded, a schematic flowchart for the approach to the child with persistent diarrhea may be applied. The main etiologies of persistent diarrhea may be investigated, based on the features of diarrhea and their predominant or selective intestinal dysfunction (Figure 10-7). A step-by-step diagnostic approach is important to minimize the invasiveness to the child and the overall costs, while optimizing the yield of the diagnostic workup.

THERAPY

Persistent diarrhea associated with impaired nutritional status should always be considered a serious disease, and therapy should be promptly started. Treatment of persistent diarrhea can be schematically divided into general supportive measures, nutritional rehabilitation, and drug treatment. The latter includes specific therapy targeted to individual etiologies and treatment aimed at counteracting fluid secretion and/or promoting restoration of disrupted intestinal epithelium.

Because death in most instances is caused by dehydration, replacement of fluid and electrolyte losses is the major early intervention. Rehydration is best performed through the oral route with oral rehydration solution (ORS).[60] Recent studies provide data on the efficacy of hypotonic ORSs rather than isotonic solutions.[84,85] The addition of amino acids to glucose-based ORSs, or the substitution of rice gruel or cereal for glucose, has been proposed to create a "super ORS," which may provide advantages over the pure sugar and electrolyte conventional WHO ORS.[86,87] Amylase-resistant starch (pectin), added to an ORS, is not digested and absorbed in the upper bowel but reaches the colon, where bacteria break it down to short-chain fatty acids, having fluid absorptive effects.[88,89] It may be added to ORS to specifically counteract secretory diarrheas.

In malnourished children, nutritional rehabilitation is essential, also when an enteric infection is documented. Exclusion diets are usually administered with the double purpose of overcoming food intolerance, which may be the primary cause of persistent diarrhea or its complication. The sequence of elimination should be graded from less to more restricted diets, that is, cow's milk protein hydrolysate to an amino acid–based formula or vice versa, depending on the severity of the child's conditions. If the latter are severe, it may be convenient to start with amino acid–based feeding formula. Even though a disaccharidase deficiency is no longer recognized as a major cause of persistent diarrhea, hypolactasia is often secondary to intestinal damage and malnutrition, irrespective of its primary etiology. A lactose-free diet should be started in all children with persistent diarrhea and is included in a treatment algorithm designed by the WHO.[90] Lactose is generally withdrawn and replaced by maltodextrins or a combination of other carbohydrates. Sucrose-free formulas are indicated in sucrase-isomaltase deficiency.

A sufficient number of calories should always be provided. Caloric intake may be progressively increased to 50% or more above the Recommended Dietary Allowance for age and sex. In children who do not tolerate high feeding volumes, caloric density may be increased by adding fat

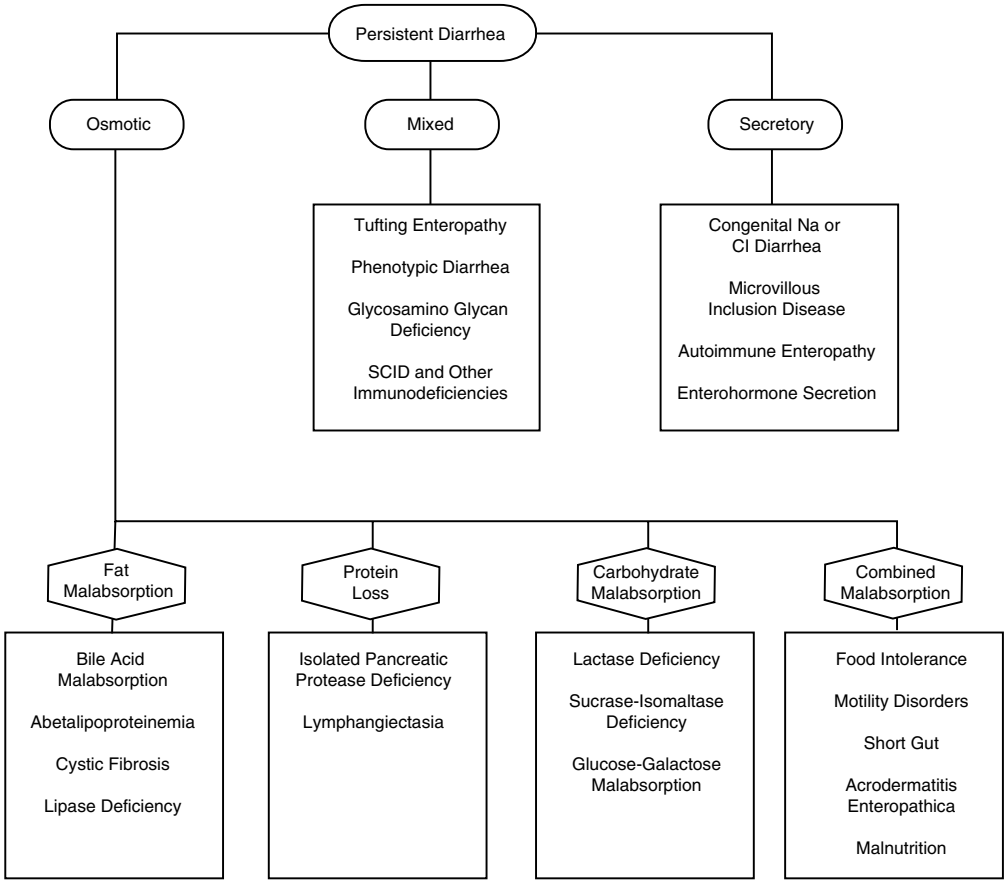

FIGURE 10-7 Scheme of specific etiologies of persistent diarrhea according to its pathophysiology. Assessment of the secretory, osmotic, or mixed mechanism of diarrhea and of predominant nutrient malabsorption may help identify the primary etiology, thereby directing the diagnostic workup through specific investigations. SCID = severe combined immunodeficiency.

or carbohydrate. However, formula osmolality should be checked and the intestinal absorbing capacity should be monitored by digestive function tests. In children with steatorrhea, medium-chain triglycerides may be the main source of lipids because they are easily absorbed.

In several cases, clinical nutrition should be considered; this includes enteral or parenteral nutrition. Enteral nutrition may be performed via a nasogastric or gastrostomy tube and is indicated in a child who is not able to be fed through the oral route either because of primary intestinal diseases or because of extreme weakness. Continuous enteral nutrition is effective in children with a reduced absorptive function. The rationale of continuous enteral nutrition is based on the increased ratio of time to the absorptive surface. A reduced surface functioning for extended time increases daily nutrient absorption. In children with extreme wasting, enteral nutrition may not be sufficient. In such cases, parenteral nutrition may be a life-saving procedure. Parenteral nutrition should be undertaken at an early phase, as soon as other, less invasive, nutritional approaches have been unsuccessfully attempted.

Nutritional rehabilitation has a general beneficial effect on general condition, intestinal function, and immune response. Continuous enteral nutrition was used in children with HIV and intestinal malabsorption and was effective in increasing their body weight while restoring intestinal absorptive function and inducing a rise in CD4 cell number.[23] Micronutrient and vitamin supplementation is part of nutritional rehabilitation and prevents further problems, especially in malnourished children from developing countries.[28,29]

Specific therapy includes anti-infectious drugs and immunosuppression. When a specific infectious agent is detected, specific treatment should be undertaken. In case of bacterial intestinal infection, antibiotic therapy can be administered.

In *Rotavirus*-induced diarrhea, oral administration of human immunoglobulins has been demonstrated to be effective both in immunocompetent and immunocompromised children and should be considered for treatment in severe or protracted diarrhea.[91,92] Human serum immunoglobulins, available in preparations for intravenous use, may be administered through the oral route at a dose of 300 mg/kg of body weight in a single oral dose. The rationale of passive immunotherapy is based on the demonstration of neutralizing antibodies against all viruses of medical importance in the preparations for intravenous use.[91]

Diarrheal diseases are consistently associated with modifications of intestinal microflora. An attempt at modifying intestinal microflora may be worth considering, even when an infectious cause is suspected but not proved. Two distinct strategies are available: the administration of probiotic bacteria and the administration of antibiotics. Large and reliable meta-analyses demonstrated an efficacy of probiotic administration both in the prevention and treatment of acute and protracted diarrhea.[93,94] Alternatively, empiric antibiotic therapy may be undertaken in children with either small bowel bacterial overgrowth or with suspected bacterial diarrhea. A specific indication for persistent diarrhea has been shown for trimethoprim-sulfamethoxazole.[95] Metronidazole is a reasonable alternative for the broad pattern of target agents, including parasites. The so-called bowel cocktail (metronidazole, cholestyramine, and high-dose gentamicin given orally) has been proposed for severe protracted diarrhea of suspected infectious etiology, although conclusive proof of efficacy is lacking.[96]

Specific therapy with immunosuppressive drugs should be considered in selected conditions such as autoimmune enteropathy. Azathioprine, cyclosporine, and tacrolimus have been used in severe protracted diarrhea of immune origin. Autoimmune enteropathy may be successfully controlled by immunosuppression, allowing withdrawal of parenteral nutrition.[57,97]

Treatment may also be directed at modifying specific pathophysiologic processes. Most secretory diarrheas are infectious, but intestinal ion secretion is also a common mechanism of the intractable diarrhea of infancy. In these cases, the use of drugs that are able to modify intestinal ion transport may be considered. Among proabsorptive agents, the enkephalinase inhibitor racecadotril inhibits the breakdown of natural endogenous opiates (enkephalins) by intestinal tissue and has been effective in controlled clinical trials.[98,99] In severe secretory diarrheas, such as in neuroendocrine tumors, microvillous inclusion disease, and enterotoxin-induced severe diarrhea, a trial with somatostatin or its analogue, octreotide, may be considered.[100] Octreotide has been used in diarrhea secondary to neoplastic diseases and in intestinal infections. Subcutaneous administration of octreotide was effective in reducing fecal output in HIV-infected children with severe cryptosporidiosis, and a specific antagonist effect against the enterotoxic activity associated with *Cryptosporidium* has been shown in vitro.[101] Loperamide and chlorpromazine have also been used in children with severe and protracted diarrhea, but they are loaded with several major side effects, particularly in children. Growth hormone has been used as a trophic factor in the short-gut syndrome and may have an additional beneficial effect in secretory diarrhea because it inhibits chloride secretion and promotes sodium absorption through a direct effect on the enterocyte.[102–104] Growth hormone may be an ideal drug in case of severe and protracted diarrhea when both epithelial atrophy and ion secretion are associated.

However, when other attempts have failed, the only option may be parenteral nutrition or surgery, including intestinal transplant.

CONCLUSIONS

Persistent diarrhea is still a major problem worldwide, but with two distinct presentations. In developing and transitional countries, persistent diarrhea is a relatively frequent outcome of intestinal infections and is loaded with a high case-fatality ratio, mainly because of the combined effect of enteric infections, intestinal dysfunction, malnutrition, and immunosuppression. In industrialized countries, the etiologic spectrum of persistent diarrhea is broader, with infections still playing a role, particularly in children with immunosuppression, but with a progressively increasing

number of primary, and usually irreversible, intestinal diseases. Optimal diagnostic approach and general management require advanced knowledge and technology and should be carried on in tertiary care centers or through a close interaction among experts in gastroenterology, nutrition, infectious diseases, and pediatric surgery.

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CHAPTER 11

PROTEIN-LOSING ENTEROPATHY

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The loss of serum proteins across the gut mucosa can occur in association with a wide variety of gastrointestinal and nongastrointestinal disease states in children. The effect on the patient is largely the result of the balance between excessive enteric protein loss and hepatic protein synthesis, protein distribution, and degradation in the rest of the body. This chapter reviews the relevant aspects of serum protein metabolism and the alterations that occur in a number of disease states.

NORMAL ALBUMIN METABOLISM

Albumin is a water-soluble molecule (molecular weight 65,000) that maintains plasma oncotic pressure and functions as a transport protein for hormones, metals, ions, bilirubin, and a variety of other biologic molecules. Because of the nonselective nature of intestinal protein loss, serum albumin level serves as an indicator of the degree to which abnormal loss of many different proteins may be occurring.

Serum albumin concentration depends on relative rates of synthesis and breakdown and on patterns of tissue distribution. Albumin is synthesized in the liver at a rate of 120 to 200 mg/kg/d in the normal adult, with somewhat higher rates (180–300 mg/kg/d) during the first year of life.[1] The predominant factor affecting albumin synthesis is nutrient intake. Food or protein deprivation is associated with a decrease in albumin synthesis of 50% within 24 hours. Meal-stimulated increases in hepatic albumin synthesis have been demonstrated in vivo using isotopic techniques.[2] Amino acids, especially tryptophan, appear to be essential nutritional requirements.[3,4] Hormonal influences also play a role, with cortisol, thyroid hormone, and insulin stimulating albumin synthesis.[5] Experimentally, hypophysectomy and diabetes may be associated with decreased synthesis that corrects with growth hormone or insulin replacement, respectively.[6,7] A variety of proinflammatory cytokines may also decrease the synthesis of albumin as part of the hepatic "acute-phase response" to inflammation.[8,9]

Approximately one-third of total-body albumin is intravascular. Extravascular albumin is distributed throughout the skin (30–40% of the total extravascular pool), muscle, and viscera. An exchangeable pool of extravascular albumin exists that can be mobilized to conserve serum albumin levels. The exchangeable pool size is 6 to 8 g/kg for children less than 1 year of age and 3 to 4 g/kg in the older child. Malnutrition may decrease the exchangeable pool to one-third of its normal size.

CONTRIBUTION OF THE GASTROINTESTINAL TRACT TO ALBUMIN METABOLISM

Between 6 and 10% of the plasma albumin pool is degraded in a 24-hour period. Loss into the gastrointestinal tract accounts for less than 10% of albumin degradation in healthy subjects. In patients with protein-losing enteropathy, however, albumin catabolic rates are 43% higher than in healthy controls. Much of this albumin loss is at the expense of plasma albumin, with the net effect of increasing the fractional catabolic rate of the plasma pool by a factor of 3. This loss provokes a relatively modest increase in hepatic albumin synthesis (24%), suggesting a limited capacity of the liver to respond to these losses.[10,11]

The site of albumin loss at the mucosal surface is not well characterized. Postulated mechanisms include diffusion between mucosal cells, rupture of lymphatics through the mucosal surface, and leakage of protein across ulcerated mucosa.[12] An immunofluorescent study in a canine model has demonstrated that in the setting of elevated intestinal venous pressure, transmucosal albumin flux occurred exclusively at the villus tip region. At normal venous pressures, no loss was observed.[13] The sites of protein loss attributable to other mechanisms have not been characterized.

METHODS OF DIAGNOSIS OF ENTERIC PROTEIN LOSS

Methods for documenting enteric loss of plasma proteins have evolved to elucidate normal physiologic losses and alterations attributable to various diseases. Techniques employed for these studies involve either the injection of radiolabeled substances and determination of radioactivity in enteric secretions or direct measurement of an endogenous protein in enteric secretions. Ideally, the molecules employed in these techniques should distribute and be metabolized similarly to other plasma proteins; they should not be selectively secreted, digested, or reabsorbed by the gastrointestinal tract. If a radiolabel is employed, it should remain bound to its carrier and, if dissociated within the gastrointestinal tract, should not be reabsorbed.[14] The technique employed must be safe, reproducible, and cost-effective.

Radiolabeled proteins that have been used to determine enteric protein loss have included iodine 131(^{131}I) albumin, chromium 51 (^{51}Cr) albumin, and copper 67 (^{67}Cu) ceruloplasmin. Radioiodinated albumin was initially employed for a variety of physiologic studies of plasma protein distribution and metabolism and thus initially seemed to be a likely candidate for similar studies of enteric protein loss. However, free iodine is actively secreted by the salivary glands and gastric mucosa and is reabsorbed in the intestine, so it is not suitable for these studies. Because chromium is not actively reabsorbed or secreted into the intestine in appreciable quantities, ^{51}Cr-labeled albumin has been used more extensively. The labeled albumin is given as an intravenous injection, and stools are collected for several days and assayed for radioactivity.[15,16] The use of ^{51}Cr albumin has been somewhat limited in the pediatric population because of the need for a several-day stool collection, the requirement that stool not be contaminated by urine, the concern about the use of radioactive agents in young patients, and, in recent years, the unavailability of the radiopharmaceutical. The use of ^{67}Cu ceruloplasmin gives similar results to those obtained with ^{51}Cr albumin, but its use has been limited by its cost and its brief half-life.[14,17] More recently, technetium 99m (^{99m}Tc)-labeled albumin has been used to visualize scintigraphically the sites of gastrointestinal protein loss.[18,19] In these studies, serial imaging following intravenous injection of the ^{99m}Tc-labeled albumin has allowed not only the detection of excessive enteric protein loss but also localization to the stomach, small intestine, or colon. Such discrete localization has the potential to identify surgically correctable lesions in the setting of localized loss and may also facilitate specific diagnosis of etiology based on the affected portion of the gastrointestinal tract.

Studies of enteric protein loss have also employed radiolabeled chemical agents believed to behave metabolically in a fashion similar to that of plasma proteins. ^{131}I polyvinylpyrrolidone has been used for this purpose but has been faulted because of the release of iodine from the carrier molecule and because of variability in the molecular weights of the preparations employed.[20] Iron 59 (^{59}Fe)-labeled iron dextran has also been tried.[21] Chromic chloride 51 ($^{51}CrCl_3$) can be injected intravenously; it can then bind to plasma proteins in vivo to give results similar to those of studies employing injected ^{51}Cr albumin.[22]

In recent years, the measurement of fecal concentrations of α_1-antitrypsin (α_1-AT) has been used more routinely for documentation of intestinal protein loss.[23,24] α_1-AT has a molecular weight similar to that of albumin and is not actively secreted, absorbed, or digested by the gastrointestinal tract. α_1-AT can be detected in the stool following lyophilization, extraction by solubilization, and subsequent immunologic assay, with radial immunodiffusion appearing to be superior to immunonephelometry.[25] Stools can be collected over several days and α_1-AT clearance determined, or fecal concentration of α_1-AT in random single-stool specimens can be compared with control values. Variations in the extraction procedure used have led to differences in the absolute values of α_1-AT concentrations obtained by different investigators, so control values may be different, depending on the method employed.

The concentration of fecal α_1-AT correlates well with enteric protein losses in children with various gastrointestinal disorders.[26,27] This assay has been shown to correlate with disease activity and response to therapy. Clearance of α_1-AT correlates closely with determinations made by the intravenous $^{51}CrCl_3$ technique.[28,29] Spot sample determinations of fecal α_1-AT concentrations agree with measures of α_1-AT clearance done on more prolonged stool collections.[30,31] Thus, the simplicity of this test, combined with its avoidance of radioisotopes, has made the α_1-AT assay very appealing for use in clinical pediatrics. Modifications that may make the assay even easier and less expensive have been suggested.[30,32]

Although the fecal α_1-AT assay has been shown to be useful clinically, assay results should be interpreted carefully. α_1-AT is degraded at a pH of less than 3; therefore, the fecal α_1-AT assay is not reliable for assessing gastric protein loss. Small amounts of blood loss into the gastrointestinal tract will not appreciably alter fecal α_1-AT concentration, but gross hematochezia will.[26] Fecal α_1-AT concentrations may vary during infancy, depending on the age of the patient and the type of feeding.[33,34] The synthesis and secretion of α_1-AT by intestinal epithelial cells, the process of which has been documented,[35] may increase in response to the inflammatory cytokines interleukin-1 and interleukin-6.[36] These findings raise the possibility that measurements of fecal α_1-AT concentrations in the setting of underlying gastrointestinal inflammation may reflect epithelial secretion along with systemic protein loss. In spite of these considerations, the relative ease and reliability of the fecal α_1-AT assay are likely to result in its continued use clinically as the primary method for documenting enteric protein loss.

GASTROINTESTINAL DISEASES ASSOCIATED WITH ENTERIC PROTEIN LOSS

A number of disorders have been associated with excessive enteric protein loss in children. Using a pathophysiologic classification, these disorders can be divided into those owing predominantly to excessive protein loss from intestinal lymphatics and those owing to protein loss across an abnormal or inflamed mucosal surface (Table 11-1). An alternative classification scheme would be a more clinical approach, distinguishing those disorders in which enteric protein loss is the major manifestation of the illness from those in which protein loss is present but is overshadowed by other clinical manifestations of these disorders. Those diseases that are characterized predominantly by a protein-losing state are discussed below, with the focus on the contribution of enteric protein loss to their manifestations.

PRIMARY INTESTINAL LYMPHANGIECTASIA

Intestinal lymphangiectasia is characterized by diffuse or localized ectasia of the enteric lymphatics, often in association with lymphatic abnormalities elsewhere.[14] The patho-

TABLE 11–1 DISEASES ASSOCIATED WITH EXCESSIVE ENTERIC PROTEIN LOSS

LOSS FROM INTESTINAL LYMPHATICS
Primary intestinal lymphangiectasia
Secondary intestinal lymphangiectasia
Cardiac disease
Constrictive pericarditis
Congestive heart failure
Cardiomyopathy
Post–Fontan procedure
Obstructed lymphatics
Malrotation
Lymphoma
Tuberculosis
Sarcoidosis
Radiation therapy and chemotherapy
Retroperitoneal fibrosis or tumor
Arsenic poisoning
LOSS FROM AN ABNORMAL OR INFLAMED MUCOSAL SURFACE
Infection
Invasive bacterial infection (eg, *Salmonella*, *Shigella*)
Parasitic infection (eg, *Giardia*)
Clostridium difficile
Helicobacter pylori
Bacterial overgrowth
Immunologic and inflammatory disorders
Gastric inflammation
Ménétrier disease
Eosinophilic gastroenteritis
Intestinal inflammation
Gluten-sensitive enteropathy
Milk- and soy-induced enteropathy
Common variable immunodeficiency
Tropical sprue
Radiation enteritis
Graft-versus-host disease
Ulcerative jejunitis
Colonic inflammation
Ulcerative colitis
Crohn disease
Hirschsprung disease
Necrotizing enterocolitis
Vasculitic disorders
Systemic lupus erythematosus
Mixed connective tissue disease
Schönlein-Henoch purpura

genesis of these abnormal lymphatic structures is uncertain. Ectatic lymphatics may be located in the mucosa, submucosa, or subserosa, leading to excessive loss of protein and lymphocytes into the gut or the peritoneal cavity. The mechanism of this lymphatic loss is believed to be from rupture of lymphatics across the mucosa, with subsequent leakage of lymph into the bowel lumen. The resulting clinical picture is one of edema (which may be asymmetric), growth failure, and variable gastrointestinal symptoms.

The presentation of primary intestinal lymphangiectasia may occur at any time throughout infancy and childhood. Gastrointestinal symptoms, in decreasing order of frequency, include intermittent diarrhea, nausea, vomiting, and, occasionally, abdominal pain.[37] Steatorrhea may also occur. Some patients may have relatively few gastrointestinal symptoms. Edema may be symmetric and pitting if it is due to the presence of a hypoproteinemic state, or, alternatively, it may be asymmetric and nonpitting if it is due to an underlying lymphatic abnormality of the affected extremity. Lymphedema of an extremity may precede the onset of symptoms owing to gastrointestinal involvement. Lymphatic aberrations may also lead to the development of a chylothorax or chylous ascites, which should be differentiated from pleural effusions or ascites, resulting from hypoproteinemia. Primary intestinal lymphangiectasia may occur as an isolated abnormality or as part of a more generalized syndrome such as Noonan syndrome or Klippel-Trénaunay-Weber syndrome.[38,39] Intestinal lymphangiectasia has also been noted to occur in families[40] and in other syndromatic forms with variable associated features and inheritance patterns.[41,42]

In addition to the loss of albumin, patients with lymphangiectasia may have reduced levels of immunoglobulins and lymphocytes owing to the loss of lymph. Differentiation from a primary immunodeficiency with gastrointestinal disease may be difficult initially. Enteric lymphatic loss results in the predominant loss of T lymphocytes, leading to reduced delayed hypersensitivity skin test responses, prolonged homograft survival, and diminished blast transformation to mitogen stimulation in vitro.[43,44] Additional abnormalities that have been described include hyposplenism,[45] thymic hypoplasia,[46] and neutrophil dysfunction.[47] In spite of these abnormalities, patients with intestinal lymphangiectasia do not appear to be unusually susceptible to infections.

The diagnosis of intestinal lymphangiectasia is suggested by the previously mentioned clinical findings and supported by the presence of hypoproteinemia and lymphocytopenia. Hypocalcemia is also occasionally present and may produce tetany. Steatorrhea may be demonstrable on a fecal fat collection, and fecal α_1-AT excretion may be increased. Barium studies may demonstrate thickening of the jejunal folds, fluid hypersecretion, and nodular or punctate lucencies in the mucosa of the small bowel.[37,48] Lymphangiography, which may be technically difficult to perform, has shown hypoplasia of the peripheral lymphatics in the injected limb, partial or complete absence of the thoracic duct, or, rarely, entry of contrast into the bowel lumen via mesenteric lymphatics.[14] Lymphoscintigraphy using ^{99m}Tc labeling of a microcolloid has been used more recently to localize lymphatic leakage and is technically easier than lymphangiography.[49] It should be recognized that the site and extent of the intestinal lesion vary widely between patients and may sometimes be extremely difficult to document with imaging studies.

Biopsy of the small intestinal mucosa may demonstrate dilated lacteals associated with distortion of the villi if a proximal lesion is present (Figure 11-1). Occasionally, extrusion of Brunner glands into villus tips and detachment of surface epithelium with formation of a subepithelial space may mimic the histologic appearance of lymphangiectasia.[50] Because of the focal nature of some lymphatic abnormalities, a capsule biopsy of the jejunal mucosa may miss the lesion. Endoscopic abnormalities, such as scattered white plaques or the presence of chyle-like substances covering the mucosa, have been observed

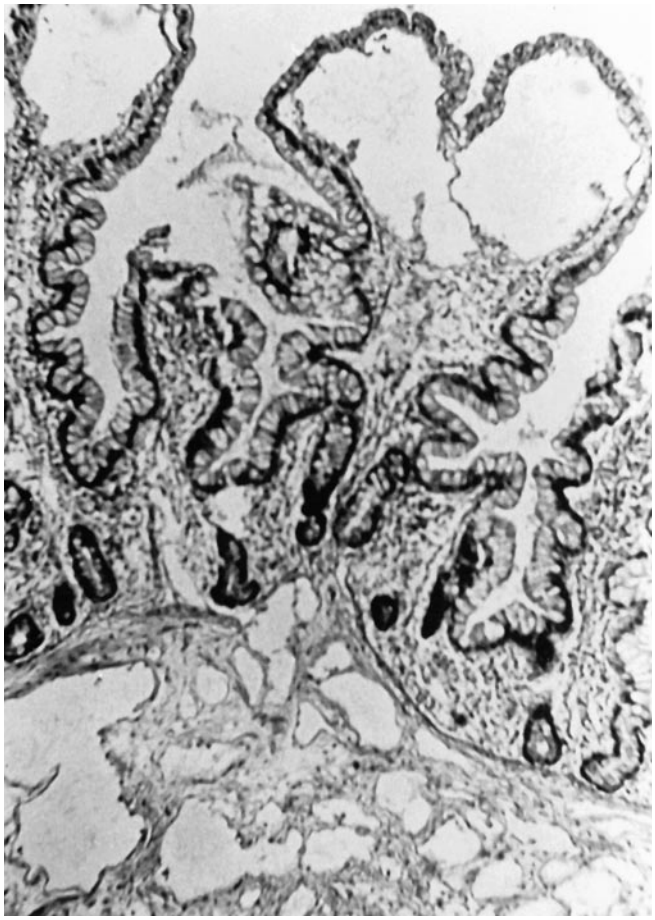

FIGURE 11-1 Microscopic view of intestinal biopsy specimen demonstrating wide villi with dilated lacteals in the mucosa and enlarged submucosal lymphatics.

and have been useful for directing biopsies at the time of endoscopy.[51,52] The endoscopic and histologic findings are less obvious if the patient has been on a low-fat diet. Endoscopic documentation of mucosal lymphangiectasia in patients without compatible symptoms is usually of no clinical significance.[53] Mucosal biopsy is not useful in patients with lymphangiectasia confined to deeper layers of the bowel wall.

The mainstay of treatment for intestinal lymphangiectasia is a low-fat, high-protein, medium-chain ($C_{6:0}$ to $C_{12:0}$) triglyceride (MCT) diet. MCTs are not re-esterified within the intestinal cell and thus bypass the enteric lymphatics and directly enter the portal system. It is believed that the reduction in dietary long-chain fats reduces lymphatic flow and pressure within the lymphatic system and decreases the amount of lymph leakage. This dietary approach can be implemented by the use of special MCT-containing formulas during infancy or by the supplementation of a low-fat diet with MCT oil in older children and adolescents. Using this approach, several authors have reported favorable effects on hypoalbuminemia, gastrointestinal symptoms, and growth.[37,54,55] This improvement may occur in spite of ongoing protein loss. The need for dietary therapy is often permanent, although occasional spontaneous remissions do occur.

Not all patients will respond completely to this dietary approach. Some patients will require additional supplementation with calcium salts and water-soluble forms of fat-soluble vitamins.[56] Low gammaglobulin levels may persist in some patients, but such patients are unlikely to require treatment because of their continuing capacity to generate specific antibodies.

Isolated reports have documented improvement in patients following localized resections of affected portions of the bowel or anastomosis of abnormal lymphatics to venous channels.[37,57] These approaches may be hampered by an inability to demonstrate radiographically such a localized lymphatic abnormality and the tendency for patients with the primary form of lymphangiectasia to have extensive lesions. Surgical shunts have been used in patients with persistent chylothorax or chylous ascites, with variable success.[58,59] The favorable response of patients with congenital chylous ascites to treatment with total parenteral nutrition suggests that this approach may be helpful in certain cases that are resistant to other forms of therapy.[59] Antiplasmin therapy has been found effective in a patient with lymphangiectasia and increased plasma fibrinolytic activity who did not respond to dietary therapy.[60] Octreotide has also shown some efficacy for the treatment of intestinal lymphangiectasia.[61]

SECONDARY INTESTINAL LYMPHANGIECTASIA

In addition to its occurrence as a primary developmental abnormality, lymphangiectasia may occasionally result from lymphatic obstruction or elevated lymphatic pressure. Cardiac lesions such as in congestive heart failure[62] and constrictive pericarditis[63] can increase lymphatic pressure. A protein-losing state has also been documented following the Fontan procedure for complex congenital heart disease.[64] Corticosteroids may have a role in the treatment of this protein-losing enteropathy after a Fontan procedure,[65] and, more recently, heparin therapy has been found efficacious in a few of these cases.[66] This clinical observation is supported by the recent suggestion of a relationship between heparan sulfate and other lamina propria glycosaminoglycans in the pathogenesis of some enteric protein-losing states.[67] Inflammatory processes that cause retroperitoneal lymph node enlargement[68] or fibrosis may lead to obstruction of enteric lymphatics. Chemotherapeutic agents or other toxic substances may directly damage the lymphatic structures.[69,70] Fleisher and colleagues have described a few patients with a steroid-responsive acquired form of intestinal lymphangiectasia with elevated immunoglobulins and sedimentation rates, suggesting an underlying inflammatory etiology.[71]

MÉNÉTRIER DISEASE

Ménétrier disease is a relatively rare disorder characterized by a marked protein-losing gastropathy associated with enlarged and thickened gastric folds in the fundus and the body of the stomach. Most pediatric patients with this dis-

order have been less than 10 years of age, and their illness has usually presented abruptly, with vomiting, abdominal pain, and peripheral edema.[72] Laboratory findings often reveal a mild normochromic normocytic anemia, eosinophilia, and hypoalbuminemia. Excessive protein loss has been demonstrated by the use of ^{51}Cr albumin excretion.[72,73] The typical upper gastrointestinal radiographic appearance has thickened gastric folds in the fundus and body of the stomach, often with antral sparing (Figure 11-2), which has been confirmed endoscopically.[74] Thickened folds have also been shown by ultrasonography.[73] Histologic examination of endoscopic or suction biopsies has shown a hypertrophic mucosa with elongated pits. Occasionally, cystic or polypoid changes and an inflammatory infiltrate have been noted.[72] Several cases of Ménétrier disease in childhood have been associated with histologic or serologic evidence of cytomegalovirus infection.[75–77] An infant with coexistent Ménétrier disease and formula-protein allergy has been described.[78]

The etiology of Ménétrier disease has not been conclusively determined. Theories have suggested the possibility of abnormal regulation of gastric epithelial growth, which might, in some cases, have been triggered by an environmental pathogen such as cytomegalovirus. Transforming growth factor-α (TGF-α), a member of the epidermal growth factor family of peptides, may be important in the pathogenesis of Ménétrier disease. Transgenic mice overexpressing TGF-α have been found to have a gastric lesion resembling Ménétrier disease.[79,80] Increased expression of TGF-α has also been demonstrated in gastric mucosa from patients with Ménétrier disease.[80] Studies such as these may help to further characterize the factors that initiate the abnormal mucosal growth that appears to occur in this disease.

The differential diagnosis of Ménétrier disease in childhood is that of a protein-losing gastropathy associated with thickened gastrointestinal folds. Initially, diagnosis can be facilitated by showing that a protein-losing state is present and that protein loss is primarily from the stomach. Because α_1-AT determinations may be inaccurate when the site of loss is the stomach, modifications of the technique or scintigraphic detection of the site of protein loss may be necessary.[81,82] Radiographically, eosinophilic gastroenteropathy may present in a similar fashion, but it usually involves the antrum and presents a distinctive histologic picture on biopsy.[83] Gastric lymphomas and Zollinger-Ellison syndrome can also produce a similar clinical and radiographic picture, but they are both very rare in children.[74] Thickened gastric folds without discrete polyps have been noted by upper gastrointestinal radiography in Peutz-Jeghers syndrome.[74] A retrospective histologic analysis of cases of Ménétrier disease has recently suggested that patients may be further subclassified on the basis of the degree of glandular hyperplasia and the presence and extent of infiltrating lymphocytes.[84] The distinction between lymphocytic gastritis and hypertrophic gastropathy may be of prognostic and therapeutic significance.

Children with Ménétrier disease usually have a self-limited illness without recurrence or sequelae, unlike adults, in whom the disease is chronic.[85] The chronic form of Ménétrier disease has also been described in a family in which two cases began in childhood.[86] Treatments for the chronic form that have been partially or completely effective include acid suppression, octreotide, and gastrectomy.[87,88] Omeprazole has been highly effective in some patients with this disorder.[89]

ENTERIC PROTEIN LOSS DURING THE COURSE OF OTHER GASTROINTESTINAL DISORDERS

Excessive enteric protein loss is common in several diseases in which symptoms attributable to protein loss are overshadowed by other consequences of the disease process. For the majority of these conditions, protein loss is due to mucosal disruption from a diffuse inflammatory process affecting the mucosa of the stomach, small intestine, or colon, alone or in combination. The following briefly reviews issues related to

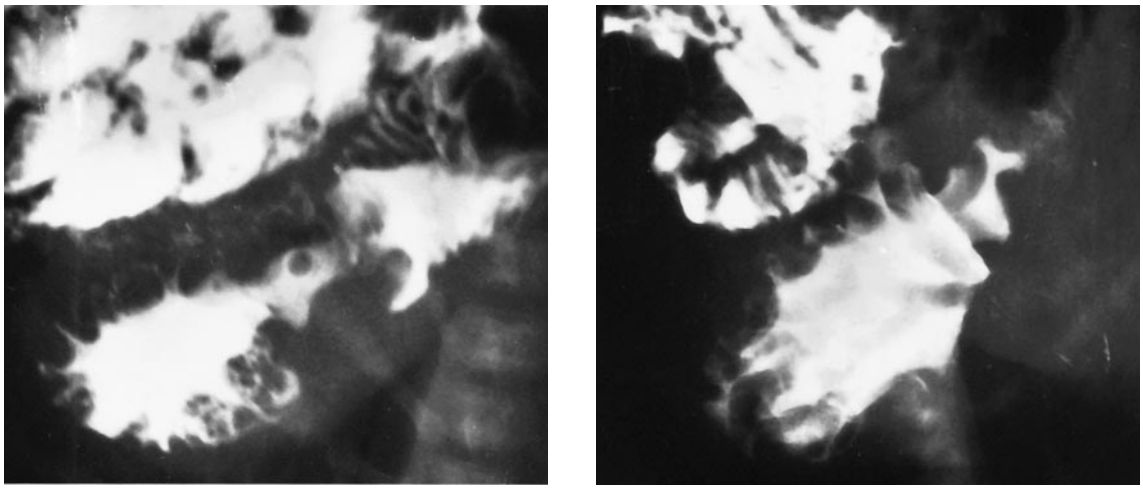

FIGURE 11-2 Upper gastrointestinal radiographs from a patient with Ménétrier disease showing marked thickening of the gastric folds.

protein loss in a number of disorders that are discussed in greater detail elsewhere in this book.

INFECTIONS

Gastrointestinal protein loss during the course of enteric infectious processes has not been extensively evaluated, but such loss can be excessive during the course of infections with *Salmonella*,[12] *Shigella*,[90] *Giardia*,[91] and other parasites.[92] Thomas and colleagues found no difference in fecal α_1-AT excretion between control patients and infants with acute self-limited gastroenteritis.[26]

Excessive gastric protein loss has been observed during the course of *Helicobacter*-associated gastritis.[93] A correlation between *Clostridium difficile* colonization and fecal α_1-AT excretion has also been detected in asymptomatic infants[94] and in symptomatic adults.[95] Excretion of ^{51}Cr albumin can be increased in patients with bacterial overgrowth of the small bowel from blind loop syndrome.[96] A decrease in protein loss was associated with prolonged antibiotic therapy in these latter cases.

INFLAMMATORY AND IMMUNOLOGIC DISORDERS

Hypoalbuminemia is a common occurrence in the course of eosinophilic gastroenteritis and milk- and soy-sensitive enteritis in infants,[26,97–99] In cow's milk– or soy-sensitive subjects, the absence of gastrointestinal symptoms in the presence of profound hypoalbuminemia and edema can be striking. A decrease in the degree of protein loss with treatment has been documented in both of these conditions. Active celiac disease has also been shown to result in increases in fecal α_1-AT.[26,27] This loss of protein not only improves with treatment, but treated celiac patients cannot be distinguished from normal subjects on the basis of fecal α_1-AT excretion. Fecal α_1-AT excretion has been used to monitor the response to gluten withdrawal as well as to monitor compliance in patients on a gluten-free diet.[100]

An elevated concentration of fecal α_1-antitrypsin has been detected in the stools of patients with probable necrotizing enterocolitis[101] and patients with graft-versus-host disease following bone marrow transplant.[102] In each of these cases, the possibility of using serial values to detect the onset and resolution of these conditions has been suggested.

Several studies have revealed increased gastrointestinal protein loss in patients with idiopathic inflammatory bowel disease (ulcerative colitis and Crohn disease).[26,31,103,104] Fecal α_1-AT excretion, fecal α_1-AT clearance, and ^{51}Cr albumin excretion are all increased in patients with inflammatory bowel disease compared with controls. Fecal α_1-AT excretion has been shown to correlate with disease severity and, to some extent, the degree of small bowel involvement but to correlate poorly with the Crohn Disease Activity Index.

Protein-losing enteropathy has been demonstrated in association with common variable immunodeficiency.[105] Thus, because hypogammaglobulinemia can be either the effect or the cause of a protein-losing state, one needs to exercise caution in discerning the etiology of hypogammaglobulinemia associated with hypoalbuminemia and enteric protein loss.

VASCULITIC DISORDERS

Excessive enteric protein loss can be associated with systemic lupus erythematosus either at diagnosis or during the course of the disease.[106] Similarly, protein-losing enteropathy has been observed in other vasculitic conditions, including mixed connective tissue disease[107] and Schönlein-Henoch purpura,[108] and during the course of vasculitis after bone marrow transplant.[109]

METABOLIC DISORDERS

Previously, metabolic disorders have not been considered among those conditions that have protein-losing enteropathy as a common clinical feature. However, improved diagnosis of metabolic disease has allowed a greater understanding of the diverse spectrum of clinical presentation of some of these illnesses. One such example is the recent elucidation of variants of carbohydrate-deficient glycoprotein syndrome.[110,111] These disorders, which may be associated with a significant protein-losing enteropathy, may be responsive to dietary therapy.[110,111] Protein-losing enteropathy has also been seen in cobalamin C deficiency and may be a feature of other underlying metabolic disease.[112]

CONCLUSION

Protein-losing enteropathy occurs in the course of a number of gastrointestinal disorders. Those in which the protein-losing state contributes significantly to clinical manifestations are relatively uncommon in children and can be identified on the basis of clinical, laboratory, radiographic, and endoscopic criteria. Scintigraphic techniques and the measurement of fecal α_1-AT can be used to document protein loss and to potentially localize the site of loss. In addition, these measurements may be useful for following the course or activity of a number of chronic inflammatory conditions during which protein loss occurs.

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CHAPTER 12

VOMITING

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Vomiting presumably conveys a survival advantage in that it promotes the rapid expulsion of ingested toxins. It is a complex behavior, which in humans is usually composed of three linked activities: nausea, retching, and expulsion of stomach contents. In 1952, Wang and Borison proposed a model for the neurohumoral control of vomiting that has stood up remarkably well to subsequent investigation.[1] These investigators identified two anatomic regions in the medulla controlling vomiting: the chemoreceptor trigger zone (CTZ) and the central vomiting center. The CTZ is located in the area postrema on the floor of the caudal end of the fourth ventricle outside the conventionally defined blood-brain barrier. Receptors in this region are activated by proemetic agents in the circulation or cerebrospinal fluid. It was proposed that efferents from the CTZ project to a central vomiting center from which the motor events of vomiting are initiated via vagal and splanchnic sympathetic efferents. The central vomiting center was thought to be located in the nucleus tractus solitarius and surrounding reticular formation of the medulla just beneath the CTZ. Input from all other sources provoking emesis—gastrointestinal, vestibulo-ocular, and higher cortical afferents—also was said to project to the central vomiting center, which then initiated and coordinated nausea, retching, and expulsion, as well as the preparatory autonomic phenomena (gastrointestinal motor events, salivation, tachypnea, and tachycardia). Ablation studies in animals, which have failed to identify an anatomically discrete central vomiting center, have suggested that the central vomiting center represents the integrated activity of the paraventricular nuclei arrayed along the central neuraxis controlling a myriad of autonomic functions. These nuclei receive input from central and peripheral afferents and together serve as a central pattern generator for the respiratory, cardiac, gastrointestinal, and somatomotor events of vomiting.[2,3]

NAUSEA AND THE GASTROINTESTINAL CORRELATES OF VOMITING

Cineradiographic evaluation of animals after administration of intravenous proemetics has revealed a series of gastrointestinal motor events preceding vomiting (the gastrointestinal motor correlates of vomiting).[4] First, there is an increase in segmental, nonperistaltic activity in the duodenum and small intestine. Gallbladder contraction occurs, and some duodenal contents regurgitate into the stomach. Next, a large-amplitude contraction originating in the mid–small bowel sweeps slowly retrograde over a period of 30 to 60 seconds, filling the gastric antrum with small bowel contents and pancreatobiliary secretions. Gastric motor activity is suppressed. By this means, diluent and buffer are added to any noxious gastric contents. Finally, a series of lesser-amplitude contractions originate in the ileum and propagate the remaining small bowel contents into the colon. Diarrhea often follows vomiting, but studies have not revealed a predictable pattern of colonic motor activity preceding this diarrhea. Just before the onset of retching, gastric contents may reflux into the distal esophagus, suggesting that relaxation of the cardia, crural diaphragm, and lower esophageal sphincter has occurred. The gastrointestinal motor correlates of vomiting are initiated by vagal and sympathetic efferents from the central pattern generator, which also coordinate the other autonomic events occurring at this time, increased salivation, increased respiratory rate, increased heart rate, and pupillary dilation.[4,5] Cholecystokinin (CCK) may be the local mediator of the gastrointestinal motor correlates. Nitric oxide may be the inhibitory mediator of gastric atony.

It is not clear that the motor correlates of vomiting actually produce nausea. Completely coordinated vomiting may occur in the absence of nausea (eg, vomiting caused by increased intracranial pressure). Furthermore, nausea may occur in the absence of vomiting (eg, following a very large meal, in motion-induced nausea, or in gastroparesis). Nausea may simply be a conscious sensation of gastric distention and/or atony. Alternatively, it could be a response to output from the central pattern generator to the cortex.[5]

SOMATOMOTOR EVENTS OF VOMITING

The vomiting act is characterized by cycles of retching followed by forceful expulsion of gastric contents through the mouth. The diaphragms descend and the external intercostal (inspiratory) muscles contract against a closed glottis. The esophagus dilates in response to the negative intrathoracic pressure. The stomach remains atonic, filled with refluxate from the small bowel. Abdominal muscle contractions begin, compressing the stomach and forcing gastric contents into the fundus and lower esophagus. The

fundus of the stomach may herniate into the thorax during this phase, effectively removing the antireflux barrier produced by abdominal pressure on the lower esophageal sphincter. With relaxation of abdominal contraction, strenuous inspiratory effort ceases and the esophagus empties back into the stomach. Several cycles of retching occur, becoming shorter, more rhythmic, and forceful until the esophagus no longer empties between cycles. The last abdominal contraction in the cycle produces expulsion of gastric contents. It occurs early, while the esophagus is still full, and is associated with elevation of the diaphragm producing positive pressure in both the thorax and abdomen. These motor events are accompanied by spinal flexion, wide open mouth, palatal elevation, upper esophageal sphincter relaxation, and forceful ejection of gastric contents.[4]

NEUROHUMORAL CONTROL OF VOMITING

Studies of the area postrema or CTZ in animals who vomit have shown that it contains specific receptors for many neuroactive compounds that can cause vomiting. These include receptors for dopamine (the site of action of apomorphine), acetylcholine, vasopressin, enkephalin, angiotensin, insulin, serotonin, endorphin, substance P, and many others.[6] A common mediator, 3′, 5′-cyclic adenosine monophosphate, may be involved in the excitatory responses of all of these stimulatory peptides because theophylline inhibits the proemetic activity of them all. Receptors of the dopamine D_2 type are present in the highest concentration, explaining the exquisite sensitivity of humans to apomorphine. The efferent output from the CTZ projects to the nearby paraventricular nuclei. Subemetic doses of stimulatory agents may simply produce the gastrointestinal motor correlates of vomiting without expulsion of contents.

Gastrointestinal vagal and sympathetic afferents carry sensory input from the gut to the nucleus tractus solitarius and the other paraventricular nuclei of the central pattern generator. A small number of vagal afferents appear to terminate in the area postrema as well. Stimulation of the central end of the cut vagus provokes a complete vomiting cycle even in the absence of the CTZ, so the role of vagal afferents to the CTZ is unclear. The widespread projections of the visceral afferents in the paraventricular area are consistent with the concept of a central pattern generator rather than a discrete vomiting center. Emesis in response to direct gastrointestinal irritants such as copper sulfate, abdominal radiation, and gastrointestinal dilation is a result of afferent vagal signals to the central pattern generator produced by local release of inflammatory mediators from damaged mucosa, with secondary release of excitatory neurotransmitters, most importantly serotonin from deep mucosal enterochromaffin cells. In motion sickness, afferent input to the central pattern generator from the vestibular organ, visual cortex, and higher cortical centers involved in integration of sensory input is probably more important than gastrointestinal afferent input.[7] Some animal experiments suggest that humoral excitation of the CTZ may play a role in motion sickness (see "Therapy," below). The anticipatory vomiting of cancer chemotherapy is mediated by afferents to the central pattern generator from higher cortical centers, in response to smells, sights, sounds, and feelings associated with chemotherapy.[8] The CTZ is almost certainly involved in intravenously administered chemotherapy, but there is increasing appreciation of the importance of gastrointestinal afferent input to the central pattern generator resulting from drug-induced mucosal damage and intestinal distention.[9] Emesis seen in association with inflammatory bowel disease is stimulated by gastrointestinal afferents in response to the locally produced prostaglandins and distention. The vomiting of pregnancy has been incompletely defined. The CTZ may mediate emesis in early pregnancy in response to luteinizing hormone, progesterone, human chorionic gonadotropin, or androgens, all of which have been reported to be elevated in women with vomiting of pregnancy.[10] The role of vagal afferents from the uterus and emotional adjustment to pregnancy in this condition has not been clarified. Vomiting associated with systemic anaphylaxis is probably mediated by the effects of histamine on the CTZ.

VOMITING SYNDROMES

Regurgitation

Effortless regurgitation of gastric contents is a characteristic symptom of gastroesophageal reflux in infants. It is not clear whether this behavior is centrally or locally controlled or whether it should be considered "vomiting" at all. Most episodes of regurgitation are not associated with nausea; retching is rare; and expulsion is not forceful or complete. When regurgitation of gastric contents causes aspiration, cough, gagging, or peptic injury, a full vomiting reflex with forceful expulsion of gastric contents may occur, probably mediated by afferents from the pharynx and esophagus. Spontaneous relaxation of the lower esophageal sphincter is the major mechanism by which gastroesophageal reflux occurs, with or without regurgitation.[11] Whether other reflex motor activity involving the abdominal or gastric musculature is required to produce regurgitation during reflux is unknown. It is not known why regurgitation is so characteristic of infant reflux compared with that of older children and adults.

Cyclic Vomiting Syndrome

This disorder is characterized by recurrent episodes of nausea and vomiting without an identifiable organic cause. Recent reports indicate that the condition may affect up to 1.9% of schoolchildren.[12] The episodes are of rapid onset, often starting during sleep or early morning. Children may vomit many times per hour, to the point of dehydration. Persisting for hours to days, but rarely more than 72 hours, the episodes are separated by completely symptom-free intervals. The episodes may end spontaneously, may cease after a period of sleep, or may progress to such severe dehydration and electrolyte imbalance that intravenous fluids, sedatives, and antiemetics are required. There are few

residua to most episodes, and the patient suddenly seems better and complains of hunger. For each individual, the pattern of inciting events and the character of the attacks are similar. Stress or minor intercurrent illness is frequently noted at the onset. The symptom-free interval ranges from several weeks to more than a year. A similarity to migraine attacks and even seizures has long been noted, and headaches of various types are present in up to 25% in some series.[13] Migraine headaches may be present in up to 47% of patients, and a family history of migraine is found in 47% of first-degree and 72% of second-degree relatives.[14] A family and personal history of irritable bowel symptoms is seen in 62%.[15] The diagnosis rests on the characteristic history, the normal physical examination, and a meticulous evaluation for other organic diseases causing recurrent episodes of vomiting. A unifying cause has not been determined. Attention has focused on the possibilities of mitochondrial deoxyribonucleic acid (DNA) mutations (because the mother often has a history of migraine), ion channel defects, abnormalities of the hypothalamic-pituitary-adrenal axis, and increased autonomic reactivity.[12] Inflammatory and vasoactive mediators (interleukin-6 and nitric oxide) released from gastric and esophageal mucosa may stimulate the central vomiting center [16].

Important considerations in the differential diagnosis of cyclic vomiting are such disorders as urea cycle defects, disorders of organic acid metabolism, gastric and intestinal motility disorders, central nervous system lesions, familial dysautonomia, obstructive uropathy, obstructive cholangiopathy, familial pancreatitis, intestinal malrotation, duplication, strictures and diverticulae of the intestines, adrenal insufficiency, and diabetes mellitus. All of these disorders may be associated with prolonged symptom-free intervals and normal physical examinations for some time. Peptic disease may cause vomiting, but the emesis is more chronic and less episodic. Diagnostic evaluations should be focused on conditions suggested by history. Symptomatic treatment should be instituted as early as possible after onset of symptoms. Lorazepam, butyrophenones, and benzamides such as metoclopramide and rectal trimethobenzamide have been used with occasional success. Propranolol, phenytoin (Dilantin), and antihistamines (especially cyproheptadine) have been used prophylactically.[17] Drugs commonly used in the treatment of migraine have been effective in treating attacks in some patients, including sumatriptan, amitriptyline, and pizotifen. The immediate initiation of intravenous fluids and nasogastric suction is helpful in some patients. Patient and parent education as to the appropriate treatment and the usual benign nature of the condition is essential. Evaluation and treatment of stress or overt psychiatric disease are also indicated.

Rumination

Rumination is the frequent regurgitation of previously ingested food into the mouth. Regurgitated food may be rechewed and swallowed or voluntarily spit out. Rumination is not accompanied by apparent nausea, retching, or forceful expulsion. It occurs most often in mentally retarded children. The origin of rumination appears to be behavioral in most instances. Because it is so often seen in institutionalized children, it is felt to be a form of self-stimulation. Indeed, rumination is reported to occur in 84% of gorillas held captive in zoos for more than 5 years.[18] The syndrome has been described in cases of child neglect, in neonates during prolonged hospitalization,[19] in children and infants with untreated gastroesophageal reflux,[20] and in older children as an associated symptom of bulimia. Except in infants with gastroesophageal reflux and in bulimic patients, the symptom often responds to increased personal attention, especially during feedings, and mild negative reinforcement.[21] If untreated, it may result in life-threatening inanition. Motility studies in a few adults have shown that rumination is produced by voluntary abdominal muscle contraction associated with a pharyngeal maneuver, which results in reduced upper esophageal pressure.[22] Manometric studies have also shown characteristic simultaneous spike-wave activity in the stomach and duodenum during rumination.[23] Differentiating this symptom from the vomiting and regurgitation associated with gastroesophageal reflux, metabolic disease, and many of the other conditions listed in Table 12-1 may require extensive diagnostic testing. However, the setting of neglect should raise the suspicion of rumination syndrome early in the evaluation.

Bulimia

Bulimia is an eating disorder characterized by recurrent episodes of binge eating followed by purging induced by vomiting, diarrhea, diet, and exercise.[24] Commonly seen in adolescent and young adult females (up to 10% of this age group), it does occur in males[25] and premenarchal females.[26] Patients describe a frightening sensation that they have lost control of themselves during vomiting and a persistent anxiety over body shape and weight. Vomiting may be induced by medications such as ipecac, hypertonic saline, or other emetogenic substances. It may be a result of self-induced gagging. It may also be promoted by forceful abdominal muscle contraction during spontaneous lower esophageal sphincter relaxations associated with belching. This characteristic is sometimes useful in manometrically discriminating the patient with bulimia from the patient with gastroesophageal reflux. These patients often vomit surreptitiously. They often come from dysfunctional families characterized by enmeshment with overly controlling parents. Parental substance abuse is common. Sexual abuse by a family member has been reported in up to 15% of cases.[27] Depression and feelings of helplessness are common. As in anorexia nervosa, complications of purging include malnutrition, electrolyte imbalance, esophageal erosion and bleeding, dental erosion, and dehydration. Gastrointestinal symptoms seen in up to 50% of patients include abdominal pain, constipation, bloating, nausea, and postprandial fullness. Pancreatitis may be falsely indicated by the presence of hyperamylasemia secondary to constant salivary gland stimulation. A careful history is the most important diagnostic tool. In cases in which recurrent vomiting is an admitted symptom of bulimia, antiemetics are of little use. Psychotherapy and antidepressants are the mainstays of therapy.

TABLE 12-1 ANTIEMETIC AND ANTINAUSEA DRUGS WITH INDICATIONS AND POSSIBLE MECHANISMS OF ACTION

CHEMICAL NAME	REPRESENTATIVE BRAND NAME	INDICATION	MECHANISM
ANTIHISTAMINES			
Diphenhydramine Hydroxyzine Dimenhydrinate Promethazine Meclizine	Benadryl Vistaril, Atarax Dramamine Phenergan Antivert	Motion sickness, mild chemotherapy-induced vomiting	Most likely labyrinthine suppression, possibly via anticholinergic effect as well as H_1 receptor antagonism in the central pattern generator
ANTICHOLINERGICS			
Hyoscyamine	Scopolamine Levsin	Prophylaxis of motion sickness	Antimuscarinic effect probably at the level of the labyrinth or central pattern generator
SUBSTITUTED BENZAMIDES			
Metaclopramide	Reglan	Chemotherapy, motility disorders, especially gastroparesis, GER	D_2 receptor blockade at the CTZ and enteric nervous system. In high dose, has 5-HT_3 activity enterically
Trimethobenzamide	Tigan	Often used during vomiting associated with acute gastroenteritis — ? efficacy. May abort some cases of cyclic vomiting	D_2 receptor blockade
Cisapride	Prepulsid	Motility disorders, GER	Enteric acetylcholine release
5-HT_3 RECEPTOR ANTAGONISTS			
Ondansetron Granisetron	Zofran Kytril	Chemotherapy Postoperative nausea and vomiting	5-HT_3 receptor blockade most important at the enteric level, but possibly some effect at the CTZ and central pattern generator
CANNABINOIDS			
Dronabinol Nabilone	Marinol Cesamet	Chemotherapy, but used less and less because of more efficacious drugs and central side effects	Unknown
BENZODIAZEPINES			
Lorazepam Diazepam Midazolam	Ativan Valium Versed	Chemotherapy—especially lorazepam. Preferred because of rapid effect and short duration	These drugs probably act via central GABA inhibition producing sedation and anxiolysis
PHENOTHIAZINES			
Prochlorperazine Chlorpromazine Perphenazine Promethazine	Compazine Thorazine Trilafon Phenergan	Chemotherapy, cyclic vomiting, acute gastritis. Rarely used in pediatrics because of extrapyramidal side effects	D_2 receptor antagonist at CTZ
BUTYROPHENONES			
Droperidol Haloperidole	Inapsine Haldol	Cyclic vomiting, intractable vomiting from acute gastritis, chemotherapy, postoperative nausea and vomiting	D_2 receptor blockade at the CTZ. Central anxiolysis and sedation
Domperidone	Motilium	Chemotherapy, motility disorders especially gastroparesis, GER	D_2 receptor blockade at enteric nervous system
CORTICOSTEROIDS			
Dexamethasone	Decadron	Mild chemotherapy-induced vomiting or in combination with other antiemetics; emesis resulting from increased intracranial pressure	Unknown, possibly decreased enteric prostaglandin synthesis

CTZ = chemoreceptor trigger zone; GABA = γ-aminobutyric acid; GER = gastroesophageal reflux; 5-HT = 5-hydroxytryptamine.

SUPERIOR MESENTERIC ARTERY SYNDROME

See Chapter 36, "The Surgical Abdomen."

VOMITING AND CHEMOTHERAPY

Vomiting is a common complication of cancer chemotherapy, which can be severe enough to produce dehydration, electrolyte imbalance, enhanced drug toxicity, and malnutrition. Other complications include Mallory-Weiss esophageal tears, bone fractures, wound dehiscence, and emotional depression. Delay or discontinuation of necessary cancer therapy may result from any of these complications. Emesis may be immediate, delayed, or anticipatory. Factors influencing the incidence of vomiting include the type, dose, route, and rapidity of administration of the chemotherapy. The patient's propensity to develop motion sickness, motivation for chemotherapy, and age are also relevant considerations, with young patients at higher risk than adult patients. Postpubertal females may have increased risk of chemotherapy-induced emesis in the luteal phase of the menstrual cycle. Combinations of drugs

have an additive effect on nausea and vomiting. On first intravenous exposure, vomiting usually occurs after a latency of about an hour. Vomiting may be brief (acute phase) or may persist for 2 to 5 days (delayed phase). Drugs most likely to produce emesis in pediatric patients include cisplatin, dacarbazine, and nitrogen mustard (> 90% likelihood of vomiting). Highly emetic agents (60 to 90% likelihood of vomiting) include nitrosoureas, actinomycin D, cyclophosphamide, and procarbazine. Less emetogenic drugs (30 to 60% likelihood of vomiting) include L-asparaginase, 5-azacitidine, daunorubicin, 5-fluorouracil, and mitomycin C. Drugs with low emetogenic potential (< 30%) include vinca alkaloids, epipodophylotoxins, oral alkylating agents, bleomycin, isophosphamide, cytosine arabinoside, and thiotepa.[8]

The CTZ is thought to be the most important receptor for intravenously administered chemotherapeutic agents. The receptor in the CTZ most closely associated with chemotherapy-induced vomiting is the dopamine$_2$ receptor, which explains the antiemetic effect of phenothiazines, butyrophenones, domperidone, and metoclopramide, which are all D_2 receptor antagonists. The mechanism by which cannabinoids produce an antiemetic effect is not known, but it may be via an antiadrenergic effect at the CTZ or by inhibition of prostaglandin synthesis. Neurokinin-1 receptor antagonists are antiemetic, acting on the dorsal vagal complex and preventing vagal motoneuron activity. Thus, they may prevent gastric fundic relaxation, an early stage in the vomiting complex.[28] Corticosteroids may interfere with prostaglandin synthesis and are most effective when used in conjunction with other antiemetics. Antihistamines and anticholinergics are relatively ineffective in cancer chemotherapy-induced emesis, but both may suppress output from the central pattern generator. Benzodiazepines have little direct antiemetic effect but are helpful in anticipatory emesis as they reduce anxiety and recall of the events surrounding chemotherapy.[17]

High-dose metoclopramide blocks not only D_2 receptors but also the central and local receptors for serotonin (5-hydroxytryptamine-3 [5-HT_3] receptor). Therapeutic agents with very high specificity for the 5-HT_3 receptor have fewer extrapyramidal effects than metoclopramide and have powerful antiemetic activity in the acute phase of vomiting induced by cisplatin, doxorubicin, cyclophosphamide, and abdominal irradiation. The 5-HT_3 receptor antagonists (ondansetron, ganisetron) used in combination with other standard antiemetics confer an advantage in the prevention of chemotherapy-induced nausea and vomiting in pediatric patients. The combination of a neurokinin receptor antagonist and dexamethasone has been shown specifically to decrease the delayed vomiting phase of chemotherapy with cisplatin.[29] Although it is assumed that the major site of action of the selective serotonin antagonists is the CTZ, a major effect of 5-HT_3 receptor antagonists is at the enteric level, where they block receptors in the peripheral ends of vagal afferents, thus blocking serotonin-stimulated vagal afferent input to the central pattern generator and reducing the perception of emetic stimuli.[8,9,30]

DIAGNOSTIC APPROACH TO THE CHILD WITH VOMITING

Vomiting is a common symptom of many disease states. The differential diagnosis of the child with vomiting varies with the age of the patient. Congenital anatomic, genetic, and metabolic disorders are more commonly seen in the neonatal period, and peptic, infectious, and psychogenic causes are more prominent with increasing age. Feeding intolerance and food refusal behavior, with or without vomiting, are common symptoms of cardiac, renal, pulmonary, metabolic, genetic, and neuromotor disorders; child abuse; and Munchausen syndrome by proxy. The physician must remain alert to the huge differential and not assume that all infants who vomit have gastroesophageal reflux. Serious disease in infancy may be missed by this approach. Suggested screening laboratory evaluation in any child with prolonged or repetitive vomiting includes complete blood count, serum electrolytes, blood urea nitrogen, urinalysis and urine culture, and stool examination for occult blood, leukocytes, and parasites. Specific indications from history and physical examination may result in obtaining other tests, such as upper gastrointestinal series; abdominal ultrasonography, computed tomography or magnetic resonance imaging of the head, tests of liver function, serum amylase, toxicology screen, pregnancy test, serum ammonia, urinary organic acids, urinary catecholamines, urinary porphyrins, and electroencephalography. Endoscopic examination of the esophagus, stomach, and duodenum is sometimes helpful if peptic disease or anatomic abnormality is suspected. Manometric evaluation of the esophagus, stomach, and duodenum is occasionally helpful in defining primary or secondary motor abnormalities causing emesis.

THERAPY

The use of antiemetic agents in infants and children without a clear understanding of the cause of vomiting is not recommended. The use of antiemetic agents is contraindicated in most infants and children with vomiting secondary to gastroenteritis, structural anomalies of the gastrointestinal tract, or surgical emergencies such as pyloric stenosis, acute appendicitis, renal stones, bowel obstruction, or an expanding intracranial lesion. There are only a few situations in which antiemetic agents are indicated and possibly effective. These include motion sickness, postoperative nausea and vomiting, cancer chemotherapy, some cases of cyclic vomiting syndrome, and gastroparesis or other gastrointestinal motility disorders.

Motion Sickness

The neurohumoral stimuli for motion sickness are not known. The traditional view that the vestibular organ is overstimulated in motion sickness is probably not a sufficient explanation. Indeed, in the dog, the area postrema appears necessary to motion-induced emesis, suggesting that circulating compounds may, in part, mediate this condition.[7] Possible mediators known to have receptors in the

CTZ include such stress-related hormones as epinephrine, antidiuretic hormone, adrenocorticotropic hormone, cortisol, growth hormone, and prolactin, all of which can be increased during motion sickness. Some investigators believe that sensory input to the cortex, which conflicts with previous sensory experiences (such as the sensory conflict created by distorting eyeglasses or flight simulators), creates increased input to the central pattern generator, bypassing the CTZ.[7] The most common drugs used for prophylaxis of motion sickness are anticholinergics such as scopolamine (Hyoscine) and antihistamines. Although the activity of these agents may be on muscarinic and histamine receptors in the vestibular apparatus and CTZ, recent work suggests that both drug types block muscarinic receptors in the cortex, pons, and nucleus tractus solitarius.[31] Thus, the effects of these drugs may be to raise the emetic threshold of the central pattern generator for input from the cortex generated by sensory conflict. The combination of scopolamine and D-amphetamine has been used with synergistic effect to combat the symptoms of nausea and vertigo accompanying the weightlessness of space adaptation syndrome. Phenothiazines, substituted benzamides, and other D_2 receptor antagonists are of little benefit in experimentally induced motion sickness. The 5-HT_3 receptor antagonists are also ineffective.[32]

Cancer Chemotherapy

Antinausea and antiemetic therapy for cancer chemotherapy has been discussed in part above. Drugs normally used in pediatric patients include the antihistamines (mild symptoms) benzamides, butyrophenones, and other D_2 receptor antagonists and 5-HT_3 receptor antagonists. Combinations of these drugs with others that facilitate their antiemetic activity—dexamethasone and benzodiazepines—are commonly used. Indications and modes of activity of many of the standard antiemetics are listed in Table 12-1. Recent studies in animals and adults have shown good control of chemotherapy-induced vomiting with opiate agonists (butorphanol) and potentiation of vomiting with the opioid antagonist naloxone. However, the known emetic effect of narcotics in most clinical experience limits the routine use of these agents. Control of anticipatory nausea includes relaxation techniques and anxiolytics such as benzodiazepines in combination with standard medications.[17]

Postoperative Nausea and Vomiting

The importance of controlling postoperative nausea and vomiting (PONV) has increased with the current emphasis on rapid postoperative discharge in the day-surgery setting. The incidence of PONV is reported to be 5% in infants and as high as 50% in children between 6 and 16 years of age.[33] The incidence has decreased with improvements in perioperative hydration, decreased use of preoperative narcotics, attention to speed in operative procedures, effective decompression of the gastrointestinal tract, attention to allaying anxiety and pain, and improved techniques in mask anesthesia to avoid intestinal tract distention.[34] Decreased insistence on taking oral fluids in the first postoperative hours has also decreased vomiting in the day-surgery setting. Despite improvements, PONV remains a problem in pediatrics, especially following tonsillectomy and strabismus surgery. The most commonly used medication for the prevention and treatment of PONV is droperidol, both for its effect on the D_2 receptor in the CTZ and its sedative effects. Experience with 5-HT_3 receptor antagonists, used singly or in combination with droperidol, in both the prevention and treatment of PONV is encouraging, especially after gastrointestinal surgery and other procedures in which gastrointestinal distention is produced, but expense is an important issue. Induction of anesthesia with propofol, a rapidly cleared, lipid-soluble, substituted phenol, has been reported to decrease PONV by 50%, probably because of its very rapid clearance.[31,33,35]

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CHAPTER 13

COLIC AND GAS

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Colic is usually thought of as a symptom, a synonym for acute and unexpected abdominal pain. In children, the term most commonly refers to a syndrome characterized by a self-limited cluster of behaviors in the first 3 months of life, presumed to be secondary to underlying gastrointestinal disturbances and signifying pain in the intestine. Older infants and children are described sometimes as "colicky," implying a predisposition to irritability and gastrointestinal upset.

Neither the presumption that the source is gastrointestinal nor that colic is a painful condition has been proven,[1,2] and our understanding of the etiology, pathophysiology, and treatment of colic is far from complete. However, systematic descriptions of colic and of crying in normal infants have increasingly clarified our understanding of the behaviors that are the core concerns and have led to a revision of the usual interpretation of most complaints of "colic." It is now reasonably clear that all of the behavioral characteristics of crying that constitute the behavioral syndrome of colic are typical of normally developing infants but that they are either more intense or occur more frequently in infants thought to have colic. As a result, most infants who present with this syndrome are otherwise normal infants at the upper end of a continuous spectrum of crying behavior. Although less well established, a small proportion of these infants will continue to have increased crying that persists after the first few months, when colic has subsided in most infants. The clinical challenge is to understand in which infants this typical normative crying pattern may be exacerbated by concurrent pathogenic processes and whether such processes are implicated in persistently crying infants.

DEFINITIONS

Colic refers to a behavioral syndrome occurring during the first 3 months of life. In about 30% of cases, symptoms persist into the fourth and fifth months.[3,4] Crying is the core symptom, but clinicians differ on what other behaviors constitute the syndrome.[5] There are three dimensions around which defining features of colic can be organized (Table 13-1).[6] The first, crying, has age-dependent and diurnal characteristics. Increased crying typically begins about 2 weeks after birth, reaching a peak sometime in the second month, and then declines to baseline levels by about 4 months of age. This crying is not random but tends to cluster in the late afternoon and evening hours. Changes in the amount of evening crying account for most of the developmental pattern of colic. The second defining dimension is the association between crying and several behavioral characteristics. Some of the crying occurs in prolonged bouts that are resistant to soothing, even with feeding. During these bouts, infants may clench their fists, flex their legs over their abdomens, arch their backs, flush, and have an active, grimacing, and ruddy face that is interpreted as a manifestation of pain (a "pain facies"). Other behaviors add to the impression that this crying is gastrointestinal in origin; the abdomen may be hard and distended, and the crying bout may include regurgitation and passing of gas per rectum. The third defining dimension is that the crying bouts are described as "paroxysmal" in that their onset can be sudden and unpredictable, beginning and ending without warning, and seemingly unrelated to other events in the environment.

These clusters of characteristics have been interpreted as features of a distinct clinical syndrome, but because they are qualitative and continuous, it is difficult to determine whether one infant has colic, whereas another does not. By far the most widely used quantitative definition of colic is the one proposed by Wessel and his colleagues known as the "rule of threes."[4] Infants are considered to have colic if they cry for more than 3 hours a day for more than 3 days a week for more than 3 weeks. Because few parents or clinicians are willing to wait 3 weeks before something is done, the third criterion is often dropped (modified Wessel and colleagues' criteria). This definition has provided a useful benchmark for comparing samples across studies in controlled trials of colic, but it has significant limitations in clinical settings. First, the amounts of crying designated by the definition are arbitrary, so there is no obvious reason why an infant who cries slightly less than 3 hours a day should be considered not to have colic, whereas an infant who cries slightly more should be considered to have colic. Second, it takes no account of parents who work hard to calm their infant compared with parents who let their infants "cry it out." Third, the definition does not take account of the quality of crying. Evidence concerning whether there is an acoustically specific colic cry remains controversial,[7–10] but it is clear that differences in the quality of cries contribute to whether an infant is brought to the clinician with a crying complaint.[7]

TABLE 13-1 BEHAVIORAL CHARACTERISTICS OF COLIC SYNDROME

DIMENSION	DEFINING FEATURE
Age dependent	Age dependent: total daily crying tends to increase in the first 2 mo, then decline in months 3 and 4
	Diurnal: crying tends to cluster in the late afternoon and evening
Behavioral	Common: crying occurs in prolonged bouts; some crying bouts are resistant to soothing
	Variable: clenched fists; legs flexed over abdomen; back arched; flushed face and skin; active, grimacing face ("pain facies"); hard, distended abdomen; regurgitation; passing gas (burping, per rectum)
"Paroxysmal"	Bouts can be sudden, begin and end without warning, and be unrelated to environmental events

However, the main reason Wessel and colleagues' definition is not very helpful clinically is the robust evidence that most cases of colic, however defined, represent infants at the upper end of a spectrum of crying in normally developing infants rather than a distinct syndrome indicative of underlying organic disease in the infant or psychopathology in the caregiver. Three lines of evidence support this basic concept of colic. First, all of the phenomena thought to be defining of colic are present in infants without colic, except that they are less in amount, duration, or intensity.[11] For example, an age-dependent and diurnal pattern of crying is typical of most infants in most caregiving settings studied,[12–14] including infants of the !Kung San hunter-gatherers of Botswana[15] and infants in Manali, India.[16] Furthermore, in otherwise well infants born 8 weeks prematurely, the crying curve peaks at about 6 weeks corrected age rather than 6 weeks after birth.[17] The other behaviors associated with crying, including crying after a feed, abdominal distention, and showing a "pain facies," also occur in infants without colic but slightly less frequently.[7] Infants with colic are more likely than controls to have crying bouts that are unsoothable, but their frequency is proportional to their overall amount of crying.[18] In sum, the crying pattern of infants with colic is continuous with, rather than distinct from, that of normal infants. The second line of evidence is that most cases of colic occur in the absence of detectable disease in the infant or the parent. Current estimates from admittedly imperfect data suggest that organic diseases are associated with colic in about 5% of cases (higher in referral settings) (Table 13-2).[2,6,19,20] Similarly, most cases of colic cannot be accounted for by parental inexperience, postpartum depression, or deficient caregiving. There is no difference in crying amounts between firstborn and later-born infants, even though first-time parents bring their infant to the physician with this concern more often.[21] Emotional lability in the third trimester is no different in mothers whose infants later have colic,[22] and they show similar affection, are as interactively sensitive, and hold and soothe their infants more compared with other mothers.[18] The third line of evidence is the remarkably consistent pattern across available follow-up studies showing that the physical and emotional outcomes for the infant with colic in low-risk families do not differ from those of controls.[23,24] However, colic appears to be a risk factor for the mother's emotional state, her confidence as a caregiver, and her perception of her infant, especially in high-risk families.[18,23,25–27]

It has been suspected for some time that a small number of infants who present with an otherwise typical colic syndrome continue to have persistent crying well beyond the first 3 months of life, when most colic has subsided.[26,27] These infants most often have associated prenatal, perinatal, and family risk factors and often manifest associated sleeping and feeding problems and breakdown in the usual mother–infant interaction patterns. Because of these features and the persistent crying, this pattern of early crying has been dubbed the "mother-infant distress syndrome."[28] In addition, however, increased crying and complaints about crying can arise after the first 3 months in infants who did not have colic previously.[29,30] Of the 1 in 16 infants whose crying is at levels consistent with "colic" at the end of 3 months of age, about half also had colic at 6 weeks ("persistent" cases), but the other half had not had colic previously. In summary, what has in the past been called "colic," quite nonspecifically, probably included at least three groups of infants: (1) those with the typical

TABLE 13-2 ORGANIC DISEASES PRESENTING WITH "COLIC-LIKE" SYNDROME

DISEASE STATES	STRENGTH OF EVIDENCE	ESTIMATED PREVALENCE*
Cow's milk protein intolerance	Strong	< 5%
Isolated fructose intolerance	Strong	Rare
Maternal drug effects (especially fluoxetine hydrochoride [Prozac])	Strong	Unknown, changing
Anomalous left coronary artery from the pulmonary artery	Strong	Very rare
Infantile migraine	Moderate	Rare
Reflux esophagitis	Moderate	Rare
"Shaken baby" syndrome	Moderate	Difficult to distinguish cause and effect
Congenital glaucoma	Weak, but suggestive	Rare
CNS abnormalities (especially Chiari type I malformation)	Weak, but suggestive	Rare
Urinary tract infection	Weak	Probably rare
Lactose intolerance	Very weak	Probably not etiologic

CNS = central nervous system.
*In primary care settings.

behavioral syndrome of early increased crying; (2) a subgroup of infants, often with additional risk factors, whose high levels of crying (and feeding and sleeping problems) persist; and (3) a new group of infants with increased crying that develops after the first 3 months. Respecting the developmental nature of these syndromes and their origins, the term *colic* will be reserved for the behavioral syndrome of early increased crying, *caregiver-infant distress syndrome* for later increased crying, and *persistent caregiver-infant distress syndrome* for those who had increased crying both early and later.[29]

An understanding of colic syndrome as a collection of behaviors that normally developing infants do rather than a specific condition that infants have forms the basis for a practical clinical approach to the problem. First, if there is no distinct syndrome, crying complaints need to be addressed regardless of whether they meet definitional criteria for colic syndrome. Second, even if most cases are explained by normal developmental processes, the importance of identifying those cases in which organic disease causes a colic-like syndrome is not diminished (see Table 13-2). Coexistent disease most likely exacerbates the pattern of increased crying that would be present even in the absence of disease. There are two important clinical consequences. First, even successful treatment of a coexisting disease may only reduce, but not eliminate, increased crying, depending on when it is introduced. Second, irrelevant treatment introduced when crying would diminish anyway may be inappropriately interpreted as effective.

NORMAL SPECTRUM OF CRYING AND COLIC

In controlled studies, a number of behavioral characteristics of infants representing the upper end of the crying spectrum with the behavioral syndrome of colic have been described. As a group, these infants do cry for more hours a day than control infants, independent of parental reporting bias.[31] Crying tends to cluster in the evening, but infants who cry more in the evening also tend to cry more during the rest of the day.[32,33] The quantitative increase is due primarily to longer crying bout lengths rather than increased crying frequency,[7] although some infants with colic may cry more frequently as well.[34] In infants who meet Wessel and colleagues' criteria,[4] not only are the daily duration and bout length longer, but also the facial activity is greater when crying. The cries tend to be perceived as more intense, urgent, grating, and sick-sounding, with higher, more variable fundamental frequencies and more dysphonation under some conditions.[7,8,35] In a significant proportion of infants whose parents complain about crying, the quantity of crying (daily duration and bout length) is the same as in infants who do not meet the criteria for colic.[7,31] Nevertheless, the quality of crying may be different, and it tends to be perceived as "sick-sounding" after meals.[7] This is not simply parents being anxious about their infants because it probably reflects acoustic differences in the structure of the cry sound.[10]

There have been occasional reports of differences in the crying pattern in some infants: formula-fed infants[33] or infants who are "switched" from breast to formula feeds[36] may have a "flatter" within the day distribution (more crying in the morning, less in the evening) and/or the age-related peak may occur earlier.[37] Unfortunately, there are no systematic studies of crying characteristics of infants whose colic has been demonstrated to be due to gastrointestinal disease. In case reports, the crying tends to be characterized as severe, the pattern of evening clustering within the day is often absent, there are almost always other symptoms and signs in addition to the increased crying, and the crying often does not resolve by the fourth month of life.[2,6,38–40]

Many of the features of crying behavior in infants with and without colic are understandable in light of the normal organization of infant behavior in the first few months of life. Infant behavior is not organized as a continuum of arousal but rather as a set of discontinuous and distinct modes of behavior reflecting the organism's "behavioral state,"[41,42] of which crying is one. Three important features of behavioral states are that (1) they are self-organizing in the sense that a state is maintained until that pattern of events occurs that results in a shift to another state, (2) they are relatively stable over time (minutes rather than seconds), and (3) a stimulus experienced in one state has a different effect when experienced in another state. In Wolff's classification, the crying state is defined in terms of persistent cry vocalizations (from whimpering to loud screaming), diffuse motor activity or a rigid extended trunk posture, resistance of limbs to passive movement, and a facial crying grimace sometimes accompanied by flushing.[42] Wolff notes that a specific aspect of the behavior of human infants not seen in other animal young is that crying and the accompanying rhythmic limb movements frequently continue after the removal of the offending stimulus.[42] Fussing is conceptualized as a state of transition, characterized by intermittent vocalizations and less intense and nonrhythmic motor activity.[42] Waking activity is characterized by bursts of generalized motor activity and by open eyes. Occasional moaning, grunting, or whimpering can occur, but it is always unsustained.

The features of vocalization, motor behavior, facial expression, and vascular reactivity that characterize crying as a self-organizing state closely parallel the clusters of behavior that are typical of colic but are distinct from the negative vocalizations of common fussing and intermittent crying. Because it is a different state, a soothing stimulus applied to an infant who has just begun to cry but whose crying state is not yet organized is more likely to abort the transition from awake activity to crying than if it were applied after the crying has become sustained. Consequently, crying vocalizations can become incorporated into a behavioral state of the child that is prolonged, self-sustaining, and resistant to soothing. This may explain why the sustained, unresponsive "crying of colic" appears to be distinct from normal crying while at the same time occurring in otherwise well infants. The behavioral state concept also helps to explain why a soothing maneuver successful for the unsustained whimpering of fussiness might fail if initiated after the infant's behavior has become organized into a crying state. In general, the problem of

colic may be the problem of understanding the conditions that provoke or terminate the crying state, not just crying vocalizations.

As with other behavioral states,[42,43] the prevalence and precipitating determinants change over developmental time as a function of physiologic reorganization of the child. As such, the rapid growth and differentiation of the central nervous system during early postnatal life are probably important determinants of colic syndrome. Infant behavioral states other than crying also undergo parallel significant changes during the second to fourth months of life, including periodic organization of sleeping and waking[42,44] and emergence of alert activity.[42,43] These organizational shifts probably reflect periods of increased stability for some states and unstable transitions to other newly emerging states. In particular, increasing stability for the waking states results in fewer disruptions by extrinsic or intrinsic perturbations. The decline in crying following the early peak at 2 months probably represents one manifestation of increasingly stable wakefulness.

The development of cognitive, affective, and motor functions makes maintenance of a stable state of noncrying wakefulness less dependent on environmental factors. For example, during the first 3 months, the infant becomes more responsive to environmental stimuli such as the human voice (compared with nonhuman sounds) and human figures (compared with visual distraction in general).[42] The onset of the social smile at about 6 weeks prolongs infant interaction with caregivers and stabilizes alert waking states. The increase in thumb sucking permitted by newly developed hand control allows the infant to achieve a quiet awake state through coordinated rhythmic motor activity.[45,46] In general, the infant's increasing competence provides more options for self-regulation of state in the second and third months. Thus, the infant's maturing, increasingly stable waking states may explain the spontaneous remission of colicky behavior as a manifestation of normal developmental processes, which most hypotheses postulating pathogenetic factors fail to do.

If changing organization of behavioral states accounts for most of the manifestations of early crying, then one might expect similar nonpathogenic changes in other states as well both in infants with and without colic. This appears to be true for a number of behavioral and physiologic systems. Infants with colic do have lesser amounts of sleep overall,[47–49] which, when adjusted for the "trade-off" with the increased crying, appears to be different only at night.[49,50] However, sleep architecture measured by all-night sleep recordings is no different in infants with and without colic either at 2 months or after colic has resolved at 7 months,[50] implying that this does not represent a disordered physiologic condition. Furthermore, sleeping less in the early weeks does not predict less sleeping in the remainder of the first year, nor is being a "high fusser" or a "high crier" in the first 3 months related to total sleep at 9 months or sleeping through the night.[51] Neither are there any differences for high, middle, or low frequencies in heart rate variability during sleep at 2 or 7 months of age, suggesting that colic syndrome is not associated with an "imbalance" of the sympathetic and parasympathetic autonomic systems.[52] Finally, despite almost twice as much crying and fussing, infants with and without colic show no differences in heart rate, vagal tone, or salivary cortisol secretion to the moderate stress of a mock physical examination.[49] The one apparent difference is that the pattern of diurnal rhythm in salivary cortisol secretion is "flatter" in infants with colic, although the overall daily secretion is the same.[49]

Considering colic as the upper end of a spectrum of otherwise normal crying behavior implies that complaints about crying will also be generated for behavior that does not meet clinical criteria, however defined, for "colic." Amounts of crying are not the only reason that it is brought to attention, in part because crying, whether increased or not, can have different meanings to caregivers. Despite no overall differences in crying, parents of firstborn infants are more likely to bring it to the attention of clinicians.[21] It is clear that some parents are not concerned even in the face of excessive amounts of crying and that the majority of crying complaints do not meet the clinical criteria for colic.[7,34] Often the amounts of crying in these latter infants do not differ significantly from control infants,[7,53] although it remains possible that the quality of the crying could be different, at least in some situations.[10] Interestingly, however, parents of infants with lesser amounts of crying may feel more feelings of anger and nervousness and of being rejected by their infants, whereas those with higher amounts do not.[53] However, infants with higher amounts of crying are more likely to have less optimal behavioral interchanges during feedings and diaper changes when infants are not crying, especially with fathers, than infants who cry less.[54]

PATHOGENESIS

Candidate pathophysiologic determinants should help explain both the early increase in crying and its subsequent decline, the most reliable features of crying in infants both with and without colic.[4,13,14,21,45,55] Second, they should help explain why crying during the day is not random but tends to cluster in the evening hours ("evening colic"). Third, bout length, but not frequency of crying, is amenable to change in normal infants.[14,45] In contrast, infants with colic lack the ability to calm, resist soothing, and have crying episodes that are difficult to terminate after they start.[56] Consequently, the mechanisms underlying colic probably relate to intrinsic or extrinsic proximal factors that tend to maintain rather than initiate crying bouts.

Feeding and Colic

The hypothesis that dietary factors have a significant role in the pathophysiology of colic stems from observations that feeding disruption or lack of calming follows feeding in infants with colic and that crying appears to lessen if the diet is changed to reduce exposure to cow's milk protein. In fact, however, the therapeutic success of a formula change is usually very difficult to distinguish from the normal developmental evolution of crying. The dietary factor most commonly implicated in colic relates to intolerance

to protein components, but plausible mechanisms underlying feeding-colic relationships could implicate both protein and carbohydrate nutrients as well as non-nutrient determinants related to motility, gut hormones, feeding behavior, and their interactions.

Nutrient Determinants

Protein Intolerance. Dietary cow's milk proteins are presumed to act as antigenic stimuli of a gastrointestinal hypersensitivity reaction. If this were true for most infants with colic, then change or modification of the ingested protein to a less antigenic form should reduce the colicky behavior. In breast versus formula comparisons, two contradictory predictions are possible, depending on the operative mechanism. On the one hand, formula-fed infants should develop colic because of the high concentration of potentially antigenic protein. On the other hand, breastfed infants are exposed to lower concentrations of antigenic protein but may be more likely to have colic owing to increased hunger and crying from insufficient milk. In controlled studies, neither prediction has been confirmed. In fact, the prevalence, pattern, and amount of crying associated with colic have been similar in both breast and formula feeders in referred and nonreferred infants.[36,55,57–59] However, if there is an earlier peak and a flatter, within the day pattern of crying,[33,37] this could be, in principle, related to the higher concentration and/or more uniform delivery of potentially antigenic cow's milk proteins.

Most likely, this lack of difference could have occurred because cow's milk protein is passed in breast milk.[60] The most likely and possibly most antigenic cow's milk proteins, β-lactoglobulin and casein, are found in human milk but in concentrations (up to 33 ng/mL) that are much lower than the mg/mL concentrations typical in infant milk-based formula.[61–63] However, potentially antigenic bovine immunoglobulin G (IgG) occurs at comparable levels in human and cow's milk samples, shows marked individual variability between mothers, and tends to be higher in the milk of mothers who report colic symptoms in their infants.[61] Finally, variables such as socioeconomic status, infant care, and attitudinal differences probably covary with the choice of feeding, making it difficult to identify independent effects attributable to diet. Nevertheless, the clinical impression of crying differences in breast- and formula-fed infants is not substantiated when such potential confounders are controlled.[36]

Intolerance to cow's milk protein could be implicated by immunologic or local toxic mechanisms. The quantity of ingested antigen, the relative permeability of the small intestine to dietary protein that is greater in infants with colic,[64] and reduced secretory immunoglobulin A typical of the infant may predispose patients to dietary antigen sensitization. True cow's milk hypersensitivity occurs in recognized clinical syndromes,[33,65,66] but there are only indirect data relating it to colic. Typical manifestations of gastrointestinal hypersensitivity (such as vomiting and diarrhea) are not part of the colic syndrome, colic is rarely encountered in other protein intolerance syndromes,[66] and there appears to be no specific immunologic marker associated with colic symptoms.[33] The incidence of family atopic history and other atopic manifestations is not increased in patients with colic.[24,55,58,67] However, the confusing spectrum of clinical presentations of hypersensitivity reactions,[33,68] the number of potential protein antigens in cow's milk (about 20), the lack of correlation between symptoms and permeability,[64] the possible contributory role of other digestive products (see below), and the variety of possible humoral or local and systemic cellular mechanisms make the confirmation of a specific immunologic relationship to colic very difficult.[6,67] Evidence of increased plasma cell content in the lamina propria of small intestinal mucosa in response to milk challenge has been reported in infants with colic and with other milk protein intolerance syndromes consistent with an immunoglobulin E–mediated hypersensitivity reaction.[69] Both immunologic and toxic mechanisms imply tissue damage in the intestine, for which the evidence is mixed. Both circulating motilin and permeability to human α-lactalbumin are increased in infants with colic, but fecal α_1-antitrypsin, hemoglobin concentrations, and fecal calprotectin levels are not.[64,70–72] The apparent inconsistency could be because motilin levels and permeability may index gut immaturity rather than pathology.[64,70] Evaluation of the role of dietary protein has been improved by increasingly careful reports of controlled diet trials. Conflicting findings and limitations in the designs of these studies continue to hamper their interpretation.[2,73] Evans and colleagues reported no symptomatic improvement in breastfed infants despite a measurable reduction in the presence of cow's milk antigen in breast milk on control days.[67] However, Jakobsson and Lothe and their colleagues did report symptomatic improvement in select subgroups of infants with colic symptoms with elimination of cow's milk from the diet of the infant or mother in formula- and breastfed infants, respectively.[74,75] In three breastfed infants, symptomatic improvement occurred in association with a reduction in detectable β-lactoglobulin in the breast milk.[62] In a subgroup of infants with severe colic dramatically responsive to casein-hydrolyzed formula, Lothe and Lindberg reported a recurrence of significant crying when given a 1-day challenge of bovine whey compared with human albumin protein.[39] A subgroup of referred formula-fed infants had fewer "colic" symptoms on a 1-week soy formula than on a standard cow's milk formula, with about 70% of these infants fulfilling study criteria for intolerance.[76] In a more generalizable but still selected double-blind study of referred breast- and formula-fed infants, a statistically significant and clinically meaningful reduction in crying (> 25%) was reported when the infants were placed on a low-allergen diet, but the improvements were most apparent (although not statistically significant) for the subgroup of breastfed infants younger than 6 weeks of age whose mothers were placed on a severely restricted low-allergen diet.[77] In a small open-label follow-up study, similar results were reported when mothers received amino acid–based supplements to provide sufficient caloric intake.[33] In the most carefully designed triple-crossover

study comparing cow's milk and hydrolyzed casein formulas, Forsyth reported a statistically significant overall reduction in crying and fussing when infants were on hydrolyzed casein formula compared with whole milk and a decrease in colic crying after the first change. However, only 2 of the 17 infants showed the predicted pattern of crying after each formula change, and the level of crying was still high (between 2 and 3 hours per day) even on casein formula.[78] An interesting and still unresolved question is whether the 3-day periods on casein hydrolysate formula in this study were sufficient to permit resolution of the process presumed to be causing the crying. In another small study of formula versus breast milk within the subject, there was a statistically significant but clinically unremarkable reduction in colic attacks with breast milk.[79] Finally, in two small unblinded and uncontrolled but carefully interpreted studies, Estep and Kulczycki described approximately 40% reductions in infant distress behavior in six formula-fed and six breastfed infants with colic who received an amino acid–based formula for 4 to 12 days, and all responded to a subsequent bovine IgG challenges with increased crying.[60] Interestingly, four of the breastfed infants whose mothers simultaneously followed a cow's milk protein elimination diet successfully returned to full breastfeeding after the formula "respite."

Although complicated and controversial, hypersensitivity to cow's milk protein probably does contribute to some cases of colic syndrome. It remains unclear as to whether it represents a specific mechanism for particular cases of colic-like syndrome or a mechanism that exacerbates the otherwise normal increased crying curve in the first few months of life (Figure 13-1). In the two studies in which the incidence of colic was mentioned, responders in diet trials of cow's milk elimination were estimated to account for 1 to 2% of births or 10 to 25% of infants presenting with colic.[76,79] In a prospective epidemiologic study of infants with cow's milk protein intolerance,[80] increased crying occurred in 20 to 40% of infants with cow's milk protein intolerance, but in only 4 of 65 infants (6%) was crying the main symptom. Compared with studies of nondietary constituents, diet studies tend to have smaller, more select samples from referral services, with a greater likelihood of additional symptoms (diarrhea, vomiting, weight loss) and more severe crying.[2] Consequently, formula changes are probably appropriate for a small proportion of infants with colic in primary care settings. It remains a challenge to determine to what extent protein intolerance explains crying problems brought to the physician and how cases in which protein intolerance is a factor may be selected for treatment.

Carbohydrate Intolerance and Intestinal Gas. Intestinal gas has long been considered a putative cause of colic because of the clinical association with abdominal distention, burping, and flatus. Intestinal gas may be derived from air swallowing, diffusion from the blood, and intraluminal production.[81] Air swallowing is the main source of gastric gas, which is predominantly nitrogen and oxygen. Intraluminal production of carbon dioxide derives from the interaction of hydrogen ions and bicarbonate in the small intestine and as a direct or indirect product of bacterial fermentation in the colon. Hydrogen and methane, the other major gastrointestinal gases, are produced by bacterial metabolism in the colon in adults and probably in infants.[81,82] Passive diffusion of gas between blood and lumen is dependent on relative partial pressures of the gases in the two compartments, with the probable result that carbon dioxide enters the stomach, nitrogen enters the small intestine and colon, and oxygen enters the colon. Gas is removed from the intestine through eructation, lumen to blood diffusion with subsequent expiration through the lungs, flatus, and bacterial catabolism in the colon.[81] Obligate air swallowing with sucking is thought to be exacerbated by poor feeding technique or inappropriately sized holes in artificial nipples. Crying itself may result in additional gastric gas, which remains after the crying episode itself has ended.[83] Lying supine may inhibit gas escape if the posteriorly placed gastroesophageal junction is covered by gastric liquid. Gastric accumulation itself may be irrelevant; symptoms could depend on passage of gas through the pylorus.[84]

Colonic gas produced in the gut is probably the major source of abdominal gas. Colonic bacteria use a range of substrates, but dietary carbohydrate (usually lactose) results in the most gas production.[85] Incomplete lactose digestion unassociated with clinical disease has been confirmed by breath hydrogen testing under normal feeding conditions in healthy infants and persists into the third month[82,86]; in fact, some proportion of all dietary carbohydrates is incompletely absorbed.[87] Amounts of colonic gas are influenced by a variety of factors affecting substrate levels and relative composition of bacterial flora and their function.

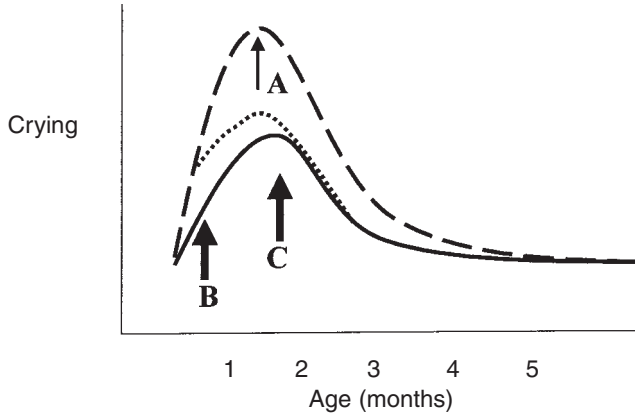

FIGURE 13–1 Probable effects of organic disease on crying pattern. The solid dark line represents the crying pattern in normal infants. If an infant has an organic condition contributing to crying, it will probably exacerbate the crying pattern (*arrow A, dashed line*). If an infant with an organic condition is appropriately treated early (*arrow B*), then the crying may still continue to increase despite appropriate treatment (*dotted line*), "rejoining" the normal crying pattern that would have occurred in the absence of the coexistent organic condition. If an infant without an organic condition is given a therapeutic trial (eg, a formula change) at a given time (*arrow C*), then crying may improve even though the improvement is unrelated to the therapy.

In principle, a primary role for colonic gas secondary to incomplete carbohydrate absorption in colic is attractive because it is consistent with the clinical pattern. The increased substrate absorption occurs at the same ages as the spontaneous reduction in crying. Evening crying could be related to the accumulation of partial, incompletely absorbed carbohydrates from the more frequent daytime feeds. The hypothesis is consistent with postfeed crying because colonic gas would still be present, even with adequate burping. However, the mechanisms by which gas produces distress are unclear. There is no significant postfeed distention in infants with colic,[7] nor is there radiologic evidence of increased intestinal gas volume.[55,83,88] Theoretically, distressing sensations could be related to changes in intestinal gas volume, producing reactive phasic gut contractions rather than a steady state of distention. Alternatively, pain may be related to motility patterns that permit retrograde gas passage from the colon.[89]

Attempts to demonstrate an etiologic role for intestinally derived gas have produced mostly negative results. Two studies have reported increased breath hydrogen excretion before and after feeding in infants whose parents reported a history of colic.[90,91] However, there is no difference in hydrogen production per gram of colonic carbohydrate (lactulose) or in mouth to colon transit,[92] nor do infants with colic differ in regard to stool pH or reducing substances.[93] In normal infants, reduced lactose intake did not reduce crying in a crossover trial.[94] There is a convincing single case of colic owing to fructose malabsorption, but the crying of this infant was severe, prolonged beyond 4 months, and accompanied by diarrhea.[40] Two trials that attempted to modify incomplete lactose absorption with oral lactase or hydrolyzed lactose milk did not reduce crying behavior.[79,95] In a large multicenter controlled trial, simethicone—a surface active agent—was ineffective, even in infants whose parents considered their symptoms to be gas related.[96] The best evidence of a possible etiologic role for incomplete lactose absorption is a randomized double-blind crossover trial that demonstrated a 1-hour reduction in crying when milk formulas were incubated for 24 hours with lactase drops in a small group of 13 infants,[97] keeping open the possibility that lactose absorption could be a factor in a few select infants.

Non-nutrient Determinants

Motility. Theoretically, alterations in intestinal motor activity could predispose the infant to colic as a direct source of abdominal distress or indirectly by affecting the distribution of intraluminal contents (including gas) in the gut. Jorup reported colonic hyperperistalsis, increased rectal pressure, and responsiveness to anticholinergics in dyspeptic infants in whom frequent stools were a prominent symptom.[88] The motility abnormalities were limited to the colon, stimulated by sucking, equally likely with breast or cow's milk, and highly variable from infant to infant. Although the frequent stools make these infants somewhat atypical, Jorup believed that most cases of colic could be accounted for by this mechanism. Newer evidence in favor of motility being implicated is the beneficial effect on colic of dicyclomine hydrochloride.[98,99] Dicyclomine may act centrally or peripherally by decreasing gastrointestinal spasm or by a direct relaxant action on smooth muscle. Unfortunately, its side effects make this drug unsuitable for use for infantile colic. A herbal tea preparation containing a number of antispasmodics (chamomile, fennel, and balm mint) has been reported to reduce colic symptoms in a controlled trial.[100] The picture remains unclear in that there are no documented abnormalities in mouth to cecum transit times in colic.[92] Furthermore, in a well-designed crossover trial to assess modifying gut motility with a fiber-enriched (soy polysaccharide) formula, the special formula reduced neither crying nor mouth to cecum or whole-gut transit.[101]

Gut Hormones. Because prostaglandins affect smooth muscle contraction and gastrointestinal motility, are present in high concentrations in breast milk, and can induce colic and diarrhea symptoms as a side effect of intravenous administration in newborns, they are candidates for proximal mediators of colic symptoms.[102–104] Which of the prostaglandins might be involved and whether the effect is local or systemic are uncertain. Most clinical syndromes in which prostaglandins are implicated involve stimulation of an intestinal secretory process with secondary motility changes. Because diarrhea is not part of the colic syndrome, a role for prostaglandins must be explained by their impact at physiologic dose levels.

A role can be proposed for several classes of hormones and transmitters found in the gut, but direct evidence is sparse. Basal motilin levels are raised in infants with colic independent of the diet, vasoactive intestinal peptide and gastrin levels are normal in colic, and all three levels are raised in other symptomatic gastrointestinal syndromes, suggesting some specificity for the role of motilin.[105] Furthermore, circulating motilin levels are elevated at birth in infants who subsequently develop colic; they are higher in formula-fed infants with colic than in breastfed infants with colic and continue to be higher throughout the first 3 months of life, but they overlap with values in noncolic controls.[70] Raised motilin levels could indicate gut disease or immaturity. By stimulating gastric emptying, small intestinal peristalsis, and shorter transit times, motilin could contribute to perceived intestinal pain or passage of intraluminal stimuli to the colon.

If dysmotility contributes significantly to colic symptoms, endogenous opioids with strong biologic effects on motility and secretion are likely to be implicated. These opioid effects should be considered in conjunction with those of cholecystokinin (CCK), which opposes the effects of opioids under many conditions. Both opioids and CCK have been implicated in the regulation of pain perception and satiety,[106] and these roles may evolve rapidly in early infancy.[107,108] Opioids may affect pain, distress, and ingestive behaviors simultaneously.[109,110] These complicated relationships may be important determinants of the developmental course of early distress behavior in human infants.

At least in infant rats, CCK is sufficient to reduce separation-induced distress vocalizations.[111] However,

CCK does not affect pain threshold in infant rats and does not, contrary to its role in adults, block morphine-induced analgesia.[112] Consequently, to the extent that the crying of infants with colic is exacerbated by separation from the mother and/or by hunger, reduced CCK might be relevant to colic behavior. Interesting evidence of possible CCK involvement in human infants with colic comes from the observation of Lehtonen and colleagues that gallbladder contractility (a function of CCK) was decreased postprandially in infants with colic, in infants who were crying at the time of examination, and in the evening.[113]

Developmental changes in the availability of melatonin and serotonin are also consistent with some of the characteristics of the crying of infants with colic.[114] The diurnal evening increase in circulating serotonin could contribute to intestinal smooth muscle contractions. Furthermore, circulating serotonin levels tend to increase during the first few months of life. Reduction in intestinal cramping could be due to the smooth muscle relaxant action of melatonin. Melatonin is present at birth,[115] after which the levels decline and then increase to develop diurnal evening elevations at about 3 months of age.[114]

Taste-Mediated Calming. Dietary intake is also implicated in proximate mechanisms to recruit physiologic systems to soothe already crying infants. In infant rats, small amounts of sucrose, polycose, corn oil, or milk formula applied to the tongue eliminate separation-induced distress vocalizations, mimicking the effect of opiates, and these responses are blocked by opioid antagonists.[109,110,116,117] In human infants, similar quieting effects have been demonstrated for sucrose and some other carbohydrates.[118–123] The sucrose effect has been shown to be due to the sweetness (or possibly positive hedonic properties) of the taste stimulus.[32,118,122] The calming effect of sucrose is specifically dependent on a normally functioning central opioid system in human infants as well.[120,124,125] The possibility that changes in a central opioid-dependent calming system might be related to early increased crying is suggested by the fact that this sucrose-induced quieting effect is gradually reduced over the first 6 weeks of life, when crying duration tends to increase.[119,126] Furthermore, the longer crying bouts typical of infants with colic implicate a less efficient distress reduction system. Indeed, when the response to sucrose taste was compared in equivalently crying infants with and without colic at 6 weeks, sucrose taste was less effective in infants with colic.[127] Consequently, developmental changes in the availability of such systems to taste stimuli, frequency of feeding, and individual differences in effectiveness may all contribute to differences in the ability to calm between normal and colicky infants. Furthermore, these findings implicate nongastrointestinal central distress reduction systems as contributory to the behavioral manifestations of colic.[128]

Biobehavioral Interactions. Gastrointestinal and behavioral systems may be complementary modulators of crying behavior. Species in which the protein and fat content of breast milk is low demonstrate low sucking rates, frequent feeding at short intervals, and continuous proximity to their source of food ("carrying species"), whereas species with high protein content in breast milk exhibit the opposite behaviors ("caching species").[129] Extrapolating from human breast milk composition, human infant sucking rates are (and caretaking should be) characteristic of carrying species. Small, frequent feeds might, for example, reduce hunger cries, result in less fermentable substrate being delivered to the colon and increased CCK being released, and elicit taste-mediated calming and increased rhythmic sucking. Indeed, !Kung San hunter-gatherers practice close mother-infant contact, constant carrying, upright posture, immediate responsivity, and frequent feeding (approximately four times per hour).[130] These features might all be expected to reduce crying behavior. Interestingly, crying in !Kung San infants also has an early peak and occurs with the same frequency but is about half the duration of crying in Western infants.[45] These findings support the concept that there is an early, maturationally based predisposition to cry but that prolonged crying bouts are amenable to change by biologic and behavioral factors associated with caregiving style.

Caregiving Style. Because colic tends to be unresponsive to maternal interventions, the concept that gastrointestinal factors rather than caregiving style or mother–infant interaction are determinants of colic tends to be accepted. However, systematic observation confirms the common wisdom that caregiving practices such as carrying and rocking are effective in shifting arousal to states of increased visual and auditory alertness and producing persistent soothing effects.[42,56] Psychologically significant interventions (eg, human voice and figures versus nonhuman sounds and visual distraction) become increasingly important as effective soothing agents during the early months.[42] In fact, the diminished effectiveness of sucrose taste at 2 and 4 weeks can be restored if the sucrose is delivered in combination with eye to eye contact from the caregiver.[131] The use of a pacifier to induce an organized rhythmic motor pattern will attenuate the crying state in response to pain[73,132] and reduce spontaneous crying.[42] However, crying owing to hunger is arrested only by gastric filling, not by sucking and swallowing.[42] In general, behaviors and environmental stimuli that involve postural change, repetitiveness, constancy and/or rhythmicity, and close proximity between mother and infant tend to maintain a noncrying state.

In two studies assessing the effects of caretaking in normal infants, crying was reduced by increased carrying and shorter interfeed intervals.[14,133] In the carrying study, crying reduction was substantial, averaging 43% for daily cry/fuss duration or 54% in the evening.[14] However, when increased carrying was tried as a therapy for colic, there was no additional benefit over that of parent counseling itself.[56] In other studies assessing the effect of modifying parental behavior, intensive parental counseling aimed at improving parental responsiveness was reported to reduce crying time by 70%, to the level seen in noncolic controls.[134] That response seemed more rapid than the response to a change of formula,[135] but this observation was not replicated.[136] In

a separate randomized controlled study, empathic interviews and advice to reduce overstimulation were reported to significantly reduce crying behavior.[137] Extensive clinical use has been made of a car-ride simulation device, but a controlled trial did not demonstrate any advantage for this technique over parental advice and reassurance.[136] As with dietary manipulations, trials of behavioral interventions have been subject to conflicting results and serious methodologic limitations, making interpretation of their significance difficult.[2] Furthermore, studied infants tend to manifest only moderately severe crying and are less likely to have been referred.[2] Consequently, firm conclusions about the effectiveness of these interventions and the selection of infants who are likely to benefit from one strategy or the other remain problematic.

Visceral Hyperalgesia and Allodynia

In light of the variety of putative mechanisms, it is clear that colic syndrome is not due to a single pathologic process, and an integrated model of pathogenetic processes is not close at hand. However, a plausible integrative hypothesis for the processes underlying colic phenomenology is that colic behavior reflects visceral hypersensitivity to otherwise innocuous intestinal stimuli, where the hypersensitivity is due to developmental processes and minor insults that alter afferent mechanisms and/or changes in the excitability of central neurons onto which these afferents project. The related concepts of visceral hyperalgesia and allodynia are analogous to mechanisms well described in the skin and possibly implicated in other gastrointestinal syndromes such as nonulcer dyspepsia, recurrent abdominal pain, and irritable bowel syndrome in which evidence of organic disease is absent.[138] Hyperalgesia refers to a reduced pain threshold and/or a greater or longer duration of response to a painful stimulus. Allodynia refers to painful or discomforting experiences owing to stimuli that do not normally produce pain or discomfort. Thus, intestinal distention, reflux of gastric contents, or changes in motility that would not normally be painful or discomforting were sensed as such.

Hypersensitivity of afferent mechanisms can occur owing to changes in the sensitivity of the primary afferent neurons in which noxious stimuli "alter the gain" of the afferent system through the action of chemical mediators that might be activated by inflammation or repeated noxious procedures.[138,139] On the one hand, this altered sensitivity changes the sensory information transmitted to secondary, dorsal horn neurons; on the other hand, it may affect reflex loops that regulate a variety of functions of the enteric nervous and gut effector cell systems, including motility, secretion, and blood flow. Afferent hypersensitivity can also be due to central hyperexcitability in dorsal horn cells. This central hyperexcitability would most likely be responsible for allodynia. These processes could be activated even in young infants. In general, excitatory pathways mature early and continue to mature postnatally. Inhibitory influences develop later. Both local inhibitory interneuronal connections in the substantia gelatinosa and the functional development of descending inhibition from the brainstem are all postnatal events, at least in the rat and probably in humans.[140]

There are a number of reasons why such mechanisms might be germane to the pathogenesis of colic. First, they provide a plausible way of understanding how such apparent discomfort can be expressed in the absence of detectable signs of disease, as is the case for most infants with colic. Second, visceral hyperalgesia and allodynia can persist long after the initial stimulus but can still be transient.[141] This finding is consistent with current evidence that colic does not have persistent effects on health and later development.[23] Third, descending cortical modulation is considered to play an important role in governing the excitability of dorsal horn neurons, especially in regard to pain perception.[138,142] This modulation inhibits excitatory tone so that in its absence, resting activity and responses to various sensory inputs to spinal dorsal horn neurons are increased. Fourth, central hypersensitivity can persist beyond the initiating stimulus (such as inflammation or repetitive noxious stimulation) and can be associated with reduction in thresholds and increases in the receptive field size of the dorsal horn neurons, allowing innocuous stimuli to excite previously unexcitable nociceptive pathways.

Such mechanisms help to explain why many disparate factors described previously (eg, protein intolerance, gas, motility differences) might cause discomfort without any one of them being wholly responsible for the behavioral manifestations of colic syndrome. Of specific interest for infants in general, but especially for those with colic syndrome, is that the maturation of these inhibitory systems is delayed relative to the functional maturity of the afferent systems.[141,143,144] Until these inhibitory systems are fully in place, infants may be predisposed to being more sensitive to otherwise innocuous stimuli. The evening clustering could be a function of the relatively increased density of sensory input to centrally hyperexcitable neurons during daylight hours. The developmental course of colic syndrome, specifically the decline in crying behavior, could be a function of modulation by increasing maturity of local inhibition owing to interneuronal synaptogenesis in the spinal cord and of descending inhibitory systems from the brainstem and cortex as the infant matures. Much less is known about the maturation of cortical pain systems, but the relative delay of inhibitory connections compared with excitatory connections appears to be a general pattern there as well.[140]

MANAGEMENT OF EXCESSIVE CRYING OR COLIC

Our current understanding of the mechanisms for excessive and prolonged crying in infants is incomplete, but the problem demands a well-conceived management strategy. Because of the continuity between the crying of infants with and without colic, the following strategies are suggested for treating all patients with excessive crying, whether or not they meet Wessel and colleagues'[4] or other diagnostic criteria. It frequently falls to the gastroenterologist to provide that management and care, which should be based on the mechanistic concepts outlined above. Early

attention should be directed to the detection of a specific disease and its treatment, while paying attention to the needs of the caregivers and the potential dangers (such as "shaken baby" syndrome) for the infant.

EARLY CRYING EXACERBATED BY SPECIFIC DISEASE PROCESSES

Treatment will depend on whether a specific disease is detected. It is rare for gastroenterologists to see such patients who have not already been considered to have a dietary intolerance or allergy or trial of various diets. By far the most common organic diagnosis is dietary protein intolerance or, more specifically, cow's milk protein intolerance. Clinical clues to this diagnosis include hyperperistalsis immediately after intake of cow's milk, persistent vomiting and/or diarrhea, weight loss, blood in the stool, and the persistence of symptoms beyond the usual peak period of crying. These symptoms favor a clinical trial of removal of milk formula from the child's diet or milk from the diet of a breastfeeding mother with or without temporary substitution of an amino acid–based formula for the infant.[60] Response should be monitored closely. If lactogenesis is carefully maintained, breastfeeding infants temporarily placed on a formula substitute can usually be returned to full breastfeeding.[60] In formula-fed infants, formulas should not be frequently switched, nor should they be changed simply for their placebo effect.[57]

EARLY INCREASED CRYING WITHOUT SPECIFIC ORGANIC DISEASE

Treatment should be focused on reducing the infant's crying and allaying the stress experienced by caregivers. Several of the measures below apply equally to children who also have a specific organic disease. With regard to reducing crying, current evidence supports the following:

1. Crying may be reduced temporarily by various behavioral interventions, but it is not likely to disappear until the infant is mature enough that his or her own regulatory controls on crying become more efficient.
2. Evidence for the effectiveness of medications is adequate only for dicyclomine hydrochloride.[98,99] However, this is no longer recommended for colic because of potentially hazardous side effects. In a single randomized double-blind placebo-controlled trial, the anticholinergic drug cimetropium bromide was used, not to prevent colic but to treat individual colic "crises," defined as inconsolable full-force crying bouts with associated behaviors (legs flexed, closed fists, meteorism) that are unresponsive to behavioral soothing measures.[145] Consequently, whereas the number of bouts were the same, the duration of bouts was reduced from 48 minutes to 17 minutes on average. The only reported side effect was increased sleepiness. Cimetropium bromide is a synthetic derivative of scopolamine, an anticholinergic agent, and its safety in infants has yet to be established. Other treatments for which there is no supportive evidence include alcohol, phenobarbitol, and/or anticholinergic combinations.[146,147] "Gripe water," the active ingredients of which are sodium bicarbonate and alcohol, may increase gastric gas through contact with gastric acid, thus stimulating burping,[148] but its effectiveness remains untested. There is no demonstrable benefit from simethicone, an "antigas" medication.[96]
3. Many commercially available devices (eg, crib shakers, heart sounds) provide stimulation analogous to that provided by more contact time with a caregiver. Temporary soothing is accomplished by anything that provides relatively constant background motion or sound. Many household devices (eg, fish tank aerators) can be used for this purpose. Placing the infant on a clothes washer or drier is contraindicated because of the danger of serious injury from falling off owing to the vibrations.

Reducing the psychological pressure on the caregiver is the most important component of management. Current evidence supports the following strategies:

1. Acknowledge the reality of the concern, regardless of the amount of crying.
2. Provide information for the parents aimed at reducing anxiety and anger at the infant attributable to not knowing basic facts about early crying and colic syndrome. First and foremost, make it clear that crying (a) increases into the second month, (b) is continuous with the behavior of infants with less crying, (c) is a universal behavioral phenomenon in normally developing infants, and (d) is not associated with organic disease (in the absence of other symptoms and signs). To quickly and efficiently provide this information about the normality of early increased crying, the National Center on Shaken Baby Syndrome (<www.dontshake.com>) sponsors educational campaigns on "the period of PURPLE crying." Each of the letters in the word "PURPLE" refers to a property of early crying that increases anxiety and angers caregivers, namely, P for peak pattern, U for unpredictable occurrence of crying bouts (or "crises"), R for resistance to soothing, P for pain-like facial grimace, L for long crying bouts, and E for evening clustering.
3. Suggest that a diary of crying and weight gain be kept.[149] A typical diurnal crying pattern and weight gain argue against underlying organic disease.
4. Determine the sources of pressure on the primary caregivers (eg, "significant other," parents-in-law, friends, or internalized cultural beliefs) and attempt to alleviate them.
5. Provide explicit "boundary" suggestions about inappropriate responses, no matter how problematic the crying. Should it seem too much to handle, it is important to stress that physically shaking the infant is never appropriate. Removing oneself from the infant and having someone to call are important alternative strategies.
6. Regular respite for the primary caregiver is best provided by the significant partner if available, especially for crying periods during the night. Other family members and baby-sitters are useful but must be educated about the "PURPLE" crying characteristics and the importance of

not shaking the infant. The primary caregiver may resist help, believing that the infant needs her in particular.
7. In severe cases, especially if the crying is occurring in the context of a fragile and otherwise challenged family, referral to specialized clinics or services is indicated. The increased crying may unmask caregivers and families with significant issues that need attention, as well as being a useful reason for the family to seek help.

INEFFECTIVE OR UNPROVEN TREATMENT STRATEGIES

Some erroneous assumptions concerning colic, derived from cultural and medical lore, have achieved the status of facts on which ill-advised management may be based:

1. Neither crying and fussing nor colic is more common in breast- or formula-fed infants. Possibly, crying in formula-fed infants peaks earlier than crying in breast-fed infants.[21] Changing from breast milk to formula does not reduce colic.
2. By including sham diaper changes, Wolff showed that wet diapers were not a sufficient cause of infant crying.[43]
3. Leaving the infants to cry out so that they learn to control their crying is not effective. Being responsive to the infant does not "spoil" the infant. The strategy of not responding to a crying infant because the infant would be "spoiled" is based on an operant conditioning/learning paradigm. To leave the infant is difficult and ineffective,[134] and there is no evidence to suggest negative consequences from responding. Indeed, pregnant women who were concerned during pregnancy about "spoiling" with too much physical contact had an increased risk of having an infant with colic.[34] Immediate responsivity and cuddling activate multisensory channels that soothe crying infants; this interaction should be responsive to the infant rather than intrusive.[137] A good functional outcome for the crying infant involves more contact, interaction, and attempts at soothing, not less, even if this increased contact does not eliminate the crying.
4. There is no systematic evidence for, and much evidence against, the proposition that maternal personality or anxiety causes excessive crying.[22,150] However, the presence of an infant with colic in an already fragile family is likely to increase the risk of negative outcomes for the infant and the family.[23,151]
5. One should not discount reports of excessive crying as attributable to bias in reporting by overconcerned or anxious mothers. Of course, maternal anxiety occurs with increased crying and colic, but there is no evidence that maternal reports of increased crying are exaggerated because of anxiety.[22]
6. Increased crying and colic are not more common in firstborns. Crying in the first- and subsequently born babies is identical, but mothers are more likely to bring the problem to a clinician when it occurs in firstborns.[21]
7. Although widely practiced, controlled evidence does not support massage therapy as an effective treatment for colic.[152]
8. To date, available studies on chiropractic spinal manipulation do not demonstrate that the natural course of the change in crying patterns is affected by treatment.[153]

POSTCOLIC CRYING SYNDROMES

The recognition that there is a small proportion (perhaps on the order of 5%[28,30]) of infants who have persistent caregiver-infant distress syndrome, presenting initially with colic and associated risk factors, and that another group with caregiver-infant distress syndrome develops colic-like crying levels after 4 months of age raises additional questions as to the management and approach to later crying problems. We know much less about infants with these conditions. There is at least some evidence that some infants with one or both of these conditions may be associated with carbohydrate malabsorption from fruit juices.[154] These infants, especially those with associated sleeping and feeding problems, may also have a poorer prognosis for later problems with hyperactivity and conduct problems during school years.[155] As with more typical infants with colic, it is difficult to distinguish those with contributory organic conditions from those whose distress reflects otherwise normal individual differences in temperament ("difficult temperament") or a more widely dysfunctional so-called "dysregulation syndrome of infancy."[156] Rational approaches to diagnosis and management for these infants remain a challenge.[157]

SUMMARY

Despite the lack of evidence for a primary etiologic role, the complaint of colic will continue to be considered gastrointestinal in origin until its mechanisms are better understood. Although the dilemma of responding to the distress of the infants and their parents in the absence of demonstrable pathogenesis remains problematic, it is clear that preventing negative secondary consequences such as shaken baby syndrome in the infant and depression or loss of confidence in caregivers is possible and should be considered a positive therapeutic achievement.

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CHAPTER 14

ABDOMINAL PAIN

John T. Boyle, MD

ACUTE ABDOMINAL PAIN

Acute abdominal pain is a popular descriptor that has evolved from the seminal definition by Sir Zachary Cope of abdominal pain of recent onset that triggers an urgent need for prompt diagnosis and active treatment.[1] The nature of the pain is usually such that the parent seeks immediate medical evaluation by a general pediatrician or emergency room physician. An acute complaint of abdominal pain precipitates at least 5% of unscheduled pediatric visits.[2] Although most children with acute abdominal pain have self-limiting conditions, the pain may herald a serious medical or surgical emergency. The diverse etiologies include acute surgical emergencies, disorders that will or may require surgical intervention, and specific intra-abdominal, extraintestinal, or systemic medical disorders. Severe acute pain may also present against a background of chronic illness. Most often, the child appears well at presentation, and the abdominal pain is accompanied by multiple complaints and is usually associated with a self-limited disease. The major challenge is to make a timely diagnosis of the acute surgical abdomen. In patients who present with acute abdominal pain to a primary care practice or community emergency room, the frequency of diseases requiring surgical intervention may be as low as 1%.[2] A physician can easily become complacent when dealing with a child with acute pain. It is important to have a consistent approach that addresses key diagnostic categories and gives parents guidelines to recognize warning signs that require re-evaluation. The primary care physician or emergency physician will most often call on the pediatric or general surgeon for consultation when concerned about a particular presentation of acute abdominal pain. There are, however, occasions when, with indefinite symptoms, there may be a tendency to wait for evolution of an acute process, in which case, the pediatric gastroenterologist may become involved in diagnosis and management.

Pathophysiology of Abdominal Pain

Abdominal pain can be perceived by autonomic sensory pathways from the abdominal viscera; somatic sensory pathways from the parietal peritoneum, abdominal wall, or retroperitoneal skeletal muscles; or somatic sensory pathways from extra-abdominal sites that share central projections with sensory pathways from the abdominal wall (referred pain).[3,4] Visceral pain is a dull or aching sensation generally perceived in one of three regions: the periumbilical, epigastric, or suprapubic midline area. Unfortunately, pain and tenderness are not always felt immediately over the site of disease. For example, the initial pain of appendicitis is usually felt in the periumbilical or epigastric areas, whereas pain caused by obstruction of the transverse colon is usually felt in the suprapubic midline area. Somatic pain, in contrast, is usually well localized and intense (often sharp) in character. An intra-abdominal process will manifest somatic pain if an inflammatory process affecting a viscus touches a somatic organ (ie, the anterior parietal peritoneum or abdominal wall). The classic example of referred abdominal pain is the shared central projections of the parietal pleura of the lung and the abdominal wall, such that abdominal pain may be the initial presentation of pneumonia. All three types of pain may be modified by a child's level of tolerance. Psychogenic and environmental factors augment or inhibit the perception of pain to varying degrees in different individuals.

Pain arising from the small intestine, regardless of the etiology, is always felt first and chiefly in the periumbilical or midepigastric area of the abdomen. Because the appendicular nerves are derived from the same thoracic nerves that supply the small intestine, it is not surprising that the pain at the onset of appendicitis is usually felt in the epigastric or umbilical area of the abdomen. The pain of disorders affecting the cecum, ascending colon, and descending colon are characteristically first felt at the actual site of the lesion because of the corresponding short mesocecum or mesocolon. An evolving change in localization of abdominal pain is often significant. Localization of pain in the right iliac fossa some hours after acute epigastric or periumbilical pain is usually due to appendicitis, although, rarely, the same sequence is seen with perforated pyloric or duodenal ulcer or in cases of acute pancreatitis. Radiation of the pain is also frequently helpful in diagnosis. In biliary colic, the pain is frequently referred to the region just under the inferior angle of the right scapula, whereas renal colic may be felt in the testicle on the same side. Testicular pain may also occur with appendicitis. A pelvic abscess, which lies close to the bladder, or an inflamed appendix that irritates the right ureter frequently causes pain on micturition. In many cases of peritonitis, intraperitoneal abscess, or abdominal distention owing to intestinal obstruction, abdominal pain will be caused or increased on inspiration.

Differential Diagnosis

Figure 14-1 divides the differential diagnosis of acute abdominal pain into three categories: (1) conditions requiring acute surgical intervention, (2) conditions that may initially be managed conservatively but will or may eventually require surgical intervention, and (3) specific intra-abdominal, extra-abdominal, and systemic conditions that require medical management. Detailed descriptions of the clinical presentation, diagnosis, and management of the specific disorders associated with acute abdomen are discussed in other chapters. The age of the patient is particularly helpful because the incidence of certain conditions is limited within a particular range of years (Table 14-1). Figure 14-2 presents an algorithm for sequential evaluation of a patient with acute abdominal pain. By thinking and working up selected key diagnoses, rare conditions will not be missed.

Is There Evidence of a Catastrophic Event Requiring Emergency Surgery?

Catastrophic events include acute generalized peritonitis, intestinal infarction, or infarction of the ovaries or testes. Such complications may follow blunt or penetrating abdominal injury, high-grade acute intraluminal intestinal

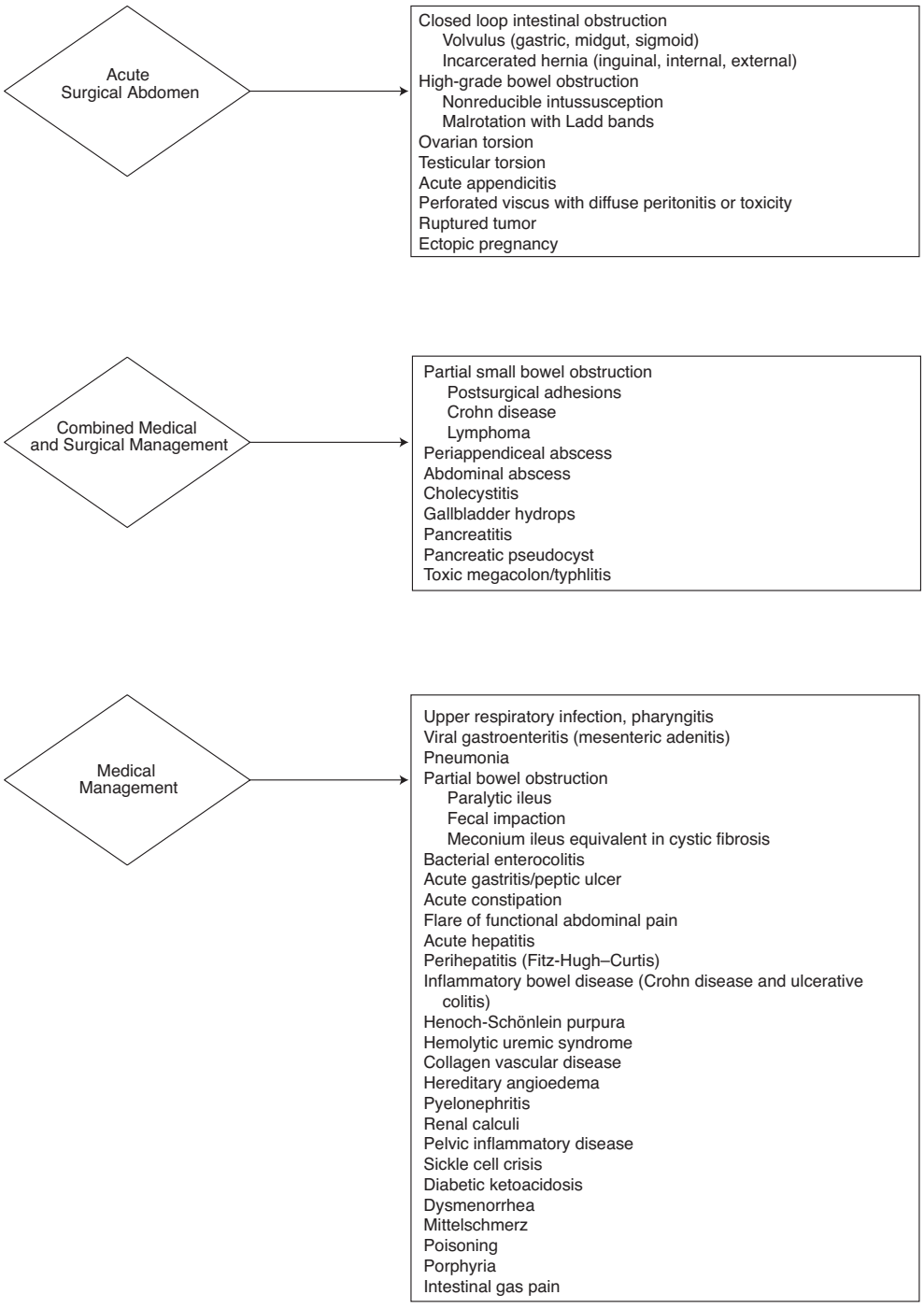

FIGURE 14-1 Differential diagnosis of acute abdominal pain.

TABLE 14-1 PRINCIPAL CAUSES OF ACUTE ABDOMINAL PAIN BASED ON AGE

NEONATE
Necrotizing enterocolitis
Spontaneous gastric perforation
Hirschsprung disease
Meconium ileus
Intestinal atresia or stenosis
Peritonitis owing to gastroschisis or ruptured omphalocele
Traumatic perforation of viscus (difficult birth)
INFANT (< 2 YR)
Colic (< 3 mo)
Acute gastroenteritis or "viral syndrome"
Traumatic perforation of viscus (child abuse)
Intussusception
Incarcerated hernia
Volvulus (malrotation)
Sickling syndromes
SCHOOL AGE (2–13 YR)
Acute gastroenteritis or "viral syndrome"
Urinary tract infection
Appendicitis
Trauma
Constipation
Pneumonia
Sickling syndromes
ADOLESCENT
Acute gastroenteritis or "viral syndrome"
Urinary tract infection
Appendicitis
Trauma
Constipation
Pelvic inflammatory disease
Pneumonia
Mittelschmerz

obstruction (intussusception), closed-loop intestinal obstruction (volvulus or incarcerated hernia), torsion of the ovaries or testes, and perforation secondary to peptic ulcer, intestinal foreign body, gallbladder hydrops, or acute cholecystitis. An obvious "sick" general appearance of a child who presents with acute abdominal pain suggests a late stage of all varieties of acute abdominal disease. A pale, ashen, diaphoretic facial appearance leaves little doubt about a serious abdominal disorder. The signs and symptoms of a catastrophic event can vary according to the time that has elapsed since the acute event has occurred. High fever is unusual in the early stages. An initial stage of generalized, continuous abdominal pain accompanied by prostration, hypothermia, retching, and vomiting is followed by a period in which abdominal pain lessens, vomiting ceases, and temperature and pulse return to normal. Sending patients home or admitting them to a general hospital ward during this "honeymoon phase" can have disastrous consequences because this stage is soon followed by a shock-like picture accompanied by high fever, abdominal distention, gastrointestinal bleeding, and generalized peritoneal signs. Well-known features of peritonitis include abdominal wall rigidity, involuntary guarding, cutaneous hyperesthesia, rebound tenderness, absent bowel sounds, positive psoas or obturator signs, and tenderness on palpation of the anterior or right lateral rectal wall during rectal examination. Rebound tenderness may or may not be a sign of a surgical abdomen. Rebound has also been reported in association with severe gastroenteritis, pneumonia, lead poisoning, and Henoch-Schönlein purpura.[1]

There is no reliable clinical, laboratory, or radiologic test that can distinguish between simple and strangulation obstruction of the small intestine.[5] Abdominal upright or decubitus plain films may show the presence of free air in the peritoneal cavity. A gasless abdomen is not uncommon in closed-loop or strangulating obstruction in which the obstructed loops are fluid filled.[6] Computed tomographic (CT) signs of strangulation include bowel wall thickening with or without target sign, pneumatosis, portal venous gas, mesenteric haziness, fluid, or hemorrhage often associated with ascites and abnormal bowel wall enhancement patterns following intravenous contrast.[7] CT may be oversensitive in diagnosing strangulation because bowel wall thickening, mesenteric haziness, fluid, and ascites are nonspecific findings that may also accompany other inflammatory processes, including appendicitis or Crohn disease.[7]

Does a Diagnosis of Acute Viral Syndrome (Gastroenteritis, Upper Respiratory Infection, Pharyngitis) Make Sense?

An acute viral syndrome is the most common cause of acute abdominal pain in any age group beyond the neonatal period.[2] Think viral syndrome when multiple symptoms, including fever, vomiting, diarrhea, decreased appetite, headache, cough, sore throat, and rhinorrhea, occur simultaneously with onset of crampy, diffuse abdominal pain. The abdomen is soft and nondistended in most cases. Although the child may perceive palpation as uncomfortable, this maneuver does not elicit localized or rebound tenderness. Bowel sounds are normal or hyperactive. Gastroenteritis may present with predominant upper gastrointestinal tract symptoms, including epigastric pain, nausea, and variable vomiting. The pain most often occurs during eating or in the immediate postprandial period. Alternatively, gastroenteritis may present with predominant lower tract symptoms with generalized, periumbilical, or lower abdominal pain associated with diarrhea. The key to diagnosis is that the pain is self-limiting and does not progress or localize.

The differential diagnosis of acute viral syndrome includes bacterial enterocolitis, food poisoning, acute infection with *Helicobacter pylori*, acute pneumonia, pyelonephritis, diabetic ketoacidosis, Henoch-Schönlein purpura, hemolytic uremic syndrome, and angioedema. Bacterial enterocolitis should be suspected by the abrupt onset of fever and diffuse abdominal pain, followed shortly by diarrhea. Small-volume, frequent stools, blood and mucus in the stool, fever greater than 102.5°F, and polymorphonuclear leukocytes in the stool suggest a bacterial rather than a viral etiology. Although the severity of the abdominal pain may simulate appendicitis, palpation of the abdomen elicits diffuse tenderness and no evidence of peritoneal irritation. Common bacterial food poisoning may present with generalized abdominal pain associated with profuse watery diarrhea (*Clostridium perfringens*), or profuse vomiting and generalized abdominal pain, followed by

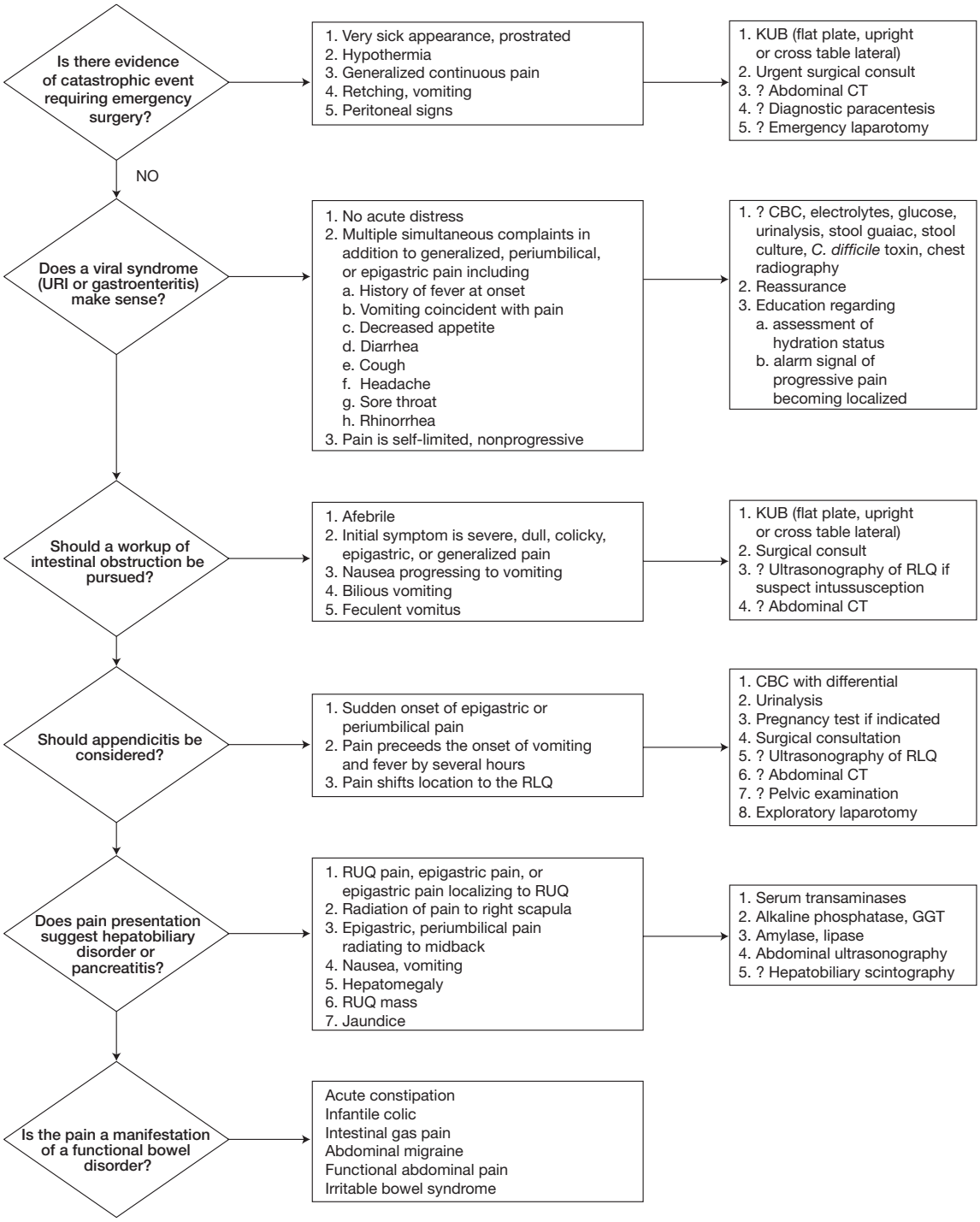

FIGURE 14-2 Approach to the patient with acute abdominal pain. CBC = complete blood count; *C. difficile* = *Clostridium difficile*; CT = computed tomography; GGT = γ-glutamyltransferase; KUB = flat plate of abdomen; RLQ = right lower quadrant; RUQ = right upper quadrant; URI = upper respiratory infection.

diarrhea (*Staphylococcus aureus*). Acute infection with *H. pylori* results in a neutrophilic gastritis with transient hypochlorhydria associated with epigastric abdominal pain and nausea. Pain is rarely severe enough to seek acute evaluation. As a general rule, serologic or stool antigen testing for evidence of *H. pylori* is not indicated in a child presenting with symptoms of viral gastroenteritis. Symptoms and signs of pneumonia are invariably present, including tachypnea out of proportion to fever, grunting respiration, cough, decreased breath sounds, and inspiratory rales. Fever, often accompanied by gastrointestinal symptoms suggestive of viral gastroenteritis, is frequent in the infant with urinary tract infection or pyelonephritis. In older children, fever accompanied by diffuse abdominal or flank pain may be the presenting symptom of pyelonephritis. Frequency, urgency, and dysuria, symptoms of cystitis, may be absent. Abdominal pain accompanied by vomiting may herald the onset of ketoacidosis in diabetes mellitus. There is usually an antecedent history of polydypsia, polyuria, and weight loss. There may be exquisite abdominal tenderness with guarding

and rigidity that may mimic peritonitis. The smell of ketones on the breath and the presence of deep sighing (Kussmaul breathing) reflect the ketoacidosis. In Henoch-Schönlein purpura, diffuse abdominal pain and vomiting with or without hematochezia may precede skin involvement by 1 week or occur 1 week after skin involvement. Diagnosis is suspected by evidence of other organ involvement, including joint pain, hematuria, and proteinuria. Intussusception occurs in 4 to 5% of children with abdominal pain. In hemolytic uremic syndrome, diffuse abdominal pain, vomiting, and hematochezia precede the onset of thrombocytopenia, coagulopathy, and oliguric renal failure by up to several weeks. At times, peritoneal signs may be prominent. Hereditary angioedema occurs in persons born without the ability to synthesize a normally functioning C1 inhibitor. Patients usually present with episodic, localized, nonpitting subcutaneous edema without urticaria, pruritus, or redness. Swelling of the intestinal wall without concurrent subcutaneous edema can lead to intense abdominal pain, sometimes with vomiting or diarrhea.

Acute abdominal pain secondary to acute infection is usually a clinical diagnosis that requires no confirmatory testing. Decision to do tests such as the complete blood count (CBC) with differential, electrolytes, blood urea nitrogen, creatinine, blood glucose, stool guaiac, stool culture, stool for *Clostridium difficile* toxin, urinalysis, or chest radiography should be based on clinical suspicion. The key to management is reassurance and education of the parents about the signs and symptoms of dehydration and the need for re-evaluation if pain progresses or localizes in the following 24 to 36 hours.

Should a Diagnosis of Intestinal Obstruction Be Entertained?

The differential diagnosis of intestinal obstruction includes closed-loop obstruction (volvulus, incarcerated hernia), high-grade or complete intraluminal obstruction (intussusception), partial obstruction (incomplete intussusception, postoperative adhesions, Crohn disease, fecal impaction), and paralytic ileus. Early diagnosis of closed-loop and high-grade intraluminal obstruction is essential to avoid intestinal ischemia. Intestinal obstruction is suggested by a history of episodic, crampy visceral pain and vomiting and is supported by physical signs of abdominal distention, diffuse pressure tenderness, visible peristalsis, and absent or high-pitched bowel sounds. In acute intestinal obstruction, the temperature, as a rule, is normal. Visceral pain is usually present from the onset and frequently comes in bouts and spasms. Frequent bilious emesis, beginning soon after the onset of epigastric pain, suggests high intestinal obstruction (malrotation with Ladd bands). If the obstruction is in the distal small bowel or colon, nausea is constant from the onset, but vomiting is usually a late symptom. In most cases, the character of the vomiting changes with time. First, the stomach contents are expelled, and then yellow-green bilious material appears. The color of the emesis gradually changes to greenish-brown and becomes "feculent" (foul smelling). Feculent vomiting is diagnostic of distal intestinal obstruction.

Causes of intestinal obstruction requiring surgery include intussusception, postoperative adhesions, and incarcerated hernia (inguinal, internal, or external). Intussusception occurs most often in infants aged 6 to 18 months and is usually ileocolic and idiopathic. Intussusception is heralded by episodic crampy abdominal pain often following signs of viral gastroenteritis or upper respiratory illness. Prior abdominal surgery or peritonitis places a child at risk for intestinal obstruction from adhesions. Adhesions can occur relatively early in the postoperative course or months or even years later. Small incarcerated indirect inguinal hernia can easily escape detection if the whole abdomen is not observed and palpated, especially in obese patients. A firm, discrete mass can be palpated at the internal inguinal ring and may or may not extend into the scrotum. The testes may appear dark because of pressure on the spermatic cord causing congestion.

Small bowel obstruction can be diagnosed on an abdominal plain film with the demonstration of dilated loops of small intestine with air-fluid levels and no or little colonic gas, whereas colonic obstruction appears as colonic distention. Typically, in intussusception, no stool or air-fluid levels are seen in the cecum. In suspected obstruction, serial abdominal radiographs reveal progressive bowel distention and disappearance of gas from the distal bowel. Unfortunately, the plain film is diagnostic in only 46 to 80% of cases of small bowel obstruction.[5] The lower percentage probably reflects the radiographic findings at the patient's initial presentation, whereas the higher percentage includes patients who received follow-up studies. Ultrasonography can readily detect distended fluid-filled bowel loops, which certainly suggests the possibility of obstruction, but defining the location, type, and cause of the obstruction is extremely operator dependent. Also, where gaseous distention predominates or if the child resists abdominal compression because of pain, ultrasonography may be technically limited. If the question is to rule out ileocolic intussusception, the sensitivity of abdominal ultrasonography has been reported to be close to 100%, even in relatively inexperienced hands.[8,9] The position of the leading edge can be determined, the presence or absence of a lead point can be ascertained, and the presence or absence of blood flow within the intussusception can be identified with Doppler examination. The presence of flow on Doppler interrogation has been shown to predict radiographic reducibility by barium or air and diminish the danger of perforation during reduction. Abdominal CT has significantly advanced the evaluation of small and large bowel obstruction, especially in the acute situation in which high-grade or possibly strangulating obstruction is being encountered.[7] The abdominal CT diagnosis of small bowel obstruction requires a dilated proximal small bowel and collapsed distal bowel. Although CT may miss low-grade partial small bowel obstruction (eg, secondary to Crohn disease), incomplete obstruction rarely results in strangulation and, therefore, can be managed conservatively, at least initially. The diagnosis of closed-loop obstruction (volvulus or incarcerated hernia) by CT may be difficult to ascertain.

Causes of intestinal obstruction requiring surgery must be distinguished from paralytic ileus, which generally presents with increasing abdominal distention, minimal abdominal pain and tenderness, nausea, and increased frequency of flatus and loose stools. Vomiting is uncommon. Bowel sounds are characteristically diminished or absent. Paralytic ileus may be seen in a number of clinical settings, including hypokalemia, uremia, lead poisoning, drug therapy that interferes with gastrointestinal motor function, postsurgical period, posttraumatic shock, and viral gastroenteritis. Radiographs in a child with paralytic ileus demonstrate multiple, small air-fluid levels throughout the abdomen, but serial films show either no worsening or gradual improvement of the bowel gas pattern. The abdominal CT finding that suggests paralytic ileus is small bowel dilatation associated with a colon that is distended by gas and fluid.[7] Although fecal impaction is a frequent complication of chronic fecal retention, complete obstruction is rare. Partial obstruction from fecal retention responds to a combination of serial enemas and a large volume of polyethylene glycol (PEG) electrolyte solution given by nasogastric tube with or without manual evacuation of the distal rectum under general anesthesia. Distal ileal obstruction syndrome is a complication of cystic fibrosis that may result in partial small bowel obstruction from inspissation of intestinal contents in the distal ileum. The obstruction usually responds to a large volume of PEG electrolyte solution given by nasogastric tube or Gastrografin enemas.

Should Appendicitis Be Considered?

The first symptom of appendicitis is characteristically epigastric or periumbilical pain. The awakening out of sleep by acute abdominal pain in a previously well child is a common presentation of acute appendicitis. The temperature at the onset of acute appendicitis is usually normal but may rise to 100 or 100.5°F within a few hours. Similarly, vomiting usually begins a few hours after the onset of abdominal pain. Frequent vomiting may occur at the onset of acute appendicitis if the distal tip of the appendix distends acutely behind a proximal appendiceal concretion. Diarrhea is not a common symptom associated with uncomplicated appendicitis. Characteristically, over time, the pain shifts to the right lower quadrant. The most reliable sign of acute appendicitis is localized tenderness in the right lower quadrant. In fact, the localization of pain and tenderness on physical examination depends on the anatomic position of the appendix. In the case of the retrocecal appendix, pain may be localized to the lateral abdomen or flank. Alternatively, pain associated with retrocecal appendicitis may never localize. An appendix pointing to the left lower quadrant may present with suprapubic tenderness.

An elevated total white blood cell count (WBC) in the range of 11,000 to 17,000/mm^3 is seen in approximately 80% of patients, but the specificity of leukocytosis for acute appendicitis is poor.[10] It is important to note that a normal WBC and differential should not delay surgical exploration in a child with localized right lower quadrant tenderness. A WBC that is higher than 20,000 mm^3 suggests an acute bacterial infection or intra-abdominal abscess.[11] The plain abdominal radiograph is most often normal in children with appendicitis. Conversly, patients with a right lower quadrant process of any etiology, including appendicitis or gastroenteritis, may present with air-fluid levels in the right lower quadrant, indicative of a localized ileus. Unless the conventional abdominal radiograph reveals a calcified appendicolith (seen in 10% of patients with appendicitis), it is too nonspecific to help in the diagnosis of appendicitis.[12]

Many surgeons now advocate imaging studies to improve diagnostic accuracy and decrease the need for hospital admission to observe patients with suspicion but a lower probability of appendicitis. High-resolution graded compression ultrasonography is an excellent test for detection of acute nonperforated appendicitis.[13] Appendicitis is suspected by visualization of a rigid, noncompressible, aperistaltic, tubular structure in the appropriate location. In children, the sensitivity and specificity of sonography as applied to the diagnosis of appendicitis are very high, reported at 94% and 89%, respectively, with an overall accuracy of 91%.[14] Ultrasound visualization of the appendix must be interpreted in light of the clinical findings. False-negative results may occur for a number of reasons, including a lack of patient cooperation, inadequate compression to displace bowel gas, and operator inexperience. Abdominal CT can also be performed quickly, does not require graded compression, requires less initial experience in interpretation, and is highly accurate in both the diagnosis and exclusion of appendicitis.[15] On CT, an inflamed appendix is fluid filled, often contains a fecolith, and shows "stranding" or inflammatory changes in the periappendiceal fat.[12]

Many cases of appendicitis progress to perforation without the occurrence of vomiting. A large percentage of very young children will have perforated by the time of presentation. Immediately following perforation, abdominal pain may improve, and the temperature may become normal or even subnormal. Within 1 to 2 hours, however, there are usually signs of generalized rather than localized peritonitis accompanied by frequent vomiting, pallor, tachycardia, and fever of 101°F or greater. Secretory diarrhea may be a predominant symptom following a perforated appendix if the inflammatory mass lays against the sigmoid colon. Following appendiceal perforation, the child characteristically prefers to lie still. Any movement usually evokes pain and irritability. Bowel sounds are absent. Following perforation, the diagnostic accuracy of ultrasonography decreases because of guarding and focal ileus. Perforation may be suspected by visualization of asymmetric mural thickening, areas of increased intramural echogenicity, and fluid in the right paracolic gutter with adjacent atonic bowel loops. Abdominal CT is more sensitive, more specific, and less operator dependent for assessing a perforated appendix. CT signs of perforation include periappendiceal phlegmon or abscess.[15]

The differential of right lower quadrant abdominal pain includes Crohn disease, small bowel obstruction, pyelonephritis, renal colic, acute salpingitis (pelvic inflammatory disease), ovarian torsion, dysmenorrhea, ruptured

ovarian cyst, mittelschmerz, typhlitis, ectopic pregnancy, and mesenteric adenitis. Acute onset of Crohn disease should be suspected if there is right lower quadrant mass and diarrhea. Children with urolithiasis rarely present with the excruciating pain of stone passage seen in adults. Colicky pain in the abdomen or flank is more common. Hematuria, either microscopic or macroscopic, occurs in the vast majority of children. The presence of fever greater than 101°F suggests pyelonephritis and salpingitis in addition to a perforated appendix. Urinalysis should be performed in all patients with right lower quadrant abdominal pain, flank pain, or pain radiating into the groin. Pelvic examination with appropriate examinations for sexually transmitted diseases is indicated in an adolescent female who has just completed a menstrual period and presents with lower abdominal pain and fever. The patient may report an increased vaginal discharge or irregular bleeding. A complication of salpingitis that evokes clinical signs of peritonitis and shock is a ruptured tubo-ovarian abscess. Typical primary dysmenorrhea consists of crampy, dull, midline, or generalized lower abdominal pain at the onset of the menstrual period. The pain may coincide with the start of bleeding or precede the bleeding by several hours. Associated symptoms include backache, thigh pain, diarrhea, nausea, vomiting, and headache. Endometriosis must be considered when there is chronic, cyclic, undiagnosed pelvic pain in teenagers. Unilateral abdominal pain at the midpoint of the menstrual cycle (time of ovulation), with or without spotty bleeding for 24 hours, is characteristic of mittelschmerz. Typhlitis should be considered in a neutropenic patient receiving antineoplastic drugs who presents with right lower quadrant abdominal pain, fever, diarrhea, nausea, and vomiting. Localized tenderness may progress rapidly to diffuse signs of peritonitis as a result of intestinal perforation. Urine or serum pregnancy testing should be performed in adolescent females of reproductive age with lower abdominal pain. Mesenteric adenitis is a commonly used term to describe clustering of inflamed lymph nodes in the region of the terminal ileum in patients undergoing appendectomy. Mesenteric adenitis should not be considered a separate diagnosis but rather a sequela of viral or bacterial gastroenteritis.

Does the Pain Presentation Suggest a Hepatobiliary Disorder or Pancreatitis?

Epigastric, right upper quadrant, or initial epigastric pain localizing to the right upper quadrant should suggest hepatobiliary disease, pancreatitis, or, rarely, acute peptic ulcer disease. Screening tests include serum transaminases, alkaline phosphatase, γ-glutamyltransferase, amylase, and lipase, as well as possible abdominal ultrasonography and hepatobiliary scintography.

Hepatobiliary disorders that may present with acute abdominal pain include viral hepatitis, biliary colic, acute calculous cholecystitis, cholangitis, acalculous cholecystitis, gallbladder hydrops, and perihepatitis (Fitz-Hugh–Curtis syndrome). Acute hepatitis is suspected if epigastric or right upper quadrant abdominal pain is accompanied by flu-like symptoms, including low-grade fever, anorexia, nausea, vomiting, malaise, and fatigability associated with palpation of tender hepatomegaly. Clinical manifestations of gallstone disease include biliary colic, acute cholecystitis, and cholangitis. Biliary colic is triggered by a gallstone(s) obstructing the cystic duct. The pain of biliary colic frequently follows a meal and can be localized to the right upper quadrant or epigastric areas. Sustained pain rises to a plateau of intensity over 5 to 20 minutes and gradually resolves over a 1- to 6-hour period. The patient tends to be restless, and the position does not help the pain. Pain lasting longer than 6 hours suggests acute cholecystitis. Cholecystitis implies an chemical inflammatory process within the gallbladder triggered by prolonged obstruction of the cystic duct. Referred pain to the dorsal lumbar back near the right scapula, nausea with some vomiting, and low-grade fever (< 101°F) are common. As inflammation worsens, the pain becomes more generalized in the upper abdomen and is increased by deep inspiration (Murphy sign: production of pain by deep inspiration or cough while the physician's fingers are compressing the abdomen below the right costal margin in the midclavicular line) and jarring movements. A common bile duct stone should be considered if the patient is jaundiced. Cholangitis should be suspected in a patient who has right upper quadrant pain, shaking chills, and a spiking fever greater than 102.5°F. A rigid abdomen or rebound tenderness suggests local perforation or gangrene of the gallbladder. Acute acalculous cholecystitis is acute inflammation of the gallbladder in the absence of stones. It is rare in children but has been associated with systemic illness or enteric infections. Acalculous cholecystitis should be included in the differential of a patient with the simultaneous onset of high fever and pain symptoms suggesting biliary colic. Gallbladder hydrops, or acute noncalculous, noninflammatory distention of the gallbladder, has been associated with Kawasaki disease, Henoch-Schönlein purpura, and scarlet fever. In addition to right upper quadrant pain, the distended gallbladder may be palpated. Perihepatitis is a complication of pelvic inflammatory disease in adolescent females that presents with severe right upper quadrant pain and tenderness produced by inflammation of the liver capsule. Fever may or may not be present. The syndrome has been associated with both *Neisseria gonorrhoeae* and *Chlamydia trachomatis*.

In pancreatitis, onset of pain is usually insidious over several hours. Constant epigastric or upper quadrant pain with or without radiation to the back, which is aggravated when the patient lies down, is an indication to check pancreatic enzymes. The pain may be referred to the left scapula or be generalized in both upper abdominal quadrants. Vomiting may be severe and protracted. A low-grade fever (< 101°F) may be present. The abdomen may be distended but is rarely rigid. Rebound tenderness is rare. Bowel sounds may be decreased.

Is the Acute Abdominal Pain a Manifestation of a Functional Bowel Disorder?

The three main considerations are acute constipation, aerophagia, and flare of functional abdominal pain. Acute

constipation may complicate a viral illness that causes decreased bowel motility and results in dietary changes. Rectal pain produced by anal fissure may be a cause of constipation. Abdominal pain is usually left-sided or suprapubic, antedated by decreased frequency or volume of usual bowel movements for several days. Acute constipation may be accompanied by sensations of urgency, tenesmus, and rectal pain. Abdominal examination may reveal distention or hard feces in a palpable colon.

In emergency rooms, acute constipation is frequently given as a cause of acute abdominal pain after abdominal plain film performed to screen for obstruction is interpreted by the radiologist as showing a moderate or large amount of stool. In fact, abdominal plain film has a low specificity for diagnosing constipation. In the absence of a confirming history and digital rectal examination showing a rectum full of stool, a child with acute abdominal pain should not be treated for acute constipation based on an abdominal radiograph.

Intestinal gas is also overplayed as a cause of acute abdominal pain. In the absence of a history of excessive air swallowing and distention, parents should not be told that acute abdominal pain is the result of intestinal gas. As with constipation, the abdominal plain film has a low specificity for diagnosing excessive intraluminal gas.

A significant percentage of children who present to emergency rooms with acute abdominal pain have a flare of chronic functional abdominal pain, described below. A history of chronic pain, a normal abdominal examination, and the absence of alarm signals suggest a flare of functional abdominal pain. Emergency physicians should reassure the patient and parents regarding normal examination and resist initiating further workup that might confuse management initiated by the patient's primary caregiver or pediatric gastroenterologist.

CHRONIC ABDOMINAL PAIN

Chronic abdominal pain is one of the most commonly encountered symptoms in childhood and adolescence. The definition of "chronic" has evolved from the seminal definition by Apley of recurrent paroxysmal abdominal pain in children between the ages of 4 and 16 years that persists for greater than 3 months duration and affects normal activity.[16] Chronic abdominal pain has been reported to occur in 10 to 15% of children.[17–19] At least as many children experience chronic pain but maintain normal activity and rarely come to the attention of the physician.[17–19]

The Pediatric Rome group has proposed that chronic abdominal pain can be subcategorized based on clinical presentation: (1) isolated paroxysmal abdominal pain, (2) abdominal pain associated with symptoms of dyspepsia, (3) abdominal pain associated with altered bowel pattern, and (4) abdominal migraine.[20] Symptoms of dyspepsia include pain associated with eating, epigastric location of pain, nausea, episodic vomiting, early satiety, occasional heartburn and acid regurgitation, and excessive belching. Symptoms of altered bowel pattern include diarrhea, constipation, or a sense of incomplete evacuation with bowel movements. The differential diagnosis of each subcategory of chronic pain includes a heterogeneous group of anatomic, infectious, noninfectious inflammatory, and biochemical organic disorders. Yet, although the exact prevalence figures are unknown, the most common cause in each subcategory is functional abdominal pain. The modifier "functional" is used in gastroenterology if no specific structural, infectious, inflammatory, or biochemical cause for the abdominal pain can be determined. The vast majority of children classified by Apley as having "recurrent abdominal pain" had functional abdominal pain.

Pathophysiology of Functional Abdominal Pain

There is general agreement that functional pain is genuine and not simply social modeling or imitation of parental pain or a means to avoid an unwanted experience (eg, school phobia or malingering). The etiology and pathogenesis of the pain are unknown. Many physicians conceptualize functional pain as "nonorganic." Yet there is a growing body of evidence that the pain is the result of disordered brain-gut communication involving both the efferent and afferent pathways by which the enteric and central nervous systems communicate. It is not clear whether the different subcategories of functional abdominal pain result from a heterogeneous group of disorders or represent variable expressions of the same disorder. The frequent occurrence of upper and lower bowel symptoms in the same patient (particularly nonulcer dyspepsia and irritable bowel in an adolescent) suggests that the latter scenario may indeed be the case. Although there is no evidence that the etiology of functional pain in children differs from functional pain in adults, the tendency of children to outgrow functional pain suggests that self-limiting developmental factors may be involved in the pathophysiology of pain in children.

The prevailing viewpoint is that the pathogenesis of the functional pain involves the interrelationship between altered gastrointestinal motility and visceral hypersensitivity.[21] Motility disturbances have been described in children, including altered intestinal transit, enhanced rectal contractility to cholinergic agonists, clusters of jejunal pressure activity that coincide with pain, lower rectal compliance, and altered rectal contractile response to a meal.[22–24] Visceral hypersensitivity in children with functional abdominal pain is supported by reports of enhanced awareness of balloon distention in the rectum and pain associated with physiologic stimuli such as the intestinal migrating motor complex.[25,26] A common link among these various motor and sensory phenomena is the autonomic nervous system. In some patients, associated symptoms including headache, dizziness, motion sickness, pallor, temperature intolerance, and nausea suggest a generalized dysfunction of the autonomic nervous system. In fact, abnormalities of sympathetic cardiac, vasomotor, and sudomotor function by autonomic testing have been described in patients with functional pain.[27] In adult studies, there is growing evidence that the initiating factors for autonomic dysfunction are found in the central nervous system, namely, the limbic system and the thalamus. Furthermore, there is increasing

evidence that serotonin (5-hydroxytryptamine [HT]) and its receptors (particularly the 5-HT_3 and 5-HT_4 receptors) play a major role in the pathogenesis of functional pain.[28]

In a child with visceral hypersensitivity, painful sensations may be provoked by physiologic phenomena or concurrent physical and psychological stressful life events. Examples of physiologic phenomena that may trigger pain include postprandial gastric or intestinal distention, gastric emptying, intestinal contractions or the migrating motor complex, intestinal gas, or gastroesophageal reflux. Intraluminal physical stress factors that may trigger pain include aerophagia, simple constipation, lactose intolerance, minor noxious irritants such as spicy foods, *H. pylori* gastritis, celiac disease, or drug therapy. Systemic physical or psychological stress factors may also provoke or reinforce the pain behavior by altering the conscious threshold of gastrointestinal sensory input in the central nervous system. Acute or chronic physical illness may unmask functional pain. Psychological stress factors may include death or separation of a significant family member, physical illness or chronic handicap in parents or a sibling, school problems, altered peer relationships, family financial problems, or a recent geographic move.

The concept of visceral hypersensitivity can be better understood by examining the role of lactose intolerance as a trigger of functional abdominal pain. There does not appear to be a difference in the incidence of lactose intolerance between patients with functional abdominal pain and age-matched patients without pain. Yet Barr and colleagues have reported a qualitative improvement in pain symptoms in 70% of intolerant children treated with a lactose-free diet.[29] They observed that such children lack an awareness of intolerance to lactose because there is no temporal relationship between lactose ingestion and abdominal discomfort. These results suggest that lactose intolerance is not directly the cause of the pain but the trigger that unmasks visceral hypersensitivity (perhaps by luminal distention) in susceptible patients. That lactose is but one provocative stimulus in such patients is supported by observations that a lactose-free diet does not induce complete resolution of the pain or alter the natural history of the condition.[29]

Recently, there has been progress in defining a subgroup of adults with symptoms of irritable bowel syndrome (IBS) developing after an episode of infective gastroenteritis.[30] The role of inflammation in the pathogenesis of functional abdominal pain must also be considered in view of the frequent finding of mild nonspecific histologic inflammatory changes at all levels of the gastrointestinal tract in patients with functional abdominal pain.[31,32] It may be speculated that such mild inflammatory changes that persist after gastroenteritis may be the cause of visceral hypersensitivity or altered intestinal motility. Immune responses alter neural and endocrine function, and, in turn, neural and endocrine activity modifies immunologic function.[33] Activated immunocompetent cells such as monocytes, lymphocytes, macrophages, serotonin-containing enterochromaffin cells, and mast cells that take up residence in the intestinal tract may secrete a repertoire of cytokines and inflammatory mediators that can lead to profound changes in enteric neural function. The main symptoms of postinfective irritable bowel (ie, diarrhea and loose stools) may reflect the prokinetic and secretory effect of 5-HT and inflammatory mediators derived from enterochromaffin cells and lymphocytes.[30] The possibility that some aspect of personality, behavior, coping style, or emotional state influences immune responses may also have implications in functional abdominal pain. The enteric or central nervous system may also modulate intestinal immune responses. Activation of the sympathetic nervous system causes leukocytosis, sequestration of lymphocytes, and inhibition of natural killer cell activity.[33] Sensory neurons also contain a variety of neurotransmitters and neuropeptides that can affect lymphocyte function, including substance P, vasoactive intestinal polypeptide, angiotensin II, calcitonin gene–related peptide, and somatostatin.

There also appears to be a genetic vulnerability because of the high frequency of pain complaints in family members.[16] Recent studies suggest that patients with functional abdominal pain who make their way to a subspecialty setting commonly exhibit "internalizing" behavior characterized by anxiety, mild depression, withdrawal, and low self-esteem.[34,35] Such a behavior profile may be primary and part of the genetic vulnerability of such patients. Alternatively, it has been postulated that such internalizing behavior is fostered within a family structure characterized by maternal depression, enmeshment, overprotectiveness, rigidity, and a lack of conflict resolution.[36] A third possibility is that the internalizing behavior is a common psychological adaptation to both organic and nonorganic chronic conditions.[35] Whether primary or secondary, the behavior pattern of the child and the family structure may both influence how the disorder is experienced and acted on.

The morbidity associated with recurrent abdominal pain is not physical but results from interference in normal school attendance and performance, peer relationships, participation in organizations and sports, and personal and family activities. Liebman found that only 1 of 10 children with functional abdominal pain attended school regularly and that absenteeism was greater than 1 day in 10 in 28% of patients.[37] A common misconception is that pain is the direct cause of the morbidity. In fact, the environmental consequences of the pain probably contribute significantly to the morbidity. Fordyce and colleagues observed that although pain does not originate from its consequences, much pain behavior is accounted for and modified by its consequences.[38] As described below, the usual parental, school, and medical management of recurrent abdominal pain is focused on symptom relief, which reinforces the pain behavior with attention, rest, and medication. This approach fails to reinforce nonpain responses such as normal activity.

Diagnosis of Chronic Abdominal Pain

Because the exact etiology and pathogenesis of the pain are unknown and no specific diagnostic markers exist for any group, functional abdominal pain is too often perceived as a diagnosis of exclusion. Yet it is the clinical presentation, together with a well-structured medical history and physical examination, that usually indicates that functional

abdominal pain is the likely diagnosis in an individual child presenting with chronic abdominal pain. Rather than a shotgun approach to rule out all potential infectious, inflammatory, structural, and biochemical causes of a particular pain presentation, diagnostic evaluation should be driven by an index of suspicion based on pertinent alarm signals in the history and physical examination. In clinical practice, functional abdominal pain should not be a diagnosis of exclusion. Primary care physicians should be able to make a primary diagnosis of functional abdominal pain without resorting to a large battery of biochemical or radiography tests. Management of functional pain is facilitated by early diagnosis, parental education and reassurance, and clear delineation of goals of therapy. The major outcome variable in the management of functional abdominal pain in children is lifestyle, not cure of the pain.

One reason why primary care physicians have difficulty making a positive diagnosis of a functional abdominal pain is that there is rarely a clear distinction between acute and chronic abdominal pain. A parent's decision to consult a physician is usually based on the age of the child, the severity of the pain, and the effects of pain on the child's lifestyle. Primary caregivers must often deal with the evolution of pain from the initial acute presentation to a chronic or recurring problem. A stepwise series of diagnostic studies is often initiated during early stages of the pain when an organic etiology is considered to be more likely. Empiric therapy with nonopioid analgesic medications, antispasmodic and anticholinergic agents, and gastric acid–reducing agents may be tried before time criteria for functional abdominal pain are met. Parents tend to become more frustrated and anxious, particularly if they perceive that a serious disorder is being missed or if the physician implies that the primary factors that influence the perception of pain are cognitive and emotional. Parental uncertainty only increases the stressful environment that provokes or reinforces the pain behavior.

Thus, the concept of functional abdominal pain must be introduced into the differential diagnosis of abdominal pain in children before the 3-month time criteria for duration of pain are met. Functional abdominal pain lacks a symptom-based diagnostic marker. None of the following have been shown to help the physician discriminate between organic, psychosomatic, and functional abdominal pain: frequency of pain; character of pain; location of pain; pain awakening patient at night; associated gastrointestinal symptoms, including anorexia, nausea, episodic vomiting, increased gas, or altered bowel pattern; or associated extraintestinal symptoms, including fatigue, pallor, headache, and arthralgia. Similarly, there is no evidence that anxiety, depression, behavior problems, or recent negative life events discriminate between organic, psychosomatic, and functional abdominal pain. Because there are no prospective studies on natural history or incidence, it cannot be stated that the duration of pain itself, beyond 3 months without an organic diagnosis, supports a diagnosis of functional pain.

Although there are no evidence-based data, clinical experience suggests that subclassifying pain presentations may facilitate the choice of testing by narrowing the differential diagnosis (Figure 14-3). Children with abdominal pain may be subclassified by one of four clinical presentations: (1) abdominal pain associated with symptoms of upper abdominal distress, (2) abdominal pain associated with altered bowel pattern, (3) isolated paroxysmal abdominal pain alone, and (4) cyclical pain syndrome. Cyclical pain refers to episodes of intense acute midline pain lasting several hours to a few days with intervening symptom-free intervals lasting weeks to months. Functional abdominal pain should be presented as the most common cause of all four clinical presentations. The frequent occurrence of upper and lower bowel symptoms in the same patient is not uncommon.

Establishing a Working Diagnosis of Functional Abdominal Pain

The key variables that point toward a functional diagnosis are a normal physical examination, other than abdominal pressure tenderness, and absence of alarm signals for an organic disorder. Even with a normal physical examination, further diagnostic testing is definitely indicated in the presence of the following alarm signals: involuntary weight loss, growth retardation, significant vomiting, significant diarrhea, gastrointestinal blood loss, associated fever, arthritis, rash, symptoms of a psychiatric disorder, or a family history of inflammatory bowel disease. Alarm signals in the physical examination include evidence of linear growth deceleration, localized tenderness in the right upper or lower quadrants, localized fullness or mass effect, hepatomegaly, splenomegaly, spine or costovertebral angle tenderness, perianal fissure, perianal fistula, visible soiling, and guaiac-positive stools. Although a family history of patients with functional pain who consult physicians is more likely to be positive for parental health complaints, including marital discord, psychiatric illness, and past surgery, this cannot be used to discriminate between functional and organic pain.

Diagnostic testing is indicated when alarm signals or abnormal physical findings suggest a possibility of an organic disorder. No studies have evaluated the value of common laboratory tests (CBC, erythrocyte sedimentation rate [ESR], comprehensive metabolic panel, urinalysis, stool parasite analysis) to distinguish between organic and functional pain. Diagnostic testing may also be considered to reassure the parent, patient, or physician that the most likely diagnosis is functional pain. The physician may also need to do testing to rule out organic disease in the patient in whom pain continues to severely affect lifestyle despite a functional diagnosis. Clinical experience suggests that subclassifying pain presentations may facilitate the choice of testing by narrowing the differential diagnosis (see Figure 14-3).

Establishing a working diagnosis of functional pain and initiating conservative therapy before time criteria are achieved does not preclude an ongoing, focused, diagnostic workup. Synonyms of functional pain that may be useful for individualizing diagnosis in a given patient are functional dyspepsia for pain with upper abdominal symptoms, IBS for pain associated with altered bowel pattern, func-

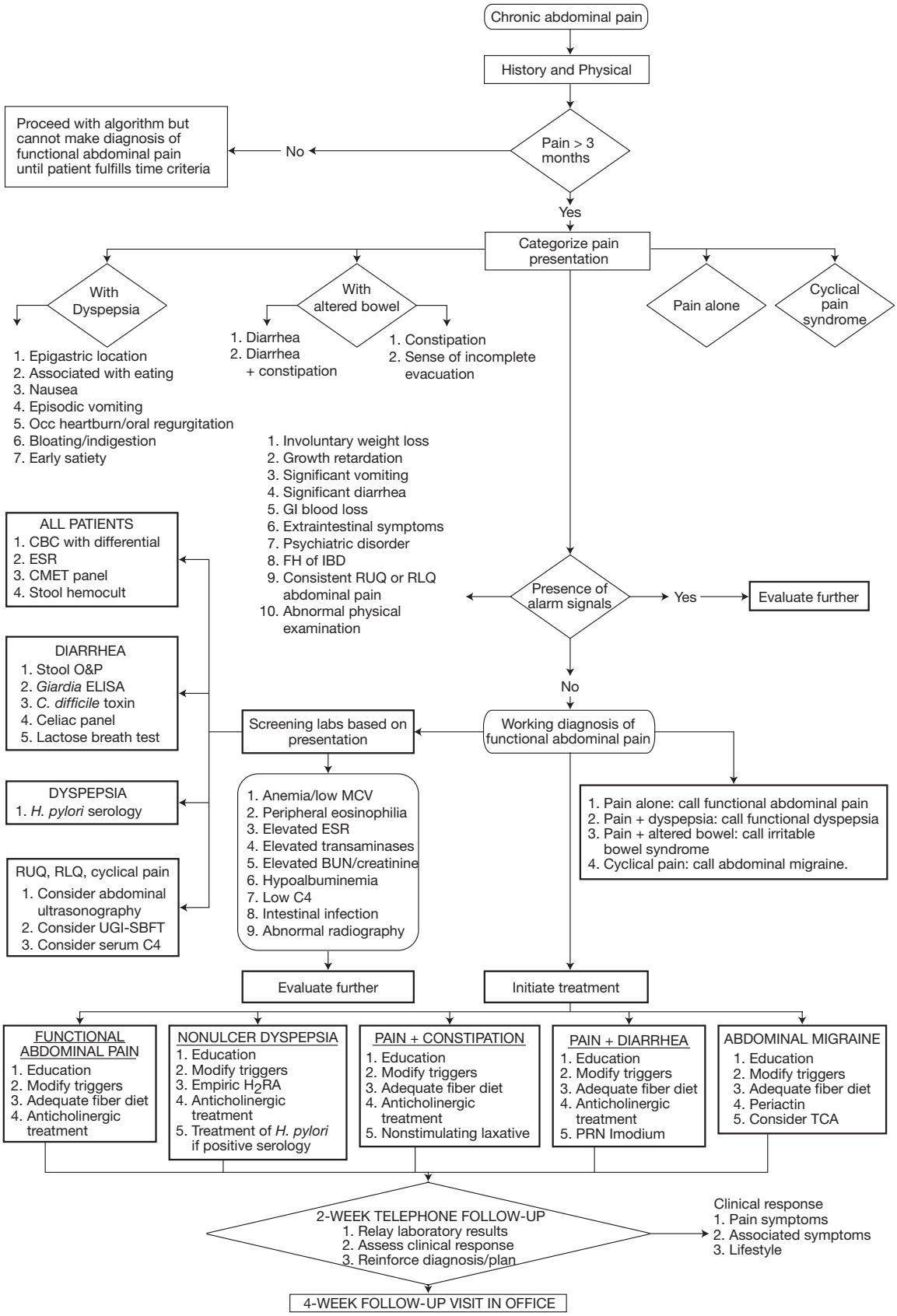

FIGURE 14-3 The author's algorithm for the evaluation and management of chronic abdominal pain. BUN = blood urea nitrogen; CBC = complete blood count; *C. difficile* = *Clostridium difficile*; CMET = comprehensive metabolic panel (Chem 12); ELISA = enzyme-linked immunosorbent assay; ESR = erythrocyte sedimentation rate; FH = family history; GI = gastrointestinal; *H. pylori* = *Helicobacter pylori*; H_2RA= histamine$_2$ receptor antagonist; IBD = inflammatory bowel disease; MCV = mean corpuscular volume; O&P = ovum parasite examination; PRN = as needed; RLQ = right lower quadrant; RUQ = right upper quadrant; TCA = tricyclic antidepressant; UGI-SBFT = upper gastrointestinal small bowel follow-through .

tional abdominal pain for patients with isolated paroxysmal abdominal pain alone, and abdominal migraine for cyclical acute pain episodes. Two of the following features are required for diagnosis of abdominal migraine: (1) a headache during episodes, (2) photophobia during episodes, (3) associated classic unilateral migraine headaches that may or may not be associated with abdominal pain, (4) a family history of migraine, and (5) visual, sensory, or motor aura antedating acute pain.[39,40]

Diagnostic Evaluation Based on Subcategories of Chronic Abdominal Pain

Chronic Abdominal Pain Associated with Symptoms of Dyspepsia. Table 14-2 lists the differential diagnosis in patients with chronic abdominal pain and upper gastrointestinal symptoms. The key to deciding on the extent of initial workup is the presence or absence of significant vomiting. A reasonable focused laboratory evaluation in all patients includes a CBC with differential, ESR, *H. pylori* serology and/or stool antigen, hepatic panel, and pancreatic enzyme measurement. In cases in which recurrent vomiting is a significant part of the history, an upper gastrointestinal series with small bowel follow-through and abdominal ultrasonography should be considered to rule out gastric outlet disorder, malrotation, partial small bowel obstruction, small bowel Crohn disease, gallstones, pancreatic pseudocyst, hydronephrosis secondary to ureteropelvic junction obstruction, and retroperitoneal mass.

Gastroesophageal reflux disease should be suspected when heartburn and acid regurgitation are prominent parts of the history. Gastroparesis following a viral infection may begin within 7 days following resolution of acute viral illness (especially post rotavirus) and lead to chronic epigastric pain associated with persistent nausea and episodic vomiting.[41,42] Diagnosis is confirmed by demonstrating delayed gastric emptying by scintigraphy. Recurrent epigastric or right upper quadrant pain associated with tender hepatomegaly suggests chronic hepatitis. Biliary colic is episodic, severe, constant pain in the right upper quadrant or epigastrium that persists for 20 minutes to 2 hours and that is usually triggered by eating. Choledocholithiasis is confirmed by abdominal ultrasonography. Gallbladder dyskinesia remains a controversial primary diagnosis to explain chronic dyspepsia. Diagnosis should be suspected in patients with protracted symptoms suggesting biliary colic, a positive family history of gallstones, normal abdominal ultrasonography, and hepatobiliary scintigraphy with delayed ejection fraction after cholecystokinin infusion.[43] Dramatic improvement has been reported in children after elective cholecystectomy.[44,45] Experience in adults has been less dramatic, with only 47% of patients becoming completely asymptomatic.[46] Adult norms for ejection fraction have been used to assess pediatric patients. In relapsing pancreatitis, recurrent severe epigastric pain persists for days and may radiate to the back. Endoscopic retrograde cholangiopancreatography is indicated only if there is biochemical or radiologic evidence of recurrent pancreatitis or biliary-type abdominal pain following cholecystectomy. Continuous pain, especially in the context of multisystem complaints, is an alarm signal for possible psychiatric disease. Eating disorder should also be considered in any young patient with significant weight loss.

TABLE 14-2 DIFFERENTIAL DIAGNOSIS OF RECURRENT ABDOMINAL PAIN ASSOCIATED WITH SYMPTOMS OF DYSPEPSIA

Associated with upper gastrointestinal inflammation
Gastroesophageal reflux disease
Peptic ulcer
Helicobacter pylori gastritis
Nonsteroidal anti-inflammatory drug ulcer
Crohn disease
Eosinophilic gastroenteritis
Ménétrier disease
Cytomegalovirus gastritis
Parasitic infection (*Giardia, Blastocystis hominis*)
Varioliform gastritis
Lymphocytic gastritis/celiac disease
Henoch-Schönlein purpura
Motility disorders
Idiopathic gastroparesis
Biliary dyskinesia
Intestinal pseudo-obstruction
Other disorders
Obstructive disorders from Table 14-1
Chronic pancreatitis
Chronic hepatitis
Chronic cholecystitis
Ureteropelvic junction obstruction
Abdominal migraine
Psychiatric disorders

H. pylori gastritis and nonsteroidal anti-inflammatory drugs (NSAIDs) are the most important exogenous factors associated with peptic ulcer in adults. However, in children, clinically significant ulceration occurs infrequently with NSAID use or *H. pylori* gastritis.[31,47] The pathogenic mechanisms distinguishing those individuals at risk have not been identified. A careful history is required to ensure that NSAID consumption is detected in any patient being evaluated for recurrent abdominal pain with dyspepsia. The incidence of *H. pylori* infection in children increases with age, is inversely related to socioeconomic class, and increases in families in which an adult has had either an ulcer or documented *H. pylori* infection.[48] In the absence of peptic ulcer disease, the relationship between *H. pylori* infection and abdominal pain remains unclear. Although there are no evidence-based data to establish a clear link between *H. pylori* gastritis without ulcer and abdominal pain associated with symptoms of upper abdominal distress,[47] most gastroenterologists will treat a symptomatic child who has been identified as *H. pylori* positive. The rationale is that *H. pylori* may act as a physical trigger of functional dyspepsia in selected patients. Some authors have concluded that the most cost-effective approach is to test serologically for *H. pylori* and to treat all infected cases. However, many investigators have pointed out that commercially available serologic assays do not appear to have the necessary sensitivity or specificity to screen pediatric

patient populations.[47] Empiric treatment of *H. pylori* should be considered only in patients with an elevated immunoglobulin (Ig)G antibody and is not recommended for patients with a positive IgM or IgA antibody. It is not unreasonable to avoid antibody testing altogether and consider treatment only in patients with endoscopically proven infection who have not responded to treatment of functional dyspepsia.

Upper endoscopy should be considered in untreated patients with alarm signals, patients who fail to respond to time-limited gastric acid reduction therapy for functional dyspepsia, and patients in whom symptoms recur after attempting to step off seemingly effective therapy. Upper endoscopy is the gold standard to rule out infectious and inflammatory disorders in the upper gastrointestinal tract. Recognizable objective findings by gross endoscopic examination include superficial erosions, ulcer, stricture, antral nodularity associated with *H. pylori* gastritis, gastric rugal hypertrophy associated with Ménétrier disease and cytomegalovirus gastritis, and the small, heaped up, volcanic-like mounds, pocked with a central crater, associated with chronic varioliform gastritis. Objective histologic findings may help to diagnose reflux esophagitis, eosinophilic gastroenteritis, cytomegalovirus gastritis, *H. pylori* gastritis, Crohn disease, and celiac disease. In the absence of gross ulcer or histologic evidence of *H. pylori*, superficial antral gastritis or duodenitis is of questionable clinical significance and should not dissuade a diagnosis of functional dyspepsia. There is no evidence in children that nonspecific superficial antral gastritis or duodenitis progresses to peptic ulcer. A diagnosis of postviral gastroparesis or gallbladder dyskinesia should not be entertained without first ruling out upper gastrointestinal tract inflammation and infection by upper endoscopy.

Chronic Abdominal Pain Associated with Symptoms of Altered Bowel Pattern. Altered bowel pattern may include a change in the frequency and/or consistency of stools (diarrhea or constipation), pain relieved with defecation, straining or urgency, a feeling of incomplete evacuation, passage of mucus, or a feeling of bloating or abdominal distention. Table 14-3 lists the major differential of chronic abdominal pain associated with an altered bowel pattern. The key to deciding on the extent of initial workup is the volume of diarrhea, evidence of gross or occult blood in the stool, and the presence of encopresis. In patients with diarrhea, a focused laboratory evaluation should include a CBC with differential, ESR, stool for *Giardia* antigen, stool for ovum parasites, and stool for *C. difficile* toxin. Alarm signals, including evidence of gastrointestinal bleeding, tenesmus, pain or diarrhea repeatedly wakening the patient from a sound sleep, involuntary weight loss, linear growth deceleration, extraintestinal symptoms (fever, rash, joint pain, recurrent aphthous ulcers), a positive family history of inflammatory bowel disease, iron deficiency anemia, and an elevated ESR are indications to pursue a diagnosis of inflammatory bowel disease by colonoscopy and barium contrast upper gastrointestinal series with small bowel follow-through. Lactose intolerance should be considered as a potential primary etiology of chronic abdominal pain in the presence of diarrhea. A trial of a lactose-free diet or performance of a lactose breath hydrogen test is prudent in children with pain associated with loose bowels, bloating, and increased flatulence. Diarrhea associated with encopresis suggests chronic fecal retention and megacolon. Serologic testing for celiac disease should be considered in patients with pain and an altered bowel pattern, especially in patients with iron deficiency anemia or secondary amenorrhea. Large-volume diarrhea is also an indication to pursue colonoscopy to rule out microscopic inflammation, which may alter colonic motility and absorptive function, including lymphocytic, collagenous, or eosinophilic colitis.[49–51]

TABLE 14-3 DIFFERENTIAL DIAGNOSIS OF RECURRENT ABDOMINAL PAIN ASSOCIATED WITH ALTERED BOWEL PATTERN

Idiopathic inflammatory bowel disorders
Ulcerative colitis
Crohn disease
Microscopic colitis with crypt distortion
Lymphocytic colitis
Collagenous colitis
Infectious disorders
Parasitic (*Giardia*, *Blastocystis hominis*, *Dientamoeba fragilis*)
Bacterial (*Clostridium difficile*, *Yersinia*, *Campylobacter*, tuberculosis)
Lactose intolerance
Complication of constipation (megacolon, encopresis, intermittent sigmoid volvulus)
Drug-induced diarrhea, constipation
Gynecologic disorders
Neoplasia (lymphoma, carcinoma)
Psychiatric disorders

Table 14-4 lists the indications for colonoscopy in children with chronic abdominal pain and an altered bowel pattern. The accuracy of colonoscopy in diagnosing inflammatory conditions of the colon is superior to barium enema because of the direct visualization of the mucosal surface and the ability to obtain biopsy and culture specimens. Intubation of the terminal ileum can also aid in the diagnosis of Crohn disease. Recognizable objective findings by gross examination with a flexible endoscope include edema, erosions, ulceration, pseudomembranes (discrete yellow plaques on the colonic mucosa), and polyps. Subjective gross endoscopic findings, including erythema, increased vascularity, and spontaneous friability,

TABLE 14-4 INDICATIONS FOR COLONOSCOPY IN PATIENTS WITH RECURRENT ABDOMINAL PAIN AND ALTERED BOWEL PATTERN

Evidence of gastrointestinal bleeding
Profuse diarrhea
Involuntary weight loss or growth deceleration
Iron deficiency anemia
Elevated acute-phase reactants (sedimentation rate, C-reactive protein)
Extraintestinal symptoms suggestive of inflammatory bowel disease (fever, rash, joint pains, recurrent aphthous ulceration)

become meaningful only in the context of histology because they are subject to more interobserver variation in interpretation. Objective histologic findings include (1) cryptitis, crypt abscesses, and crypt distortion with branching and dropout, suggesting ulcerative colitis or Crohn disease; (2) noncaseating granuloma specific for Crohn disease; (3) fibrosis and histiocyte proliferation in the submucosa suggesting Crohn disease; and (4) epithelial and intraepithelial lymphocytes or eosinophils with or without subepithelial collagen thickening in lymphocytic colitis, eosinophilic colitis, and collagenous colitis, respectively. Mild superficial increases in interstitial lymphocytes or eosinophils in the absence of crypt distortion or significant diarrhea are nonspecific and should not dissuade the physician from making a positive diagnosis of irritable bowel syndrome.

Chronic Isolated Paroxysmal Abdominal Pain. Table 14-5 lists the major differential of recurrent paroxysmal periumbilical abdominal pain in children. It is often important to try to see the patient during an attack of pain. The Carnett test may help to determine whether pain is arising from the abdominal wall or has an intra-abdominal origin.[52] The site of maximum tenderness is found through palpation. The patient is then asked to cross arms and assume a partial sitting position (crunch), which results in tension of the abdominal wall. If there is greater tenderness on repeat palpation in this position, abdominal wall disorders such as cutaneous nerve entrapment syndromes, abdominal wall hernia, myofascial pain syndromes, rectus sheath hematoma, or costochondritis should be suspected. Discitis, which is an osteomyelitis of the vertebral end plate, may present as a combination of back and abdominal pain.[53] The condition is usually associated with intermittent fever, an elevated peripheral WBC, and an elevated ESR. Unrecognized constipation should be suspected if a left lower quadrant or suprapubic fullness or mass effect is appreciated on abdominal examination and rectal examination reveals evidence of firm stool in the rectal vault or soft stool in a dilated rectal vault with evidence of perianal soiling. Often a history of constipation or encopresis is unknown to the parent. Parasitic infections, particularly *Giardia lamblia*, *Blastocystis hominis*, and *Dientamoeba fragilis*, may present with chronic pain in children in the absence of altered bowel pattern. Alarm signals are also indications to evaluate for Crohn disease or rare disorders such as polyarteritis nodosa, intestinal ischemia, and eosinophilic gastroenteritis, and angioneurotic edema can be indistinguishable from Crohn disease on clinical grounds. Suspicion of polyarteritis nodosa rests on evidence of extraintestinal disease, particularly renal involvement. Mesenteric vein obstruction should be considered in adolescents using oral contraceptives. Clinically, it can present gradually with progressive abdominal pain over a period of weeks. Pneumatosis is usually a late finding. The clinical presentation of eosinophilic gastroenteritis depends on the depth of the infiltration by the eosinophilic process. Submucosal disease can become manifest with abdominal pain and signs of obstruction. Any region of the gastrointestinal tract can be involved. Angioneurotic edema can be heralded by recurrent episodes of pain in the absence of cutaneous or oropharyngeal edema.[54] The family history is usually positive for allergy. Recurrent fever associated with generalized abdominal pain and peritoneal signs suggests the possibility of familial Mediterranean fever. Appendiceal colic is a controversial cause of chronic abdominal pain.[55,56] Appendiceal spasm has been postulated to be caused by inspissated casts of fecal material within the appendix. A number of anecdotal surgical reports have described complete resolution of pain symptoms following elective appendectomy. Appendiceal colic should be suspected in patients with recurrent acute episodes of well-localized abdominal pain and tenderness, most commonly in the right lower quadrant, demonstrated on several examinations. Ureteropelvic junction obstruction is well known to present with recurrent episodes of crampy periumbilical pain, but in all cases reported in the literature to date, the pain has been associated with vomiting.[57] Dull, midline, or generalized lower abdominal pain at the onset of a menstrual period suggests dysmenorrhea. The pain may coincide with the start of bleeding or precede the bleeding by several hours. Gynecologic disorders associated with secondary dysmenorrhea include endometriosis, partially obstructed genital duplications, ectopic pregnancy, and adhesions following pelvic inflammatory disease. Cystic teratoma has been described in prepubertal patients presenting with right or left lower quadrant pain. The vast majority of such patients have a palpable abdominal mass. Benign ovarian cysts in adolescent females do not cause recurrent abdominal pain. Acute intermittent porphyria is a rare disorder characterized by the temporal association of paroxysmal abdominal pain and a wide variety of central nervous system symptoms, including headache, dizziness, weakness, syncope, confusion, memory loss, hallucinations, seizures, and transient blindness.[58] Acute

TABLE 14-5 DIFFERENTIAL OF RECURRENT ABDOMINAL PAIN PRESENTING AS ISOLATED PAROXYSMAL ABDOMINAL PAIN

OBSTRUCTIVE DISORDERS
Crohn disease
Malrotation with or without volvulus
Intussusception with lead point
Postsurgical adhesions
Small bowel lymphoma
Endometriosis
Infection (tuberculosis, *Yersinia*)
Vascular disorders
Eosinophilic gastroenteritis
Angioneurotic edema
APPENDICEAL COLIC
DYSMENORRHEA
MUSCULOSKELETAL DISORDERS
URETEROPELVIC JUNCTION OBSTRUCTION
ABDOMINAL MIGRAINE
ACUTE INTERMITTENT PORPHYRIA
MENTAL DISORDERS (FACTITIOUS DISORDER, CONVERSION REACTION, SOMATIZATION DISORDER, SCHOOL PHOBIA)
FUNCTIONAL ABDOMINAL PAIN

intermittent porphyria is often precipitated by a low intake of carbohydrate or by specific drugs such as barbiturates or sulfonamides.

Focused laboratory evaluation might include CBC with differential and ESR to screen for occult systemic inflammatory condition. Decision to do stool ovum parasite examination is dependent on the incidence of *G. lamblia*, *B. hominis*, and *D. fragilis* within the community. The most valuable diagnostic test in a patient with symptoms suggesting obstruction is an upper gastrointestinal series and small bowel follow-through. Rare conditions such as lymphoma, angioneurotic edema, mesenteric vein thrombosis with ischemia, eosinophilic gastroenteritis, and pseudoobstruction will also be suggested by barium contrast radiography. Abdominal ultrasonography and abdominal CT have low diagnostic yield for picking up appendiceal abnormalities with recurrent right lower abdominal pain. Colonoscopy and ileoscopy should be performed to rule out Crohn disease in such patients if bloodwork or upper gastrointestinal small bowel follow-through suggests the possibility of inflammatory disease. Elective laparoscopy with planned appendectomy should be considered in patients with chronic right lower quadrant pain and negative infectious, inflammatory, and anatomic evaluation. Head CT to rule out intracranial space-occupying lesions should be considered in patients with recurrent abdominal pain and headache.

Treatment of Functional Abdominal Pain

Management of all four presentations of functional abdominal pain begins with a positive diagnosis and explanation of suspected pathophysiology and goals of therapy. Specific treatments include identification and modification of physical and psychological stress factors, dietary modification, drug therapy, and active psychological support. Hospitalization is rarely indicated for patients with functional abdominal pain.

Positive Diagnosis, Explanation of Suspected Pathophysiology, and Goals of Therapy. A positive diagnosis is based on normal physical examination and absence of alarm signals in the history, as described above. Focused laboratory and/or radiograph evaluations are based on subcategorizing pain presentation. It is important to emphasize that functional pain is the most common etiology of chronic abdominal pain in children and that the pain is real. Although the exact etiology and pathogenesis of functional abdominal pain in children are unknown, a substantial body of evidence suggests that it is caused by a disturbance of the autonomic nervous system, which results in altered communication between the gut and the brain. The prevailing viewpoint is that the pathogenesis of the pain involves visceral hypersensitivity and altered conscious awareness of gastrointestinal sensory input, with or without disordered gastrointestinal motility. Many parents and children can conceptualize the pain as a "headache" within the abdomen. Parents and child must be told that the primary goal of treatment is resumption of a normal lifestyle, not eradication of abdominal pain. Goals of treatment include regular school attendance, school performance to the child's ability, participation in desired extracurricular activities, normal weight gain and growth, and a normal sleep pattern. Reassurance that functional pain disorders will not affect future health can have positive therapeutic effects. Many patients lose their symptoms spontaneously after a positive diagnosis, suggesting that allaying the patient's and/or parents' fears may remove a significant stress factor triggering symptoms.

Modify Triggers of Pain. The first goal is to identify, clarify, and possibly reverse physical and psychological stress factors (see above) that may have an important role in the onset, severity, exacerbations, or maintenance of pain. In some cases, painful sensations may be provoked by physiologic phenomena, including postprandial gastric or intestinal distention, gastric emptying, intestinal contractions or the migrating motor complex, intestinal gas, or gastroesophageal reflux. Concurrent physical and psychological stressful life events may also trigger flares of pain. Intraluminal physical stress factors that may trigger pain include aerophagia, simple constipation, lactose intolerance, minor noxious irritants such as spicy foods, *H. pylori* gastritis, celiac disease, or drug therapy. Systemic physical or psychological stress factors may also provoke or reinforce the pain behavior by altering the conscious threshold of gastrointestinal sensory input in the central nervous system. Acute or chronic physical illness may unmask functional pain. Psychological stress factors may include death or separation of a significant family member, physical illness or chronic handicap in parents or a sibling, school problems, altered peer relationships, family financial problems, or a recent geographic move.

Equally important is to reverse environmental reinforcement of the pain behavior. Parents and teachers must be engaged to support the child rather than the pain. Regular school attendance is essential regardless of the continued presence of pain. In many cases, it is helpful for the physician to communicate directly to school officials to explain the nature of the problem. School officials must be encouraged to be responsive to the pain behavior but not to let it disrupt attendance, class activity, or performance expectations. Within the family, less social attention should be directed toward the symptoms. Consultation with a child psychiatrist or psychologist may be indicated when there is concern about maladaptive family coping mechanisms or if attempts at environmental modification do not result in return to a normalized lifestyle.

It is important to address symptoms of mental disorders that may contribute to the pathogenesis of pain symptoms. Failure to treat attention-deficit/hyperactivity, anxiety, or depression will adversely affect pain management. Anxiety may be primary, part of adjustment to an identifiable stress, or associated with panic disorder. Symptoms of anxiety include irritability, exaggerated startle response, poor concentration, worry, hypervigilance, motor restlessness, nervousness, difficulty sleeping, school phobia, fear of separation, and being easily fatigued. Depressive mood is suggested by insomnia, hypersomnia, anorexia, overeat-

ing, low energy, poor concentration, tearfulness, low self-esteem, poor concentration, feelings of hopelessness, and recurrent thoughts of death.

Dietary Modification. The role of dietary modifications in the management of functional pain disorders is not established. Postprandial symptoms in functional dyspepsia may be improved by eating low-fat meals or by ingesting more frequent but smaller meals throughout the day. A high-fiber diet is recommended for both diarrhea-predominant and constipation-predominant irritable bowel and isolated functional pain. The goal for fiber intake in grams is calculated by adding the patient's age + 5. Excessive fiber in the diet may result in increased gas and distention and actually provoke pain. Malabsorption of dietary carbohydrates may act as provocative stimuli in functional abdominal pain. Most often, the patient does not perceive a temporal association between ingestion of a particular sugar and the abdominal pain. Avoidance of excessive intake of milk products (lactose), carbonated beverages (fructose), dietary starches (corn, potatoes, wheat, oats), or sorbitol-containing products (vehicle for oral medication, sugar substitute in gum and candy, ingredient in toothpaste, and a plasticizer in gelatin capsules) is not unreasonable. Confirmation of lactose intolerance by a lactose breath hydrogen test should be considered before recommending prolonged lactase enzyme replacement therapy or commercial milk products that have been pretreated with lactase enzyme. Excessive gas in patients with irritable bowel syndrome can be managed by advising the patient to eat slowly, to avoid chewing gum, and to avoid excessive intake of carbonated beverages, legumes, foods of the cabbage family, and foods or beverages sweetened with aspartame.

Medications. There are no evidence-based data to support antisecretory therapy in pediatric patients with functional dyspepsia. Response rates in controlled clinical trials using antisecretory agents, both H_2 receptor antagonists and proton pump inhibitors, in adults with functional dyspepsia range from 35 to 80% compared with placebo response rates of 30 to 60%.[49] Meta-analyses of these trials suggest that acid reduction therapy is 10 to 30% more effective than placebo in relieving symptoms of ulcer-like (predominant abdominal pain) dyspepsia.[59] Conversely, there is no evidence that symptoms of nausea or bloating are relieved by antisecretory therapy. Given that acid reduction therapy may be beneficial in a subset of patients, it is not unreasonable to treat pediatric patients with ulcer-like dyspepsia with 4 to 6 weeks of an H_2 receptor antagonist. Patients who fail to respond or who relapse with step-down therapy should have upper endoscopy to establish a firm diagnosis of functional dyspepsia. If a firm diagnosis of functional dyspepsia is established by upper endoscopy, it is not unreasonable to continue acid inhibition therapy in patients who initially responded to shot-term empiric treatment but had recurrence of pain symptoms with attempts at step-down therapy. Short-term step-up to a proton pump inhibitor may be tried in patients who previously did not respond to an H_2 blocker. Metoclopramide, the only promotility agent available in the United States, has not been studied in pediatric patients and has only limited testing in adults with functional dyspepsia. It is not unreasonable to treat dysmotility-like dyspepsia (strong component of nausea, early satiety, and bloating) with a time-limited course of metoclopramide, but the high incidence of adverse central nervous system side effects and extrapyramidal symptoms associated with metoclopramide makes it risky for long-term use. As stated above, although *H. pylori*–eradication therapy is not established to be effective in adults with functional dyspepsia, the available data clearly do not rule out the possibility. Thus, most pediatric gastroenterologists still will treat documented *H. pylori* in functional dyspepsia. There are no evidence-based data to support the use of antispasmodic or antinauseant drugs to treat dyspepsia.

There are also no evidence-based data on the effects of pharmacologic therapy in pediatric patients with IBS. Synthetic opioids such as loperamide and diphenoxylate or the bile salt binding agent cholestyramine may be helpful in treating diarrhea associated with IBS. Loperamide is preferred over diphenoxylate because it does not traverse the blood-brain barrier. Fiber supplements such as psyllium, methycellulose, or polycarbophil are effective in treating both constipation and diarrhea, but their value in relief of abdominal pain associated with IBS is controversial. Nonstimulating laxatives such as PEG powder, mineral oil, milk of magnesia, and lactulose are effective adjuncts in treating constipation-predominant IBS. Antispasmodic or anticholinergic agents are commonly used in clinical practice to treat visceral abdominal pain, although efficacy is controversial. Only enteric-coated peppermint oil capsules (with possible smooth muscle–relaxing properties) have been shown to be superior to placebo for reducing functional pain by a randomized, double-blinded control study.[60] The duration of therapy at which time pain response was assessed, however, was only 2 weeks. Excessive gas can be managed by advising the patient to eat slowly, to avoid chewing gum, and to avoid excessive intake of carbonated beverages, legumes, foods of the cabbage family, and foods or beverages sweetened with fructose or sorbitol. Simethicone or activated charcoal may help individual patients.

In uncontrolled, retrospective case series, prophylactic cyproheptadine and propranolol have been reported to reduce the frequency of attacks of abdominal migraine.[61,62]

Although there is a lack of formal randomized, placebo-controlled trials, there has been a recent surge in using antidepressant and psychotropic agents to treat both diarrhea-predominant IBS and functional dyspepsia in adults.[63] Anecdotally, this class of drugs appears to be effective in adults with or without psychiatric abnormalities, especially low-dose tricyclic antidepressants. These drugs may act as "central analgesics" to raise the perception threshold for abdominal pain or down-regulate pain receptors in the intestine. There are as yet no data on treatment of pediatric patients.

There has been a recent surge in the development of novel drugs for IBS in adults, such as 5-HT_3 receptor antagonists and 5-HT_4 agonists aimed at modifying gas-

trointestinal motor activity and restoring normal visceral sensation. A significant beneficial effect of the 5-HT_3 antagonist alosetron has been reported in diarrhea-predominant adult women with IBS.[64] A significant beneficial effect of the 5-HT_4 agonist tegaserod has been reported in constipation-predominant adult women with IBS.[65]

Direct Psychological Support. Consultation with a child psychiatrist or psychologist may be indicated when there is concern about maladaptive family coping mechanisms or if attempts at environmental modification do not result in return to a normalized lifestyle. Referral for psychological treatment can be proposed as part of a multispecialty treatment package to help the patient manage the pain symptoms better. It is critical that the psychologist or psychiatrist initially focus on illness behavior and expand psychotherapeutic treatments as indicated only as the patient or parents begin to see the benefits of referral.

Cognitive behavioral therapies add strategies such as cognitive restructuring to behavioral interventions such as teaching relaxation and behavior management techniques. For example, a therapist would evaluate a patient's cognitive interpretation of bodily sensations and teach how cognition impacts affective experience and behavior. The perception that abdominal pain is a sign of impending physical disease must be countered both to address functional disability and to reassure the family that a functional diagnosis is credible. Attribution styles can also be examined for distortions. Patients are taught to treat their beliefs as hypotheses to be tested rather than accept their beliefs as inherently valid. Cognitive behavioral interventions targeting children's competence in social roles may be a useful adjunct to other medical treatment in reducing illness behavior. In addition, parents are trained to behaviorally reinforce appropriate coping behavior. There are evidence-based data that cognitive behavioral treatment helps to reduce pain and improve functioning. Cognitive behavioral therapy has been compared to standard supportive care of children with functional abdominal pain.[66–68] Both groups demonstrated reductions in pain at 3 months; however, those receiving cognitive behavioral treatment were more likely to be pain free at 6-month (55.6% vs 23.8%) and 12-month follow-up (58.8% vs 36.8%). These findings are very encouraging, although replication by different investigators is still needed.

Hospitalization. Hospitalization is rarely indicated for patients with functional abdominal pain. Fifty percent of patients experience relief of symptoms during hospitalization. However, no data have been presented that the natural history of the pain is affected. Hospitalization does not enhance the fundamental goals of environmental modification. More commonly, it will reinforce pain behavior.

Prognosis of Functional Abdominal Pain in Children

There are no prospective studies of the outcome of any of the various presentations of functional abdominal pain. Once functional abdominal pain is diagnosed, subsequent follow-up rarely identifies an occult organic disorder. Interestingly, pain resolves completely in 30 to 50% of patients by 2 to 6 weeks after diagnosis. This high incidence of early resolution suggests that the child and parent accept reassurance that the pain is not organic and that environmental modification is effective treatment. Nevertheless, more long-term studies suggest that 30 to 50% of children with functional abdominal pain in childhood experience pain as adults, although in 70% of such individuals, the pain does not limit normal activity.[69–71] Thirty percent of patients with functional abdominal pain develop other chronic complaints as adults, including headaches, backaches, and menstrual irregularities. Based on a small number of patients, Apley and Hale have described several factors that adversely influence prognosis for a lasting resolution of pain symptoms during childhood, including male sex, age at onset less than 6 years, a strong history of a "painful family," and greater than 6 months elapsed time from the onset of pain symptoms to an established functional diagnosis.[72]

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CHAPTER 15

ABDOMINAL MASSES

Robert H. Squires Jr, MD

Identification of an abdominal mass in a child elicits a barrage of diagnostic considerations that range from benign conditions to life-threatening malignancies. An urgent need arises to establish the diagnosis to provide an accurate prognosis and treatment plan for the child and family.

Approximately 60% of abdominal masses identified by physical examination in childhood are attributable to organomegaly, with the remainder representing anomalies of development, neoplasms, or inflammatory conditions.[1] Important clues to the diagnosis include the age of presentation (Table 15-1) and symptomatic complaints. Conditions associated with pain or gastrointestinal dysfunction generally present to medical attention early in their course, whereas asymptomatic masses may be well tolerated by the host for years before clinical detection. Thus, developmental anomalies such as an omental cyst are usually present in the young infant but may not be recognized for years or decades. The classification of abdominal masses by age at presentation is arbitrary, and overlap is obvious, but the approach is clinically useful.

GENERAL PRINCIPLES

A meticulous physical examination is essential for early detection of an abdominal mass.[2] An individual will never receive more frequent serial abdominal examinations by trained medical personnel than in the early years of life during periodic well-child checks, yet this is an age at which examinations are challenged by the uncooperative or reluctant child. It is estimated that 0.5% of newborn infants have a renal anomaly,[3] yet careful diligence on the part of the examiner is needed to detect an abnormality early. As many abdominal masses are asymptomatic, the examiner's fingers must both feel and "think" of what lies beneath them.

The examination begins with careful inspection. The child must be reassured and comfortable. For the infant or small child, the best examination table may be the mother's lap. The abdomen must be fully exposed. Signs of asymmetry or discomfort are identified. If the child is old enough to specify a sensitive area of the abdomen, the examination should begin some distance away from the area of discomfort and gradually work closer to the area of concern. The initial approach to the abdomen is best with light and then deep palpation of the left lower quadrant. By slowly moving the examining fingers cephalad to the left costal margin, abnormalities of the colon, bladder, kidney, adrenal gland, mesentery, and spleen can be identified. The process is repeated beginning in the right lower quadrant to detect abnormalities of the cecum, ileum, right colon, kidney, adrenal gland, gallbladder, and liver. Beginning the abdominal examination in the lower quadrants allows for the detection of marked enlargement of the liver or spleen, which can be otherwise missed. The rectus muscle can make it difficult to detect a mass in the midline, particularly in the older child. Therefore, a bimanual examination along the midabdomen can detect a subtle fullness. If a mass is identified, the examination should focus on physical characteristics such as tenderness, firmness, mobility, and whether the surface is smooth or irregular.

Radiologic assessment is an essential component of the evaluation of an abdominal mass. A collaborative relationship with a pediatric radiologist is helpful to determine the most productive diagnostic approach.[4] Abdominal radiographs are of limited value when a palpable mass is present but will detect calcification within the mass. Ultrasonography (US) is often the most useful initial test. It has many advantages, particularly in children, including portability of the instrument, absence of ionizing radiation, no requirement for sedation, and the ability to discriminate between a solid or a cystic mass. If the mass involves the urinary system, intravenous pyelography will provide information regarding renal anatomy and function, and voiding cystourethrography will identify abnormalities of the bladder, vesicoureteral reflux, and posterior urethral valves. Radionuclide scintigraphy is of limited value but may be useful to evaluate renal anatomy and function. A Meckel scan can identify gastric mucosa contained within a Meckel diverticulum or an intestinal duplication. Computed tomography (CT) with enteric and intravenous contrast offers superior anatomic detail of an abdominal mass, whether it is in the peritoneal or retroperitoneal space. Although CT technology is noninvasive, many children require intravenous sedation and analgesia to obtain an adequate study, and general anesthesia is occasionally indicated. Magnetic resonance imaging (MRI) is particularly useful in the evaluation of vascular malformations and retroperitoneal tumors that displace, obstruct, or invade important vascular structures.

TABLE 15-1 ABDOMINAL MASSES IN INFANTS AND CHILDREN

- NEONATES
- Retroperitoneal—kidney
  - Hydronephrosis
  - Multicystic dysplastic kidney
  - Autosomal recessive polycystic kidney disease
  - Autosomal dominant polycystic kidney disease
  - Mesoblastic nephroma
  - Renal vein thrombosis
- Retroperitoneal—other
  - Adrenal abscess
  - Fetus in fetu
- Pelvic
  - Hydrometrocolpos
  - Ovarian cyst
- Gastrointestinal
  - Intestinal duplication, malrotation, obstruction
  - Sacrococcygeal teratoma
- INFANTS AND CHILDREN
- Retroperitoneal
  - Wilms tumor
  - Neuroblastoma
  - Pancreatoblastoma
  - Rhabdomyosarcoma
  - Lymphoma
  - Ewing sarcoma
  - Germ cell neoplasm
- Liver—benign solid tumors
  - Adenoma
  - Mesenchymal hamartoma
  - Focal nodular hyperplasia
- Liver—malignant tumors
  - Hepatoblastoma
  - Hepatocellular carcinoma
  - Germ cell neoplasm
  - Angiosarcoma
  - Intrahepatic mesenchymal tumor
  - Embryonal rhabdomyosarcoma
- Liver—vascular lesions
  - Capillary hemangioendothelioma
  - Solitary cavernous hemangioma
- Liver—cystic hepatobiliary disease
  - Choledochal cyst
  - Caroli disease
  - Caroli syndrome
  - Congenital cysts
- Alimentary tract
  - Stomach
    - Carcinoma
    - Leiomyosarcoma
    - Rhabdomyosarcoma
    - Myosarcoma
    - Fibrosarcoma
  - Small bowel
    - Anomalies: duplication, Meckel, malrotation
    - Lymphoma
  - Colon
    - Fecal mass
    - Adenocarcinoma
- Omentum and mesentery
  - Cysts
  - Mesenteric fibromatosis
  - Inflammatory pseudotumor
  - Liposarcoma
  - Leiomyosarcoma
  - Fibrosarcoma
  - Mesothelioma
  - Metastatic tumor
- ADOLESCENTS
- Retroperitoneal
  - Renal cell carcinoma
- Pelvic
  - Hematocolpos
  - Ovarian cyst
  - Teratoma
  - Germ cell tumor
  - Choriocarcinoma
  - Gonadoblastoma
  - Embryonal carcinoma
- Liver
  - Hepatocellular carcinoma

ABDOMINAL MASSES IN THE NEONATAL PERIOD

Detectable masses in neonates likely originate in the genitourinary system.[5] Hydronephrosis and multicystic dysplastic kidney (MDK) make up 50 to 75% of abdominal masses reported in the neonatal period.[1] Physical findings associated with renal masses include isolated anomalies of the external ears or developmental features associated with in utero low urine output such as Potter facies.

RETROPERITONEAL MASSES

Hydronephrosis constitutes 25% of neonatal abdominal masses and is the most common renal mass in the newborn period.[5,6] It occurs as a consequence of a dilated collecting system. Renal damage results from either infection owing to stasis or increased intrapelvic pressure. Obstruction to the urinary tract occurs primarily in three locations: (1) ureteropelvic junction, (2) ureterovesical junction, and (3) bladder outlet or urethra.

Ureteropelvic junction obstruction is a common cause of unilateral hydronephrosis. In the majority of cases, the cause is unknown. However, abnormal angulations, intra-abdominal adhesion, abnormal valve, aberrant vessel, ureteral stenosis, or hypoplasia can be found. Other causes for unilateral hydronephrosis include ureterocele, periureteral diverticulum, and ectopic distal ureteral insertion.[1] When unilateral hydronephrosis is identified, the contralateral kidney is often at increased risk for developmental anomalies, including renal absence and multicystic dysplasia. Other congenital anomalies associated with hydronephrosis include imperforate anus, spinal dysraphism, and congenital heart disease.

Bilateral hydronephrosis suggests distal obstruction and is seen in males with posterior urethral valves. These folds, called the cristae urethralis, are membranes that arise from the verumontanum in males, extend distally to attach to the anterior wall of the urethra, and result in bladder outlet obstruction. Male children will present with a distended bladder, poor urinary stream, dribbling, or urosepsis. Occasionally, the initial presentation will be renal failure, failure to thrive, or urinary ascites. A defined obstructing structural lesion is not the only cause of bilateral hydronephrosis. Myoneural dysfunction, myelomeningocele, and prune-belly syndrome are also associated with two abnormal kidneys.

Regardless of the etiology, physical examination usually reveals a large, rounded and smooth, cystic flank mass or masses. If the obstruction is near the bladder, as seen in ureterovesical junction obstruction, the hydroureter may also be felt. When the obstruction is at or distal to the bladder outlet, as in posterior urethral valves, it is possible to palpate the kidneys, both hydroureters, and the distended bladder. US is the best initial test to evaluate suspected hydronephrosis. If present, a voiding cystourethrogram can be diagnostic for sites of obstruction and for the presence of posterior urethral valves. Diuretic renography using mertiatide (MAG3) with furosemide also allows for reliable diagnosis of obstruction.[7] Intravenous urography is not recommended in the neonate.[8]

Although neonatal hydronephrosis is detected more frequently, many cases appear to resolve spontaneously, which suggests that a structural obstruction may not be present in many neonates.[6,9] If a structural abnormality is suspected, treatment is directed to correct the underlying condition.[10] Appropriate antibiotic therapy for the patient with urosepsis is indicated. Patients with significant compromise of renal function require prompt decompression of the obstructed urinary system. If bilateral hydronephrosis is present, a Foley catheter in the urinary bladder may provide adequate drainage initially. Depending on the site of obstruction, however, percutaneous nephrostomy may be needed to drain the upper tracts.

MDK is the most common form of cystic disease in infants and accounts for 20% of all urinary tract malformations. It is usually sporadic, but, occasionally, an autosomal dominant pattern of inheritance is noted.[11,12] Whereas most tumors involve one kidney, 15% are bilateral. The affected kidney is usually nonfunctional.[13] The vast majority of cases are discovered before the child reaches the second birthday.

The condition usually presents as an asymptomatic flank mass discovered by the physician on routine physical examination or by the caretaker who notices an unusually large abdomen. The kidney contains cysts of variable size throughout the organ, which can be palpated as a multilobulated and immobile cystic mass. Other anomalies associated with MDK include esophageal atresia, imperforate anus, and tracheoesophageal fistula. Respiratory distress and gastric outlet obstruction owing to the large abdominal mass have been described.[14] Examination of the mass by US reveals the diagnostic "grape cluster" pattern. It is important to evaluate the contralateral kidney. If the involved kidney is small or hypoplastic, there is a higher incidence of renal abnormalities of the uninvolved kidney.[15] Although the lesion is not premalignant, treatment usually involves removal of the affected kidney to avoid problems with hypertension, abdominal pain, infection, or mass effect. The outcome is generally good when only one kidney is involved. However, chronic renal insufficiency or renal failure is associated with bilateral disease.[16]

Autosomal recessive polycystic kidney disease (ARPKD), also known as infantile polycystic disease, has an incidence of approximately 1 to 2 per 10,000 births.[5,17] The condition is characterized by bilateral renal enlargement caused by generalized dilation of the collecting tubules and is invariably associated with congenital hepatic fibrosis. A mutation in the *PKHD1* gene, located on the short arm of chromosome 6, is thought to be responsible for this condition.[18] Fibrocystin, a potential receptor protein that acts in collecting duct and biliary differentiation, is the transcription product of the *PKHD1* gene.

Clinicians have traditionally identified four classifications of the condition, based primarily on age and symptoms. Infants with the prenatal form, which is the most severe, are often delivered stillborn as a result of massive cystic kidneys and pulmonary hypoplasia. Potter facies (wide-set eyes, beaked nose, low-set ears, prominent fold arising from the inner canthus), usually associated with renal agenesis, can also be seen in this severe form of ARPKD.[5] The neonatal form of ARPKD is less severe, but the children will develop renal failure months or years later. Hepatic involvement is often minimal. Children with the infantile and juvenile forms of the disease have the onset of renal insufficiency later in life but are more predisposed to develop significant liver disease. In these patients, liver biopsy reveals portal areas to be expanded by an increased number of dilated ductules surrounded by fibrous tissue. The dilated ductules may later become cystic. These original four classifications of ARPKD are now felt to have considerable clinical variability and intrafamilial differences regarding onset of renal insufficiency.[17] Different subtypes can be observed within the same family.[19] However, the age at presentation, development of hypertension, and degree of hepatic involvement are often similar among siblings.

Autosomal dominant polycystic kidney disease (ADPKD), also known as adult polycystic disease, is increasingly recognized in infants.[3] Ultrasonographic features that distinguish it from ARPKD include the presence of renal cysts within the enlarged renal masses. A number of extrarenal anomalies associated with ADPKD include endocardial fibroelastosis, intracerebral vascular anomalies, pyloric stenosis, and hepatic fibrosis. The clinical course is highly variable, with some children asymptomatic whereas others progress early to end-stage renal failure.[20] An ADPKD gene (*PKD1*) has been identified on chromosome 16 and accounts for 85% of the pedigrees.[21] A second gene (*PKD2*), located on chromosome 4, accounts for the remainder. Identification of the gene products structure and function may provide clues to pathogenesis.[22]

Mesoblastic nephroma is the most common renal tumor in the neonatal period.[23] This generally benign tumor has also been called a congenital Wilms tumor, fetal renal hamartoma, or leiomyomatous hamartoma. It presents as a massive flank mass with accompanying hematuria, hypertension, and vomiting and is cured by nephrectomy. Rarely, it presents with atypical features such as polyhydramnios and hypercalcemia.[24] There are reports of metastasis to the brain, skeletal structures, lungs, and heart.[5,25,26] Following nephrectomy, the prognosis is good.[27]

Renal vein thrombosis (RVT) results in infarction of variable amounts of renal parenchyma.[1] Factors that predispose the newborn to develop RVT include hemoconcentration from dehydration, polycythemia, and low perfusion

states with secondary venous congestion, local tissue swelling and hypoxia, and cellular disruption and hemorrhage. Maternal factors that predispose the newborn to RVT include maternal diabetes, toxemia, and the use of medications such as steroids and thiazide diuretics during pregnancy.[28] This condition can occur in utero without any identifiable predisposing illness.[28] In the older patient, RVT is associated with nephrotic syndrome.[29]

Presenting features may include a palpable flank mass, hematuria, thrombocytopenia, and a consumptive coagulopathy.[30] However, this classic presentation is infrequent. More often, the infant presents with minimal symptoms, such as peripheral edema or signs of a hypercoagulable state with thromboses at extrarenal sites.[29] US reveals a swollen kidney with decreased echogenicity, which suggests focal or diffuse disruption of renal parenchyma. Use of Doppler technology can assess flow within the renal vein and will detect the thrombus.[31] Diuretic renography using mertiatide is also used.[32] MRI is an alternative to evaluate the patency of the renal vein, although venography remains the gold standard to diagnose RVT.[33]

Management should focus initially on treatment of the underlying factors that precipitated the RVT. Improved hydration and perfusion may interrupt thrombus progression. Use of anticoagulation therapy such as heparin or streptokinase is not well defined.[29] However, it is likely to be most useful when RVT is associated with inferior vena cava thrombosis, extension of the thrombus to the contralateral renal vein, or evidence of recurrent pulmonary emboli. Thrombectomy may be needed for bilateral RVT.

Other Retroperitoneal (Nonrenal) Masses

Neonatal adrenal abscess is a rare condition that most likely begins with a hemorrhage into the adrenal gland. Signs of adrenal hemorrhage include shock, an abdominal mass, anemia, and prolonged jaundice, but the condition may be deceptively asymptomatic.[34] A differential diagnosis for the physical and radiographic findings in this condition would include lymphatic cyst, neuroblastoma, Wilms tumor, renal duplication, and hydronephrosis. Rarely, an extrapulmonary sequestration of the lung will mimic an adrenal abscess.[35] A delay in diagnosis can be fatal. Treatment involves percutaneous or surgical drainage.

Fetus in fetu is an extremely rare event that occurs in approximately 1 in 500,000 deliveries. The location of the entrapped fetus is most often in the upper abdominal retroperitoneal space, although other extra-abdominal sites have been reported. The mass is usually painless, and the diagnosis is made with either plain radiography revealing a vertebral column and an axial skeleton[36] or prenatal US.[37]

Pelvic Masses

Hydrometrocolpos is a rare condition that presents as a mass in the lower midline of the abdomen.[38] It can be associated with a number of syndromes, such as Bardet-Biedl, McKusick-Kaufman, and oral-facial-digital.[39] The uterus may be palpable as a nodule at the top of the vaginal mass (hydrocolpos), or the uterus itself may be enlarged as well (hydrometrocolpos). An imperforate hymen is the most common cause and is revealed as a bulging hymen detected on physical examination. In the absence of an imperforate hymen, vaginal and cervical stenosis or atresia should be considered in the differential diagnosis. Complicated anatomic problems may need assessment prior to surgical correction attempts, using techniques such as US, cystography, excretory urography, or barium enema.[40]

Cysts and neoplasms are rare in infancy, although the incidence increases throughout childhood. Cystic lesions are usually follicular and likely attributable to ovarian stimulation by maternal hormones.[1] Eighty-five percent of ovarian cystic lesions are benign. Solid lesions are more likely to be malignant. In the newborn, ovarian lesions are displaced out of the pelvis and present as an abdominal mass.

Gastrointestinal Lesions

Intestinal duplication can occur at any level, but the most common site is in the area of the terminal ileum.[41] A typical duplication mass is located on the mesenteric border of the adjoining bowel and is lined with intestinal or gastric mucosa. On palpation, the mass is soft, compressible, and mobile. Symptoms that typically develop include intestinal obstruction, perforation, or hemorrhage.[42] US is the best initial examination and can be diagnostic. Contrast radiography of the intestinal tract and a Meckel scan to identify ectopic gastric mucosa are two additional studies that may provide useful information.[43]

Intestinal distention can also simulate an abdominal mass, especially if there is an anatomic blockage. A volvulus secondary to malrotation can present with bilious vomiting, distention, and a mass. Intussusception, with its classic sausage-shaped mass, is rare in infancy.[44] Other common causes of intestinal obstruction in neonates include meconium ileus and meconium plug. If scybala are palpable on abdominal examination in the newborn or young infant, then Hirschsprung disease should be considered.

Other Neoplasms

Sacrococcygeal teratoma is the most common neoplastic abdominal mass in the neonatal period. It has a large external component and is obvious at birth. The vast majority are benign, but a delay in diagnosis is associated with malignant transformation.[45,46]

ABDOMINAL MASSES IN INFANTS AND OLDER CHILDREN

Retroperitoneal Masses

Nephroblastoma or Wilms tumor is an embryonal renal neoplasm and is the most common childhood abdominal malignancy (Figure 15-1).[23,47,48] The incidence remains fairly constant at 500 cases annually in the United States, which is 8 cases for every 100,000 children less than 15 years of age or an approximate risk of 1 case per 10,000 infants.[23,49] A typical patient is a 4 year old who presents with an asymptomatic abdominal mass discovered by a parent while bathing the child or by a physician as an incidental finding during an abdominal examination.[48,50] Infrequently, a left-sided varicocele owing to tumor com-

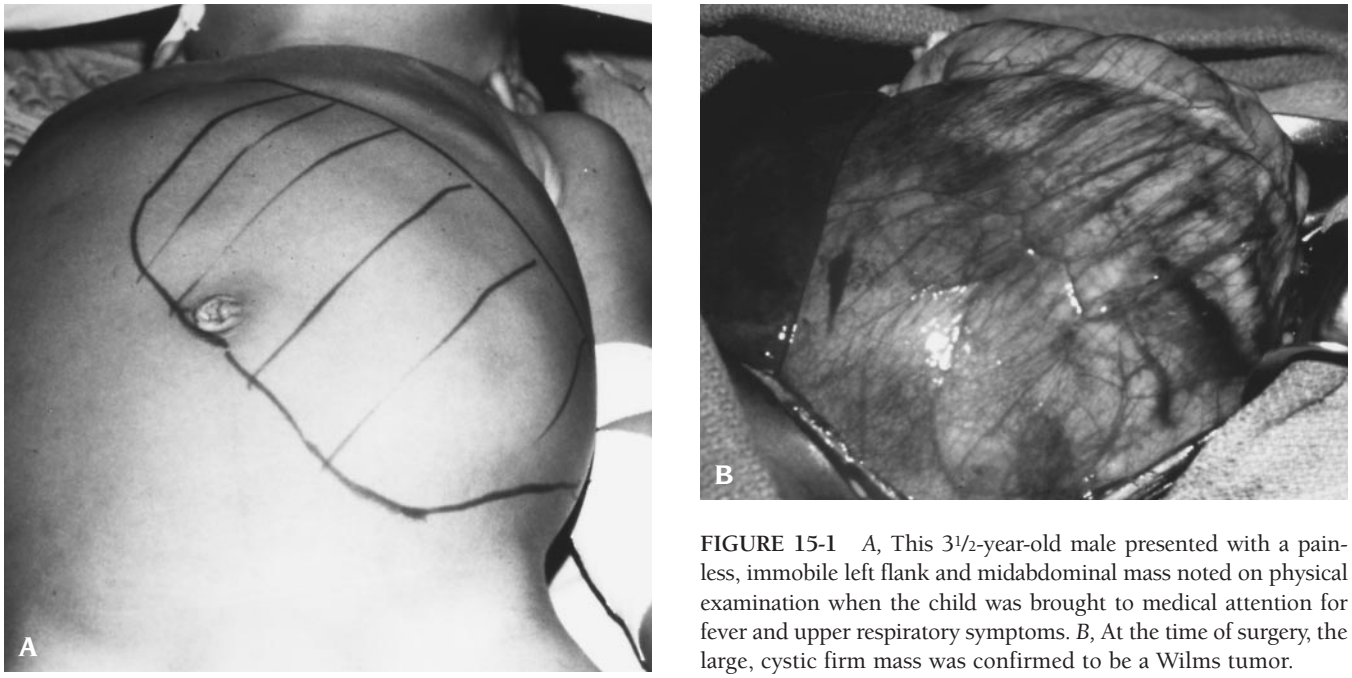

FIGURE 15-1 *A,* This 3½-year-old male presented with a painless, immobile left flank and midabdominal mass noted on physical examination when the child was brought to medical attention for fever and upper respiratory symptoms. *B,* At the time of surgery, the large, cystic firm mass was confirmed to be a Wilms tumor.

pression of the left renal vein may be seen. Malaise, weight loss, and anemia are uncommon presenting signs or symptoms. A large, unrecognized tumor that is ruptured during play may present as an abdominal catastrophe. The incidence of Wilms tumor is increased in children who manifest a variety of extrarenal anomalies, which include aniridia, hemihypertrophy, genitourinary anomalies, neurofibromatosis, Beckwith-Wiedemann syndrome, hypospadias, cryptorchidism, gonadal dysgenesis, and duplication of the renal collecting system.[48] Microscopic hematuria following minimal abdominal trauma should also raise the suspicion of a Wilms tumor.

The cause of Wilms tumor is unknown, but associations with three specific suppressor genes are recognized.[51] Deletions of the short arm of chromosome 11 are thought to set the stage for oncogenesis when certain gene products are not present.[52] The *wt1* gene deletion is associated with aniridia, the *wt2* gene deletion is seen with Wilms tumor and Beckwith-Wiedemann syndrome, and the *wt3* gene deletion is associated with bilateral Wilms tumor.[48]

Physical examination reveals a palpable immobile flank mass with a smooth contour, which is painless unless rupture of the tumor or intraparenchymal hemorrhage has altered the basic nature of the tumor. Evidence of hypertension may be present as a consequence of increased rennin or compression of the vasculature by the tumor.[53] A careful inspection of the eyes, extremities, and external genitalia may provide evidence of the associated findings discussed above.

US identifies the kidney as the origin of the mass and can assess the patency of the inferior vena cava and the presence of renal vein involvement.[48] CT of the chest will identify lung metastasis, which will be present in 8 to 15% of patients with Wilms tumor. MRI may be useful to evaluate for renal vein invasion and to identify contralateral renal involvement.[3]

Treatment involves a combination of surgery, chemotherapy, and radiation.[47] Complete surgical excision is the primary focus of treatment. The tumor must be handled with care during the surgical procedure to prevent rupture. The National Wilms Tumor Study Group reports a 90% survival rate in patients with favorable histology and with disease that is limited to the kidney, which can then be completely excised.[54] This compares with 54% survival rates for patients with distant metastasis or bilateral renal involvement. A number of clinical variants of nephroblastoma, including cystic nephroma and cystic partially differentiated nephroblastoma, are separated from Wilms tumor based on differences in gross and histologic appearance (Figure 15-2).[55] Nephrectomy alone appears to be adequate therapy.

Neuroblastoma is a malignancy involving neural crest cells that arise within the adrenal medulla or anywhere along the chain of sympathetic ganglia from the neck to the pelvis (Figure 15-3).[48,56] This is the most common solid tumor of infancy, and 60 to 75% develop within the abdomen.[57] Approximately 500 new cases are reported each year.[47,58] Half of the cases are diagnosed in patients under 2 years of age, and 90% occur in the first 8 years of life.[57] The tumor is twice as common in boys as in girls.

Clinical presentation is variable and related to the site of the primary tumor. It is often first recognized as an immobile abdominal mass that can be either asymptomatic or painful. Symptoms may include fever, weight loss or failure to gain weight, abdominal pain and distention, and anemia. The tumor may secrete vasoactive intestinal polypeptide or other catecholamines that result in intractable diarrhea and hypokalemia. Less common manifestations include opsoclonus-myoclonus syndrome and Horner syndrome. Neuroblastoma is associated with a number of conditions, including Beckwith-Wiedemann syndrome, Hirschsprung disease, fetal alcohol syndrome, and Waardenburg syndrome.[57]

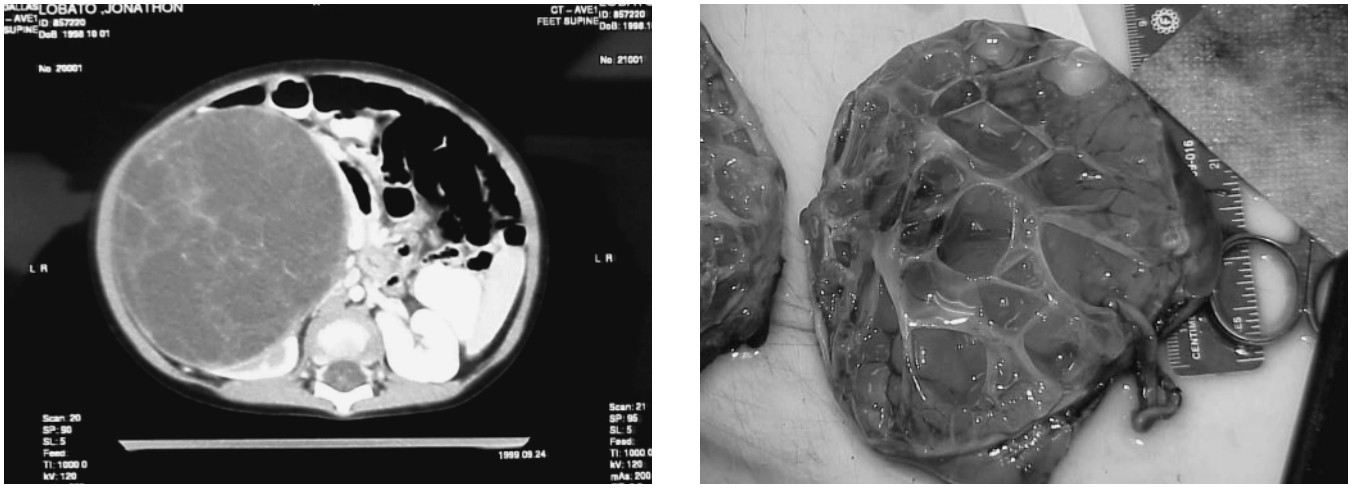

FIGURE 15-2 Cystic nephroma (multilocular cyst of the kidney). The computed tomographic scan on the left reveals a large, thin-walled cystic structure that occupies most of the right midabdomen in this 4-year-old female who presented with a painless abdominal mass. The pathologic specimen on the right is characteristic of a cystic nephroma with thin septa between the multiple cysts.

Because 50 to 75% of patients have advanced disease at the time of presentation, an effort to screen 1-year-old children was initiated with the hope of early detection, but this strategy was not helpful.[59] The tumor disseminates by direct extension, lymphatic involvement, or hematogenous spread. Metastasis to the bone may result in debilitating pain and refusal to walk. Unilateral proptosis with orbital ecchymosis is characteristic of central nervous system involvement. Paraplegia may result if the tumor extends through the spinal foramina. Pulmonary metastases are rare.[47,58]

Treatment is based on the stage of the lesion. The most important independent variables that determine outcomes are age and stage of disease. Infants younger than 1 year of age and no evidence of remote disease (eg, stages I, II, and III) have the best survival rates.[47,58] Primary tumor excision provides the most successful treatment, and survival under these circumstances is good.[56] Other favorable prognostic factors include thoracic location and histology that reflects a high degree of maturation.[50] An elevated serum lactate dehydrogenase, serum ferritin, or neuron-specific enolase is a poor prognostic factor.[57]

Genetic abnormalities are identified in about 80% of tumors, the most common being deletions on chromosome 1.[60,61] As the prognosis has not changed significantly in the last two decades, novel treatment strategies continue to be investigated.[56,58] The molecular biology of neuroblastoma is characterized by somatically acquired genetic events that lead to gene overexpression (oncogenes), gene inactivation (tumor suppressor genes), or alterations in gene expression. New efforts to define risk stratification by a combination of clinical features and biologic factors may lead to more specific treatment strategies.[62]

Pancreatoblastoma is a rare pancreatic tumor that generally affects infants and young children. It presents as a palpable abdominal mass accompanied by abdominal pain, anorexia, vomiting, and weight loss. Jaundice is rare. Elevation of serum α-fetoprotein (AFP) occurs only with liver metastases. Interestingly, almost half of cases reported are in Asians. Complete surgical resection is the treatment most commonly associated with long-term survival.[63,64]

Other tumors can present in the retroperitoneal space. Some are benign mesenchymal tumors such as hemangioma, lymphangioma, and lipoma. Malignant tumors can also occur, with examples including rhabdomyosarcoma, lymphoma, Ewing sarcoma, and germ cell neoplasms.[65] Significant retroperitoneal lymphadenopathy is not detectable on palpation but may be found unexpectedly on radiographic studies and does not always herald malignancy. Enlarged retroperitoneal nodes can be seen in some patients with celiac disease.[66]

Benign Liver Masses

Hepatic adenoma is a rare benign encapsulated tumor derived from hepatic epithelium.[47,67] The finding consti-

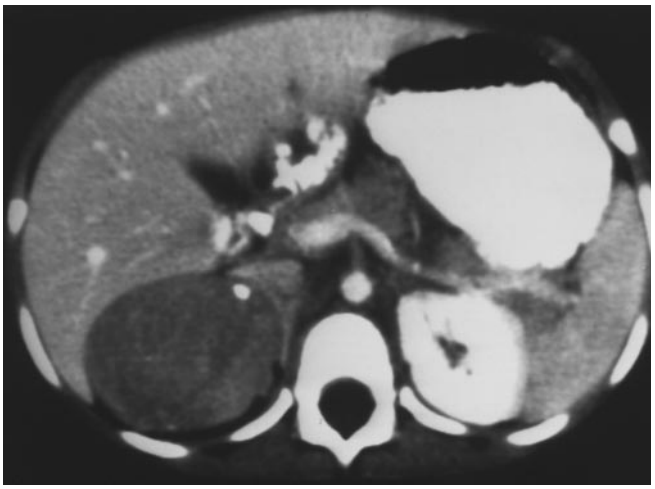

FIGURE 15-3 A computed tomographic (CT) scan of the abdomen was performed on this 18-month-old male child who presented with abdominal pain and fever. Physical examination revealed a painless mass in the right abdomen. The CT scan demonstrated a solid mass above the right kidney that was subsequently found to be a neuroblastoma.

tutes 2 to 5% of all benign tumors in childhood[47] and is commonly seen in adult women during the reproductive years. In a child, the presentation may vary among the following scenarios: (1) an asymptomatic mass noted as an incidental finding on a sonogram, (2) a sudden abdominal crisis secondary to hemorrhage or rupture of the tumor, (3) a finding in a patient with known risk factors for developing the tumor. Spontaneous rupture or hemorrhage occurs in up to 25% of cases.[47] Risk factors associated with adenoma formation include glycogen storage disease types I and III, familial diabetes mellitus, use of birth control pills or androgenic steroids, hereditary tyrosinemia, and adenomatous polyp syndromes.[68,69] Liver tests and AFP levels are usually normal.[69] There are no reports of significant parenchymal insufficiency or portal hypertension associated with these lesions.[70]

Radiographic features on US reveal either hyperechoic or hypoechoic lesions, depending on the fat content of the tumor.[71] Findings on CT will vary depending on the presence or absence of hemorrhage. Sufficient overlap exists between hepatocellular adenoma and hepatocellular carcinoma to prevent a definitive diagnosis based on imaging alone.[72]

Hepatic adenomas, although not malignant, do have some potential for malignant transformation.[67] Factors associated with increased risk for malignancy include lesions greater than 5 cm, glycogen storage disease, and use of oral contraceptives.[47,70] Surgical excision of the adenoma should be considered if the lesions increase in size, if there is a predilection for spontaneous hemorrhage, if premalignant risk factors are present, or if more malignant diagnoses cannot be excluded. A wedge resection or lobectomy is the surgery of choice.

Mesenchymal hamartoma (also called a giant lymphangioma, hamartoma, or bile cell fibroadenoma) is a benign hepatic tumor that results from a developmental anomaly of periportal mesenchyme and contains connective tissue stroma combined with bile ducts, liver cells, and angiomatous components (Figure 15-4).[73,74] It occurs almost exclusively in children less than 2 years of age. Children usually present with abdominal distention, respiratory distress owing to a mass effect, and/or congestive heart failure from arteriovenous shunting. Some children can be asymptomatic.

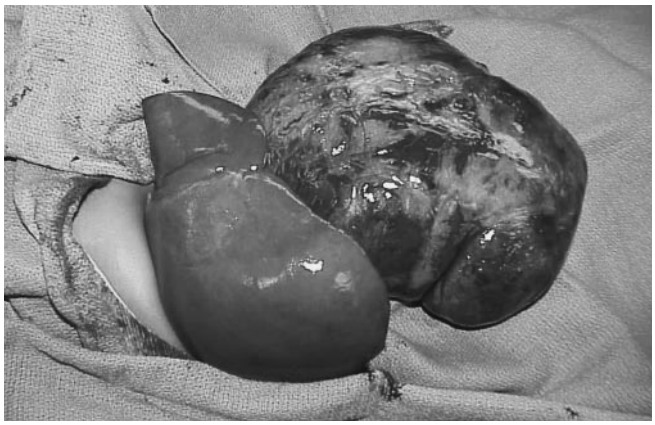

FIGURE 15-4 An 8-month-old child presented with fever, fretfulness, and vomiting. Guarding was noted on abdominal examination and radiographic studies identified a subhepatic mass. The surgical specimen revealed a large mesenchymal hamartoma.

US will often reveal a cystic mass that is contained within the right hepatic lobe. The CT scan defines boundaries of the lesion and helps determine resectability. As some tumors regress spontaneously, a conservative approach has been advocated. However, complete resection with extensive histologic sampling is suggested by some following reports of an apparent association with an undifferentiated embryonal sarcoma, which is highly malignant.[75]

Focal nodular hyperplasia is rare and always benign and usually presents as an asymptomatic incidental finding on a diagnostic study.[76] It occurs most commonly in young women. The lesion likely develops as a consequence of hyperplastic growth in response to altered blood flow in the liver parenchyma adjacent to a preexisting arterial malformation. There is an association with micronodular cirrhosis. Acute abdominal pain may develop owing to torsion or rupture of the lesion with bleeding.[76]

A CT study reveals a large homogeneous, hypodense mass and a low-density central area that corresponds to a scar that is seen in one-third of cases.[77] After intravenous gadolinium administration, lesions are enhanced when compared with normal liver and the central scar becomes hyperintense owing to concentration of the contrast. Calcifications are rare but described. Imaging findings are not specific, and there is overlap with conditions such as fibrolamellar carcinoma; therefore, a biopsy is usually indicated.[47,78] The diagnostic feature is the presence of medium to large thick-walled muscular vessels contained within fibrous bands.

Malignant Liver Masses

Hepatoblastoma (HBL) almost always arises in an otherwise normal liver (Figure 15-5).[79,80] The incidence of HBL is between 0.5 and 1.5 cases per million children.[80] The median age at presentation is 16 months, although cases in adolescents and adults are described. Most patients present with modest symptoms such as weight loss, anorexia, and anemia and a painless mass in the right upper quadrant. The etiology is unknown. There appears to be an increased risk for the development of HBL for patients with Beckwith-Wiedemann syndrome, Meckel diverticulum, diaphragmatic or umbilical hernias, Wilms tumor, fetal alcohol syndrome, familial adenomatous polyposis, and very low birth weight infants.[81,82] Chromosomal abnormalities are reported and involve chromosomes 11, 20, and 8.

Aminotransferase levels are normal in approximately two-thirds of patients with HBL, and most are not icteric. However, all children with HBL show elevations of AFP. Levels of AFP can be used to monitor the course of the disease, but the height of AFP elevation is not related to prognosis.[83] In addition, histology and tumor size have no bearing on the ultimate prognosis. A tumor that is confined to one lobe, usually the right, and is resectable carries a good prognosis. Poor prognostic factors include vascular invasion and distant metastases.[83] Abdominal and chest CT studies are useful to predict resectability.

Complete surgical resectability remains the most important prognostic factor.[84] At the time of diagnosis,

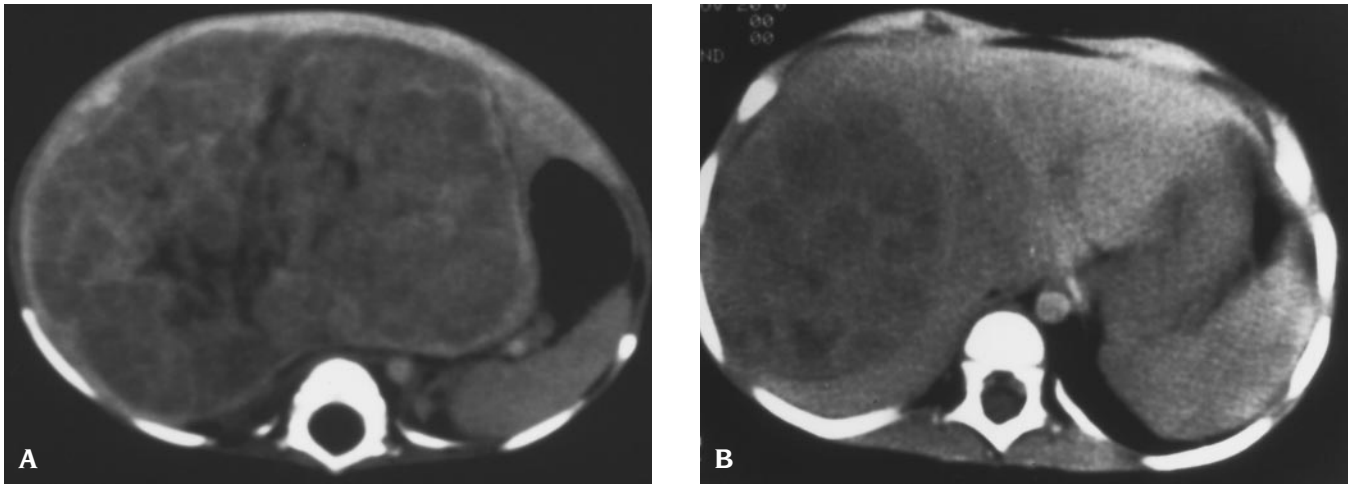

FIGURE 15-5 A 1-year-old male infant presented for routine physical examination and was found to have firm hepatomegaly. A sonogram confirmed an intrahepatic mass, and the α-fetoprotein was elevated. *A,* Computed tomographic scan of the abdomen confirms a large, unresectable hepatoblastoma. *B,* Following chemotherapy, the tumor has dramatically decreased in size.

approximately 50% are resectable, 40% are localized to one lobe but are not resectable, and 10% of the patients have distant metastases, usually to the lung.[83] Antineoplastic agents such as cisplatin and doxorubicin, as dual therapy or in combination with other chemotherapeutic medicines, are used to treat unresectable HBL.[85] Some patients who present with initially unresectable lesions subsequently become candidates for complete surgical excision following chemotherapy. With the current protocols, up to 65 to 75% of patients may be cured.[86] Orthotopic liver transplant is an option for patients whose tumor remains unresectable despite chemotherapy.[87,88]

Hepatocellular carcinoma (HCC) is a rare epithelial neoplasm that occurs primarily in older children and adolescents.[47,89] HCCs account for 0.5 to 2% of all pediatric tumors. Patients with HCC are more likely to present with signs and symptoms such as abdominal pain, fever, anorexia, malaise, and hepatomegaly.[90] The relationship between chronic hepatitis B and the subsequent development of HCC is well established.[90] However, other conditions also place the patient at an increased risk for development of HCC and include hemochromatosis, hepatitis C, α_1-antitrypsin deficiency, hereditary tyrosinemia, porphyria cutanea tarda, glycogen storage disease, and hypercitrullinemia.[91] In adults, the vast majority of patients have well-established cirrhosis at the time of diagnosis of HCC. In children with hepatitis B, however, many only have evidence of inflammation and regeneration and do not have cirrhosis.[92,93] Patients with polymorphisms of the uridine diphosphate–glucuronosyltransferase *UGT1A7* gene may be at increased risk for development of HCC.[94]

CT of the abdomen reveals a large hypodense mass with central areas of low density corresponding to tumor necrosis. After intravenous contrast, HCC appears hyperdense and is accompanied by an enhancing thin rim around the tumor. Vascular invasion of either portal or hepatic vein branches is characteristic and identified by CT in 70% of patients.[92]

Unfortunately, most children present with advanced disease at the time of diagnosis, with metastases to the lung present in up to 40% of cases.[90,93] As with HBL, complete tumor resection remains the only realistic chance for cure.[95] Chemotherapy is reserved for unresectable lesions, when a delay in surgical resection is anticipated, and as adjuvant chemotherapy following surgical resection.[96,97] Liver transplant is a potential alternative for some patients. Unfavorable factors include very large tumor size, positive hepatitis B status, nonfibrolamellar histologic type, and local or metastatic spread.[98]

Other hepatobiliary malignancies may occur in children. Germ cell tumors are reported, and resection is curative.[99] Angiosarcoma is rare, usually occurs in girls between 3 and 5 years of age, and presents with a rapidly expanding mass. These lesions can develop following treatment and apparent cure of hemangioendothelioma. Surgical resection is the only curative therapy.[100] Intrahepatic mesenchymal tumors represent 2% of all malignant mesenchymal tumors in children and 6% of primary hepatic tumors in childhood. Complete resection is needed to cure the disease, and chemotherapy is reserved for more extensive tumors.[101,102] Embryonal rhabdomyosarcomas arise in the extrahepatic biliary tree, and prognosis is dismal because complete resection is seldom possible, chemotherapy is ineffective, and radiation is, at best, palliative (Figure 15-6).[101,102]

VASCULAR LESIONS OF THE LIVER

Capillary hemangioendothelioma is a massive arteriovenous connection, which typically presents as high-output congestive heart failure and hepatomegaly in infants less than 5 months of age.[99,103,104] A bruit is sometimes heard over the epigastrium. Few clinical symptoms are noted in some patients owing to slow growth of the lesion.[105] Anemia and platelet trapping within the tumor may result in the Kasabach-Merritt syndrome. This tumor is associated with other congenital anomalies, including bilateral Wilms tumor, hemihypertrophy, Beckwith-Wiedemann syndrome, and meningomyelocele.[99,106,107]

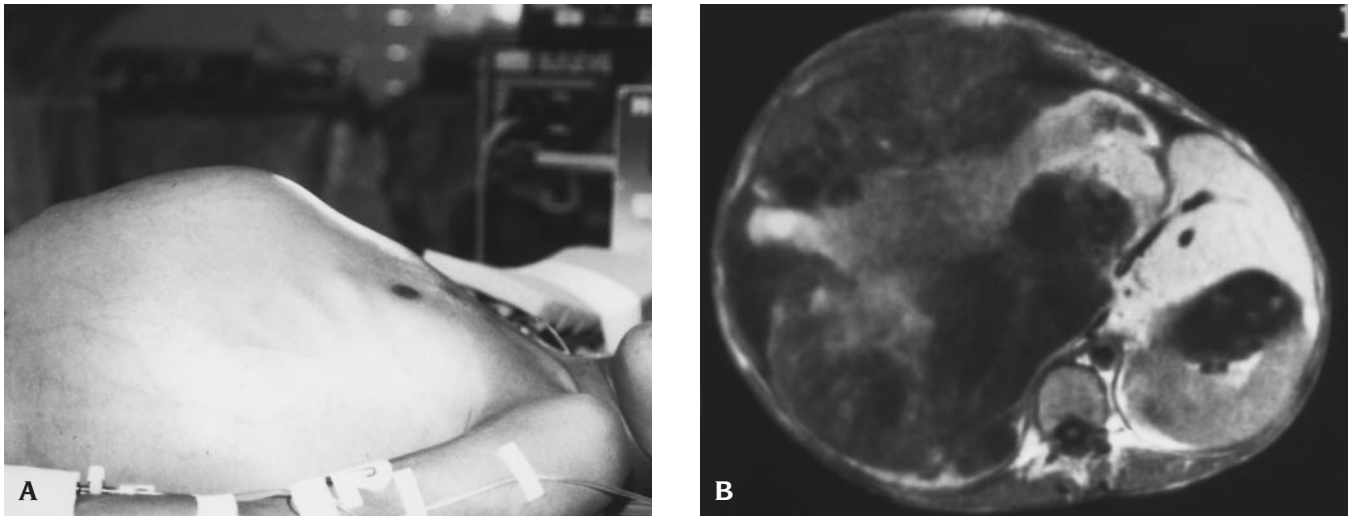

FIGURE 15-6 *A,* This 4-year-old child presented with abdominal distention, anorexia, weight loss, and vomiting. On physical examination, the child had evidence of decreased muscle mass and a hard painless mass in the right upper quadrant. *B,* An abdominal computed tomographic scan demonstrates extensive replacement of hepatic parenchyma by a mass, which was identified as an embryonal sarcoma.

A plain kidney, ureter, bladder film often identifies an enlarged liver shadow, and calcification of the tumor may be present in up to 30% of cases.[105] Doppler analysis of this vascular lesion may show high flow velocities. Specific enhancing characteristics, using helical CT and MRI, allow for the best characterization of hemangioma in most cases.[105]

The natural history of the capillary hemangioendothelioma is spontaneous resolution.[99] Digitalis and diuretics may be needed to support the child in high-output heart failure. Thyroid hormone therapy may be necessary owing to increased catabolism of endogenous thyroid hormone by the hemangioendothelioma. Steroids will elicit a clinical response for some patients, but improvement may take weeks. For severe or recalcitrant cases, treatments include antineoplastic drugs, steroids, and interferon-α, used alone or in combination.[104,108] Complete surgical resection or selective hepatic artery embolization can be lifesaving in some cases.[99] Patients with multifocal lesions are usually not surgical candidates, and they rely on embolization of the tumor or hepatic artery ligation to provide symptomatic relief while awaiting spontaneous resolution.[109] Hepatic transplant is rarely necessary.[110]

Solitary cavernous hemangioma tends to be localized within one lobe, usually the right.[111] Angiography shows a large feeding artery. The risk to the patient is rupture with hemoperitoneum. If the lesion is localized, then surgical excision is preferred. Otherwise, steroids, hepatic artery embolization, or surgical ligation is helpful.[112]

Hepatobiliary Disease

Choledochal cysts present with hepatomegaly and a palpable mass in up to 60% of patients (Figure 15-7).[113] This condition should be considered in any patient with evidence of cholestasis, with or without jaundice. The risk of malignant transformation increases with age, reaching a 12.5% overall risk. Factors that likely play a role in the development of malignancy include intracystic lithiasis, prolonged bile stasis and infection, chronic inflammation, reflux of pancreatic fluid, and genetic factors.[114] Surgical excision is not by itself fully protective against future development of cancer.[115] Liver transplant is considered for extensive intrahepatic cysts.

Caroli disease is a rare malformation consisting of multifocal nonobstructive cystic or saccular dilations of the intrahepatic bile ducts.[116] It may be an isolated finding (type 1, Caroli disease) or associated with congenital hepatic fibrosis (type 2, Caroli syndrome). Symptoms include fever, abdominal pain, jaundice, and hepatosplenomegaly. The median age at onset of symptoms is 5.5 months, whereas the median age at diagnosis is 12 months. Congenital renal mal-

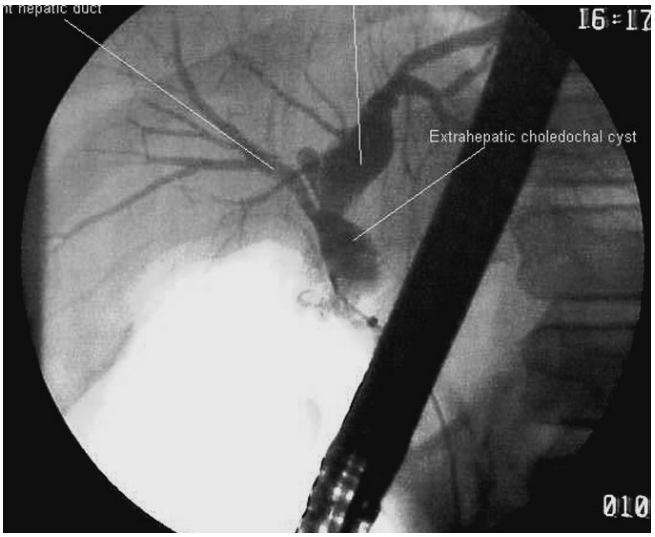

FIGURE 15-7 A 7-year-old female presented with a history of jaundice and abdominal pain for 3 days. History revealed that similar episodes had occurred at least twice in the past. At the time of the visit, she was anicteric with a liver 2.0 cm below the right costal margin. A sonogram suggested a choledochal cyst that was confirmed on endoscopic retrograde cholangiopancreatography. A left lobectomy was performed owing to the involvement of the left intrahepatic bile duct.

formations occur concomitantly in 80% of children and include ARPKD, renal tubular ectasia, and other forms of cystic diseases of the kidneys and pancreas. For children with ADPKD, the liver abnormalities may be diffuse throughout the liver or, less commonly, localized and confined to the left lobe. Prognosis depends on the frequency and severity of cholangitis.[117,118]

Congenital cysts are usually large enough to be detected on physical examination, and abdominal US characterizes the lesion as a cyst. Vague, nonspecific abdominal pain associated with abdominal distention may be the dominant clinical symptoms initially; however, hemorrhage, perforation, or torsion of the cyst can bring the patient to immediate medical attention. Treatment depends on the size and location of the cyst and is usually achieved by complete resection or drainage using a Roux-en-Y intestinal limb.[1]

Masses of the Alimentary Tract

Stomach. Tumors that involve the stomach include leiomyosarcoma, rhabdomyosarcoma, myxosarcoma, teratoma, and fibrosarcoma. Lymphoma is usually associated with post-transplant lymphoproliferative disease. Gastric carcinoma accounts for less than 5% of childhood carcinomas.[39] Presenting symptoms include anorexia, weight loss, hematemesis, and, occasionally, abdominal pain. Upper gastrointestinal contrast radiography or fiberoptic endoscopy usually identifies the lesion. Abdominal CT will demonstrate the extent of the disease and assess nodal involvement.[119]

Small Bowel. Congenital anomalies of the small bowel, which include malrotation, duplication, and Meckel diverticulum, can present in this age group with symptoms that range from vague abdominal pain to pernicious vomiting or intestinal hemorrhage. The most common malignant small bowel tumor is a lymphoma, which accounts for 5 to 10% of all non-Hodgkin lymphomas.[120,121] Patients are rarely asymptomatic and will present with fever, weight loss, altered stool pattern, and an abdominal mass. CT and US are used to evaluate the extent of the disease. A combination of surgery and chemotherapy can obtain a good remission rate for localized primary intestinal lymphoma. Intestinal perforation that complicates intestinal lymphoma in children brings a poor prognosis.[122]

Colon. Scybala, in the constipated child, can be confused for a mass arising from the pelvis or within the left lower quadrant. A careful abdominal and rectal examination will rapidly identify stool in the colon and thus avoid additional expensive and invasive tests. Adenocarcinoma is the most common colon malignancy in children, but only 1% of colon malignancies occur under 30 years of age.[119] Conditions associated with increased risk for colon cancer in children include familial polyposis syndromes and ulcerative colitis. Abdominal CT is needed to assess staging of the tumor.

Masses of the Omentum and Mesentery

Omental and mesenteric cysts likely result as a consequence of obstructed or ectopic lymphatics.[123] The lesions present most often in children and young adults and are often found incidentally, and up to 90% of patients have minimal or no symptoms. When present, symptoms can vary between a vague, nonspecific "pulling" sensation with mild abdominal discomfort and fullness and an acute abdominal crisis resulting from intestinal obstruction, volvulus, or rupture of the cyst. An abdominal mass is not always palpable but, when present, is a soft, thin-walled, freely mobile mass that is typically located within the mesentery near the terminal ileum (Figure 15-8).

Abdominal CT and US will demarcate the extent of the cyst. Omental cysts should be completely resected, but recurrences are reported.[122]

Mesenteric fibromatosis is a rare benign intra-abdominal tumor that has an aggressive tendency to infiltrate surrounding structures.[124,125] It is often associated with Gardner syndrome, previous trauma, or prolonged estrogen intake but can occur as a primary condition in the absence of predisposing factors. Possible presenting complaints include abdominal pain, a nontender abdominal mass, weight loss, or evidence of intestinal obstruction or perforation. The condition represents 12% of the soft tissue tumors in children. Spontaneous regression occurs in less than 20% of cases and most likely occurs in congenital cases. Cytogenetic analysis may improve the classification of disease in the future.[126,127]

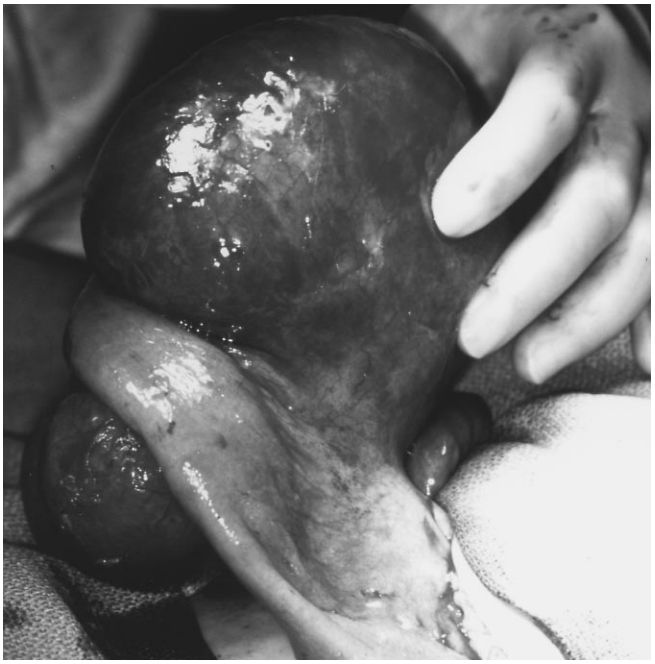

FIGURE 15-8 A 12-year-old female presented with chronic, intermittent abdominal pain that would occasionally awaken her at night. Nonbilious, nonbloody emesis occurred two to three times per week. Abdominal examination suggested a mass in the right lower quadrant and a sonogram demonstrated a cystic lesion. The surgical specimen confirmed a large mesenteric cyst on the mesenteric border of the terminal ileum.

Complete surgical resection is the treatment of choice, but complete resection may not be possible, and local recurrence is not uncommon. Clinical features that favor the likelihood of recurrence following resection include (1) presentation at an age greater than 5 years, (2) incomplete surgical resection, (3) dermoid present on an extremity, (4) microscopic evidence of tumor at the resection margins, (5) mitotic index of 5 or more per 10 high-power fields, and (6) areas of necrosis and inflammation within the tumor. [125]

Peritoneal metastases are associated with a wide variety of primary diagnoses and will have a mass-like appearance on abdominal CT.[128] Primary tumors are rare and generally include liposarcoma, leiomyosarcoma, fibrosarcoma, and mesothelioma.[129]

Inflammatory conditions can present as an abdominal mass; examples include an abscess from a ruptured appendix or Meckel diverticulum, pelvic inflammatory disease, or Crohn disease. The mass is usually tender and sometimes fluctuant and can be identified on abdominal or rectal examination. Abdominal US and contrast-enhanced CT are useful if more definition of the mass is required. An inflammatory pseudotumor is a firm, painless, well-circumscribed, nonencapsulated mass that commonly adheres to and infiltrates surrounding viscera.[130] The etiology is obscure, but such pseudotumors are associated with an inflammatory response in conditions such as Hodgkin disease, Castleman disease, and peptic ulcer disease. There are no reports of malignant change. Multiple local recurrences may plague the patient following initial surgical resection.

MASSES IN ADOLESCENTS

Many of the conditions previously described may occur in adolescents. However, some occur more commonly in this age group. Hematocolpos may not become evident until the onset of menses, when the female patient presents with no menstrual bleeding, constipation, difficulty voiding, or recurrent urinary tract infections.[1] Ovarian cysts and tumors can present as an asymptomatic abdominal mass, or the patient may complain of a dull, poorly localized discomfort. Occasionally, an abdominal crisis brings the child to medical attention as a consequence of ovarian torsion (Figure 15-9). Over 85% of cystic ovarian lesions are benign, and a teratoma (dermoid) is the most common cystic lesion. Physiologic follicular cysts occur in 12- to 14-year-old adolescents. If surgery is needed, enucleation of the cyst will preserve ovarian tissue.[1] Overall, 17% of ovarian masses in children are malignant.[1] A solid lesion noted on pelvic US should be a warning sign that a malignant lesion may be present. Potential malignant ovarian tumors include germ cell tumor, dysgerminoma, choriocarcinoma, gonadoblastoma, endodermal sinus tumor, and embryonal carcinoma (Figure 15-10). Approximately 20% of malignant ovarian tumors involve epithelial tumors (serous and mucinous cystadenocarcinoma), mesenchymal tumors (androblastoma, granulosa cell tumor), and stromal tumors. Because most ovarian lesions are benign, ovary-sparing operations should be performed whenever feasible.[131] Renal cell carcinoma occurs at a mean age of 14 years, and patients will present with flank pain and gross hematuria.[132,133] Survival is 60% in those patients with complete resection because these tumors are resistant to chemotherapy. Prognosis is poor with metastatic disease.

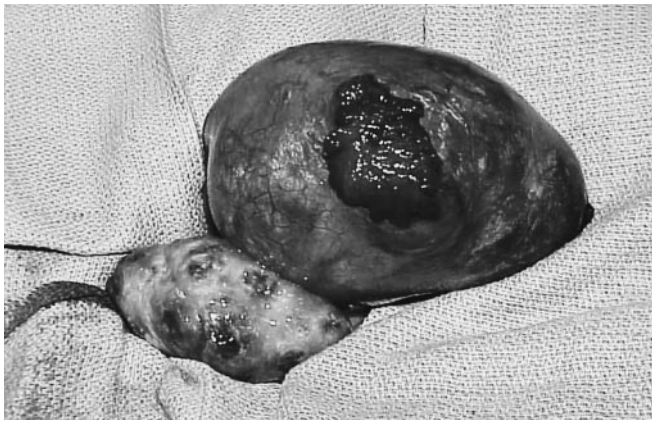

FIGURE 15-9 A 13-year-old female presented to the emergency room with sudden, intense left lower quadrant pain. An ultrasound examination suggested an ovarian mass. This surgical specimen is an ovarian torsion. The fimbria on the tubal portion can be seen.

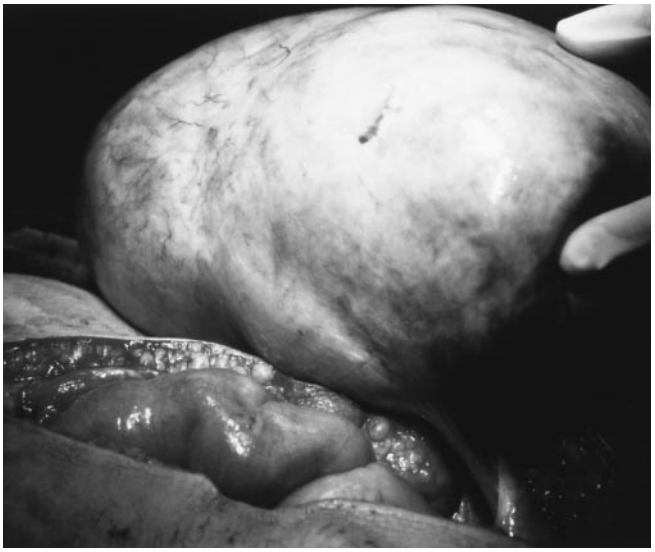

FIGURE 15-10 This large ovarian teratoma was delivered from the abdomen of a 15-year-old female who presented with acute lower abdominal pain and vomiting. A mass arising from the pelvis was noted on physical examination.

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CHAPTER 16

GASTROINTESTINAL BLEEDING

1. *Upper Gastrointestinal Bleeding*

Mark A. Gilger, MD

Upper gastrointestinal (UGI) bleeding in children is an unusual but potentially serious problem. Hematemesis is always a frightening ordeal for the patient and parents. Fortunately, it is rarely life threatening and usually stops. Although the causes of UGI bleeding have remained unchanged, the treatment, such as the use of octreotide and endoscopic therapy, has improved. UGI bleeding is considered a "red flag" sign of possible peptic injury and always warrants further investigation.[1] The purpose of this chapter is to outline a rational approach to children with UGI bleeding and discuss diagnostic and therapeutic interventions.

EPIDEMIOLOGY

UGI bleeding is uncommon in children. Using the Pediatric Endoscopy Database System–Clinical Outcomes Research Initiative (PEDS-CORI), Bancroft and colleagues found that hematemesis accounts for only about 5% (327 of 6,337) of indications for upper endoscopy in children.[2] In children hospitalized in the intensive care unit, UGI bleeding is considerably more common, with an incidence ranging from 6 to 25%.[3,4] However, even in this critically ill population, life-threatening UGI bleeding occurred in only 0.4% of children.[5]

DEFINITIONS

UGI bleeding refers to bleeding occurring above the ligament of Treitz. Hematemesis indicates vomiting with frank red blood and generally denotes a more rapidly bleeding lesion. Coffee ground emesis is secondary to the coagulative effect of gastric acid on blood. It is usually slower and from a more benign bleeding source. Melena refers to black, tarry stools. The dark black color is probably caused by hematin, the oxidative product of heme produced by intestinal bacteria. Melena can be produced by relatively small volumes of blood (50–100 mL) in the stomach. Melena may persist for 3 to 5 days and thus cannot be used as an indication of ongoing bleeding.[6] In general, most UGI bleeding in children is benign and stops without intervention.

IS IT BLOOD?

There are a variety of important considerations in a child with suspected UGI bleeding. The first is to determine if the suspicious material, whether seen in emesis or stool, actually contains blood. In children, many common beverages and foods may give the appearance of blood if vomited. For example, red food coloring, as found in Jello, Kool Aid, and other red foods, such as tomatoes and strawberries, may give the appearance of blood in emesis. UGI bleeding also may cause melanotic stools. Once again, foods such as spinach and licorice and certain medications such as bismuth and iron may cause the stool to appear black.

Determination of blood in gastric contents is best accomplished using a bedside technique to detect hemoglobin (ie, Gastroccult, SmithKline Diagnostics, Inc, San Jose, CA). Fecal material can also be tested for the presence of hemoglobin (ie, Hemoccult, SmithKline Diagnostics).[7] The principle in both products is based on the oxidation of guaiac by hydrogen peroxide to a blue quinone compound. Guaiac is a colorless compound that turns blue when placed in contact with substances that have peroxidase activity and are then exposed to hydrogen peroxide. The paper is impregnated with guaiac, a natural resin extracted from the wood of *Guaiacum officinale*. If the fecal material contains blood, the peroxidase activity of the heme portion of hemoglobin will catalyze the oxidation of guaiaconic acid when hydrogen peroxide (the developer) is placed on the paper. The test is simple and convenient but has limitations. The guaiac oxidation reaction is not specific for blood but rather for peroxidase activity. Many foods, such as red meat and certain vegetables (melons, grapes, radishes, turnips, cauliflower, and broccoli), contain enough peroxidase activity to cause a positive guaiac reaction.[7,8] Fortunately, plant peroxidases are relatively unstable and, despite the potential, rarely cause a false-positive reaction.[7] Iron no longer produces a false-positive Hemoccult reaction.[7]

IS THE BLEEDING GASTROINTESTINAL?

UGI bleeding is often manifest as blood per os. There are a variety of potential nongastrointestinal bleeding sources above the ligament of Treitz. For example, epistaxis, blood-tinged sputum, and oropharyngeal bleeding (ie, tonsils, adenoids, tongue, and gingivitis) will all produce blood per os. In some cases, the source is obvious, such as with epistaxis or visible injury to the oropharyngeal mucosa. However, the bleeding source could be the lungs or upper airway. It must be recognized that sputum is often poorly expectorated in young children, leading to the assumption that the bleeding is gastrointestinal. Pharyngeal bleeding may be difficult to identify with routine inspection of the oral cavity. Arteriovenous malformations of the tongue and posterior pharynx must be considered, as well as bleeding from the salivary glands.

APPROACH TO UGI BLEEDING

The initial evaluation of any child with gastrointestinal bleeding must include a brief history followed by a rapid assessment of the physical condition, with particular attention to the vital signs and the patient's level of consciousness (Figure 16.1-1). If the bleeding is severe, therapy may need to begin before the location of the bleeding can be ascertained. Significant gastrointestinal bleeding will be initially manifest by tachycardia, whereas hypotension occurs later, an ominous signal of impending cardiovascular collapse. Immediate therapy is aimed at correction of volume loss and anemia, which should include aggressive fluid and blood resuscitation. If the patient remains unstable after receiving a blood transfusion of approximately 85 mL/kg or greater, emergency exploratory surgery is indicated. Surgical consultation is mandatory in any case of severe UGI bleeding. Sources such as varices, ulcers penetrating into an artery, or mucosal tears into arterial vasculature must be considered in severe UGI bleeding in children. Careful attention must be paid to the child's fluid volume status. Be aware that overexpansion of blood volume may worsen variceal bleeding.

Once the child is hemodynamically stable, further evaluation can proceed in a controlled manner (see Figure 16.1-1). Initial laboratory evaluation should generally include a complete blood count with platelets and reticulocyte count, a protime and partial thromboplastin time, and a blood type and crossmatch (Table 16.1-1). Additional laboratories to assess liver function (alanine aminotransferase, aspartate aminotransferase, and albumin) and kidney function (blood urea nitrogen and creatinine) may prove useful. For example, abnormal liver enzymes and a low albumin or an elevated protime can be an indication of chronic liver disease. An elevated blood urea nitrogen can be an indication of UGI bleeding because azotemia may be the result of intestinal absorption of the blood and hypovolemia.[9,10]

Gastric aspiration is a simple, useful indicator of UGI bleeding. A bloody aspirate indicates active bleeding, usually gastric or esophageal. A clear aspirate does not eliminate a duodenal bleeding source. In a national survey of

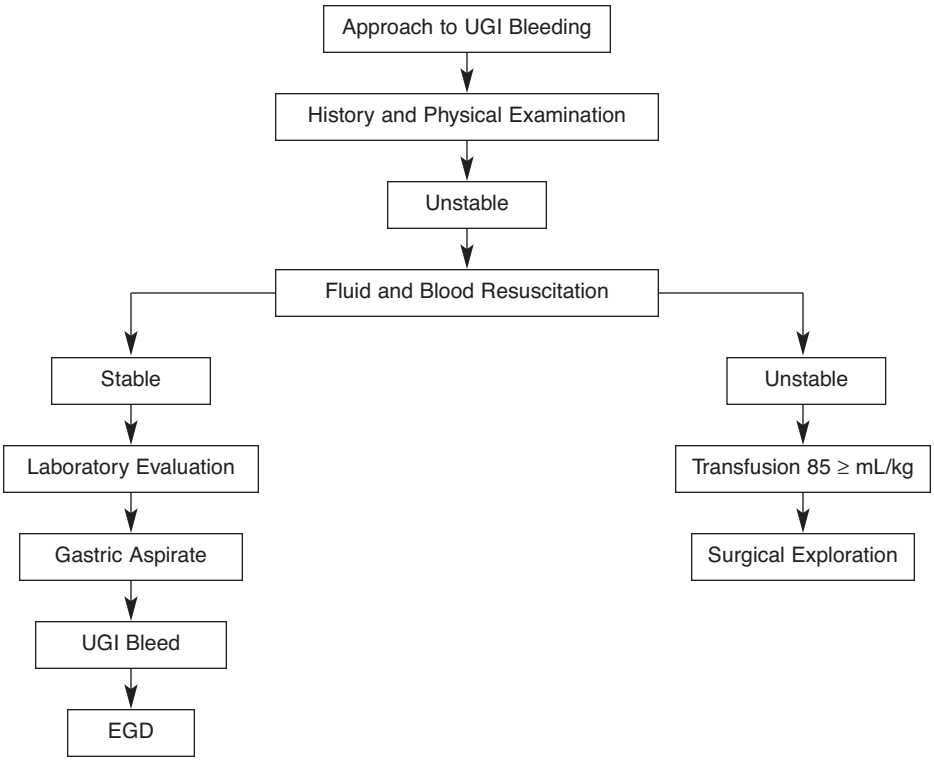

FIGURE 16.1-1 An approach to the diagnosis and treatment of upper gastrointestinal (UGI) bleeding in children, emphasizing initial stabilization followed by a controlled diagnostic evaluation. Adapted from references 13 to 17, 43, 63, and 86. EGD = esophagogastroduodenoscopy.

TABLE 16.1-1 COMMON LABORATORY STUDIES USEFUL IN THE EVALUATION OF UPPER GASTROINTESTINAL BLEEDING IN CHILDREN

Complete blood count with differential
Reticulocyte count
Protime and partial thromboplastin time
Blood type and crossmatch
Alanine aminotransferase, aspartate aminotransferase, albumin
Blood urea nitrogen, creatinine

UGI bleeding in adults, Gilbert and colleagues reported that a bleeding source was identified in 16% of patients with a negative gastric aspirate.[11] Iced saline lavage is no longer recommended. It has been shown to be ineffective in slowing UGI bleeding in animal models and has the theoretic potential of causing hypothermia and electrolyte abnormalities in infants.[12]

After the rapid assessment of vital signs, a thorough physical examination can reveal stigmata of underlying disease, suggesting a source of bleeding (see Figure 16.1-1). For example, a variety of dermatologic lesions are similar to those seen in the gastrointestinal tract. Skin findings such as a caput medusa, spider angiomata, and jaundice may indicate liver dysfunction. Other dermatologic findings, such as hemangioma and telangiectasia, may be indicative of lesions in the gastrointestinal tract. Cutaneous palpable purpuras are suggestive of Henoch-Schönlein purpura. Other physical findings, such as hepatosplenomegaly, may indicate cirrhosis.

ETIOLOGY BY AGE GROUP

UGI bleeding in children is best considered by age because certain causes are unique to particular age groups. Table 16.1-2 lists the potential etiologies of UGI bleeding in children by age group.[13–22]

NEONATE

Hematemesis in the first few days of life is suggestive of swallowed maternal blood.[23] Fortunately, maternal blood can be differentiated from that of a newborn using the Apt test for fetal hemoglobin.[23] The test can be performed using emesis or stool. One milliliter of emesis is mixed with 5 mL of water. The mixture is centrifuged to produce a clear, pink supernatant. One milliliter of supernatant is added to 4 mL of 1% sodium hydroxide. After 2 minutes, if the solution remains pink, it is fetal hemoglobin, whereas a yellow-brown color indicates maternal hemoglobin A.

The well newborn may present with hematemesis with or without other manifestations of bleeding, such as epistaxis or easy bruising. In this scenario, hemorrhagic disease of the newborn secondary to vitamin K deficiency must be considered.[24] This problem is easily remedied by the intramuscular or intravenous administration of vitamin K. Failure to correct the bleeding should be followed by laboratory evaluation to assess for other bleeding disorders, such as clotting factor deficiencies, von Willebrand's disease, and other bleeding diatheses.[25] Vitamin K deficiency can also be the result of underlying fat malabsorption, such as cystic fibrosis and cholestatic syndromes, although these problems are rare in the neonatal period. In the breastfed infant, maternal blood can be ingested from a cracked or irritated breast. Sensitivity to dietary proteins, especially milk and soy, can be manifest as hematemesis, although it more commonly presents as streaks of blood and mucus in the stool.[26] Unusual causes of UGI bleeding in this age group include milk-protein sensitivity,[18,19] hypertrophic pyloric stenosis,[20] and anatomic deformities such as a web of the stomach and duodenum.[21,22]

Significant UGI bleeding in the neonate is usually associated with critical illness, such as shock. The neonatal gut is highly susceptible to mucosal injury during the relative intestinal ischemia produced by shock. Stress gastritis and ulcers of the stomach and duodenum can result and are potential causes of significant UGI bleeding (see Table 16.1-2).

INFANT

Stress gastritis and ulceration with secondary UGI bleeding are well recognized in the critically ill infant.[3–5] Although fairly common, clinically significant bleeding is unusual, with estimates ranging between 0.4% and 2% of children.[3,4]

Acid-peptic disease, such as esophagitis, gastritis, and ulcer, although uncommon, does occur and can cause UGI bleeding in this age group.[27] The bleeding is generally minor. Hematemesis is rarely the only manifestation and is usually accompanied by irritability with feeding, feeding refusal, and regurgitation.[27]

TABLE 16.1-2 ETIOLOGIES OF UPPER GASTROINTESTINAL BLEEDING IN CHILDREN BY AGE GROUP, IN RELATIVE ORDER OF FREQUENCY

NEWBORN	INFANT	CHILD–ADOLESCENT
Swallowed maternal blood	Stress gastritis or ulcer	Mallory-Weiss tear
Vitamin K deficiency	Acid-peptic disease	Acid-peptic disease
Stress gastritis or ulcer	Mallory-Weiss tear	Varices
Acid-peptic disease	Vascular anomaly	Caustic ingestion
Vascular anomaly	Gastrointestinal duplications	Vasculitis (Henoch-Schönlein purpura)
Coagulopathy	Gastric/esophageal varices	Crohn disease
Milk-protein sensitivity	Duodenal/gastric webs	Bowel obstruction
	Bowel obstruction	Dieulafoy lesion, hemobilia

Adapted from references 3 to 5, 13 to 17, 43, and 63.

Vascular anomalies often appear on the skin and soft tissue during infancy. They can be divided into tumors and vascular malformations. Hemangiomas are the most common tumor, being found in 4 to 12% of white infants (Table 16.1-3).[28,29] Fortunately, vascular anomalies of the gastrointestinal tract are considerably less common, although the exact prevalence is unknown.[28] Symptomatic hemangiomas of the gut are rare, but when they do occur, they are commonly associated with skin lesions. The gastrointestinal bleeding can be significant, even requiring transfusion (Figure 16.1-2).[28] Most hemangiomas require no treatment and will spontaneously regress with time. In severe, multifocal, or large hemangiomatous lesions, treatment with prednisone may be useful. Lesions that do not respond to corticosteriods may be treated with interferon alfa-2b.[30]

Mechanical obstruction of the UGI tract can be a cause of hematemesis in this age group. Bleeding may be caused by mucosal injury from the mechanical shearing forces, such as Mallory-Weiss syndrome or gastric prolapse.[31–34] Anatomic abnormalities, such as duplication cysts, can cause UGI bleeding. Duplications containing gastric mucosa can ulcerate and bleed. Antral duplications have been reported to cause hypergastrinemia with ulceration and bleeding.[35] Obstructive lesions such as hypertrophic pyloric stenosis,[20] duodenal web,[21] and antral web[22] have all been reported as causing UGI bleeding in infants.

Children and Adolescents

Vomiting is common in children, usually the result of infection. It should come as no surprise that the consequence of forceful vomiting, such as a Mallory-Weiss tear, is likely the most common cause of minor UGI bleeding in children (see Table 16.1-2). Peptic mucosal injury, such as esophagitis, gastritis, and ulceration, may result in UGI bleeding. Although erosive esophagitis is actually quite common, being reported in 34.6% of neurologically normal children undergoing upper endoscopy,[36] secondary UGI bleeding is not nearly so frequent. As in adults, a variety of nonsteroidal anti-inflammatory medications, such as aspirin, ibuprofen, naproxen, and ketorolac, can cause gastric mucosal injury, resulting in UGI bleeding.[37–39] Gastric infection with *Helicobacter pylori* can result in peptic ulceration with bleeding.[40] Rare cases of mucosa-associated lymphoid tissue lymphoma (MALToma) have occurred secondary to *H. pylori* infection, which may also result in UGI bleeding.[17]

TABLE 16.1-3 CLASSIFICATION OF VASCULAR ANOMALIES IN CHILDREN

Tumors
Hemangiomas
Hemangioendotheliomas
Congenital hemangiomas
Malformations
Capillary
Venous
Lymphatic
Arterial/arteriovenous

Adapted from references 17, 28 to 30, and 72.

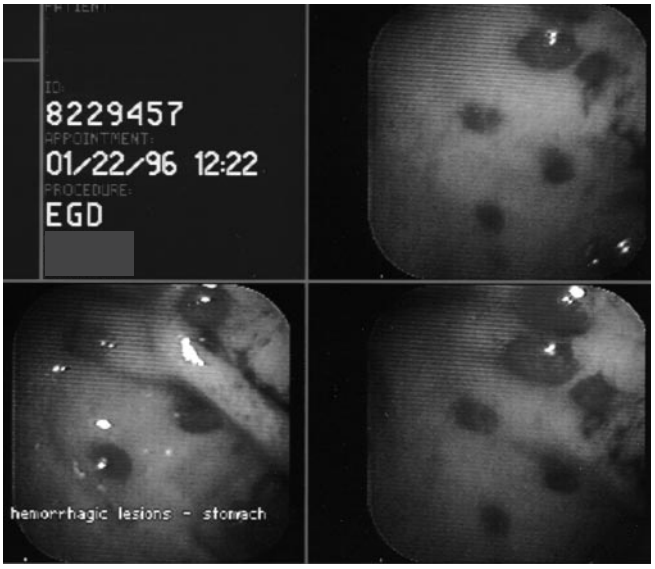

FIGURE 16.1-2 Multiple hemangiomas seen in the stomach of a 3-month-old girl with hematemesis.

Variceal bleeding is the most common cause of severe UGI bleeding in children.[41] Most variceal bleeding is esophageal, secondary to the high-pressure, turbulent flow in the thin-walled superficial vessels of the distal esophagus.[42] Acute variceal bleeding in children stops spontaneously in about 50% of patients, with rebleeding found in 40%.[43] The lifelong risk of variceal bleeding appears to be about 50%.[43,44] The clinical presentation is usually either hematemesis or melena. In some children, nonspecific abdominal pain may precede variceal bleeding for up to 48 hours.[45]

Ingestion of foreign bodies and caustics has the potential to cause UGI bleeding.[13,46] In general, bleeding from a foreign body implies that either a sharp object (eg, safety pin, needle, razor) has been ingested, causing a mucosal tear, or the ingested object is chronic and has caused an ulcer.[46,47] Bleeding caused by a caustic ingestion is usually a late sign of the ingestion, which has caused a stricture.

Vascular anomalies are a rare cause of UGI bleeding (see Tables 16.1-2 and 16.1-3). Minor UGI bleeding has been found with gastric hemangioma and Dieulafoy lesions.[48] Very rare but massive UGI bleeding has been noted with an aortoesophageal fistula.[49]

A variety of other causes of UGI bleeding have been reported in children. These include gastric or duodenal vasculitis, such as Henoch-Schönlein purpura,[50] Munchausen syndrome by proxy,[51] pharyngeal leeches,[52] ruptured pancreatic pseudocyst,[53] and bleeding from gastric polyps in children with Menkes disease.[54] Certain gastric tumors have the potential to ulcerate and bleed, such as leiomyosarcoma and teratoma.[55] Crohn disease with UGI involvement must be considered as a cause of UGI bleeding in this age group.[13,56,57]

DIAGNOSIS

Radiographic Studies

In general, radiographic studies have a limited role in the diagnosis of UGI bleeding. A plain x-ray film is useful in identifying unsuspected foreign bodies, with free air sug-

gesting bowel perforation and bowel obstruction. Barium studies are of little value because they cannot detect superficial mucosal lesions and can obscure the mucosa during endoscopy. Abdominal ultrasonography is useful in the assessment of portal hypertension or if large vascular anomalies are suspected.[17] Doppler flow can identify evidence of cirrhosis and portal blood flow dynamics.

Angiography

In cases of massive UGI bleeding, angiography offers an alternative to endoscopy for both diagnosis and treatment. The rule of thumb is that the bleeding must be at least 0.5 mL/min to be detected by angiography.[58] Hemobilia is a very unusual although appropriate indication for angiography over upper endoscopy.[17] Angiography also provides a therapeutic approach, such as the placement of "coils" for embolization of the bleeding vessel.[59,60] Transjugular intrahepatic portosystemic shunts offer an alternative to surgical therapy in some children with variceal bleeding. Experience is still limited in children, but this angiographic technique holds promise.[61]

Nuclear Medicine

Technetium-labeled bleeding scans and sulfur colloid scans are helpful in the diagnosis of obscure bleeding in the small bowel. However, in UGI bleeding, upper endoscopy is far superior for evaluation of bleeding above the ligament of Treitz.

Endoscopy

Esophagogastroduodenoscopy (EGD) is the preferred method to evaluate the UGI tract for a source of bleeding. Hematemesis is considered a "red flag" sign and is an indication for early EGD.[1] EGD is generally indicated for assessment of acute UGI bleeding requiring transfusion or unexplained recurrent bleeding.[17] EGD can determine the source of the bleeding in 90% of cases.[13] EGD is particularly useful in the diagnosis of mucosal lesions such as gastritis, esophagitis, peptic ulcers, and Mallory-Weiss tears. In a recent review of 6,337 EGDs using the PEDS-CORI database, Bancroft and colleagues found that 20% had esophageal inflammation, 17% had gastric mucosal abnormalities, 6% had peptic ulcers, 6% had varices, 2% had Mallory-Weiss tears, and 8% had nonspecific mucosal abnormalities.[2] This differs somewhat from the reports of others, such as Cox and Ament, who reported that 20% of children undergoing EGD had duodenal ulcer, 18% had gastric ulcer, 15% had esophagitis, 13% had gastritis, and 10% had varices.[62] It is important to recognize that most UGI bleeding in children stops spontaneously; thus, emergency endoscopy is indicated only when the findings will influence a clinical decision, such as the need for medical or surgical therapy.[13] EGD is contraindicated if the patient is clinically unstable, such as in shock, hypovolemia, myocardial ischemia, or profound anemia.[13,17]

TREATMENT

The initial goal in the treatment of any child with UGI bleeding is to provide hemodynamic stability, such as adequate oxygen delivery, fluid and blood resuscitation, and correction of any coagulopathy or metabolic or electrolyte abnormality (see Figure 16.1-1). It is only after the patient has been appropriately stabilized that medical and endoscopic treatment can begin.

Medical Therapy

Table 16.1-4 lists the medications useful for treatment of UGI bleeding.[63] Treatment consists of either acid suppression or vasoactive agents. Initial treatment for suspected acid-peptic disease is appropriate because this is a common cause of UGI bleeding in children.[63–69]

In children with severe UGI bleeding, medications to reduce splanchnic blood flow, such as octreotide and vasopressin, can be useful. Although both drugs are generally well tolerated in children, octreotide is the drug of choice because it is effective and has minimal side effects.[42,43]

Endoscopic Therapy

A variety of endoscopic therapies are available for the treatment of UGI bleeding. These include electrocoagulation (heater probe, monopolar probe, and bipolar electocoagulation [BICAP] probe[70]), laser photocoagulation,[71] argon plasma coagulation,[72] injection of epinephrine and sclerosants,[73] band ligation,[48] and mechanical clipping.[74] Unfortunately, there is little published experience with these techniques in children. As a result, which approach is best remains unknown. The argon plasma coagulator is an attractive tool because it fits through small pediatric endoscopes and has a controlled depth of penetration.[72] Laser therapy is commonly applied in adults, but there is a significant learning curve and high potential for full-thickness wall injury.[13,71] Injection therapy is appealing because it is easily performed, is inexpensive, and uses a sclerotherapy needle, all of which are familiar to the pediatric endoscopist. The combination of injection (ie, epinephrine) and BICAP is likely the most commonly performed endoscopic treatment of UGI bleeding in children, although no data exist. In this technique, the bleeding is extinguished with injection of 1:10,000 epinephrine followed by BICAP (Figure 16.1-3).

Endoscopic treatment of esophageal varices includes either injection sclerotherapy or variceal banding. Injection sclerotherapy has been used for the treatment of

TABLE 16.1-4 MEDICATIONS FOR TREATMENT OF UPPER GASTROINTESTINAL BLEEDING IN CHILDREN

ACID SUPPRESSION
Antacids
H_2 receptor antagonists
Proton pump inhibitors
VASOCONSTRICTION
Octreotide
Vasopressin
CYTOPROTECTION
Sucralfate
Misoprostol

Adapted from references 5, 13 to 17, 63 to 69, 76, and 77.

variceal hemorrhage for over 40 years, even in children.[75] Control of bleeding is achieved in over 90% of cases.[76,77] Varices are eradicated in over 80% of cases.[43,77] Although simple and attractive, complications after sclerotherapy are common, such as strictures, recurrence of the varices, and recurrent bleeding.[43] Variceal banding is a considerably more recent development, first introduced in 1989 as an adaptation of the treatment for hemorrhoids (Figure 16.1-4).[78] In meta-analysis studies in adult patients, there is no difference between sclerotherapy and banding in the control of bleeding or mortality.[79] Although there are no comparative studies between sclerotherapy and banding in children, there is an increasing body of literature to suggest that banding is the preferred method in children.[43,80–82] Banding appears to be much better tolerated in children compared with sclerotherapy, with less retrosternal pain and no fever.[43,79] Banding equipment has not yet been adapted for use in infants and small children because the banding device remains too large. Although variceal banding is effective in controlling bleeding, rebleeding occurs in up to 80% of patients.[83,84]

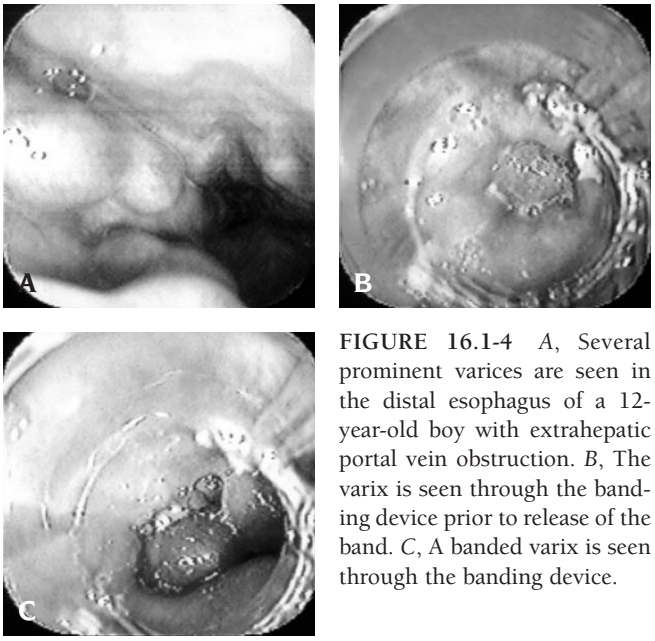

FIGURE 16.1-4 *A*, Several prominent varices are seen in the distal esophagus of a 12-year-old boy with extrahepatic portal vein obstruction. *B*, The varix is seen through the banding device prior to release of the band. *C*, A banded varix is seen through the banding device.

Surgery

In any situation in which the risk of bleeding is high, surgical consultation should always be obtained prior to the interventional technique (see Figure 16.1-1). Exploratory laparotomy is reserved for uncontrollable bleeding. In general, surgery is most common with a posterior duodenal ulcer with arterial bleeding, bowel perforation with bleeding, and gastroesophageal varices.[17] In a review of a 45-year experience with 43 children requiring surgery for peptic ulcer disease and secondary UGI bleeding, Arazow and colleagues found that 38 were in the era before histamine receptor antagonists.[85] They found that only two children in the "proton pump inhibitor era" required surgery, one for bleeding and one for obstruction.

Surgical intervention for gastroesophageal varices requires a portosystemic shunting procedure, such as a mesocaval shunt, distal splenorenal shunt, or central portocaval shunt.[86] The esophageal transection and devascularization, or Sugiura procedure, is a rare but potentially lifesaving surgery for bleeding esophageal varices.[87] de Ville de Goyet and colleagues reported a portal revascularization surgery for extrahepatic venous obstruction.[88] In this surgery, the superior mesenteric vein is connected to the left hepatic vein bypassing the portal venous obstruction. It has been successful in seven children to date. In children in whom the left intrahepatic vein is patent and accessible, this surgery is a unique treatment option.

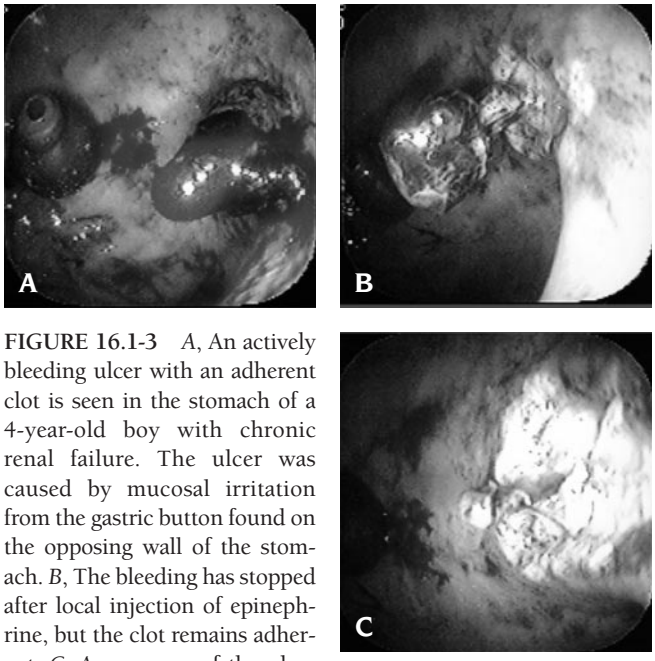

FIGURE 16.1-3 *A*, An actively bleeding ulcer with an adherent clot is seen in the stomach of a 4-year-old boy with chronic renal failure. The ulcer was caused by mucosal irritation from the gastric button found on the opposing wall of the stomach. *B*, The bleeding has stopped after local injection of epinephrine, but the clot remains adherent. *C*, Appearance of the ulcer base after coagulation with a bipolar electrocoagulation probe and removal of the clot.

CONCLUSIONS

UGI bleeding in children is an unusual but occasionally devastating problem. Fortunately, it is rarely life threatening in children and usually stops spontaneously. Treatment options, such as the use of octreotide and endoscopic therapy, have markedly improved. However, even though new medications and exciting interventional techniques exist, one must first do no harm.

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2. Lower Gastrointestinal Bleeding

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Lower gastrointestinal (LGI) bleeding is defined as bleeding with an origin distal to the ligament of Treitz. Although the passage of blood rectally in a child is very alarming to parents and is therefore promptly brought to the physician's attention, blood losses are usually mild to moderate and self-limited. The clinical presentation of LGI bleeding depends mainly on the rate and quantity of blood loss and may range from stools positive for fecal occult blood test to a life-threatening hemorrhage presenting with profound shock.

LGI bleeding can be revealed in four ways: (1) hematochezia, that is, the passage of bright red blood per rectum, either isolated or mixed with stools, indicating an origin low in the gastrointestinal tract, most commonly the colon. However, this finding is less reliable in infants, given their relatively shorter intestinal transit time. Owing to the quantity and cathartic action of blood through the intestine, massive upper gastrointestinal bleeding may present with hematochezia; (2) melena, that is, the passage per rectum of black, tarry, and foul-smelling stools, indicating a source of bleeding from above the ileocecal valve. Melena can also be seen in cases of bleeding from the proximal large bowel provided that the colonic transit time is slow; (3) occult gastrointestinal bleeding, with symptoms limited to pallor or fatigue, detected by discovery of iron deficiency or iron deficiency anemia or by testing for the presence of fecal blood; and (4) symptoms of severe blood loss such as malaise, tachycardia, or even profound shock without any objective sign of bleeding.

Proper history taking and thorough physical examination are of paramount importance to help differentiate the numerous diagnostic possibilities of LGI bleeding in children. The spectrum of disease to be considered is very different from that of adults. The pediatrician should never rush toward diagnostic workup at the expense of basic care of the child. Caution is necessary for the use of diagnostic tests, especially in very young children. The main parameters to take into account for the differential diagnosis are (1) age, because many causes of LGI bleeding are specific to certain age groups; (2) location of the bleeding in relation to the characteristics of the stools; (3) amount of blood passed; and (4) condition of the patient, that is, the presence or absence of associated symptoms and physical signs.

The objectives of this chapter are to review the nature of bleeding and the initial assessment, history taking, physical examination, and diagnostic investigations to be performed in a child presenting with LGI bleeding. The main specific causes of LGI bleeding are reviewed by age from birth to adolescence. Rare causes, occult LGI bleeding, and LGI bleeding in developing countries are also presented. Specific treatment of the various underlying causes of LGI bleeding is beyond the scope of this chapter.

NATURE OF BLEEDING

Is It Blood?

Many substances ingested by children, either foods or medicines, may cause the stool to appear bloody or dark or to test positive for the presence of blood.[1] A red or purple tinge to the stools can mimic hematochezia, especially in cases of concomitant diarrhea, because of the ingestion of artificial food colorings used in drinks, breakfast cereals, syrup medications, and gelatin desserts or of tomato skins, peach skins, and beets. Melena may be confused with dark- or black-colored stools owing to iron preparations, bismuth subsalicylate, spinach, dark chocolate, purple grapes and grape juice, blueberries, and cranberries.[2]

The history will help determine if it is truly blood that has been passed, but if questions remain as to the nature of the red streaks or the black discoloration, stool analysis can rapidly bring the answer. Fecal occult blood testing has been shown as an efficient screening aid for detecting gastrointestinal blood loss.[3] However, fecal blood testing devices may lack specificity or sensitivity. False-positive results can be due to the ingestion of red meat or peroxidase-containing fruits and vegetables such as tomato, cherry, turnip, broccoli, radish, cantaloupe, or cauliflower. False-negative results can be observed in case of ingestion of large doses of vitamin C or, because of a dry stool sample, outdated reagents or the prior conversion of hemoglobin to porphyrin by the intestinal microbiota of the child.[3]

Is It Blood from the Child?

Ingestion of maternal blood at delivery or from a fissured nipple during breastfeeding can result in melena in an otherwise healthy-appearing baby. Blood can be identified as of maternal origin by means of the Apt-Downey test of a stool sample. The Apt-Downey test differentiates maternal from fetal hemoglobin by their different colorimetric responses to denaturation with sodium hydroxide.[4] Factitious reporting of LGI bleeding or addition of blood to stool samples has been described in Munchausen syndrome by proxy.[5,6] This factitious illness alleged by a parent, usually the mother, should be suspected when extensive testing yields no cause in an otherwise healthy child whose clinical examination is repeatedly normal.

Is It Blood from the Gastrointestinal Tract?

Blood exogenous to the gastrointestinal tract is a possible cause of pseudo-LGI bleeding. Swallowed blood postadenoidectomy or post-tonsillectomy, or from epistaxis or traumatic nasopharyngeal lesions owing to the passage of a nasogastric tube, may, in cases of vigorous bleeding, be the cause of melena. In a pubertal female patient with apparent hematochezia, the onset of menarche should be considered. Hematuria may also be mistaken for hematochezia.

Is It Blood from the Lower Gastrointestinal Tract?

As mentioned above, hematochezia may be seen in cases of severe upper gastrointestinal bleeding, whereas melena may be secondary to LGI bleeding. The presence of hyperactive bowel sounds is suggestive of upper gastrointestinal bleeding. The placement of a nasogastric tube and aspiration and blood testing of gastric contents are of great help to determine the location of gastrointestinal bleeding, especially in patients with hemodynamic instability and in the absence of frank hematemesis. The presence of esophageal varices is not a contraindication to the use of this test. A gastric aspirate negative for blood is very much in favor of bleeding originating from beyond the ligament of Treitz in the small bowel or colon. However, a bleeding duodenal ulcer or hemobilia (hemorrhage into the biliary tract) cannot be completely ruled out if no reflux of blood occurs from the duodenum to the stomach. In case of doubt, esogastroduodenoscopy will enable recognition of the bleeding source from the upper gastrointestinal tract.

INITIAL ASSESSMENT

The most important step in the initial management of a child with LGI bleeding is the rapid assessment of the degree of volume loss and the initiation of fluid resuscitation if needed. Therefore, hemodynamics is the initial focal point. Vital signs are taken, and the child's skin and mucous membranes are inspected for pallor and signs of shock. If the child looks healthy and has no past history of disease that could lead to LGI bleeding, and if blood loss is minor and hemodynamic condition is unquestionably normal, admission to hospital is not necessary, and a diagnostic workup can be performed on an outpatient basis. However, close follow-up is necessary in cases of worsening or recurrence of bleeding.

Tachycardia is a very sensitive indicator of severe blood loss, whereas slow capillary refill and hypotension are ominous signs of hypovolemia and shock. Symptoms of hemodynamic instability should prompt urgent placement of two large-bore intravenous catheters and may lead to transferring the patient to the intensive care unit. Supplemental oxygen is provided if necessary. Blood is drawn for a complete blood count (hemoglobin, hematocrit, platelet count), clotting studies, and routine chemistry. Blood typing and crossmatching should also be performed so that transfusion can be given without delay if needed.

HISTORY

Family History

A first-degree relative history of allergy, inflammatory bowel disease, familial adenomatous polyposis, hereditary hemorrhagic telangiectasia, Ehlers-Danlos syndrome, or bleeding disorders is strongly suggestive of the same disease in the presenting child.

Child History

Omphalitis, sepsis, and umbilical catheterization in the neonatal period should be sought because of the risk of portal vein cavernoma and secondary portal hypertension, as well as abdominal surgery, especially resection of the small bowel and/or right colon in the neonatal period or early infancy, previous episodes of bleeding from the gastrointestinal tract or other site, hematologic abnormalities, and liver disease.

Attendance at a day-care center is associated with a higher risk for bacterial or viral gastrointestinal infection, whereas recent travel to an endemic area is suggestive of amebiasis. Recent use of antibiotics is a risk factor for antibiotic-associated diarrhea and pseudomembranous colitis.[7] Exposure to contaminated foods (chicken, eggs, unpasteurized milk) enhances the risk for outbreaks of bacterial gastrointestinal infection.

The occurrence of LGI bleeding soon after weaning and introduction of cow's milk or soy protein formula in the diet strongly suggests the occurrence of cow's milk or soy protein allergy.

Age at Onset of LGI Bleeding

Age is a very important component of the history for finding the most common causes of LGI bleeding. However, some causes of LGI bleeding can be seen in different age groups, such as malrotation with midgut volvulus, allergic proctocolitis, intussusception, Meckel diverticulum, lymphonodular hyperplasia (LNH), or Henoch-Schönlein purpura. Anal fissure and infectious colitis can even be observed in any age group, from birth to adolescence. Table 16.2-1 shows the main causes of LGI bleeding in relation to age.

Characteristics of LGI Bleeding

A careful analysis of the aspect of the hemorrhage passed per rectum will help to localize the site of bleeding in the LGI tract. The amount of blood (ie, streaks, drops, teaspoonful, cupful) is often difficult to determine precisely and is overestimated by the parents because of their anxiety. Hematochezia limited to the outside of the stools or spots of red blood coating the stools or found in the diaper, on the toilet tissue, or in the toilet bowl imply bleeding from an anal or rectal origin. Hematochezia mixed through the stool suggests a colonic source for the bleeding located higher than the rectum, whereas hematochezia mixed with mucus and loose stools suggests colitis. Maroon-colored stools are strongly suggestive of a vigorous hemorrhage arising from the distal small bowel. Currant jelly stools are potentially indicative of ischemic bowel lesions, such as those seen in cases of intussusception or midgut volvulus. In case of

TABLE 16.2-1 PRINCIPAL CAUSES OF LOWER GASTROINTESTINAL BLEEDING IN RELATION TO AGE

NEWBORN (BIRTH–1 MO)	INFANT (1 MO–2 YR)	PRESCHOOL AGE (2–5 YR)	SCHOOL AGE (> 5 YR)
Necrotizing enterocolitis	Anal fissure	Anal fissure	Anal fissure
Malrotation with volvulus	Infectious colitis	Infectious colitis	Infectious colitis
Allergic proctocolitis	Allergic proctocolitis	Polyp	Polyp
Hirschsprung disease enterocolitis	Intussusception	Meckel diverticulum	Henoch-Schönlein purpura
Hemorrhagic disease of the newborn	Meckel diverticulum	Henoch-Schönlein purpura	Inflammatory bowel disease
	Lymphonodular hyperplasia	Hemolytic uremic syndrome	
	Malrotation with volvulus	Lymphonodular hyperplasia	
	Hirschsprung disease enterocolitis		
	Intestinal duplication.		

melena, special attention should be given to the darkness of the stools: in general, the more proximal the hemorrhage is in the gastrointestinal tract, the darker the stool.

Symptoms Associated with LGI Bleeding

Selective questioning should also point to the presence of concomitant transit abnormalities (constipation or diarrhea) and association with abdominal and/or anorectal pain to help the physician narrow down possible causes of LGI bleeding. Table 16.2-2 shows the main associated gastrointestinal symptoms in relation to the underlying causes of LGI bleeding.

PHYSICAL EXAMINATION

Findings in the physical examination are very helpful in elucidating the cause of the LGI bleeding. Failure to examine properly the anus, perineal area, and rectum, as well as the skin and mucous membranes, will result in missing many obvious causes of LGI bleeding and performing unnecessary tests. Main physical findings in relation to the underlying diseases are shown in Table 16.2-3. Fever suggests the presence of an infectious disease or inflammatory disorder. Carefully assessing growth is necessary because failure to thrive may be suggestive of an underlying chronic disease such as Hirschsprung disease or inflammatory bowel disease.

DIAGNOSTIC INVESTIGATIONS

The main advantages and potential disadvantages of the various diagnostic investigations available for children with LGI hemorrhage are reviewed below. The specific diagnostic investigations allowing diagnosis of the main causes of LGI bleeding in children are listed in Table 16.2-4.

Laboratory Investigation

Laboratory tests must be individually tailored to the patient's history, associated symptoms, and physical signs.[2] Complete blood count, clotting studies, and routine chemistry are performed unless history taking and physical examination allow the cause of LGI bleeding in the patient to be determined without doubt. Hypereosinophilia is very suggestive of allergy or parasitic infection. The presence of iron deficiency anemia suggests a history of chronic blood loss. Determination of erythrocyte sedimentation rate and/or C-reactive protein is useful when infectious colitis or an inflammatory disorder is considered for the cause of LGI bleeding. Liver function tests are necessary when liver disease and portal hypertension are suspected to be responsible for LGI bleeding. Renal function should be assessed and urine analysis performed when an underlying renal disease is suspected. The determination of the ratio of blood urea nitrogen to creatinine has been proposed by some authors to help localize the origin and importance of gastrointestinal bleeding.[8] A high ratio of blood urea nitrogen to creatinine would be in favor of upper gastrointestinal bleeding. However, this measurement has not been confirmed on a routine basis as a valuable tool in the clinical setting.

In cases of bloody diarrhea, stool culture and stool examination for virus, ova and parasites, and *Clostridium difficile* toxin are necessary.

TABLE 16.2-2 PRINCIPAL ASSOCIATED GASTROINTESTINAL SYMPTOMS IN RELATION TO THE UNDERLYING CAUSE(S) OF LOWER GASTROINTESTINAL BLEEDING

AMOUNT OF BLOOD LOSS	APPEARANCE OF BLEEDING	CHARACTERISTICS OF STOOLS	PAIN	UNDERLYING DISEASE
Small	Red	Hard	Yes (anorectal)	Anal fissure
Small to moderate	Red	Loose	Variable (abdominal)	Allergic proctocolitis, infectious colitis, hemolytic uremic syndrome, IBD
Small to moderate	Red	Normal, coated with blood	No	Polyp
Moderate	Red to tarry	Normal	Yes (abdominal)	Henoch-Schönlein purpura
Moderate	Red to tarry, currant jelly	Normal	Yes (abdominal)	Intussusception
Moderate	Red to tarry	Loose	Yes (abdominal)	Hirschsprung disease enterocolitis
Large	Red to tarry	Normal	No	Meckel diverticulum, angiodysplasia

IBD = inflammatory bowel disease.

TABLE 16.2-3 PRINCIPAL PHYSICAL FINDINGS IN RELATION TO THE UNDERLYING CAUSE(S) OF LOWER GASTROINTESTINAL BLEEDING

LOCATION	PHYSICAL FINDING	UNDERLYING DISEASE
Abdomen	Hepatosplenomegaly, ascites, dilated venous channels on the abdomen, caput medusa	Portal hypertension
	Abdominal mass	Intussusception, IBD, intestinal duplication
Perineal area	Anal fissure	Constipation, Crohn disease
	Skin tag, fistula, abscess	Crohn disease, chronic granulomatous disease, immunodeficiency syndromes
	Hemorrhoids, rectal varicosities	Portal hypertension, constipation (adolescent)
	Rectal mass at digital rectal examination	Polyp
Skin and mucous membranes	Eczema	Food allergy
	Purpura	Henoch-Schönlein purpura, hemorrhagic disease, hemolytic uremic syndrome
	Jaundice, palmar erythema, spider angioma	Liver cirrhosis
	Digital clubbing	Liver cirrhosis, IBD
	Pyoderma gangrenosum	Ulcerative colitis
	Erythema nodosum	Crohn disease
	Telangiectasia	Hereditary hemorrhagic telangiectasia
	Soft tissue tumor (skull, mandible)	Gardner syndrome
	Café au lait spots	Turcot syndrome
	Pigmentation of the lips, buccal mucosa, face	Peutz-Jeghers syndrome
	Alopecia, onychodystrophy, hyperpigmentation	Cronkhite-Canada syndrome
	Breast hypertrophy	Cowden disease
	Bluish soft nodules	Blue rubber bleb nevus syndrome
	Soft tissue hypertrophy	Klippel-Trénaunay syndrome
Eye	Iritis	IBD
Joint	Arthritis	Henoch-Schönlein purpura, IBD
Growth	Failure to thrive	IBD, Hirschsprung disease
	Very short stature, webbed neck, widespread nipples	Turner syndrome

IBD = inflammatory bowel disease.

Radiographic Examination

If the patient's history and physical examination are suggestive of either an obstructive or ischemic underlying process, plain supine and upright films of the abdomen are urgently indicated to look for air-fluid levels, dilated loops of bowel, or pneumoperitoneum. Barium enema has absolutely no role in the initial evaluation of a patient with hematochezia or melena and will delay any further endoscopic or scintigraphic evaluation. Indication for barium enema is limited to the very few situations when other attempts have failed to localize any potential source of bleeding.

Ultrasonography

Ultrasound examination of the abdomen is very useful in the emergency setting, when an acute abdominal disorder with obstruction and/or ischemia is suspected, or when an abdominal mass is present. In cases of intussusception diagnosed by abdominal ultrasonography, most radiologists favor the use of air rather than barium enema to confirm the diagnosis and reduce intussusception.

Anoscopy

This is the first-step examination when the child presents with hematochezia suggestive of anal or rectal origin, as detailed above. It allows a prompt diagnosis of anal fissure or hemorrhoids. It should be emphasized that the presence of an anal lesion does not exclude a more proximal lesion that may be responsible for LGI bleeding.

Proctosigmoidoscopy

Proctosigmoidoscopy is the first procedure to consider in children with hematochezia suspected of rectosigmoid origin, as detailed above, because it can identify the source of bleeding with much more accuracy and specificity than barium enema.[9] Proctosigmoidoscopy is helpful for the detection of anal fissure, hemorrhoids, polyp, colitis, and inflammatory bowel disease.[10] Biopsy specimens can be harvested during the procedure.

Colonoscopy

Colonoscopy is indicated when proctosigmoidoscopy fails to find the cause for LGI bleeding, as well as in patients with melena after an upper tract lesion has been ruled out by a negative nasogastric aspirate and/or upper endoscopy and when examination of the terminal ileum is necessary or polypectomy has to be performed. Proper preparation of the colon is very important to allow satisfactory examination of the mucosa. Colonoscopy is unnecessary in children with an acute-onset bloody diarrhea, in whom infections should be ruled out with the appropriate stool specimens and cultures, and is contraindicated in children with suspected intestinal obstruction or ischemia.[2] Other contraindications for colonoscopy include fulminant colitis or toxic megacolon, suspicion of perforation or peritonitis, pneumatosis intestinalis, and suspicion of intussusception. Colonoscopy offers the opportunity to provide direct access to biopsies, polypectomy, and coagulation of bleeding lesions.

TABLE 16.2-4 DIAGNOSTIC INVESTIGATIONS TO BE PERFORMED FOR IDENTIFYING THE MAIN CAUSES OF LOWER GASTROINTESTINAL BLEEDING IN CHILDREN

DISEASE	DIAGNOSTIC INVESTIGATION(S)
Newborn period: birth to 1 mo	
Necrotizing enterocolitis	Physical examination, plain radiographs of the abdomen
Malrotation with midgut volvulus	Plain radiographs of the abdomen, ultrasonography, upper gastrointestinal series, barium enema
Allergic proctocolitis	Diet history taking, exclusion of the allergen(s) from the diet, skin prick tests, total IgE and RAST, proctosigmoidoscopy
Hirschsprung disease enterocolitis	Barium enema, rectal manometry, rectal biopsies
Hemorrhagic disease of the newborn	Clotting studies
Infancy: 1 mo to 2 yr	
Anal fissure	Physical examination, anoscopy
Infectious colitis	Stool culture, stool examination for virus, ova, and parasites
Intussusception	Ultrasonography
Meckel diverticulum	Radionuclide scanning, exploratory laparoscopy or laparotomy
Lymphonodular hyperplasia	Proctosigmoidoscopy, biopsy, barium enema
Intestinal duplication	Ultrasonography, CT, upper gastrointestinal series, barium enema
Preschool age (2–5 yr)	
Polyps	Proctosigmoidoscopy, colonoscopy
Henoch-Schönlein syndrome	Physical examination
Hemolytic uremic syndrome	Complete blood count (anemia, thrombopenia, schizocytes), renal function (renal insufficiency)
School age (> 5 yr)	
Inflammatory bowel disease	Ultrasonography, small bowel follow-through or CT, esogastroduodenoscopy, colonoscopy, biopsy
Vascular causes	
Hemorrhoids	Physical examination, anoscopy
Angiodysplasia	Colonoscopy, angiography
Dieulafoy lesion	Colonoscopy, angiography
Telangiectasias	Colonoscopy
Miscellaneous	
Diversion colitis	Proctosigmoidoscopy, biopsy
Jejuno- or ileocolic perianastomotic ulceration	Barium enema, proctosigmoidoscopy, biopsy
Neoplasia	Colonoscopy, biopsy
Solitary rectal ulcer syndrome	Proctosigmoidoscopy, biopsy

CT = computed tomography; Ig = immunoglobulin; RAST = radioallergosorbent test.

Enteroscopy

Advances in instrumentation have allowed endoscopic evaluation of part or all of the small intestine. Recent introduction of long enteroscopes allowed endoscopic investigation of the small bowel to localize sites of occult bleeding between the ligament of Treitz and the ileocecal valve. For now, only a few models are in limited use.

Capsule Endoscopy

Obscure gastrointestinal bleeding, either occult or overt, is certainly the most frequent indication for capsule endoscopy. Despite some drawbacks, capsule endoscopy is a big step forward in the diagnosis of obscure gastrointestinal bleeding of presumed small bowel origin.[11] In a study of adult patients with LGI bleeding and negative colonoscopy and gastroscopy, capsule endoscopy was superior to enteroscopy in the identification of bleeding abnormalities in the small intestine (68% vs 32%). Capsule endoscopy was safe and well tolerated.[12] The wireless capsule can make diagnoses beyond the reach of enteroscopes.

Radionuclide Scanning

Abdominal scintigraphy with technetium (Tc) 99m pertechnetate (Meckel scan), which rapidly binds to the gastric mucosa, has been very useful in making the diagnosis of heterotopic gastric mucosa contained in Meckel diverticulum or in intestinal duplication in children, with an 85 to 90% sensitivity. The Tc 99m pertechnetate red blood cell scan (bleeding scan) requires that a sample of the patient's own red cells be labeled with Tc 99m pertechnetate and reinjected into the bloodstream. The site of bleeding can be visualized provided that the bleeding rate is 0.5 mL/min or higher.[13]

Angiography

An ongoing bleeding rate of 0.5 mL/min or greater is also needed for angiography to identify the bleeding source accurately through the visualization of luminal extravasation. Angiography can identify a potential bleeding site in 50% of children.[14] Angiography also offers the benefit of local therapy infusion of vasopressin or selective arterial embolization in tertiary centers with the technical expertise to perform supraselective catheterization. Angiography may cause very serious complications such as arterial spasm, arterial thrombosis, contrast reactions, and acute renal failure. It should therefore be limited to the very few patients with active LGI bleeding and negative esogastroduodenoscopy and colonoscopy. The introduction of bleeding scans using pertechnetate has markedly decreased the role of angiography as a primary test in severe LGI bleeding.

Intraoperative Endoscopy: Laparoscopy

Intraoperative endoscopy was the only available method of total gastrointestinal tract examination.[15] The two main indications for intraoperative endoscopy were the localization of unknown sites of gastrointestinal bleeding and the search for small bowel hamartomatous polyps of the Peutz-Jeghers syndrome for polypectomy and/or segmental resection. Enterotomy should be avoided as often as possible to limit the incidence of wound infection complications. Laparoscopy has been proposed for the definitive diagnosis and treatment of gastrointestinal bleeding of obscure origin in children negative for gastroscopy, colonoscopy, and Tc 99m pertechnetate scan.[16]

The development of capsule endoscopy in the near future will more than likely reduce dramatically the indication for intraoperative endoscopy and laparoscopy.

SPECIFIC CAUSES OF LGI BLEEDING

Age is the first parameter to take into account to find the cause of LGI bleeding in the neonatal period, as well as in infancy, childhood, and adolescence. However, some causes of LGI bleeding can be encountered in at least two or even more different age groups. An algorithm for managing LGI bleeding in children is shown in Figure 16.2-1.

Newborn Period (Birth to 1 Month)

Necrotizing Enterocolitis. Necrotizing enterocolitis results from a loss of the protective mucosal barrier of the intestine damaged by ischemia, thus allowing bacteria of the intestinal microbiota to invade the bowel wall and possibly enter the bloodstream.[17] It is the first diagnosis to rule out in a neonate presenting with rectal bleeding (see Chapter 42, "Necrotizing Enterocolitis"). Necrotizing enterocolitis is associated with prematurity, low birth weight, asphyxia, and sepsis, all of which predispose the infant to intestinal ischemia. Affected infants usually have an average birth weight of 1,500 g and a mean age of 30 to 32 weeks. However, up to 10% of all cases of necrotizing enterocolitis occur in full-term infants.[18]

Symptoms include systemic instability with hypothermia, apnea, bradycardia, and lethargy, associated with

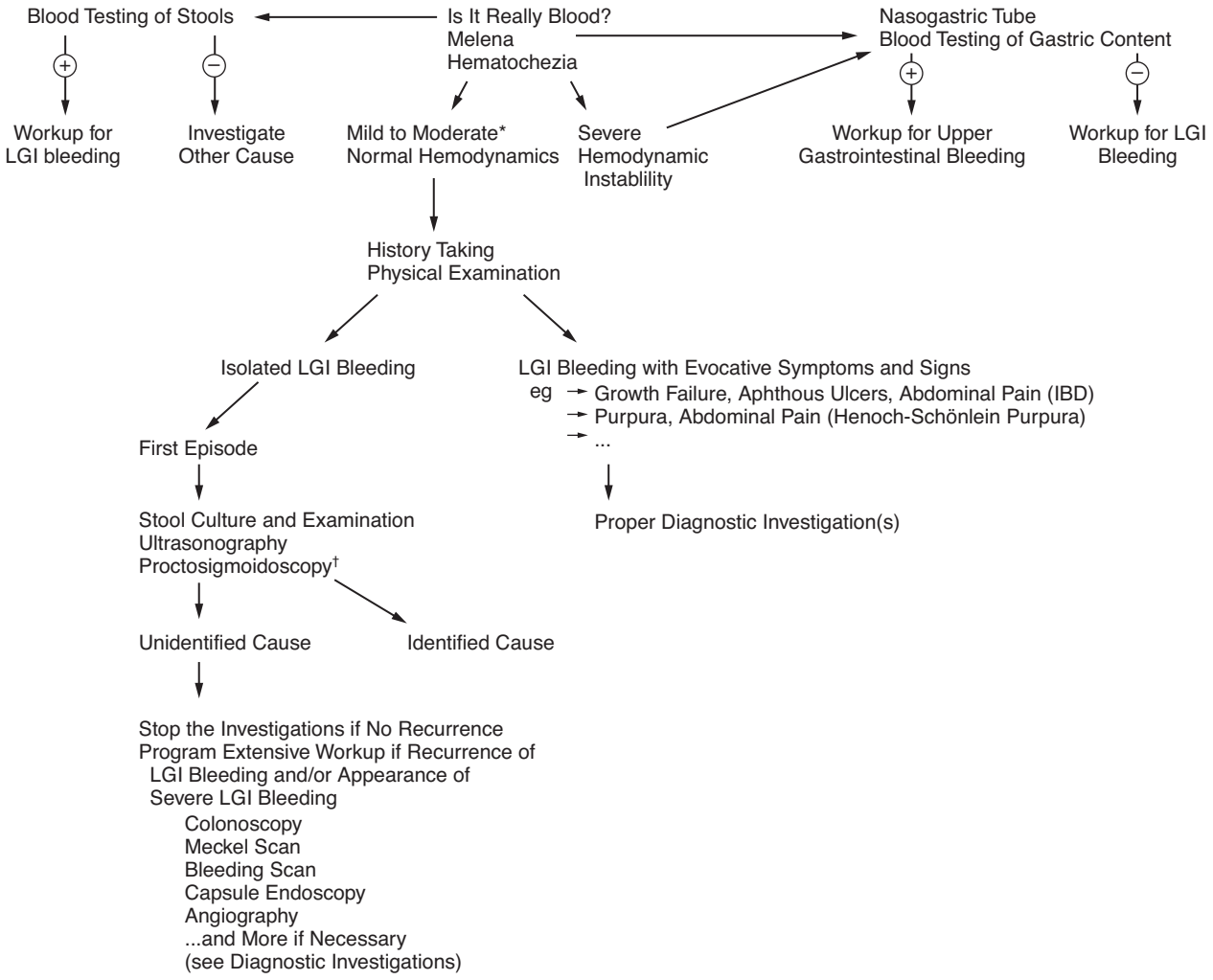

FIGURE 16.2-1 Algorithm for managing lower gastrointestinal (LGI) bleeding in children. IBD = inflammatory bowel disease. *In case of doubt, nasogastric intubation for blood testing of gastric content performed without any delay. †In the absence of symptoms and signs suggestive of obstruction or ischemia.

abdominal distention, feeding intolerance, increased gastric residuals, bilious vomiting, and bloody stools.[19] Physical examination may reveal decreased bowel sounds, abdominal tenderness, and erythema of the abdominal wall. Proctosigmoidoscopy or colonoscopy is absolutely contraindicated in this context to avoid any risk of subsequent iatrogenic perforation. Radiologic examination of the abdomen may show dilated bowel loops, bowel wall thickening, pneumatosis intestinalis (ie, gas in the bowel wall—the hallmark radiologic finding of the disease), or even pneumoperitoneum in cases of intestinal perforation. Medical and/or surgical management depends on the severity and/or course of the disease.

Malrotation with Volvulus. Malrotation with midgut volvulus is most commonly seen in the neonatal period, with an incidence of 1 case in 6,000 live births,[20] and constitutes a surgical emergency. Symptoms are suggestive of bowel obstruction and include bilious vomiting, pain, and abdominal distention. Melena can be associated and results from mucosal injury secondary to ischemia of the volvulized bowel.[1] Depending on the clinical situation, the child may undergo surgery directly. Otherwise, a definitive diagnosis can be made by a barium enema localizing the cecum either in the right upper or left upper abdominal quadrant. An upper gastrointestinal series can also show that the duodenum does not cross the midline and that the remainder of the small intestine lies to the right of the midline. Doppler ultrasonography is of great interest for the diagnosis of midgut volvulus by showing clockwise rotation of the superior mesenteric vein around the superior mesenteric artery ("whirlpool" sign).[21] Once the diagnosis is made, prompt surgical correction is performed to prevent necrosis of the small intestine.

Allergic Proctocolitis. Food-induced proctocolitis usually occurs in the first few weeks or months of life and is most often secondary to cow's milk or soy protein hypersensitivity. Infants usually have occult or gross blood in their stools with or without mucous stool or diarrhea.[22] Aside from occasional apparent pain on defecation and eczema in a few cases, infants with food-induced proctocolitis generally appear healthy and have normal weight gain. No single laboratory or biochemical test is either sensitive or specific enough to be diagnostic of allergic colitis (see Chapter 44, "Enteropathy"). A positive family history is helpful. Diagnosis of allergic colitis can be established by the response to elimination of cow's milk or soy protein through the use of an extensively hydrolyzed formula and standardized rechallenge.[22] When some doubt remains as to the presence of food allergy, proctosigmoidoscopy in conjunction with the evaluation of multiple mucosal biopsy specimens may be helpful for diagnosis. Focal erythema and hemorrhagic erosions are frequently seen at endoscopy. Biopsies show an infiltration of the mucosa and lamina propria with eosinophils. By 1 year of age, the infants usually tolerate an unrestricted diet, and the long-term prognosis is excellent.

Proctocolitis related to cow's milk protein allergy may also occur in exclusively breastfed infants because of sensitization to cow's milk proteins entering into the mother's milk. Evolution after exclusion from the maternal diet of the protein is usually simple.[23] Sensitization to other trophallergens via mother's milk (eg, egg, fish, peanuts) has also been described.

Hirschsprung Disease Enterocolitis. The typical pattern of presentation for Hirschsprung disease is severe constipation, abdominal distention, vomiting, and feeding intolerance in a young male infant, usually under the age of 3 to 6 months. However, 10 to 30% of patients with Hirschsprung disease present with LGI bleeding from enterocolitis, manifested as occult blood-positive or frankly bloody stools, associated with fever, abdominal distention, and sepsis (see Chapter 46, "Hypomotility Disorders").[24]

Hemorrhagic Disease of the Newborn. Hematochezia or melena may be a manifestation of a hemorrhagic disease of the newborn, starting usually between 2 and 5 to 7 days of life and is often associated with bleeding from other origin, that is, mucocutaneous or urinary. Prolonged prothrombin time can be observed in relation to vitamin K deficiency resulting from failure to administer vitamin K at birth, maternal treatment with phenobarbital or phenytoin, and untreated fat malabsorption (cystic fibrosis). Severe bacterial sepsis can lead to disseminated intravascular hemolysis and cause upper gastrointestinal and/or LGI bleeding. Patients with a specific clotting disorder (hemophilia A and B, von Willebrand disease, idiopathic thrombocytopenic purpura) are also prone to gastrointestinal bleeding. However, spontaneous bleeding from the LGI tract is unusual in this situation, and an underlying bleeding source is identified in most cases.[25]

Severe esogastroduodenitis and/or gastroduodenal ulcer can also be observed in patients with maternal and/or neonatal stress and can lead to hematochezia or melena. In case of doubt, in particular in a neonate presenting with pallor and a shock-like appearance, the placement of a nasogastric tube can easily point to bleeding from the upper gastrointestinal tract.

Infancy (1 Month to 2 Years)

Anal Fissure. Anal fissure is probably the most common cause of LGI bleeding in infants and young children, although statistics concerning the actual incidence of this condition are impossible to obtain because most cases go unreported. Streaks of bright red blood on the surface of otherwise formed stools or spots of red blood in the diaper or on the toilet tissue are very suggestive. Anal fissure results from a superficial tear of the squamous lining of the anal canal, which is usually caused by the passage of hard, large stools.[2] The fissure is often located in the midline (6 and 12 o'clock). In most cases, anal fissure is secondary to constipation (see Chapter 46.1, "Idiopathic Constipation").[26] Because defecation may be very painful, the child usually reacts by withholding stools, thereby increasing

constipation and creating a vicious cycle. Diagnosis is made on the patient's history and thorough inspection of the anal canal. Treatment usually consists of stool softeners associated with topical analgesic ointments.

Painless anal fissure is very suggestive of Crohn disease, especially when deep and eccentric to the midline, and is associated with perineal lesions (fistula, skin tags). However, Crohn disease is very rare in infancy and mainly concerns children over 8 to 10 years of age, as well as adolescents (see Chapter 41.1, "Crohn Disease"). It should be kept in mind that regardless of the age group, anal fissure may be the result of sexual abuse, especially in boys.[27] Careful questioning is necessary to exclude this possibility.[1] Other causes of perineal disease that may lead to bleeding include chronic granulomatous disease and immunodeficiency syndromes.

Infectious Colitis. Any enteroinvasive infection is capable of disrupting mucosal integrity, resulting in LGI hemorrhage (see Chapter 9, "Acute Diarrhea," and Chapter 38, "Infections"). Bacterial pathogens causing infectious enterocolitis are numerous and include *Salmonella*, *Shigella*, *Campylobacter jejuni*, *Yersinia enterocolitica*, and *Escherichia coli* (especially the O157:17 variant). Frankly bloody mucous diarrhea occurs most commonly, with or without abdominal pain and fever. Stool cultures and stool examination for ova and parasites are necessary not only in cases of bloody diarrhea but also when the child exhibits unexplained prolonged LGI bleeding without diarrhea. *Entamoeba histolytica* is the most important parasitic pathogen. When routine stool parasitic examinations are negative, proctosigmoidoscopy with tissue biopsy may be required to make a proper diagnosis. Amebiasis presenting as rectal bleeding without diarrhea has been described in childhood.[28] In the older child, *Y. enterocolitica* may mimic the presentation of inflammatory bowel disease. Pseudomembranous colitis owing to *C. difficile* infection is mainly observed during the use of antibiotics or up to 3 to 4 weeks after the treatment has ceased. It should be remembered that this condition may occur without known ingestion of antibiotics. Patients commonly have watery diarrhea with blood and mucus and may experience fever and abdominal pain. Acute hemorrhagic colitis owing to *Klebsiella oxytoca* has been described after the use of oral or parenteral ampicillin, amoxicillin, or cephalosporin.[29] *Aeromonas hydrophilia* has also been implicated in gastrointestinal bleeding.[30] About 25% of the patients infected with *A. hydrophilia* develop diarrhea with blood and mucus that can last up to 1 month. Although the presentation of the LGI bleeding is rarely associated with significant hemodynamic changes, bleeding may be massive in patients with underlying coagulopathy or immunocompromised status.

Rotavirus and Norwalk virus may cause mild bloody diarrhea, but they are generally not responsible for overt blood losses in the stool. Cytomegalovirus is a possible cause of significant LGI hemorrhage and is prominent in immunocompromised patients, especially those infected with human immunodeficiency virus (see Chapter 39, "Gastrointestinal Manifestations of Immunodeficiency").[31]

Intussusception. Intussusception usually occurs in patients 4 to 10 months of age, with 65% of cases occurring before 1 year and 80% by 2 years of age.[1] Intussusception is often idiopathic or associated with lymphoid hyperplasia of the terminal ileum. The majority of cases occur in the region of the ileocecal valve, and no lead point can be precisely identified. In older children, a lead point, including polyp, Meckel diverticulum, intestinal duplication, or neoplasm, especially lymphoma, is more likely to be found. Intussusception may also be a complication of Henoch-Schönlein purpura, cystic fibrosis, and Peutz-Jeghers syndrome.

A previously healthy, well-nourished infant typically presents with episodes of colicky abdominal pain and vomiting, followed by passage of "currant jelly stool," representing a mixture of blood, mucoid exudate, and stool. However, intussusception should be considered in the differential diagnosis of children passing any type of bloody stool.[32] Clinical examination may reveal a palpable sausage-shaped abdominal mass. The presence of LGI bleeding suggests that venous congestion with ischemia has already occurred in the affected area of the bowel. Such bowel compromise is usually seen only after 6 to 12 hours of symptoms of colicky abdominal pain and vomiting. The child may then pass a normal stool and show marked improvement. However, pain reappears shortly thereafter, lasts a couple of minutes, and recurs at regular intervals. Eventually, the infant will become pale and apathetic.

Diagnosis is confirmed by abdominal ultrasonography. The triad of vomiting, abdominal pain, and bloody stools is not consistently present, especially in infants below 4 months of age. The intussusception is ileocolic in location in 80 to 90% of cases, although ileoileal intussusception may also occur on an idiopathic basis. Hydrostatic or pneumatic enema techniques allow reduction of the intussusception in 80% of cases.[20]

Meckel Diverticulum. Meckel diverticulum is an anomalous remnant of the vitelline duct present in the terminal 100 cm of the ileum that results from incomplete obliteration of the omphalomesenteric duct. It is the most common congenital abnormality of the gastrointestinal tract, with an incidence ranging from 1 to 4% and a male-to-female ratio of 2:1.[33,34] Approximately 50% of the diverticula contain heterotopic tissue, with gastric mucosa being by far the most common type. A few diverticula contain pancreatic tissue. Most cases of Meckel diverticulum are fully asymptomatic and are found incidentally at the time of surgery or autopsy.[35] Among patients with complications, 60% are less than 2 years of age.[1]

The typical presentation of Meckel diverticulum in childhood is LGI bleeding resulting from the ulceration of adjacent ileal mucosa by acid-secreting heterotopic gastric mucosa contained in the diverticulum.[33] LGI bleeding is often brisk and painless and may present as self-limited recurrent bleeding in an otherwise healthy child or life-threatening acute massive lower hemorrhage. Meckel diverticulum represents the most common cause of significant LGI bleeding in infants and young children. Provided that gastric mucosa is present, the diagnosis of Meckel

diverticulum can be made with an 85 to 90% sensitivity by a radionuclide Tc 99m pertechnetate scan showing the presence of heterotopic gastric mucosa in the right lower quadrant of the abdomen.[36] It has recently been shown that in many patients, the inflamed Meckel diverticulum can be identified on abdominal ultrasonography or Doppler ultrasonography.[37] False-positive results of Tc 99m pertechnetate scintigraphy have been reported in patients with intussusception, hydronephrosis, arteriovenous malformation, and inflammatory bowel disease. If obtaining a technetium 99m pertechnetate scan or any other diagnostic procedure is going to incur a significant delay, with ongoing bleeding in the patient, exploratory laparotomy remains the only way to confirm a suspected Meckel diverticulum. Laparoscopy can be an alternative diagnostic and therapeutic modality of choice to exploratory laparotomy in patients suspected of Meckel diverticulum.[38]

Locations of heterotopic gastric mucosa other than Meckel diverticulum may be found anywhere in the gastrointestinal tract, from the tongue to the rectum, but are extremely rare in children, especially in the hindgut. However, bleeding from gastric heterotopia in the rectum has been described during infancy, as well as obstruction with recurrent intussusception responsible for episodes of hematochezia.[15,39] The treatment of choice is surgical excision.

Lymphonodular Hyperplasia. LNH of the colon is characterized by multiple yellowish nodules that are enlarged lymphoid follicles. The appearance of LNH is readily seen on either an upper gastrointestinal series or a barium enema, as well as endoscopically and microscopically on biopsy. LNH is a common intestinal phenomenon observed in children below the age of 10 years undergoing investigational studies of the intestinal tract. Two retrospective studies showed that LNH of the colon was present in 14% and 33% of children undergoing colonoscopy, respectively.[40,41] Thus, LNH is a common endoscopic bystander on the mucosa of the lower gastrointestinal tract.

The etiology of LNH remains unknown. Some studies attempting to correlate specific gastrointestinal symptoms with the presence of LNH have been inconclusive, prompting several authors to conclude that LNH is a normal finding in children.[42,43] LNH is nowadays thought to be an allergic response to parasites, yeasts, food antigens, or other unknown antigenic stimulants.[40] If detected on the colon, it seems more suggestive of gastrointestinal food allergy.[41,44] Food allergy should be investigated properly in this situation: a history of allergy in the patient and the patient's family, immunoglobulin (Ig)E levels, skinprick tests, and radioallergosorbent test. Regardless of their etiology, hyperplastic lymphoid nodules disrupt the normal mucosal architecture, which leads to mucosal thinning. Ulceration may occur over the follicles and lead to hematochezia.[45] LNH is therefore primarily associated in childhood with abdominal pain and hematochezia.[46] The only syndromic association appears to be IgA deficiency with giardiasis and chronic diarrhea. Lymphoma associated with LNH has been reported in adults, but in children, LNH is a nonmalignant process.[47] LNH probably represents a normal response of lymphoid tissue in children to a variety of stimulations, which accounts for its frequent identification. LNH resorbs slowly as the child enters adolescence, similarly to the resorption of adenoid and tonsillar tissues seen in the same period. Hence, LNH becomes a very unlikely source of LGI bleeding in older children, those over the age of 7 years.[46]

Intestinal Duplication. Intestinal duplication is most often found in the small bowel. Similar to Meckel diverticulum, duplication of the bowel often contains ectopic gastric mucosa, which may result in local peptic ulceration and LGI bleeding.[48,49] Bleeding may also be due to stasis and bacterial overgrowth causing subsequent local ulceration or to ischemic necrosis of the bowel secondary to intussusception or enlargement of the duplication.[50] Rectal bleeding and proctalgia have been reported in a child with a diverticular rectal duplication with heterotopic gastric mucosa.[48] However, clinical presentation of duplication of the bowel as an abdominal mass or intestinal obstruction is more common than LGI bleeding. Ultrasonography or computed tomography may suggest the diagnosis, but only laparotomy is definitive. Treatment is surgical resection of the affected segment, thus avoiding complications described with other digestive duplications: infection, ulceration, bleeding, or malignant changes during later life. A recent advance has been laparoscope-assisted resection of intestinal duplication cysts.[51]

Other main causes of LGI bleeding in infants from 1 month to 2 years of age are allergic proctocolitis, malrotation with volvulus, and Hirschsprung disease enterocolitis (see Table 16.2-1).

Preschool Age (2 to 5 Years)

Polyps. Polyps of the colon and rectum in children do not carry the serious implications of polyps in adults, with the exception of familial polyposis coli, in which there is a very high risk of malignant change before 20 years of age or even during late childhood or adolescence (see Chapter 45, "Intestinal Tumors"). Intestinal polyps represent the most frequent cause of significant LGI bleeding after 2 years of age, usually presenting with isolated, recurrent, and painless hematochezia, small in amount, and no hemodynamic change.

Juvenile polyps account for more than 95% of all polyps found in children. They are hamartomatous and have very low, if any, malignant potential.[52,53] However, it has recently been emphasized that careful histologic examination of juvenile polyps should be performed because of an increased incidence of potential malignant changes in children presenting with multiple polyps and polyps located in the ascending colon.[54] The vast majority of juvenile polyps are solitary (five polyps or less in the entire large bowel) and mainly occur in the left side of the colon, with a predominance in the rectosigmoid area. However, they may occur throughout the colon. If rectosigmoidoscopy fails to demonstrate the presence of polyps, colonoscopy should be performed thereafter. Recurrent or

multiple juvenile polyps can be seen in juvenile polyposis coli or generalized juvenile polyposis. Juvenile polyposis coli refers to multiple juvenile polyps found only in the colon, whereas in generalized polyposis coli, polyps are found throughout the gastrointestinal tract. Juvenile polyposis coli and generalized polyposis coli have been shown to be associated with adenomas, thus raising the question of possible malignant change.[54] Peutz-Jeghers syndrome is a condition inherited as autosomal dominant and characterized by hamartomatous gastrointestinal polyps and abnormal brown pigmentation of the lips, oral mucosa, and skin. Abdominal pain owing to mechanical blockage or intussusception is the most common symptom. Almost all patients have small intestinal polyps, but some also have polyps in the colon, which may cause hematochezia. Cowden disease and Cronkhite-Canada syndrome are extremely rare and are characterized by the presence of hamartomatous and/or inflammatory polyps.

Adenomatous polyps, the common type in adults, are much less frequent than juvenile polyps, but they represent a premalignant condition. They are found in familial polyposis coli, Gardner syndrome, and Turcot syndrome. Total colectomy is necessary before adulthood. LGI bleeding is rarely seen as a complication of colonoscopy following polypectomy (see Chapter 67.3, "Ileocolonoscopy and Enteroscopy").[55] Bleeding may occur immediately or may be delayed for more than 2 weeks after the procedure. A vast majority of postpolypectomy bleeding cases resolve spontaneously without requiring blood transfusion or further intervention.

Henoch-Schönlein Purpura. Henoch-Schönlein purpura primarily involves the skin, gastrointestinal tract, joints, and kidney. The peak age at onset ranges from 3 to 7 years. The typical clinical picture is characterized by an urticarial rash on the buttocks and lower extremities, followed by large joint arthralgia and papular purpuric lesions. Gastrointestinal manifestations occur in 45 to 75% of cases and include vomiting, colicky abdominal pain, melena, and/or bloody stools resulting from diffuse mucosal hemorrhage.[56] Intussusception associated with Henoch-Schönlein purpura can also cause melena or hematochezia. In up to 15% of patients, gastrointestinal bleeding and other gastrointestinal symptoms precede the appearance of skin lesions by as many as 7 to 10 days.[56] Urinalysis is necessary to check for blood and/or albumin. Forty percent of patients may have recurrence of any of the symptoms within 6 weeks of the initial onset of the disease. Recurrence is more common in older patients.

A variety of systemic vasculitides can precipitate gastrointestinal hemorrhage but are rarely observed in children: polyarteritis nodosa, Churg-Strauss syndrome, or systemic lupus erythematosus.[57–59] Vascular inflammation leads to mucosal ischemia and ulceration, resulting in abdominal pain and/or gastrointestinal bleeding.

Hemolytic Uremic Syndrome. Hemolytic uremic syndrome is characterized by microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure. Symptoms of acute hemorrhagic colitis with abdominal pain, vomiting, fever, and bloody diarrhea precede the illness in approximately 50% of cases. LGI bleeding results from small vessel occlusion and mucosal ischemia of the intestine. Infection with *E. coli* O157:H7, an enterohemorrhagic *E. coli* that produces verotoxin, is considered the most important causative event in both sporadic and epidemic cases of hemolytic uremic syndrome.[60] Intussusception and colonic perforation have rarely been described.[61] Systemic involvement may involve the liver and the pancreas (pancreatitis).[61] Gastrointestinal manifestations of hemolytic uremic syndrome resolve, usually without sequelae. Prognosis depends on the severity of renal involvement. The severity of the gastrointestinal prodrome reflects the severity of the extraintestinal acute microangiopathic process and the resulting renal long-term outcome.[62]

Other main causes of LGI bleeding in children from 2 to 5 years of age are anal fissure, infectious colitis, Meckel diverticulum, and LNH (see Table 16.2-1).

School Age (Above 5 Years)

Inflammatory Bowel Disease. Approximately 25% of all new cases of inflammatory bowel disease in the population occur in individuals younger than 20 years of age.[63] In this population, rectal bleeding and/or bloody diarrhea are present at diagnosis in 90 to 95% of patients with ulcerative colitis and 25% of patients with Crohn disease (see Chapter 41, "Inflammatory Bowel Disease").[64] Rectal bleeding as an isolated presenting feature is very unusual in Crohn disease as opposed to ulcerative colitis. Acute major LGI bleeding is uncommon in adult patients with inflammatory bowel disease.[65] To our knowledge, no pediatric data are available. Profuse bleeding leading to shock and requirement for blood transfusion seems to be very rare in children and adolescents with inflammatory bowel disease, apart from very severe attacks of ulcerative colitis.

Other main causes of LGI bleeding in children from 5 years of age to adolescence are anal fissure, infectious colitis, polyps, and Henoch-Schönlein purpura (see Table 16.2-1).

RARE CAUSES

Many rare causes of LGI bleeding have been reported in children. They can be arbitrarily classified as from vascular and miscellaneous origin (Table 16.2-5).

Vascular Abnormalities

Hemorrhoids: Colonic and Anorectal Varices. Hemorrhoids are very unusual in children. If present, portal hypertension should be suspected.[66] Varices most commonly occur in the esophagus and stomach in patients with portal hypertension, with less frequent involvement of the small intestine and rarely of the colon. However, a prospective study has shown that one-third of the children with portal hypertension had hemorrhoids or anorectal varices that were most often totally asymptomatic. The incidence of both hemorrhoids and anorectal varices was twofold

TABLE 16.2-5 RARE CAUSES OF LOWER GASTROINTESTINAL BLEEDING IN CHILDREN

VASCULAR ABNORMALITIES
Hemorrhoids
Colonic and rectal varices
Angiodysplasia
Dieulafoy lesion
Telangiectasia
Hereditary hemorrhagic telangiectasia
Turner syndrome
Other vascular lesions
Angioma
Hamartoma
Hemangioma
Hemangioendothelioma
Blue rubber bleb nevus syndrome
MISCELLANEOUS
Diversion colitis
Jejuno- or ileocolic perianastomotic ulceration
Neoplasia
Graft-versus-host disease
Solitary rectal ulcer syndrome
Traumatic rectal lesions

higher in case of extrahepatic than in intrahepatic disease.[67] Hemorrhoidal bleeding usually presents with bleeding on defecation. The hemorrhoidal bleeding may be significant, but in the absence of bleeding diathesis, it rarely causes hemodynamically compromising hemorrhage. The presence of rectal, perirectal, and colonic varices can be successfully assessed by endoscopic ultrasonography. Treatment is advised only for symptomatic patients, and injection sclerotherapy is satisfactory for the majority.[66]

Angiodysplasia. There is little experience of angiodysplasia of the colon in the pediatric population. Identification of vascular lesions can be made either endoscopically or angiographically. The endoscopic appearance of angiodysplasia (or vascular ectasias) has been described as flat or slightly raised lesions that range from 2 to 10 mm in diameter and are red in color. In children, intestinal perforation has also been reported.[68] The severity of bleeding can vary in the same patient at different times. Most bleeding episodes stop spontaneously. The probability of recurrent hemorrhage is unpredictable, and approximately 50% of patients will rebleed. A recent study clearly showed in children that colonoscopy found an apparently normal mucosa in half of the patients and a vascular anomaly in the other half, the nature and the extent of which could not be detailed. Mesenteric arteriography detected all cases of angiodysplasia.[69]

The diagnosis is usually not considered in children, which leads to a significant delay in diagnosis, as was true in a pediatric series of nine patients.[68] Unlike in the elderly and the cases reported in the literature, the left hemicolon was the most frequently involved area.[68]

A variety of modalities have been described to treat angiodysplasia colonoscopically, with the most widely accepted techniques involving some degree of thermal ablation, but surgical resection remains the first-line therapeutic modality.

Dieulafoy Lesion. Dieulafoy lesion is a very unusual cause of gastrointestinal hemorrhage in children.[70] Among Dieulafoy lesions, 80% are located in the stomach, with the majority of the remaining 20% located in the duodenum.[71,72] However, cases describing similar lesions located in the more distal small bowel and the colon have been reported. Pathologically, these lesions are characterized by a congenitally abnormal enlarged arteriole running within the submucosa. The diagnosis is most often made because of massive and recurrent bleeding.[73] The endoscopic diagnosis is difficult unless the procedure is performed during active bleeding. In adults, the descriptive reports of colonic and small bowel Dieulafoy lesions are mainly based on angiographic descriptions of a documented bleeding site. Therapy with injection techniques, coagulative therapy, and banding can all be efficient in stopping bleeding. If unsuccessful, embolization therapy or surgery should be considered.

Telangiectasias. In the autosomal dominant condition of hereditary hemorrhagic telangiectasia (Osler-Weber-Rendu disease), gastrointestinal hemorrhage is very unusual before typical skin and mucous lesions are noticeable. The most common presenting sign is epistaxis, which is reported in 78 to 96% of cases.[74] Approximately 80% of patients have a family history of bleeding. A few cases of telangiectasias of the colon have been described in patients with Turner syndrome.

Connective tissue disorders, especially Ehlers-Danlos syndrome type IV (ecchymotic) and pseudoxanthoma elasticum, are associated with intestinal bleeding as a consequence of fragile vascular epithelium.[75]

Other Vascular Lesions. A variety of vascular lesions may be found either in isolation or in association with systemic diseases. These lesions include angioma, hamartoma, hemangioma, hemangioendothelioma, and blue rubber bleb nevus.

Blue rubber bleb nevus syndrome, or Bean syndrome, is a rare systemic disorder characterized by cutaneous and gastrointestinal vascular malformations that lead from occult blood loss with severe anemia and iron deficiency to overt life-threatening gastrointestinal bleeding. Blue rubber bleb nevus syndrome belongs to the group of vascular venous malformations. It occurs sporadically most of the time but can be inherited as an autosomal dominant disease. Patients with blue rubber bleb nevus syndrome present with typical skin lesions, with some lesions having a rubber-like appearance.[76] Bluish, soft, compressible skin nodules, especially noticeable on the soles of the feet and the palms of the hands, are pathognomonic of the syndrome. In the absence of massive bleeding, a conservative treatment using endoscopic laser coagulation or bipolar electrocoagulation will be sufficient. Resections are otherwise necessary, but additional lesions may subsequently develop.[77]

A complex malformation known as the Klippel-Trénaunay syndrome is a capillary-lymphaticovenous malformation that results in limb hypertrophy and can extend into the pelvis and colon, resulting in hematochezia.

Miscellaneous

Diversion Colitis. Whatever the underlying reason, surgical isolation of colonic mucosa from the normal fecal stream may provoke inflammation and ulceration. Theories to explain diversion colitis include bacterial overgrowth, the use of antibiotics, the presence of intraluminal toxins, and, more importantly, diminished production of local short-chain fatty acid leading to impaired colonocyte metabolism. Reported symptoms include rectal bleeding, mucoid discharge, tenesmus, and abdominal pain.[78] Massive hemorrhage has also been reported. Endoscopic and histologic findings may be indistinguishable from inflammatory bowel disease.[79] Biopsies usually show nonspecific acute and chronic inflammation and/or nodular hyperplasia.[80] The presumed pathogenic mechanism of local short-chain fatty acid deficiency is supported by the resolution of symptoms and gross injury following administration of short-chain fatty acid enema.[78] However, other authors were unable to reproduce similar results.[81] Restoration of normal fecal flow results in complete resolution of diversion colitis.

Jejuno- or Ileocolic Perianastomotic Ulceration. This is a pediatric entity following ileocolic or jejunocolic anastomosis after intestinal resection in infancy and early childhood that may occur many years after surgery.[82] Gross or occult rectal bleeding, with or without abdominal pain and diarrhea, has been described. The cause of anastomotic ulceration remains unknown.[83] Bacterial overgrowth, blind loop formation, and ischemia have all been considered a possible mechanism, but there is no evidence confirming any of these hypotheses. Perianastomotic ulceration is likely to reflect a process of chronic inflammation and repair. Surgical resection has been necessary in most of the published cases.[82]

Neoplasia. Gastrointestinal tumors revealed by LGI bleeding are very uncommon in children. Carcinoma of the colon and rectum has been reported in patients of all pediatric age groups, and the youngest known living patient was 9 months old at the time of diagnosis.[84] Abdominal pain and vomiting were the main revealing symptoms, associated with frank rectal bleeding or melena. Familial adenomatous polyposis syndromes represent premalignant conditions and are obvious risk factors for the occurrence of adenocarcinoma of the colon and rectum, as well as ulcerative colitis after 10 years of the disease.

Leiomyoma of the colon is rarely found in the pediatric population and can be revealed by LGI bleeding.[85] Histologic differentiation from leiomyosarcoma may be difficult. Gastrointestinal stromal cell tumors are considered visceral sarcomas arising from the gastrointestinal tract, and there is evidence to suggest that they originate from the interstitial cells of Cajal. Cases of rectal and colonic stromal cell tumors have been reported.[86] A case of bloody diarrhea has been described in a single patient with colonic ulcerations related to fatal histiocytosis X.[87]

Graft-versus-Host Disease. Enteric graft-versus-host disease, in both its acute and less common chronic form, may rarely present in children with hemodynamically significant LGI hemorrhage. Bleeding may be exacerbated by an associated coagulopathy.

Solitary Rectal Ulcer Syndrome. Solitary rectal ulcer syndrome is a benign chronic ulcerative disease that is very unusual in childhood.[88] Symptoms include dyschezia, tenesmus, mucous discharge, pain located in the perineal area, rectal prolapse, and rectal bleeding. Most patients present with mild rectal bleeding, although major gastrointestinal blood loss requiring multiple transfusion has been reported.[89] A relationship between solitary rectal ulcer syndrome and chronic constipation is often reported. The postulated mechanism seems to be excessive straining efforts during which high intra-abdominal pressure forces the anterior rectal mucosa firmly into the contracting puborectalis muscle. The anterior rectal mucosa is frequently forced into the anal canal and, as a consequence, becomes strangulated, causing congestion, edema, and ulceration. Rectoscopy may show a unique superficial ulcer with an exudative base, which may vary in diameter from 5 mm to 5 cm. Histopathologic diagnosis of solitary rectal ulcer is based on the fibromuscular obliteration of the lamina propria stroma with misorientation of smooth muscle cells.[88]

Traumatic Rectal Lesions. Rectal prolapse per se, most often secondary to constipation, may cause rectal bleeding. A foreign body inserted into the rectum is a very rare cause of LGI bleeding in children. Sexual abuse should then be ruled out.

OCCULT LGI BLEEDING

Large amounts of blood can be lost into the gastrointestinal tract and remain occult. In most cases, occult LGI bleeding is revealed by symptoms limited to pallor or fatigue or failure to thrive. It is further detected by discovery of iron deficiency or iron deficiency anemia and is confirmed by repeated positive testing for the presence of fecal blood. A very careful history taking and thorough physical examination is of crucial importance to resolve adequately the differential diagnosis of the patient. In the absence of any clinical clue, that is, associated symptom and/or physical finding suggesting a precise cause for LGI bleeding, esogastroduodenoscopy and colonoscopy are performed. If endoscopy fails to identify a source of bleeding, abdominal scintigraphy with Tc 99m pertechnetate or Tc 99m pertechnetate red blood cell scan and angiography will not be helpful because in cases of occult LGI bleeding, the bleeding rate is very unlikely to be 0.5 mL/min or higher.

Ultrasonography and small bowel follow-through or computed tomography may help to find abnormalities suggestive of Crohn disease, especially in adolescents presenting with occult LGI bleeding and an elevated erythrocyte sedimentation rate or C-reactive protein. Preliminary data in adults strongly suggest that capsule endoscopy is a very promising tool for adequate diagnosis of obscure LGI bleeding of presumed small bowel origin.

LGI BLEEDING IN DEVELOPING COUNTRIES

The profile of recurrent LGI bleeding in children has been reported by several authors in developing countries, especially from India. Colonic polyps were the most common lesion (50%), followed by amebic colitis (25%), solitary rectal ulcer (10%), and polyposis syndrome (13%).[90] Sigmoidoscopy alone could establish the diagnosis in almost all cases. Juvenile polyps were by far (90–95%) the main cause of colonic polyps, most often located in the rectosigmoid region.[91] Rare cases of ulcerative colitis, tuberculous colitis, and allergic colitis have also been described.[92] In children of school age (mean age 7 years), colitis and colorectal polyps were reported to represent 42% and 41% of all cases of LGI bleeding, respectively, in an Indian tertiary university hospital.[93] The causes of colitis were mainly infectious (60%), pseudomembranous (15%), and allergic (11%). The authors concluded that the spectrum of LGI bleeding was similar to that of developed countries.

CONCLUSION

The causes of LGI are numerous and depend strongly on the age of the child. Anal fissure secondary to constipation, intussusception, Meckel diverticulum, juvenile polyps, and inflammatory bowel disease is most commonly found in the pediatric age. The pediatrician should always keep in mind the importance of adopting a rational approach for the differential diagnosis of LGI bleeding. It should be reemphasized that adequate history taking and detailed clinical examination are two essential steps before performing any diagnostic investigation.

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CHAPTER 17

GROWTH FAILURE

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Growth failure and malnutrition are inextricably linked. The relationship between the two can be illustrated in many ways, from cocausal to cyclic, and analyzed on many levels, from the molecular to the epidemiologic. Growth failure is the principal manifestation of malnutrition in childhood, and chronic diseases of the gastrointestinal tract and digestive system frequently lead to both malnutrition and impairment of growth.

Growth and nutritional status can be assessed in six principal ways:

1. Anthropometry: measurement of the dimensions of the body and/or its parts
2. Clinical examination: detection of abnormalities associated with specific nutrient deficiencies
3. Biochemistry: measurement of circulating or tissue levels of nutrients; their carriers, precursors, or metabolites; and markers of inflammation and growth
4. Body composition: eg, assessment of fat and fat-free mass, bone density
5. Dietary assessment: prospective and retrospective food intake measurements
6. Functional tests: eg, muscle strength; cardiac, visual, and nerve function.

Each assessment has a normal range and reference standards, but no single assessment provides a single index. The assessments need to be considered together to completely describe the growth and nutritional status of the child.

The aim of this chapter is to focus on the basic biology of growth, how to measure it, the factors that may disturb it, and the manifestations of growth failure. This is followed by a review of the regulation of growth during the main phases of early life: intrauterine, infancy, childhood, and puberty.

Growth failure and malnutrition are common in developing countries, and many seminal studies of the relationship between the two have been undertaken there. We have focused much of this chapter on data obtained from this part of the world. However, the general principles involved in understanding the factors that regulate and impair growth, particularly dietary factors, are equally applicable to children who suffer malnutrition from insufficient nutrients in the developing world as they are to those in the developed world who do not grow optimally because of gastrointestinal disease.

MEASUREMENT OF GROWTH

Measurements

Growth is an increase in the mass and dimensions of the body and comprises ponderal and linear components. To assess growth, we must be able to measure both components accurately. Standardization of techniques and calibration of equipment ensure accurate and reliable measurements.

Ponderal growth is assessed by measuring change in weight. Instruments include the balance beam scale and the electronic scale. Ideally, the child should be measured naked in the early morning. In general, ponderal growth measurements are more accurate than linear measurements because the techniques employed are easier, but whichever scales are used, they must be calibrated regularly. Other measures of ponderal growth that are commonly assessed include skinfold thickness and mid–upper arm circumference (MUAC). Skinfold thickness is generally measured in the triceps and subscapular areas. Calipers are held perpendicular to the skin, and the skinfold is pinched and elevated with the free hand. The caliper jaws are opened, closed over the skinfold, and released, and the measurement is recorded. MUAC and subscapular skinfold thickness are both assessed with the left arm hanging down and the elbow extended at a point midway between the olecranon and the acromion. MUAC is measured with a narrow, nonstretching tape applying only light pressure to the arm to avoid biting into the tissue. Skinfold thickness is most commonly used to assess subcutaneous fat as an index of total-body fat. Subcutaneous fat, however, varies between body sites[1] and can form a variable proportion of total-body fat. Thus, combinations of measures of two skinfold thicknesses from different sites or one skinfold thickness with arm circumference measurement (as "arm fat area") have been used to improve correlation with total-body fat,[2] but

this correlation varies between 0.4 and 0.9 and is particularly poor in preschool children. MUAC encompasses not only fat but also muscle and bone. It is a sensitive indicator of malnutrition, particularly when combined with height. It is a useful tool for community nutritional assessment of preschool children because it remains relatively constant between 2 and 5 years of age and is therefore age independent during that time.

Linear growth is more difficult to measure accurately and is assessed either as supine length or standing height. Supine length is best assessed in children less than 2 years using a baby board with supports for the head and feet. An assistant is required who holds the baby's head in firm contact with the headboard so that the line between the center of the ear hole and the lower border of the eye socket (Frankfort plane) is vertical. The measurer then straightens the child's legs by gripping the ankles and takes the reading. The standing height of children over 2 years is measured using a stadiometer. The child should be in his/her bare feet with heels together and buttocks and shoulder blades against the stadiometer, looking straight ahead, with a headboard resting at right angles against the highest point of the head. The measurer should ensure that the Frankfort plane is horizontal and apply gentle pressure to the mastoid process to extend the head, checking that the heels have not lifted off the baseboard.[3] Assessing height accurately in the 2- to 3-year age group is difficult, and to obtain accurate measurements, it is appropriate to measure supine length even in children up to 5 years old. The US National Center for Health Statistics (NCHS) reference data, however, are based on length to 2 years old and height above this age. Because length is 0.5 to 1.5 cm greater than height, it is recommended that if the former is measured over 2 years old and the NCHS reference is used, then 1.0 cm should be subtracted. Length or height should be reported to the nearest 0.1 cm, but it should be remembered that the measurement error is nearly 0.5 cm. The rate of change of height (velocity) has been promoted as a better expression of linear growth than height alone,[4] but an inherent lack of precision in estimating velocity may limit its reliability in assessing growth in short children.[5]

Assessing body proportions by measuring subischial leg length, as a derivative of sitting height, or knemometric length (distance between knee and heel) has been employed to measure long bone growth and its contribution to total linear growth. However, these measurements are more technically difficult than length or height assessment, and their relationship to total linear growth is not clear because different parts of the skeleton appear to grow at different rates and times.[6] Assessing body proportions is useful, however, when assessing linear growth in children who cannot stand.

Indices and Reference Populations

Growth is measured using a combination of height, weight, age, sex, and other anthropometric variables, including MUAC. Single anthropometric measurements, however, are uninterpretable, and indices (eg, weight for height) are combinations of measurements that allow growth data to be compared within and between groups (eg, a single weight is meaningless unless related to a child's height or age). In turn, growth indices must be related to a reference population for meaningful interpretation. The World Health Organization (WHO) has endorsed the use of that population defined by the NCHS as a reference.[7] However, the use of references based on a population of infants and children from one country to assess the growth of children in another country (eg, a developing country) has proved controversial. Differences in the genetic potential for growth are often quoted as a rationale for having country- or region-specific growth references. However, although genetic differences do exist, environmental factors have the larger effect on the potential for growth. Martorell, who looked at the heights of school-age children from different socioeconomic groups in different countries, demonstrated this (Figure 17-1).[8]

The WHO has acknowledged that reference data will be used as standards and recommends that care be taken to choose references that resemble standards.[7] It further acknowledges that because the mean heights of young children of many affluent populations differ little among ethnic groups, it should be possible to construct a standard that reflects the growth potential of all children throughout the world. The WHO chose the 1977 NCHS reference because the population on which it was based lived in a healthy environment, was well nourished, and had probably met its full growth potential. As a standard, its limitations must be recognized. The growth curves were originally constructed in 1975 from four sources. The 0- to 23-month data of recumbent lengths came from the Fels Research Institute Longitudinal Study of 1923 to 1975. The infants

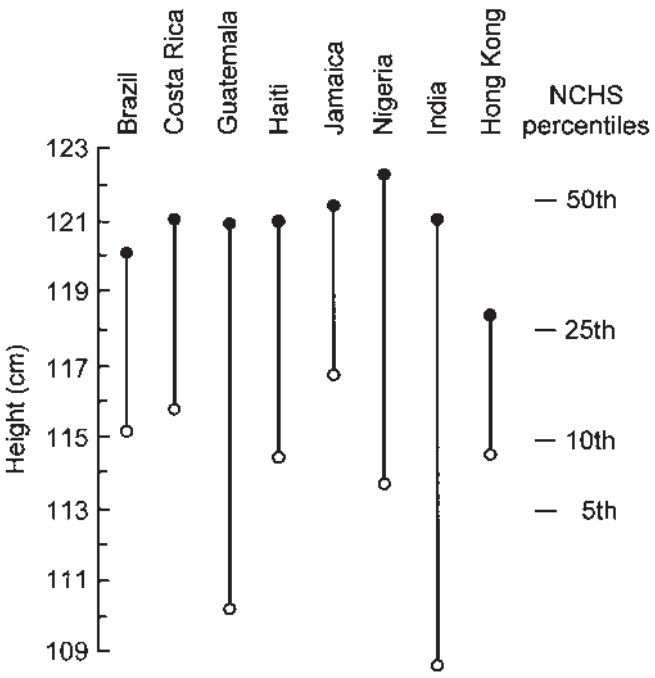

FIGURE 17-1 Mean heights of 7-year-old boys of high (●) and low (○) socioeconomic status in representative countries. Reproduced with permission from Martorell R.[8] NCHS = National Center for Health Statistics.

included in this data set were predominantly formula-fed and were from a relatively restricted genetic, socioeconomic, and geographic background. The 2- to 18-year-old data of standing heights came from three US surveys from 1960 to 1975. Across most populations, there is little difference in mean growth in height or in the distribution around the mean, but the inclusion of both healthy and sick, breast- and formula-fed infants in this reference should be remembered, particularly when comparing individuals or particular groups against the reference, for example, breastfed infants (see below). With these limitations in mind, NCHS data should perhaps be used as a tool to identify children at risk of malnutrition rather than as a standard to be attained or as a means to label children as malnourished.[9] An expert committee of the WHO has recommended the development of a new reference for infants and children, which will be a complex and costly undertaking.[10] The Centers for Disease Control and Prevention (CDC) recently published new childhood growth percentiles to replace the 1977 NCHS percentiles in the United States to provide a more up-to-date reference.[11] The new CDC percentiles may better represent current growth patterns but may still misdiagnose the normalcy of growth in young exclusively breastfed infants.[12]

Nutritional status can be assessed at specific time points by employing one or more anthropometric indices and comparing them against a reference. This can de done in one of three ways: as a deviation from the median of the reference expressed as standard deviation (SD) scores, as a percentile of the reference population, or as a percentage of the median reference value. For the analysis of data, SD or z-scores are recommended because they lend themselves to easy mathematical manipulation and statistical analysis.[9]

Growth, however, is expressed as rate of change in weight, height, and any other anthropometric variable (velocity) and can be assessed only by comparing indices over time against the reference population. In assessing individual children, it must be emphasized that plotting a child's weight and height over time allows assessment of the child's own growth curve in relation to a reference population. Indices examined at one time point without reference to earlier recordings make it impossible to determine whether the child is following steadily along a growth percentile, moving downward, or catching up.

Commonly used derived indices include weight for height, weight for age, and height for age. Deficits in different indices reflect different underlying processes and can indicate different causation. Weight can be lost and gained quickly in response to environmental insults, whereas height cannot be lost. "Wasting" and "stunting" are terms coined to reflect these different processes. Wasting is a deficit in weight for height and results either from a failure to gain weight or from weight loss. It can develop rapidly and be reversed rapidly and reflects a process occurring in the relatively recent past.

Body mass index (BMI), calculated as weight (kg)/height^2 (m), is commonly used as a "fatness" index in adults and has recently been recommended as an indicator of nutritional status in children after infancy.[7] The median values of BMI in children vary markedly throughout childhood, particularly in the first year of life and at puberty. BMI has been noted recently to be different in normal Chinese and Caucasian infants.[13] In the United Kingdom, boys attain 16% and 45% of their adult height and weight, respectively, between the age of 12 years and adulthood, resulting in a large change in median BMI from 16 to 21 years.[14] This additional weight is predominantly composed of muscle and bone rather than fat and is therefore relatively unaffected by the factors that determine fat accretion (food intake, disease). Children with a BMI z-score < –2 or > +2 are generally considered undernourished and overnourished (obese), respectively.

Stunting is a deficit in height for age and signifies slowing of skeletal growth. In general, it reflects a chronic process. The prevalence of nutritional deficits varies with age, and low weight for height often peaks in the second year of life, whereas low height for age starts earlier and decreases by 3 years. Interpretation of these indices must take into account age. Thus, a low height for age among 1 year olds reflects current health and nutrition, whereas among 6 year olds, it suggests a past problem but may also indicate concurrent stunting in the same population among younger children. Weight for age encompasses both weight for height and height for age. As an index of nutritional status, it has limitations; for example, a child with a low weight for age could be stunted and have a relatively normal weight for height.[15] In younger children, low weight for age may reflect the prevalence of low weight for height but in older age groups is more likely to be associated with a low height for age (Figure 17-2).

One of the major uses of derived growth indices is to predict subsequent health problems, especially morbidity, mortality, intellectual development, work capacity, reproductive performance, and risk of chronic disease. However, prediction does not necessarily indicate causation. There

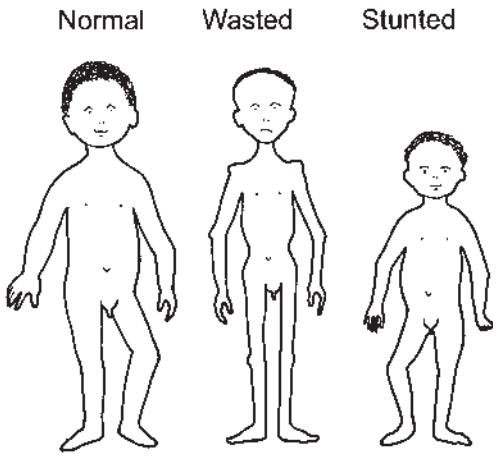

FIGURE 17-2 Comparison of a normal, a wasted, and a stunted child, all aged 1 year. Reproduced with permission from Waterlow JC.[15]

was a strong exponential association between weight for age and mortality rates in a meta-analysis of six longitudinal studies of children, but its capacity to predict death was low.[16] Its predictive value was highest in populations with high morbidity and mortality, indicating that malnutrition increases case-fatality rates rather than the incidence of disease. Weight for height is a better index of acute risk than weight for age and therefore of more value in identifying children who need nutritional treatment.[17] A cutoff of < 70% of the reference median is commonly used as an indicator for admission to hospital in developing countries to treat severe malnutrition.

UNICEF employs the terms "underweight," "wasted," and "stunted" to describe types of childhood malnutrition. It defines children as being moderate/severely underweight, wasted, or stunted as those > 2 SD below the reference (NCHS) median weight for age, weight for height, and height for age, respectively. Using these definitions, 28% and 32% of under 5 year olds in developing countries are currently estimated to be moderate/severely underweight or stunted. Almost half of all malnourished children live in South Asia, where the prevalence of moderate/severely underweight children is 48% (Figure 17-3).[18]

Combinations of indices can also give clues to causation. In areas of low prevalence of nutritional deprivation, where stunting occurs and weight for height is maintained, endocrine causes or skeletal dysplasias, as opposed to chronic disease, in which weight for height is often also low, are commonly responsible.

GROWTH: INFANCY, CHILDHOOD, AND PUBERTY MODEL

Linear growth is a complex process occurring in three distinct phases: infancy, childhood, and puberty.[19] The infancy phase is a continuation of the high fetal growth rate, with a rapid decline to 3 years of age. The onset of the childhood phase is heralded by an abrupt increase in linear growth rate and thereafter continues with a lower, more slowly decelerating velocity. A third distinct phase occurs at puberty before linear growth ceases and adulthood is reached. These three phases contribute differently to overall linear growth. Maximal rate of growth occurs during the infancy phase, but the slower but longer childhood phase is responsible for two-thirds of postnatal linear growth. The pubertal phase is associated with a second increase in growth velocity but is relatively short-lived and contributes least to the overall sum of linear growth.

The control of the infancy phase is poorly understood but is primarily a function of the intrauterine environment and postnatal nutrition. The onset of the childhood phase, normally in the second half of the first postnatal year, is influenced by the action of growth hormone (GH), which particularly regulates long bone growth in the legs. GH continues its influence throughout childhood and adolescence, but sex steroid secretion during puberty superimposes a further spurt in linear growth on the decelerating childhood phase. The etiology and reversibility of stunting are best considered with reference to the infancy, childhood, and puberty model (Figure 17-4). Changes in onset and duration of these phases and the effect of nutritional insults and interventions during them can best be understood within this context.

Ponderal Growth and Changes in Body Composition during Infancy and Childhood

Body composition can be assessed in a number of ways but is best viewed as a "two-component" model that consists of fat mass and fat-free (or lean body) mass. Human beings are among the fattest of mammals,[20] and the high level of fatness in infancy is particularly striking. The principal function of body fat is believed to be insulation, but high

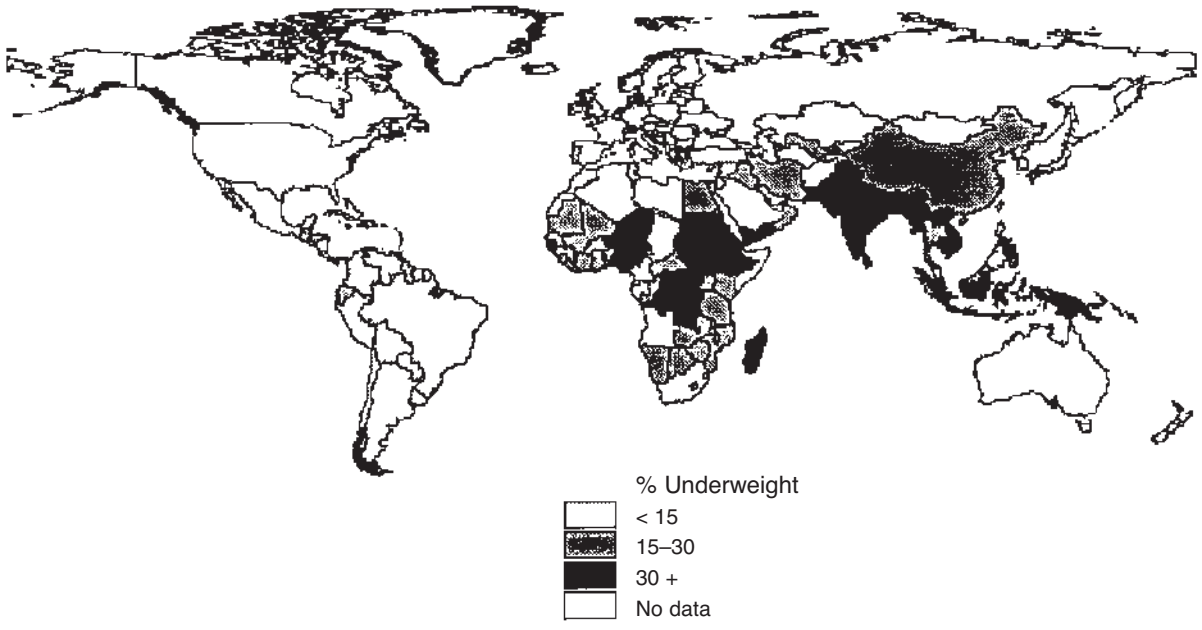

FIGURE 17-3 World map of underweight children. Reproduced with permission from UNICEF.[18]

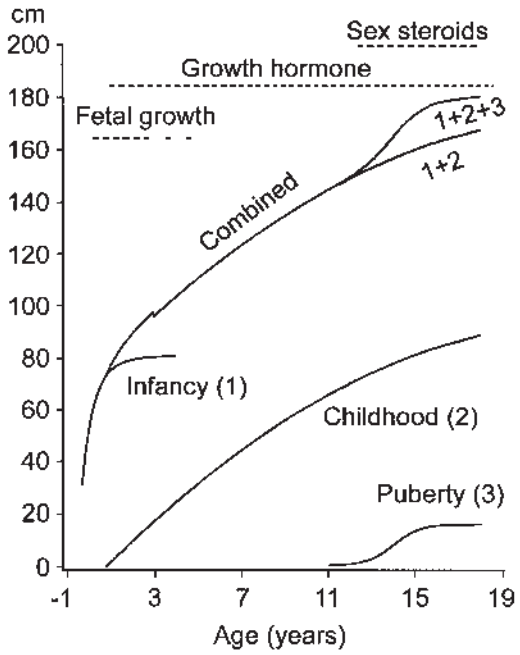

FIGURE 17-4 The infancy, childhood, and puberty model of growth. Reproduced with permission from Karlberg J.[19]

fatness in infancy probably evolved as an energy reserve to meet the relatively high energy demands of the large human brain and the risk of inadequate feeding, particularly around the time of weaning.[20] This underlines the importance of adequate ponderal growth for a variety of physiologic and biochemical functions and the adverse effects of inadequate ponderal growth (such as stunting). Epidemiologic studies in developing countries have consistently shown that inadequate growth and energy reserves (as indicated by weight or height) represent an important independent factor risk for morbidity and mortality[21] and contribute between 56 and 83% to infectious disease mortality in childhood.[16]

Typical changes in the body composition of children throughout early life can be summarized using the concept of the "reference child."[22] In the last trimester of gestation, appreciable quantities of lipid are deposited such that the reference child is born with around 14% of body weight as fat. Body fat continues to increase to about 25% of body weight at 6 months of age and then falls to a nadir of around 13% in boys and 16% in girls in late childhood. Intergender differences in body composition are present in childhood, with girls typically slightly fatter than boys. These gender differences become more pronounced during adolescence. Between the ages of 10 and 20 years, boys typically gain 33 kg of fat-free mass, but girls gain only 16 kg, and the difference between the sexes in body fat percentage therefore becomes more pronounced as they get older (Figure 17-5). Undernutrition is typically associated with depletion of both fat-free mass and fat mass. Overnutrition/obesity is associated with increased fat-free mass and increased fat mass.[22]

There is substantial variability in body composition at all ages, and secular trends in body composition have occurred. Modern children are considerably fatter than the reference child, but the general age-related changes noted above are still present.[23] These age-related changes in body fatness probably reflect changes in the need for an energy reserve of adipose tissue. Changes in body composition result from changes in the balance of lipogenesis and lipolysis. Regulation of this balance throughout childhood is complex and not fully understood but probably depends on the balance between the actions of insulin, GH, and insulin-like growth factor (IGF)-1 and IGF -2.[24]

Ponderal growth has a genetic component. Evidence for this includes the concordance between body size and composition of monozygotic twins, even when reared separately.[22] However, environmental influences are also important determinants of ponderal growth. Adiposity depends on energy balance, which is the difference between energy intake and total energy expenditure (made up of resting energy expenditure, diet-induced thermogenesis, and energy expended on physical activity). A positive energy balance promotes fat gain, but in developed countries, increases in energy intake may not be the principal cause of the secular trends toward increasing fatness that have been observed recently.[25] Rather, these have probably resulted from a reduction in energy expenditure secondary to reduced habitual physical activity in childhood, even in infancy. In developed countries, there is little evidence of a relationship between dietary composition and body composition, and the latter seems to vary largely independently of the former.[24]

However, inadequate food intake is the principal cause of inadequate ponderal growth in developing countries, and in undernourished patients, inadequate energy and protein intake make a major contribution to abnormalities in body composition and to inadequate ponderal growth—hence the increased risks of morbidity and mortality noted above.[21]

One dietary factor that seems increasingly important for ponderal growth in developing countries is parental feeding style (PFS). PFS shows substantial variation between and within developing countries, and this is strongly related to ponderal growth.[26] In particular, more "laissez-faire" PFS can predispose to inadequate food

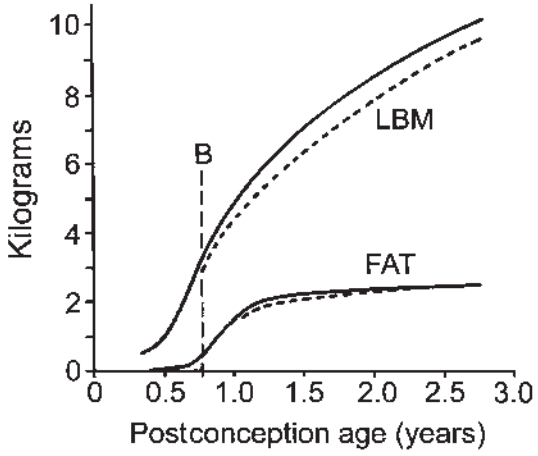

FIGURE 17-5 Average values for lean body mass (LBM) and fat in fetus and infant. Boys —; girls - - -. Reproduced with permission from Forbes GB. Human body composition. New York: Springer-Verlag; 1987.

intake and hence compromise ponderal growth. It is of note that interventions that promote more supportive and responsive feeding styles appear to have had some success in improving ponderal growth in developing countries. In developed countries, an overly controlling PFS might predispose children to obesity by impairing their innate capacity to regulate energy intake.[27]

Prenatal Influences

Intrauterine growth has a significant influence on postnatal growth and must be considered in the assessment of growth during infancy. Birth size is a reflection of gestational age, with those born prematurely, but with an appropriate weight for gestation, usually demonstrating normal postnatal growth. Infants with a low weight for gestational age are termed small for dates or intrauterine growth retarded (IUGR). The relative degree of retardation of linear and ponderal growth can suggest causation. Those infants with length affected less than weight usually have a good postnatal growth prognosis and reflect a short-term insult to intrauterine growth in late pregnancy (eg, placental insufficiency). Those with length and weight equally affected, however, reflect a more chronic process, and the prognosis for optimal postnatal growth is much poorer. Thus, chronic fetal undernutrition, chronic maternal illness or malnutrition, toxin ingestion (eg, alcohol, tobacco), or genetic abnormalities can lead to proportionally small babies.

GH secretion is high during fetal life but does not influence the linear growth of the fetus. GH-deficient children are only 1 to 2 cm shorter on average than normal infants at birth.[28] GH receptors are present in cartilage but may be immature. Whether this small discrepancy in linear growth is a result of this immaturity or a secondary metabolic action of fetal GH remains to be established. Anencephalic and athyroid fetuses do not demonstrate growth retardation, indicating that both pituitary GH and thyroid hormone are not vital determinants of intrauterine growth.[29] Placental factors (eg, lactogen and somatomedins) may well influence intrauterine growth and need further study. Placental size and function clearly influence birth weight.

The incidence of low birth weight (< 2,500 g) in 1995 was 15.3% or 21.3 million newborns worldwide, of which 20.4 million were born in developing countries.[30] IUGR, defined as birth weight below the 10th percentile of the birth weight for gestational age reference curve,[10] in developing countries alone represents 30 million newborn infants per year or 23.8% of births. IUGR is associated with increased mortality, and the strength of the association is greatest in the neonatal period but also extends postneonatally. Infants weighing 2,000 to 2,499 g were approximately 4 and 10 times more likely to die in the neonatal period and 2 and 4 times more likely to die in the postneonatal period than those born weighing 2,500 to 2,999 g and 3,000 to 3,499 g, respectively.[31] IUGR babies can demonstrate catch-up growth postnatally. Data from developed countries indicate that partial catch-up growth can occur by 2 years of age and thereafter maintain that achieved place in the growth distribution until adulthood. Achieved adult sizes are, on average, 5 cm shorter and 5 kg lighter than controls.[32] In developing countries, where the postnatal environment might be less favorable, the effect of IUGR is similar in absolute terms. The influence of BMI at birth for growth-retarded children on subsequent catch-up growth is unclear. However, birth length and predicted target height (a function of midparental heights indicating genetic potential) influence catch-up growth and explained half of the variation in catch-up growth in one study.[33] The effect of birth length predominated up to 2 years of age; thereafter, target height dominated up to 8 years. Pubertal catch-up growth in these children was small and was not influenced by fetal experience. Overall, the difference in final height of these children was primarily attributable to the difference in the magnitude of catch-up growth during the first 6 months of life, confirming that this is the critical period for catch-up growth.

Thrifty Phenotype (Barker) Hypothesis

IUGR is increasingly recognized as a major determinant of some chronic adult diseases, in addition to its effect on growth and early mortality. Recognition of the long-term adverse effects of the intrauterine and early infantile environment on later disease susceptibility has led to the formulation of the thrifty phenotype (Barker) hypothesis.[34–36] This proposes that impaired fetal and early infantile growth affects susceptibility to chronic adult degenerative disease.

Birth weight is a relatively easily obtained index of growth for use in epidemiologic studies, yet it is a crude measure of the quality of the intrauterine environment. Associations between low birth weight and later hypertension, ischemic heart disease, and non–insulin-dependent diabetes in adulthood have been reported from retrospective studies, mainly in developed countries.[37–39] Low weight at age 1 year has also been associated with an increase in the prevalence of non–insulin-dependent diabetes or impaired glucose tolerance in adult life.[37] Prospective studies are now in progress, and early results from developing countries suggest a link between low birth weight and the development of insulin resistance,[40] which could account for some of these reported findings. However, much remains to be clarified, particularly which aspects of the uterine or early infantile environment are related to later disease susceptibility[41] and which nutritional or metabolic mechanisms explain these associations.[42] Birth weight could be a proxy for other factors that are more difficult to measure directly. Other characteristics of size at birth (eg, degree and distribution of adiposity, birth length, and rate of postnatal growth) may be more important.

Animal models have generated further insights into these associations.[43,44] The offspring of rat dams fed low-protein diets during pregnancy demonstrated permanent changes in hepatic enzyme activity and insulin and glucagon sensitivity. Continuation of the protein restriction postnatally was associated with increased longevity compared to the offspring of dams that were solely protein restricted during pregnancy (ie, catch-up growth reduced longevity).

A recent epidemiologic follow-up study of Finnish men with detailed anthropometric records has shown that the highest risk of coronary artery disease mortality occurred

in boys born thin at birth but who demonstrated ponderal catch-up growth in childhood.[45] Ponderal growth can be generated with relative ease in the malnourished child. Linear growth is more difficult to induce and requires a more prolonged intervention, and few malnourished infants in developing countries reach their full linear growth potential. Improving a child's ponderal growth in an environment of high infant mortality owing to infectious disease will reduce that child's risk of morbidity and mortality owing to infectious disease and thus is desirable. It is difficult to assess the effect on subsequent mortality and morbidity of improving a child's ponderal growth in an environment of already low infant mortality. What is clear is that further thought and study are needed into the long-term effects of nutritional interventions in infancy in growth-retarded children.

Prevention of IUGR has proved difficult, and a systematic review of 126 randomized controlled trials evaluating 36 interventions demonstrated that most did not have any effect. Cessation of smoking, balanced protein/energy supplementation, and antimalarial chemoprophylaxis were found most likely to be beneficial.[46] Near-normal fetal growth rates can occur in even severe maternal malnutrition, indicating that factors regulating placental and fetal growth are robust and resistant up to 36 weeks gestation. This is reflected in the similarity of mean birth weights at 36 weeks across populations. At 34 to 36 weeks of gestation, fetal growth slows owing to space constraints within the uterus; thereafter, maternal malnutrition can have a marked effect on birth weight.

Dietary supplementation of mothers during pregnancy might therefore be expected to reverse IUGR. A meta-analysis of controlled clinical trials, however, demonstrated only modest increases in maternal weight gain and fetal growth.[46] Targeting of specific populations of women has proved more encouraging. Rural Gambian women have low weight gain in pregnancy, lose body fat, and have a high ratio of fetal weight to total weight gain in pregnancy, particularly during the wet season. High-energy groundnut biscuit supplementation (4.3 MJ/d) for 82 days significantly increased weight gain during pregnancy (by 580 g) and birth weight (by 136 g) and significantly reduced odds ratios for stillbirths and all deaths up to 28 days postpartum. This was achieved through a reduction in the number of babies who were small for their dates rather than born preterm.[47]

Maternal height and prepregnancy weight are also important determinants of birth size,[46] but their effects could, in large part, be due to an intergenerational effect of maternal birth size. Recent data from Guatemala demonstrated that maternal birth size was a significant predictor of child's birth size; for every 100 g increase in maternal birth weight, there was a 29 g increase in the child's birth weight, and for every 1 cm increase in mother's birth length, the child's birth length increased by 0.2 cm.[48]

Infancy Phase of the Infancy, Childbirth, and Puberty Model

Infant birth size is linked to that of the mother rather than that of the father, whose influence on growth becomes more apparent with time. If there is a large disparity between the size of the father and that of the mother, then the likelihood increases that the baby's growth will cross growth reference percentiles either up or down. Approximately two-thirds of normal children cross percentiles in the first 18 months of life. By the age of 2 years, growth "converges to the mean," genetic potential predominates as its principal determinant, and the percentile trajectory leading to midparental height is achieved.

The infancy phase of growth represents a continuation of the fetal phase with a rapid deceleration of growth until 3 years of age, when the childhood phase of growth predominates. Growth during infancy may continue to be influenced by the same factors that determined growth in utero, with nutrition being a preeminent factor. Human milk has long been recognized as the optimal food for babies, and complementary feeding is generally recommended as starting at around 6 months.[7] Longitudinal studies of infant growth in developing countries indicate that stunting occurs between the ages of 4 months and 2 years (see below), coinciding with the transition from breastfeeding to complementary foods. The energy density of weaning foods has therefore been proposed as a factor in the etiology of stunting.

The timing of this transition has recently been questioned[49] and is of considerable importance, particularly in developing countries. The increased risk of disease associated with the introduction of microbially contaminated solid foods must be balanced against the risk of malnutrition from prolonged exclusive breastfeeding—"the weanling's dilemma."[50] For a sound recommendation as to the optimal timing of complementary feeding, an understanding of the growth of breastfed babies is required.

The common observation of apparent growth faltering of breastfed babies beginning at 3 to 4 months is based on the use of reference growth curves that were constructed from data from predominantly formula-fed infants. Breastfed babies have a differently shaped growth curve and gain less weight in the second half of the first year of life than formula-fed babies do. Gaining less weight, however, does not necessarily imply that breastfed infants are not meeting their energy or nutrient requirements. Studies confirm that breastfed infants have significantly lower energy intakes than formula-fed infants do,[51] but this is not due to inadequate maternal milk production. Breastfed babies regulate their own milk intake[52] and may not consume all of the milk in the breast during a feed. Despite lower energy intakes and weight gain, babies exclusively breastfed to 4 to 6 months of age have similar motor development and lower rates of infection than those who are formula-fed.[53] Thus, NCHS growth curves may reflect the "overfeeding" of formula-fed babies rather than the "underfeeding" of those exclusively breastfed, and the growth of breastfed babies in developing countries may be more appropriately compared with that of breastfed babies of affluent populations.

The weight gain of breastfed babies in developing countries is similar to that of breastfed babies from more affluent populations[54] to 6 months of age, although attained weight differs owing to differences in birth weights. Ponderal

growth faltering thereafter occurs at 6 months in those predominantly but not exclusively breastfed and at 9 months in those exclusively breastfed to 6 months.[55] Linear growth, however, is poorer, and breastfed babies from developing countries are generally shorter than those from developed countries. Infant linear growth is not exclusively a function of nutrition, and when maternal height was controlled for, the difference in length between Honduran and American breastfed babies disappeared.[56]

It seems, therefore, that the growth rate of breastfed infants in developing countries is similar to that of breastfed infants of more affluent populations. Introduction of complementary feeds before 6 months did not influence infant growth in a pooled sample of 453 breastfed babies from six industrialized countries.[57] Even if growth faltering occurs, complementary feeding may not improve growth. The introduction of hygienic precooked complementary food at 4 months of age did not improve growth before 6 months of age in a study of breastfed babies of low-income primiparous mothers in Honduras.[58] The provision of a zinc-fortified complementary food between 4 and 12 months of age to infants of urban slum dwellers in India produced only a modest increase in weight gain, between 6 and 9 months only, and no significant effect on length gain. The prevalence of dysentery and fever was increased in the supplemented group, and breastfeeding decreased.[59] The study, of course, was not designed to examine for these outcomes if complementary foods were delayed until 6 months of age; thus, one cannot conclude from this study that exclusive breastfeeding for 2 more months (beyond 4 months of age) would have any benefit.

The timing of the introduction of complementary feeds, however, cannot be made on consideration of dietary intakes and growth alone. Infant morbidity, mortality, and development, as well as maternal considerations (eg, nutritional impact and length of lactational amenorrhea), must all be included. It may well be that the optimal age of transition varies between populations. In affluent populations, the benefit-to-risk ratio for complementary feeding at a particular age will differ from that of a population in a developed country owing to the lower risk of contaminated complementary feeds. The report of an expert consultation for the WHO recently pointed to the paucity of good data to resolve this issue. They concluded that exclusive breastfeeding to 6 months confers benefit to the infant and to the mother but can lead to iron deficiency anemia; insufficient data precluded the assessment of other potential risks of exclusive breastfeeding such as growth faltering, particularly in populations with severe maternal malnutrition and IUGR. They made a population-based recommendation of exclusive breastfeeding for 6 months, with introduction of complementary foods and continued breastfeeding thereafter.[60]

Childhood Phase

Linear growth velocity rapidly decelerates during the first year of life and is a product of the declining influence of the infancy phase and the onset of the childhood phase of growth. That contribution owing to the infancy phase ceases by 3 years of age. This deceleration is not constant, and the onset of the childhood phase between 6 and 12 months of age is defined by an abrupt and temporary increase before continuing to decelerate. The onset of the childhood phase represents that time when GH begins to influence linear growth (Figure 17-6).[61] In children with isolated GH deficiency, this abrupt onset is lost.[28] Long bone growth is particularly dependent on GH and makes up the majority of growth in the childhood phase compared with the infancy phase, when truncal growth accounts for the majority of linear growth.[61]

The trigger for the onset of the childhood phase is not understood. In Swedish children, it is independent of social class, age at cessation of breastfeeding, and midparental height, but it is influenced by growth rate immediately prior to onset.[62] The age at onset of the childhood phase will influence attained height subsequently; growth between 6 months and 3 years of age is negatively related to the age at onset.[61] Later onset at the childhood phase is common in populations of children with disturbed growth patterns (eg, malnourished children from developing countries or children with a chronic disease such as celiac disease).[63] This delay in the onset of the childhood phase causes growth faltering and has been proposed as a determining factor in attainment of final height, particularly in developing countries.[64] The reasons behind this delay in onset remain to be identified, but because the incidence of faltering clearly reflects socioeconomic conditions, it seems that environmental factors are more important than genetic factors. This view is supported by the observation that the growth rates of affluent members of society in these same

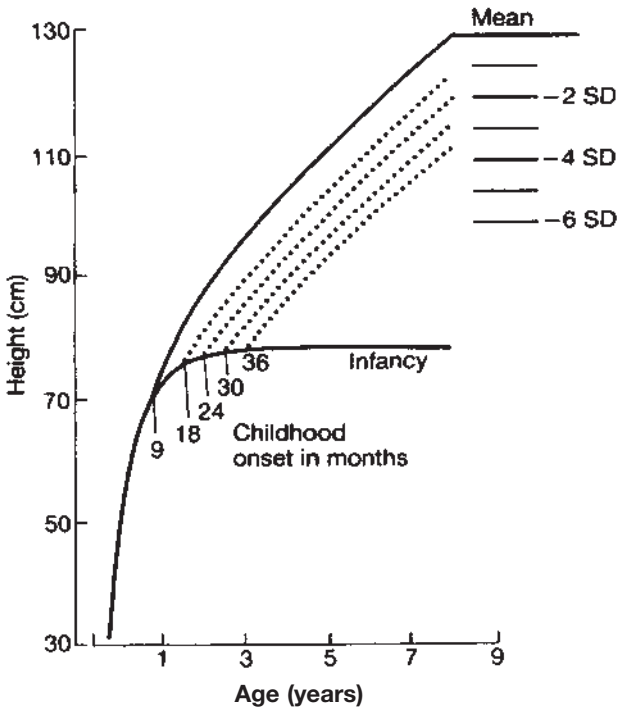

FIGURE 17-6 The effect of late onset of the childhood phase of growth on subsequent linear growth assuming normal action of the infancy component. Normal action of the childhood phase (–) and delayed onset as specified (····). Reproduced with permission from Karlberg J.[61]

developing countries do not falter or show delayed onset of the childhood phase. This phase, especially the first 6 months, is the critical phase for catch-up growth.[33]

Seasonality is an influence on weight gain from the first year of life, reflecting changes in food availability. In the second year of life, seasonality, for the first time, affects linear growth, which fluctuates more in those populations with a delayed onset of the childhood component.[62] As the effect of the infancy phase disappears in the third year, growth trajectory becomes more stable until puberty. This slower but longer phase is responsible for two-thirds of postnatal growth.

Puberty Phase

Puberty is the final phase of growth and development. It is marked by a period of accelerated linear growth accompanied by sexual maturation, as first described by Tanner.[65] At the end of this phase, physical sexual maturity is achieved, and adult stature and body proportions are attained.

The onset of puberty is marked by an increase in the frequency of pulsatile gonadotropin-releasing hormone (Gn-RH) secretion by the hypothalamus. The neuroendocrine control of this event is unclear but may be related to reduced sensitivity of the hypothalamic "pulse generator" to an inhibitory autofeedback mechanism.[66] Gn-RH induces release of the gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary gland, also in a pulsatile fashion. During the early stages of puberty, increased pulsatile LH secretion occurs at night only. The adult pattern of intermittent release of pulses every 60 to 120 minutes is established later in puberty. The gonadotropins stimulate the gonads to produce the sex steroids. In the male, LH acts on the Leydig cells of the testis to stimulate testosterone secretion, whereas FSH acts on the seminiferous tubules and Sertoli cells. This results in spermatogenesis and maintenance of the Sertoli cells. In the female, LH and FSH act in concert to develop the ovarian follicles, resulting in estrogen secretion and ovulation.[67]

The adolescent growth spurt is a consequence of the combined action of the sex steroids and GH. GH is secreted by the pituitary somatotrophs in a pulsatile fashion, under the regulation of growth hormone–releasing hormone (GH-RH) and somatostatin. It is postulated that GH-RH controls the amplitude of the GH peak, whereas somatostatin controls its frequency and duration.[68] During puberty, in both sexes, there is a strong positive correlation between levels of gonadal steroids and levels of GH and IGF-1, suggesting that the sex steroids have a regulatory role in growth during this period. In boys, peak height velocity occurs relatively late in puberty, when testosterone levels have risen to approximately adult levels. IGF-1 levels are also maximal at this time. In girls, increased GH secretion occurs earlier in puberty, with maximum concentrations correlating with peak height velocity. Enhanced GH concentrations during puberty in both sexes reflect increased pulse amplitude rather than an increase in the frequency of pulses. The mechanism by which the sex steroids mediate GH secretion is unclear but may involve alteration of the secretory dynamics of GH-RH and somatostatin and/or the responsiveness of the pituitary somatotrophs.

In girls, the first physical sign of puberty is breast budding (Tanner stage B2). The adolescent growth spurt starts around the same time, and peak height velocity is a relatively early event within the pubertal phase (usually occurring around stage B2–3). By the time secondary sexual development is complete, growth rate has slowed considerably.[69] Menarche typically occurs toward the end of the growth spurt. Age at menarche is negatively correlated with height increase postmenarche. In boys, the first physical sign of puberty is testicular enlargement (Tanner stage G2). Onset of puberty in boys occurs less than 1 year after girls. However, the adolescent growth spurt starts much later, at around Tanner stage G3 (when penile growth begins), with peak height velocity occurring at stage G4, with considerable growth potential left after secondary sexual development is complete.[65]

Sexual dimorphism in height is attributable to three factors: boys are slightly taller than girls during the childhood phase of growth; the adolescent growth spurt begins 2 years earlier in girls; hence, boys have an additional 2 years of childhood growth; and the adolescent growth spurt is more intense in boys (mean peak height velocity 10 cm/yr at age 14 years) than in girls (mean peak height velocity 8 cm/yr at age 12 years).[70] Thus, the male adolescent growth spurt contributes more to adult stature than does that of the female.

Extensive changes in body morphology occur during the adolescent growth phase. Leg length increases first, but overall growth is due more to an increase in trunk length, so that the ratio of trunk to leg length increases during puberty.[65] Before puberty, boys and girls have similar body fat distribution. During puberty, fat deposition is less in boys but more truncal in distribution, and their increase in weight during puberty is largely due to an increase in lean body mass.[71]

Within developed countries, there is a secular trend toward earlier maturity and greater adult stature, reflecting favorable socioeconomic conditions. A similar trend is seen within developing countries, where children are stratified according to socioeconomic status (ie, children from privileged groups tend to be taller and mature earlier than their less privileged peers). A similar trend has also been observed in children of immigrants to the United States.[72]

Intercountry adoption of children from developing to industrialized countries has provided valuable data on environmental influences on growth and maturation. Linear growth improves, but final height is compromised by earlier stunting and/or low birth weight, indicating the importance of the fetal and infantile growth phases. Catch-up growth is often cut short by early pubertal onset, particularly in girls, resulting in short final height, despite a normal pubertal growth spurt.[73] Thus, whereas the potential for pubertal growth is unaffected by early malnutrition, the realization of full genetic potential may well be. Malnourished male rats given access to unrestricted feeding had accelerated hypothalamic and testicular maturation. This effect was seen only in those refed before weaning,

perhaps indicating a critical period of hypothalamic sensitivity to changes in nutrition.[74]

In the United Kingdom, the construction of new growth charts in 1990 using cross-sectional data from a variety of sources enabled comparison with current growth standards dating from 1966.[75] Both boys and girls, at all ages, were taller and heavier in 1990. They were also heavier, although the weight differences were more marked in girls. In boys, peak height velocity was reached 6 months earlier at 13.5 years.

Obesity in prepubertal children is associated with increased growth velocity, advanced skeletal maturity, and early onset of puberty. However, obese children rarely become tall adults because obesity affects the timing rather than the magnitude of growth. Obese boys are taller than their thin peers before and during the early stages of puberty.[71] However, these height differences are lost as puberty progresses, and final heights are similar. In the Amsterdam Growth and Health Study, adolescents of both sexes who progressed rapidly through puberty were found to be more obese (BMI and skinfold thickness) than their more slowly maturing peers.[76]

Whereas obesity accelerates linear growth and advances sexual maturity, undernutrition slows growth and delays puberty. Menarche is not dependent on attained body weight in a well-nourished population.[77] However, anorexia nervosa of postpubertal onset is associated with secondary amenorrhea and in prepubertal onset with growth retardation and delayed puberty.[78] Abnormal and highly variable levels of GH, gonadotropins, and sex steroids were found in 19 cases, of which 17 had delayed puberty. Endocrine function failed to normalize after weight gain.[79]

Stunting

Stunting results from growth failure in childhood, which is commonly nutritional in origin. UNICEF estimates that 40% of the world population under age 5 years (226 million) is moderately or severely stunted. As a marker of deprivation, stunting also predicts other functional consequences of severe nutritional insults early in life. Cognitive deficits, decreased work ability, increased morbidity, and increased obstetric risks have all been associated with stunting. Stunted rural Guatemalan children had lower literacy scores, had completed fewer years at school, and scored less well in tests of intellectual function than their peers who had grown normally.[80] Adult height predicted the work capacity of Colombian sugar cane cutters[81]; shorter women with smaller pelvic sizes are at a greater risk of obstetric complications.[82,83]

Stunting is a chronic process, and it may take many months of suboptimal growth before it occurs. The degree of stunting is a product of the severity, timing, and duration of the nutritional insult. If a normal 12-month-old child stops growing completely, then he will take 6 months to fall below the –2 z-score for height for age (ie, to become stunted), whereas a 36-month-old child will take 13 months to do the same (Figure 17-7). Equally, that same 12-month-old child will take 42 months to become stunted if he reduces his growth rate to 70% of normal as opposed to stopping growing completely.[84] Stunting results from a chronic insult, and, equally, catch-up growth will have to be prolonged to reverse it; the older and the more stunted a child, then the longer that he will have to grow at an accelerated rate before full catch-up growth is achieved. In the environment in which the vast majority of stunted children reside, this is usually impossible.

In a study from Pakistan, between 75 and 83% of children were stunted by 24 months of age.[85] The stunting process (defined as height-for-age z-scores) started at 6 months of age and continued to 18 months of age, whereas weight-for-length z-scores increased from a baseline of –1 to 0 at 24 months (Figure 17-8). Other studies have found that length attained at 3 years is highly related to adult height but is independent of subsequent linear growth (ie, that early growth retardation is not reversed later).[86]

The requirement for dietary energy is highest in the first year of life, when growth velocities are high but stomach volumes are low. Yet commonly used weaning foods in many countries with a high prevalence of stunting are bulky and have energy densities too low to support optimal growth (Figure 17-9). Infections, especially gastrointestinal, are common in areas of poverty and illiteracy and contribute to malnutrition, which makes children more susceptible to further infections. This cycle of poor nutrition and infection in this critical phase of growth leads to stunting.

The relative contribution of diarrheal disease and inadequate diet to the commonly observed growth failure of children in developing countries remains uncertain.[87] At an individual level,[88] diarrheal episodes cause short-term faltering in both ponderal and linear growth, yet whether these children then catch up and whether their long-term growth failure is due to inadequate food intake or recurrent diarrhea is controversial. Malnourished rural Bangladeshi children grew equally well in the three monthly intervals in which a diarrheal episode of at least 10 days occurred at the beginning of the interval compared with an interval with no diar-

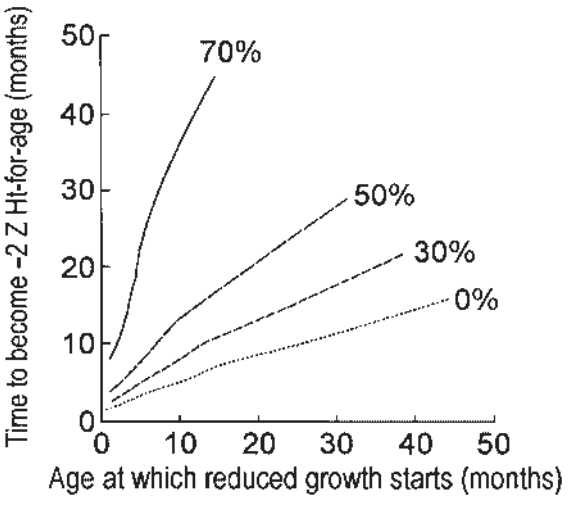

FIGURE 17-7 The time necessary for a child to fall from the median height for age to more than 2 SD below the median, if not gaining height at all (*dotted line* 0%) or gaining at 30% (*short dash*), 50% (*long dash*), or 70% (*continuous*) of the normal rate. Reproduced with permission from Golden M.[84]

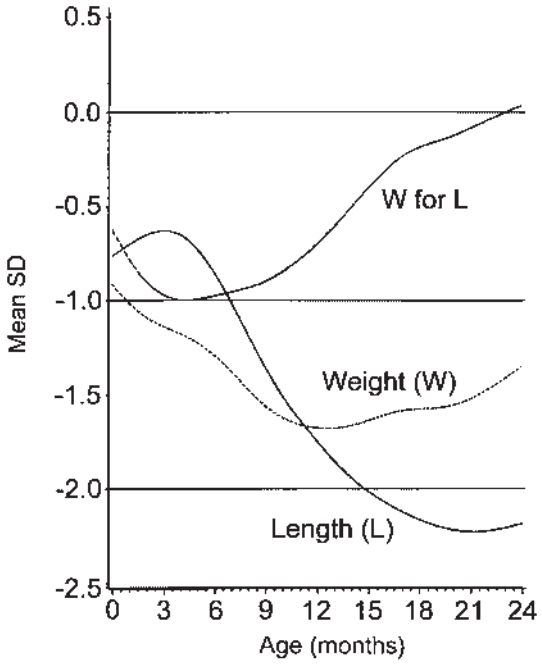

FIGURE 17-8 Mean SD values for weight, length, and weight for length against age for a pooled sample of Pakistani village and periurban slum dwellers. Reproduced with permission from Karlberg J et al.[85]

rhea.[89] In contrast, intervals with at least 10 days of diarrhea occurring in the last 45 days were associated with significantly lower weight gain than those with diarrhea-free intervals. These children were free of diarrhea for over 90% of the time but gained weight only to 74% of the NCHS median during diarrhea-free periods. The authors concluded that these children were malnourished owing to poor food intake rather than diarrhea. A review focusing on evidence of causality concluded that malnutrition was likely to predispose to diarrhea but that there was no conclusive evidence to support the hypothesis that diarrhea is a major cause of permanent growth faltering in whole communities.[90]

Recent application of noninvasive tests of mucosal integrity (eg, dual sugar intestinal permeability test) has permitted the study of the relationship between growth and mucosal injury. In The Gambia, 119 rural infants aged 2 to 15 months had their growth and intestinal permeability assessed monthly until 15 months of age, during which time diarrheal morbidity was also recorded.[91] All were breastfed until 3 to 4 months of age, during which time their growth approximated to the 50th NCHS percentile. By 14 months, both height and weight had fallen to the 5th percentile. Intestinal permeability was strongly related to mean monthly weight and length gain and predicted 39% and 43% of the observed faltering in weight and length, respectively. Intestinal permeability values were abnormal during 76% of the study period, yet the infants had diarrhea for only 7.3% of time. The intestinal mucosal histology of these children was abnormal for most of the time, and the authors concluded that this was more likely due to gastrointestinal infection than to malnutrition. Both decreased nutrient absorption and increased permeability of the mucosal barrier (allowing translocation of microorganisms, endotoxins, and food proteins causing both a local and a systemic inflammatory response) were postulated to contribute to poor growth. In The Gambia, intestinal permeability values improved with age but never reached values seen in similar age children in the United Kingdom. One hypothesis is that the permeability of the intestine is "set" early in life,[92] and the failure of nutritional interventions to correct growth faltering in subjects with early and severe malnutrition may reflect a failure to reverse this enteropathy, once established. This, of course, remains only speculation at this time. Suboptimal intestinal repair after injury was also demonstrated in 20 Gambian infants with persistent diarrhea and malnutrition who had no significant improvement in intestinal permeability 1 month into rehabilitation.[93] Intestinal biopsies demonstrate partial to subtotal villous atrophy, moderate to severe crypt hyperplasia, and marked infiltration of activated intraepithelial lymphocytes. The enteropathy associated with malnutrition has long been recognized; however, only recently has this been appreciated to be a cause and a consequence of malnutrition.

REVERSIBILITY OF WASTING AND STUNTING

Ponderal catch-up growth is relatively easy to achieve in malnourished children through appropriate dietary rehabilitation and can be spectacular (Figure 17-10). Rates of 10 to 20 g/kg/d can be generated—up to 10 times the normal rate of gain in the under 2-year-old age group.[88] The optimal macro- and micronutrient content of rehabilitation diets has long been debated. Not only must preexisting deficiencies be corrected, but energy, protein, and micronutrient content must match the potential for rapid growth; if any one constituent is limiting, growth may falter. Recent WHO recommendations summarize the requirements for energy, protein, potassium, sodium, zinc,

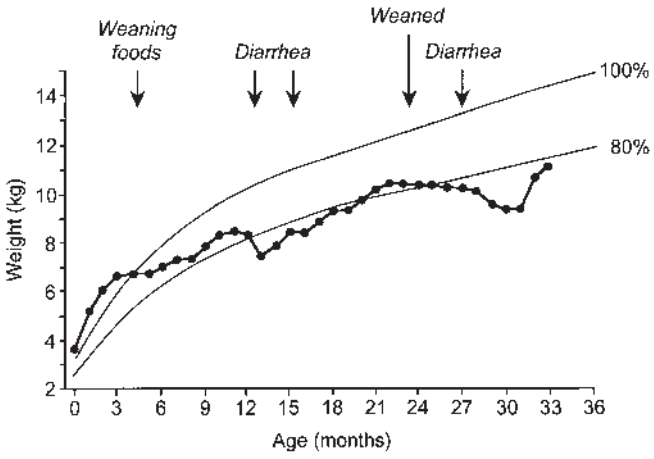

FIGURE 17-9 Weight chart of a Gambian infant. *Arrows* indicate first introduction of weaning foods, episodes of acute diarrhea, and cessation of breastfeeding. One hundred percent and 80% weight-for-age reference standards are shown. Reproduced with permission from Hoare S, Poppitt SD, Prentice AM, Weaver LT. Dietary supplementation and rapid catch-up growth after acute diarrhoea in childhood. Br J Nutr 1996;76:479–90.

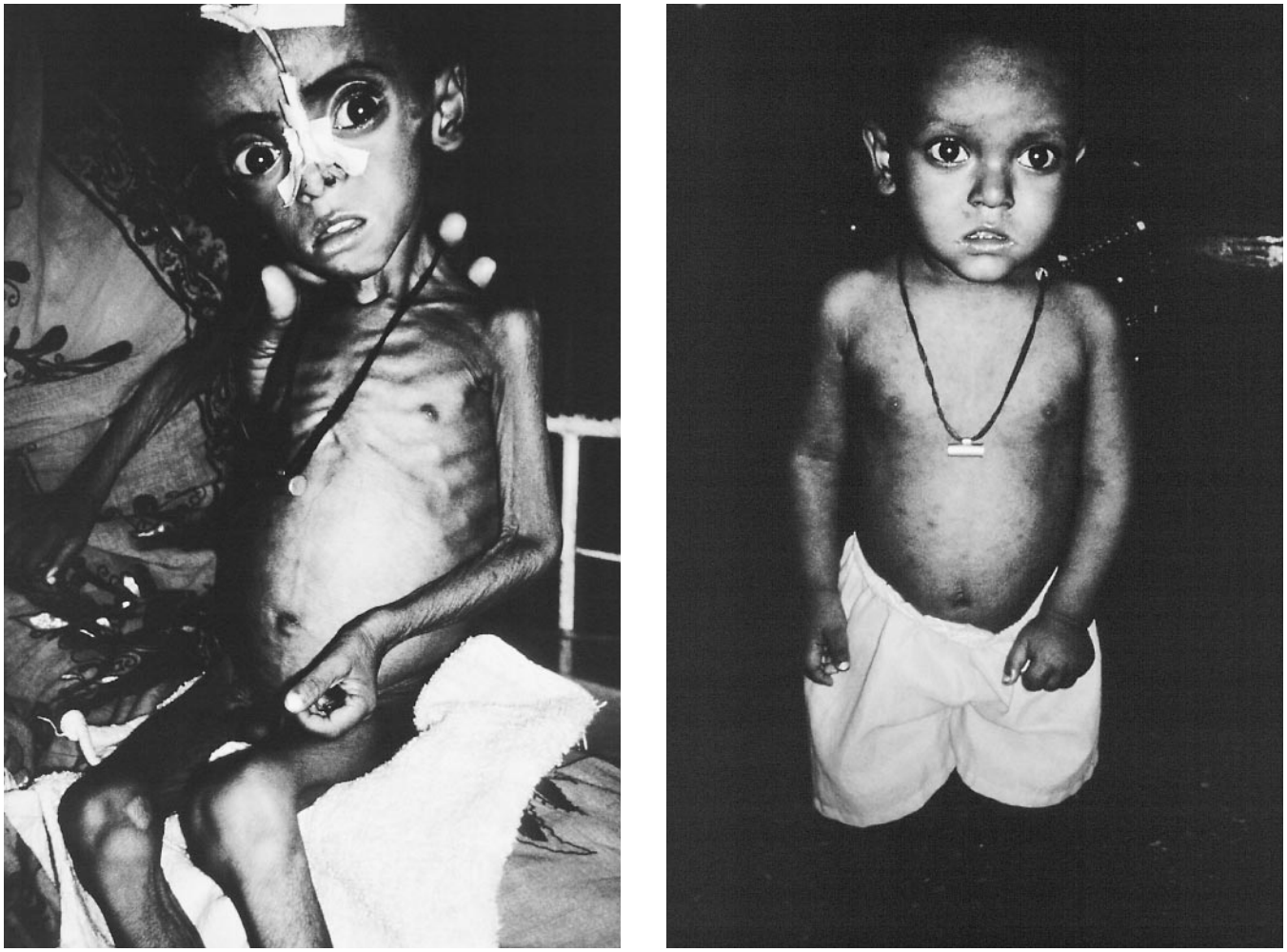

FIGURE 17-10 Nutritional rehabilitation and ponderal growth. A 2-year, 3-month-old Bangladeshi child weighing 3.3 kg (*A*) and 5 months later weighing 7 kg (*B*).

and copper, as well as other minerals and water- and fat-soluble vitamins.[94] The exact requirements of severely malnourished children for many of the micronutrients in particular and when to administer them remain to be clarified. Overzealous[95] or inappropriate timing of supplementation[96] can have potentially fatal consequences for severely debilitated, immunocompromised, septic children. Linear growth is dependent on lean tissue deposition, and the proportion of lean to fat tissue deposited is determined by both the macro- and the micronutrient content of the diet. Adequate dietary protein is necessary for protein deposition, but if it accounts for over 15% of total dietary energy, it will not increase protein deposition further and can be harmful. Zinc supplementation has been shown to increase the proportion of lean tissue deposited,[97] and in children recovering from severe protein-energy malnutrition, it improves ponderal growth[98] and immune function.[99] However, zinc supplementation of growth-retarded and presumed zinc-deficient children has had mixed effects on the promotion of linear catch-up growth. This probably reflects both the diversity of populations in which zinc supplementation was employed in terms of age, degrees of growth retardation, dietary intake, bioavailability of zinc and other growth-limiting nutrients, and the study design.

Examination of both ponderal and linear growth after a nutritional insult can throw light on the relationship between the two. The growth of 369 Jamaican children recovering from severe malnutrition (95% had a weight-for-age z-score < 60% of the NCHS median and/or nutritional edema) was retrospectively examined over a 31-day period. Only a subgroup (29%) demonstrated catch-up in height-for-age z-scores over this period, and they were the children who were most stunted at the onset. Most children did not demonstrate linear growth until they had achieved 85% of expected weight for length.[100] This suggests a threshold for length gain, but even severely malnourished children can gain in length early in rehabilitation; Figure 17-11 demonstrates weight-for-height z-scores of 141 severely malnourished Bangladeshi children plotted for six time points during rehabilitation against subsequent linear growth.[101]

Longitudinal follow-up studies commonly report residual growth failure after severe malnutrition.[102] What appears to limit subsequent linear growth is the severity and duration of the original insult, at what age it occurred, and the nature of the nutritional macro- and micronutrient rehabilitation employed. Ponderal growth reflects the recent health and nutritional status of the child, whereas linear growth reflects longer-term health and must have a

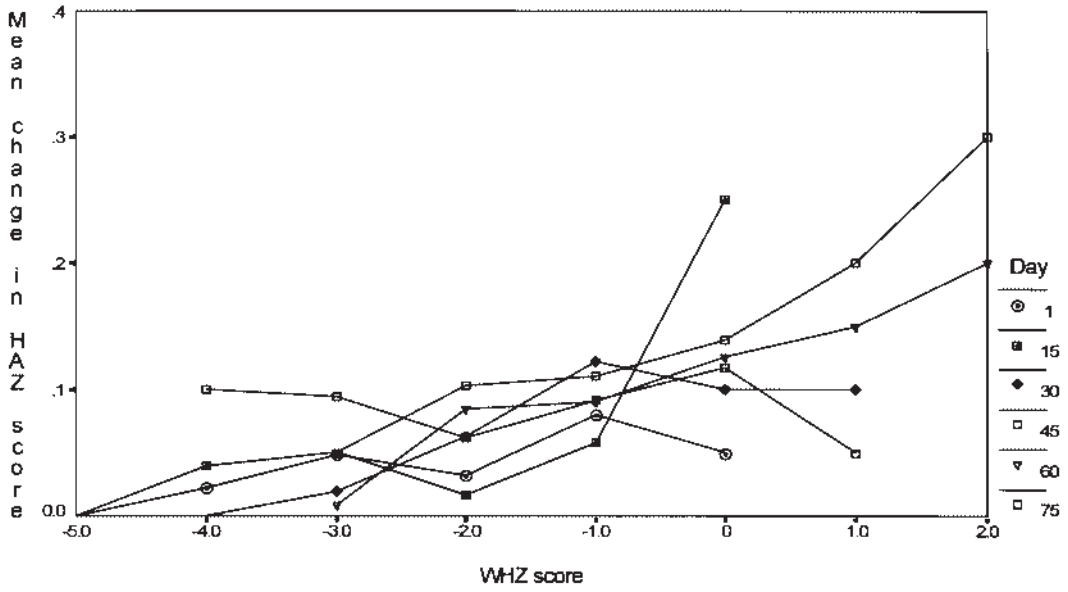

FIGURE 17-11 Linear growth of severely malnourished children. Reproduced with permission from Doherty CP et al.[102]

more sustained optimal environment to occur normally. In general, babies are born with the same mean length between and within populations of diverse socioeconomic backgrounds. The process of stunting seems to occur between the ages of 6 and 18 to 24 months and is associated with a delay in the onset of the childhood phase of growth. If a child in a developing country survives the critical growth period up to 2 years of age, then locally available foods with adequate energy density and the development of the child's immunity to environmental pathogens should allow the child to continue to grow at a normal velocity thereafter. The child will often remain at that baseline level of stunting, however, and demonstrate only marginal catch-up growth. The ability to catch-up linear growth has been demonstrated in studies of children adopted into better socioeconomic conditions.[103] Even this catch-up growth in later childhood is incomplete, and the effect on pubertal timing and final adult stature is not clear. The vast majority of children in developing countries stunted at 2 years of age will be left with a degree of stunting until adulthood.[84]

Nutritional interventions in the critical period[104] of the first 2 years of life, however, can generate catch-up growth and reverse stunting. In Guatemala, a study of food supplementation demonstrated a differential effect at different ages, with only those under 3 years of age demonstrating catch-up growth, and the linear growth of those 3 to 7 years of age did not benefit.[104] The effect of nutritional interventions on stunted adolescents, their age and duration of menarche, and their final attained height are not known.

The potential for catch-up growth will depend on the environment (eg, dietary macro- and micronutrient supply), patterns of morbidity, and the predetermined height potential for that individual child. Height potential is genetically predetermined and normally reflects parental heights in well-nourished populations. Comparing height potential for individual populations across the world, it is clear that differences are primarily due to environmental factors rather than genetic factors. However, stunted children are frequently born to stunted parents. Therefore, the parental heights in this situation are not a useful guide to potential. Animal experiments demonstrate that the progeny of rats that have been nutritionally restricted are small, and even when their offspring have been adequately nourished, it will take three generations or more for them to attain their true height potential.[105] Thus, it will probably take several generations of an optimal nutritional environment for the offspring of stunted parents to attain full genetic height potential. This process of environmental regulation of genetic potential is not clearly understood.

Age at menarche determines the length of the childhood phase of growth and therefore the potential period for catch-up growth. Menarche is frequently delayed in poorly nourished populations,[106] but the degree of this delay and thus the potential for catch-up have declined historically because diet and nutritional status have improved. In certain populations, this delay has been sufficient to allow significant catch-up growth.[107]

CONCLUSIONS

Studies from developing countries provide important observations concerning the relationship between malnutrition and growth failure. These observations are directly applicable to the developed world, especially to children with chronic gastrointestinal disease. The critical period for linear growth is under 2 years of age, and significant nutritional insults before this age are likely to have profound and long-term effects on growth. Ponderal catch-up growth is relatively easy to achieve, but nutritional interventions designed to promote linear growth must be initiated early and be sustained. Even then, potential catch-up may be limited by maternal malnutrition and intergenerational effects. An understanding of the infancy, childhood, and puberty model

of linear growth and the relative contributions of each phase of growth underlie this. Stunting is not a benign adaptation to chronic nutritional insufficiency but has serious consequences in terms of general and reproductive health, school performance and intelligence, and adult work capacity.

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CHAPTER 18

MALNUTRITION

Stephen John Allen, MBChB, MRCP (UK) Paeds, DTM&H, MD

Malnutrition is closely associated with poverty in all regions of the world.[1] It makes an enormous contribution to child morbidity and mortality in the half of the world's population that lives on less than $2 (US)/d. The wide-ranging and severe abnormalities of the gastrointestinal system that occur in severely malnourished children are well known. In addition, "tropical" or "environmental" enteropathy occurs in most children living in developing countries and may be an important cause of growth faltering. Recent studies have given new insights into the pathogenesis of the enteropathy associated with malnutrition that raise the prospects for specific interventions for prevention and management.

MALNUTRITION: A MAJOR PUBLIC HEALTH PROBLEM IN THE THIRD MILLENNIUM

The 2002 *World Health Report* focuses on identifying risks to health as the key to prevention.[1] One-fifth of the global disease burden can be attributed to the combined effects of protein-energy and micronutrient deficiency. Underweight is the most important risk factor globally for disease, with most of the burden occurring in developing countries with high mortality rates (Figure 18-1). In these countries, underweight accounts for 12.6% of all deaths in males and 13.4% in females, with an estimated 3.4 million deaths in 2000 (1.8 million in Africa and 1.2 million in Asia). The disease burden attributable to underweight is even greater than that caused by other major risk factors, such as unsafe sex, which captures the burden attributable to human immunodeficiency virus (HIV)/acquired immune deficiency syndrome (AIDS).

Malnutrition is most common among children aged younger than 5 years; the World Health Organization (WHO) estimates that in 1998, 168 million children (27% of all children under 5 years) were at least moderately underweight (defined as a weight for age z- [WAZ] score of < –2 compared with National Center for Health Statistics [NCHS] standards).[2] In prospective community studies in developing countries, 56% of all deaths in children under 5 years were associated with malnutrition.[3] Weight for age has a direct relationship with child mortality that is independent of secular and socioeconomic factors.[4] A central maxim of preventive medicine, that "a large number of people exposed to a small risk may generate many more cases than a small number exposed to a high risk,"[5] is well illustrated by malnutrition. Although the risk of mortality rises progressively with worsening nutritional status, it is important to note that over 80% of malnutrition-associated deaths occur in children with mild to moderate malnutrition because these greatly outnumber severely malnourished children.[3]

Underweight children are at increased risk of dying from common infectious illnesses such as diarrhea and pneumo-

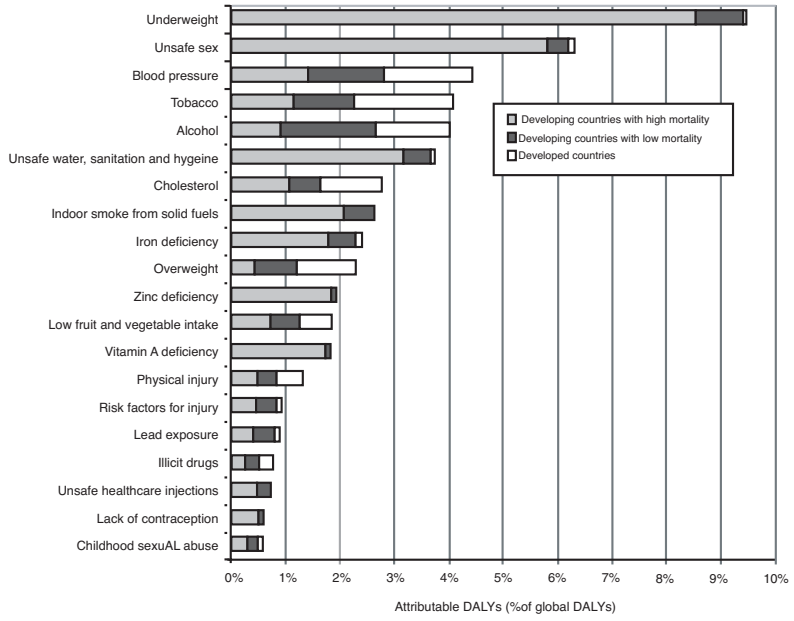

FIGURE 18-1 Underweight (weight-for-age z-score < –2) is the most important risk factor for the global burden of disease (assessed as lost disability-adjusted life years [DALYs]).[1] Reproduced with permission from the World Health Organization.[1]

nia,[6] and malnutrition is particularly common in children attending clinics and hospitals serving economically deprived populations. Underweight increased susceptibility to several major infections in a large series of children under 5 years admitted to hospitals in The Gambia.[7] Mean admission WAZ score in children with a primary diagnosis of malnutrition was 3.8 lower than that in community controls but was also between 1.4 and 2.5 lower for those with a primary diagnosis of malaria, severe malaria, pneumonia, meningitis, or gastroenteritis. Overall, case fatality rose progressively with decreasing WAZ score—from 7.2% for a WAZ score > –2 to 22.7% for a WAZ score < –4—and this relationship was seen in all of these disease categories (Figure 18-2). Weight deficits were too great to be accounted for by dehydration or anorexia during an acute illness.

A further study of 1,264 children admitted to a hospital in The Gambia specifically identified wasting as a major risk factor for mortality. Severe wasting (weight-for-height z- [WHZ] score < –3) was present in 13.1% of admissions, with the greatest frequency in 1 year olds, of which 1 in 4 were severely wasted. Case fatality was increased 3.5-fold (95% confidence interval 1.6–7.6) in severely wasted children compared with better nourished children (WHZ score > –2). Severe stunting (height-for-age z-score < –2) was present in 9.7% of admissions but was not associated with mortality (S. Allen, unpublished data, 2000). Case fatality for severe malnutrition remains high and may even reach 60%.[8] These data highlight the fact that wasting is common in children with a primary diagnosis other than severe malnutrition, and this often goes unrecognized. Specific nutritional support in these children may reduce case fatality.

The longer-term outcome after treatment of severe malnutrition is also usually poor. Mortality during follow-up was 2.3% in Bangladesh,[9] 8% in Tanzania,[10] 18% in Niger,[11] 19% in Zaire,[12] 32% in The Gambia,[13] and 36% in Kenya.[14] In survivors, the recurrence of wasting after discharge is variable, but stunting usually persists and morbidity is high. The marked variability in the reported mortality rates may reflect differences in the adequacy of nutritional rehabilitation before discharge, the education of caregivers during admission, and the length and completeness of follow-up. Furthermore, mortality may be reduced in studies in which children were followed up frequently at home for the purposes of recording outcome.

HISTORICAL PERSPECTIVE

The development of instruments to acquire per oral biopsies of the small intestine in the 1950s led to several classic studies of gut structure and function in malnutrition. However, marked differences in patient groups and study designs make comparisons between studies difficult. Most patients were young children (mostly aged 1–3 years), but the classification of malnutrition and the mix of the major types varied between studies. Classifications of nutritional deficiency are generally complicated because they combine anthropometry with clinical signs.[15] Early studies used the term "marasmus" to describe severe wasting but without edema (Figure 18-3). Kwashiorkor was used less consistently; it denoted the presence of nutritional edema in children who were usually underweight and may, or may not, have had the classic clinical signs initially described in Ghanaian children by Williams,[16] such as dermatosis, hair changes, apathy, and irritability (Figure 18-4). Marasmic kwashiorkor was used for children with edema who were severely underweight. Investigations were done in children with varying severity of malnutrition ("mild" kwashiorkor) and at different stages of rehabilitation.

This terminology was summarized in the Wellcome classification.[17] In a later classification, Waterlow emphasized the importance of distinguishing wasting, expressed as low weight for height and signifying acute malnutrition, from

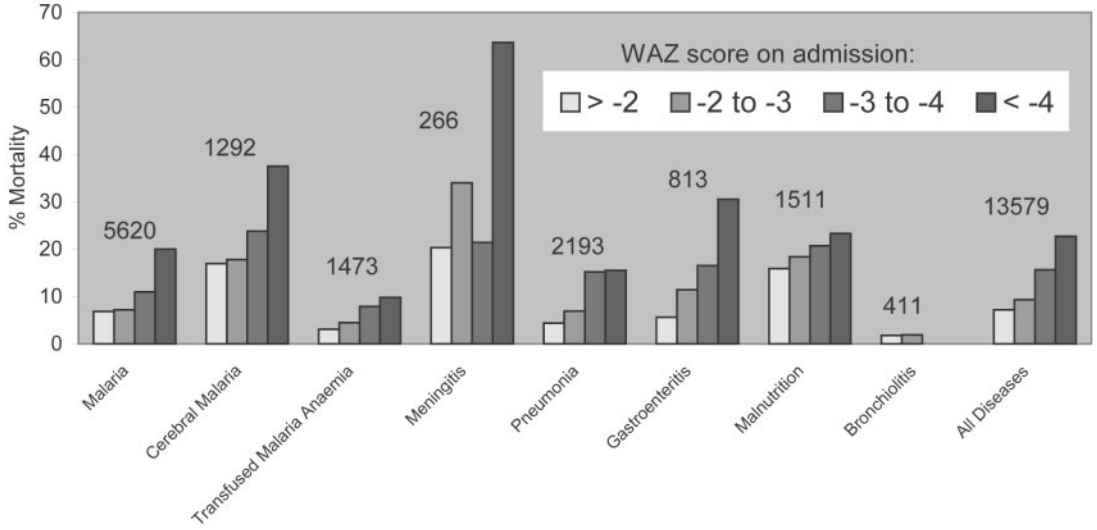

FIGURE 18-2 Low weight-for-age z-score (WAZ) was associated with increased case fatality in children admitted to hospitals in The Gambia. Underweight was common, and this relationship with mortality was seen in several major diseases, as well as in those with a primary diagnosis of malnutrition. The number of children with each diagnosis is shown in the figure. Adapted from Mann WD-C et al,[7] with permission from Blackwell Publishing.

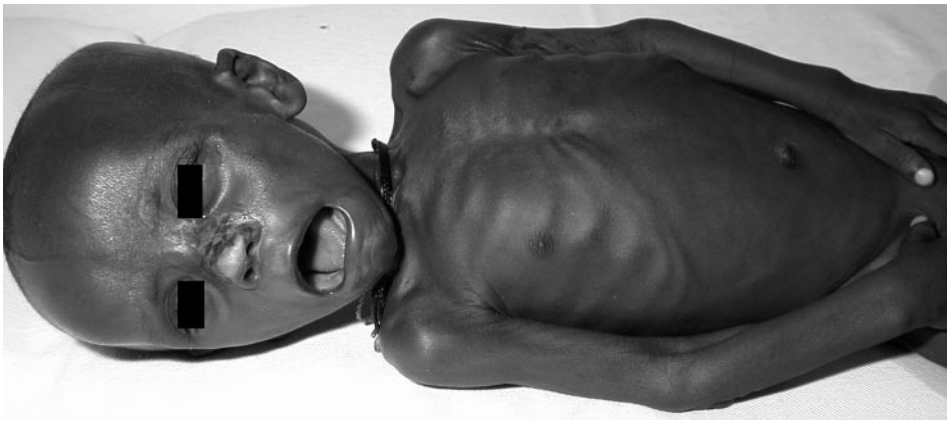

FIGURE 18-3 A severely wasted West African child who presented in 2001 with hypoglycemia, hypothermia, and dehydration.

stunting, a sign of chronic malnutrition and reflected in low height for age.[18] In more recent studies, the term protein-energy malnutrition (PEM) has been used to encompass the interrelated features of deficiency in carbohydrates, proteins, and fat, as well as vitamins, minerals, and trace elements.

In most case series, serum albumin was low or very low, and anemia was common. Micronutrient deficiencies were usually not assessed but will have differed between study populations. Researchers usually determined whether findings in malnourished children were abnormal by comparison with controls, but the choice of control group varied between studies. Some recruited children living in the same environment as the index cases, often hospital controls, who themselves may have had environmental enteropathy and moderate malnutrition. Others chose well-nourished local controls or children from developed countries.

In considering the causes of malnutrition, Bellamy highlights the complex interrelationships among infections, poor nutrient intake, and proximal causes at the household level (Figure 18-5).[19] When searching for causes of gut abnormalities in malnourished children, researchers often try to differentiate the effects of infection, especially diarrhea, from PEM per se. However, this is almost impossible to do. In addition to pneumonia and malaria, either acute or persistent diarrhea is extremely frequent in case series of malnourished children. A wide variety of bacteria and parasites are isolated from stools, but a specific organism is not identified in many children. A further difficulty is that the detection of infections is highly dependent on the methods used, which is particularly important for gut organisms such as *Giardia lamblia*, for which routine diagnostic methods are unreliable.[20] Also, attributing pathology in the gut to specific organisms isolated from stools is complicated because the same organisms are often isolated from healthy controls without diarrhea.

It is clear that effective prevention of malnutrition is likely to require multifaceted approaches that deal with several interconnected socioeconomic, public health, and disease-specific factors. The large mucosal surface area, with a high turnover rate, and the production of large amounts of fluids and enzymes make the gastrointestinal tract particularly susceptible to nutrient deficiency. However, the specific cause(s) of gut abnormalities in malnourished children remain poorly understood. In this review, emphasis is placed on those abnormalities that may be targets for therapeutic interventions either to prevent malnutrition or to improve its outcome.

MALABSORPTION

Prompted by the frequent occurrence of diarrhea and reports of steatorrhea, markedly reduced absorption of various nutrients has been demonstrated in malnourished

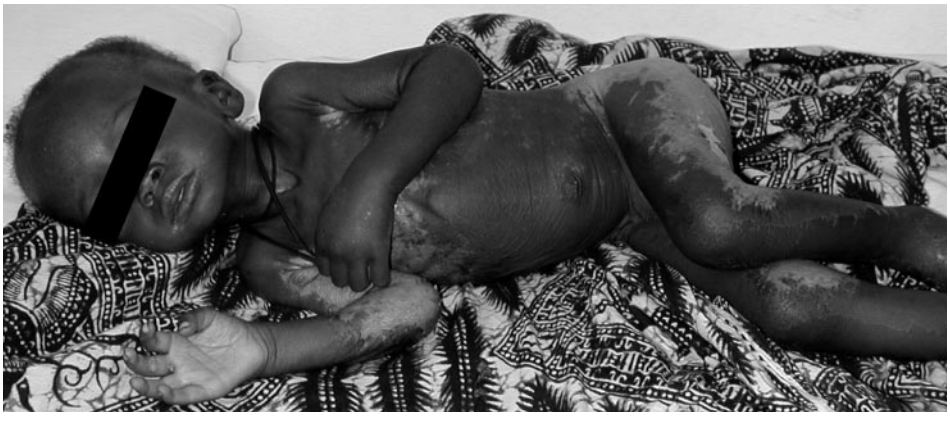

FIGURE 18-4 A West African child with marasmic kwashiorkor who presented in 1999.

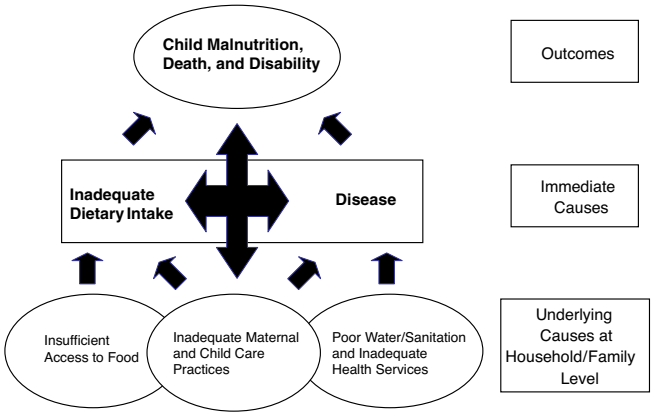

FIGURE 18-5 The vicious cycle of malnutrition, inadequate dietary intake, and disease (mostly infection) is related to underlying causes at the household and family level. Adapted from Bellamy C.[19]

children and the implications for the design of rehabilitation diets have been much debated.

Carbohydrates

Intolerance of lactose is the most consistently reported problem. Stool chromatography in 24 South African children with kwashiorkor and 3 with marasmus revealed lactose in all but 2.[21] A carbohydrate-free diet reduced stool weight and stool lactic acid content markedly in most children, including 8 children with intestinal pathogens in the stools. Reintroduction of milk increased stool output and lactic acid markedly in some children. Carbohydrate tolerance tests in a few children showed impaired lactose absorption. Lactose intolerance was also demonstrated in 14 of 17 Ethiopian boys with kwashiorkor, apparently without diarrhea[22]; 5 of 10 South African children with kwashiorkor tested after 3 weeks of hospital treatment[23]; 8 of 10 malnourished Jamaican infants, 4 of whom had edema but none had severe diarrhea[24]; 39 of 100 malnourished Indian children, of whom 7 had kwashiorkor but none had enteropathogens isolated[25]; and 21 of 43 Brazilian children with a range of nutritional deficiency.[26] Intolerant children often developed acid stools (pH < 4) and abdominal symptoms during challenge tests.[25] Flat lactose tolerance tests were also common in marasmic Brazilian children.[27] Lactose maldigestion also correlated with poor growth in breastfed Gambian infants living in the community.[28]

Malabsorption of other sugars is more varied. Glucose and galactose malabsorption occurred in about half of the Ethiopian[22] and South African children.[23] All of the Indian children[25] malabsorbed glucose, but only 1 of 20 Brazilian children did.[26] Sucrose malabsorption varied from 24 to 60% of cases.[22,24–26] Absorption of all sugars improved with clinical recovery. In the Jamaican series, absorption had increased after 6 to 16 weeks of treatment,[24] and all but 4 of the Indian children had normal sugar absorption after 3 months of nutritional rehabilitation.[25]

In keeping with these observations, variable deficiencies of mucosal disaccharidases have been reported. Half of a series of malnourished South African children had lactase deficiency, especially those with giardiasis, but sucrase and maltase levels were mostly normal.[29] Lactase, maltase, and sucrase activity was low in Ugandan children with mild to moderate kwashiorkor, with lower enzyme activity in those with more severe mucosal atrophy.[30] Disaccharidase deficiency persisted at 1 year after recovery. Lactose intolerance and lactase deficiency persisted in Ugandan children reassessed between 4 and 10 years after recovery from kwashiorkor.[31] However, mucosal histology was similar to that in adult hospital controls, and other disaccharidases were normal. Therefore, whether lactose intolerance was due to long-term mucosal damage or reflected the normal reduction of lactase activity with age in the population was unclear. Lactose-induced diarrhea in children with kwashiorkor did not significantly reduce absorption of nitrogen or fat, allowing continued milk feeding.[21]

Although sugar intolerance may be demonstrated during challenge tests and disaccharidases are shown to be deficient, lower intakes of sugar in milk feeds may be tolerated well without troublesome diarrhea.[23]

Nitrogen

Increased nitrogen losses from the gut are related to both malnutrition and gut infection. Holemans and Lambrechts studied 26 South African children with either kwashiorkor or chronic malnutrition, most of whom had hookworm infection.[32] Although the proportion of nitrogen intake excreted in stools (mean 20%) was higher than in European infants, nearly all children had adequate nitrogen retention of about 50% of dietary intake. A further study confirmed high rates of nitrogen absorption in children with kwashiorkor on milk feeds.[33] In underweight Guatemalan children with edema and a heavy burden of gut pathogens,[34] markedly decreased nitrogen absorption was correlated with the degree of protein depletion as assessed by urinary creatinine-to-height ratio. Nitrogen absorption improved rapidly with clinical recovery. Four Guatemalan children with marasmic kwashiorkor studied during the late stages of recovery absorbed about 80% of ingested nitrogen, and absorption was proportional to intake, although the children remained protein depleted.[35] However, in this study, absorption fell markedly during episodes of diarrhea.

Fat

Variable decreases in dietary fat absorption have been reported, even in children without macroscopic steatorrhea, and absorption improved slowly only during recovery. Average fat absorption was 81.8% in malnourished South African children (compared with 95% in normal children),[32] and malabsorption occurred in between 30 and 100% of malnourished children from Mexico City,[36] India,[25] and Guatemala.[34] The mean increase in plasma triglycerides after an oral margarine load was significantly lower in underweight Brazilian children than in controls.[26] In the late stages of recovery from marasmic kwashiorkor, fat absorption varied from 32 to 89% in Guatemalan children[35] and improved gradually with clinical recovery in a

Mexican series.[36] Fat absorption did not appear to be affected by episodes of diarrhea.[25,35]

Vitamin B_{12}

Initial observations of markedly reduced vitamin B_{12} absorption that was slow to improve with clinical recovery[34] were confirmed in a study of Guatemalan children with severe PEM.[37] At both admission and convalescence, absorption was reduced further in children with diarrhea. Absorption was not improved by giving intrinsic factor, suggesting mucosal dysfunction in the terminal ileum in PEM that is slow to recover, although metabolism of administered vitamin B_{12} by bacteria in the upper gut is an alternative explanation.

THE LIVER IN MALNUTRITION

The fatty infiltration in the liver in kwashiorkor is well known. Autopsies and biopsies of 10 Ugandan children showed a progression of fatty infiltration of hepatocyes beginning at the periphery of lobules and progressing to centrolobular areas.[38] In some cases, fat infiltration was so severe that the liver appeared pale yellow and normal hepatocyes could not be differentiated on microscopy. There was moderate periportal and peripheral pericellular fibrosis and cellular infiltration in the portal areas. The infiltration was mainly of lymphocytes, but eosinophils, macrophages, and neutrophils were also present. With clinical improvement, fat retreated initially from the centrolobular region, but the fibrosis persisted. More irregular patterns of fat infiltration were seen in children with concomitant severe infection,[39] and typical cases of kwashiorkor also occurred without any fatty infiltration of the liver.[38]

Abdominal ultrasonography in Jamaican children showed that hepatic steatosis was greater in children with edematous malnutrition than in those with marasmus, but all malnourished children had more hepatic fat than healthy controls did.[40] The extent of steatosis was not correlated with liver size, and fat was slow to be mobilized from the liver during recovery. Ultrasound examinations in Indian children also confirmed the presence of liver fat in malnourished children without edema.[41] The degree of hepatic steatosis was not associated with the severity of malnutrition or serum transaminases and improved in most cases with weight gain. Compared with biopsies from recovered children, autopsy specimens obtained immediately after death in Jamaican children showed several ultrastructural abnormalities, including decreased peroxisomes, consistent with increased susceptibility to free radical damage.[42]

Impairment of hepatic synthesis in malnourished children is evident by low plasma albumin. Reduced hepatic synthesis may be an important risk factor for mortality; a prolonged prothrombin time was present in 8 of 11 Nigerian children with kwashiorkor who died compared with 4 of 29 survivors.[43] One mechanism may be through decreased production of antibacterial substances such as transferrin and fibronectin, which were reduced in Nigerian children with kwashiorkor and marasmus compared with those in well-nourished local controls.[44] Aflatoxins are found frequently in kwashiorkor, and the known effects of aflatoxins in animal models raise the possibility that they contribute to liver dysfunction in malnutrition.[45]

PANCREAS AND BILE ACIDS

The extremely high rate of protein synthesis by pancreatic acinar cells in the production of digestive enzymes makes them especially susceptible to nutritional deficiency. In keeping with a general atrophy of exocrine glands,[39] children from East and Central Africa who died with kwashiorkor had a small pancreas owing to marked atrophy of the acinar cells, which had a reduced number of enzyme secretory granules. Intercalated ducts, secreting sodium- and bicarbonate-rich fluid, were relatively well preserved, but there was a generalized fibrosis.[38,39,46] Trowell and colleagues considered atrophy of the pancreatic acinar cells to be both a more constant and persistent lesion in kwashiorkor than fatty infiltration of the liver.[39] Pancreatic atrophy was common and associated with a fatty liver in Jamaican children with kwashiorkor but also occurred in marasmic children who had little liver fat.[47] Electron microscopy revealed atrophy of acinar cells with few zymogen granules and disorganization of the endoplasmic reticulum. Pancreatic fibrosis was mild in kwashiorkor and uncommon in marasmus. A further study reported ultrastructural damage of all cell types with changes in B cells, consistent with low insulin secretion.[48] Serum immunoreactive trypsinogen, a marker of either acinar cell damage or ductal obstruction, was correlated with wasting but not stunting in aboriginal children.[49] Pancreatic atrophy appeared to improve quickly with refeeding.[39]

These histologic findings correlate well with studies of pancreatic enzyme production in severely malnourished children. Pancreatic enzymes were low in Hungarian children with nutritional edema after the siege of Budapest (c. 1944).[50] Decreased amylase, trypsin, and lipase were reported in children with severe malnutrition, some with nutritional edema, in Mexico City.[51] In kwashiorkor, amylase and lipase were markedly reduced in Ugandan children,[46] and, in addition to these enzymes, trypsin and chymotrypsin were decreased in Egyptian[52] and South Africa children.[53] Production of enzymes improved promptly and to normal levels with clinical recovery.[46,49,51,53] South African children with marasmus had decreased amylase and chymotrypsin production.[53] In this study, the volume of pancreatic juice and pH in both kwashiorkor and marasmus were variable, but average values were similar to those in better nourished local controls, suggesting less impairment of the function of pancreatic ductules.

Deficiency of conjugated bile acids was the main cause of fat maldigestion, assessed by micellar lipid content of duodenal fluid, in underweight Guatemalan children with edema.[54,55] The concentration of conjugated bile acids in the duodenum was especially low in malnourished children with diarrhea. Free bile acids were increased in both cases and controls. Lipase activity was reduced but sufficient for normal lipolytic activity ($> 75 \times 10^3$ U/mL), and total pancreatic enzyme output was only mildly reduced. Micellar

lipid content and lipase activity normalized with clinical recovery, and conjugated bile acids increased to levels seen in the controls, although they remained low in children with diarrhea. Free bile acids remained high during recovery, especially in children with diarrhea. Some of the cases in this study had increased bacterial colonization of the upper gut, which may have contributed to conjugated bile acid deficiency (see below).[56] Increased free bile acids have also been reported in South African children with kwashiorkor.[57]

It is clear that several factors contribute to malabsorption, and these are likely to vary in different settings. Decreased conjugated bile acids, as a consequence of bacterial colonization of the upper gut, may be more important than deficiency of pancreatic lipase in fat malabsorption in kwashiorkor. Given the marked variability in findings between studies, it is clear that dietary rehabilitation needs to be tailored to individual children, especially those with diarrhea. However, digestion and absorption of carbohydrates, nitrogen, and fat appear to be sufficient for nutritional rehabilitation.[33,36] Milk-based diets are appropriate for most children, and the WHO has produced detailed feeding guidelines.[58] Despite decreased nutrient absorption, it is important to note that diets low in protein, fat, and sodium and high in carbohydrates are recommended during initial treatment. Dietary intake is increased during the rehabilitation phase of management, a time when absorption of many nutrients is improving.

STOMACH

Gastric histology in five South African children with kwashiorkor showed variable degrees of abnormality, including mucosal atrophy, reduced goblet cells, and increased inflammatory infiltrate in the lamina propria composed of lymphocytes, polymorphonuclear leukocytes, eosinophils, and plasma cells.[59] Abnormalities were still present 1 year later in one child after clinical recovery. Biopsies of the gastric fundus in 14 Indonesian children with a range of nutritional deficiency showed variable degrees of atrophy and chronic gastritis compared with the biopsies of healthy controls living in metropolitan Jakarta.[60]

Basal acid output was low in malnourished South African[59] and Indonesian children,[60] and 26 of 34 (76%) Bangladeshi children, mostly with marasmic kwashiorkor, had baseline hypochlorhydria.[61] Hypochlorhydria persisted despite stimulation with histamine in 4 of 20 (20%) of the South African children[59] and despite betazole stimulation in 8 (24%) of the Bangladeshi children.[61] Both basal and stimulated acid concentration had not improved at follow-up despite a marked increase in nutritional status and increased gastric juice volume.[61] In the South African cases, acid output increased only when clinical recovery from kwashiorkor and anemia was complete.[59] In some studies, hypochlorhydria was also common in children recruited as controls.[60,61] Similarly, an intubation study of Gambian infants living in rural villages reported that 4 of 29 (14%) had hypochlorhydria (gastric pH > 4).[62]

Impairment of the gastric acid barrier appears to be a common finding in children living in poverty, not only those with frank malnutrition. Clearly, it is now known that *Helicobacter pylori* infection is extremely common in young children in developing countries.[63] In a large cohort of Gambian infants, colonization determined by the urea breath test was present in 19% at age 3 months and in 84% by 30 months.[64] Also in Gambian studies, acquisition of *H. pylori* was associated with hypochlorhydria, assessed noninvasively by urine acid output following a test feed.[65] *H. pylori* may play an important role in compromising the gastric acid barrier and allowing bacterial contamination of the intestine.

SMALL INTESTINE

HISTOLOGY

Compared with findings in developed countries, studies of kwashiorkor in Uganda,[30,39,66] Kenya,[67] South Africa,[30,68,69] and Guatemala[70] reported enteropathy in all cases with a wide range of abnormalities. Typically, the intestinal wall is thin, with a smooth, atrophic mucous membrane ("tissue paper intestine").[39] Villi tend to be convoluted and ridged rather than fingerlike. Villous atrophy reduces mucosal thickness (mean crypt-to-villus ratio 1.0; normal 0.2),[30] and there may be complete villous atrophy. The brush border is irregular, and narrow and mucosal cells may be irregular or cuboidal, with irregular and displaced nuclei. Intraepithelial lymphocytes are increased. There is frequent branching of crypts. The cellular infiltrate in the lamina propria is markedly increased and consists of lymphocytes, plasma cells, eosinophils, and polymorphonuclear leukocytes.

In South African children already established on a high-protein diet, accumulation of lipid droplets within epithelial cells was prominent.[68] Electron microscopy showed variable distribution of fat—as particles enclosed by smooth endoplasmic reticulum and Golgi vesicles and as chylomicrons in intercellular spaces or in vesicles within lamina propria macrophages. Mitochondria, endoplasmic reticulum, and lysosomes appeared normal. However, in another series of South African children who were studied before starting treatment, abnormalities of epithelial cells included poorly developed microvilli, sparse endoplasmic reticulum, irregular nuclei, and disorganized cytoplasmic organelles but no accumulation of lipid.[69] Crypt cells were immature with increased mitosis, suggesting a rapid turnover. These abnormalities were consistent with impaired absorptive function. Plasma cells in the lamina propria appeared inactive. The discrepancies in findings between the two studies may have been due to biopsies being taken at different stages of management.

In children with marasmus, abnormalities of mucosal architecture similar to those in kwashiorkor are seen, again with a wide range of severity. Brunser and colleagues reported near-normal mucosal architecture except for a thinner mucosa and, at variance with kwashiorkor, reduced mitotic counts.[71] In contrast, Algerian children had thin mucosae with shortened or absent villi indistinguishable from celiac disease.[72] Studies of moderately to severely underweight Brazilian children, some with persistent diar-

rhea, reported variable shortening of villi from near-normal to subtotal villous atrophy, but all patients had increased inflammatory cell infiltrate.[26,27] Features on electron microscopy were also variable but included shortened, branched, or absent microvilli; increased intraepithelial lysosomes; irregular nuclei; and degenerative, detaching epithelial cells from the upper half of the villi—the latter associated with giardiasis.[27,73] Other epithelial cells had only minor abnormalities. Lamina propria plasma cells appeared inactive in about half of the patients. Barbezat and colleagues reported that mucosal atrophy in 3 South African children with marasmus was of a severity similar to that in 13 children with kwashiorkor and 1 with marasmic kwashiorkor,[29] whereas other authors consider mucosal lesions to be milder in marasmus than in kwashiorkor.[34,71]

Sullivan and colleagues performed detailed computerized image analysis of mucosal biopsies from 40 malnourished Gambian children with chronic diarrhea, mostly with marasmus and marasmic kwashiorkor.[74] Many had gut infections, especially with *G. lamblia*. Villi varied from normal height to absent, but nearly all biopsies revealed crypt hypertrophy and lymphocytic infiltration of the lamina propria. Intraepithelial lymphocytes were increased, especially in the crypt epithelium. The marked range of abnormalities detected is summarized in Figure 18-6A, and a typical mucosal specimen showing well-preserved villi is shown in Figure 18-6B and an atrophic mucosa in Figure 18-6C. The degree of mucosal abnormality did not correlate with nutritional status or the presence of *G. lamblia* or *Strongyloides stercoralis*, but it was difficult to distinguish the effects on the mucosa of PEM from those of diarrhea.

In kwashiorkor, improved mucosal cell ultrastructure occurring after only 48 hours of intensive supportive treatment is consistent with a rapid increase in cell protein synthesis.[69] However, repeat biopsies following clinical recovery of kwashiorkor tend to show no or minimal improvement in mucosal appearances, even up to 1 year later.[30] Schneider and Viteri reported a progressive increase in mucosal and brush border thickness and epithelial cell height as nutritional recovery progressed, but crypt mitotic activity and the degree and composition of the cellular infiltrate in the lamina propria remained unchanged.[70]

Similarly, a common finding in studies of marasmus is the persistence of the mucosal lesion. The atrophic mucosa reported in the Algerian children persisted mostly unchanged when biopsies were repeated at 3 months despite marked clinical improvement.[72] After 3 to 4 weeks of inpatient treatment, there was little change in villous volume in the majority (16; 70%) of Gambian children in whom diarrhea had resolved, weight gain was good, and mean crypt cell volume had increased.[75] Villous epithelial volume had actually decreased in three children, two of whom had failed to improve clinically. Further follow-up at 1 year after discharge in a small number of children revealed that most had diarrhea and mucosal architecture was worse than at admission. In the much longer term, between 4 and 10 years after kwashiorkor, complete recovery of the brush border and reduction in inflammatory infiltrate, at least to that seen in local controls, were observed.[31]

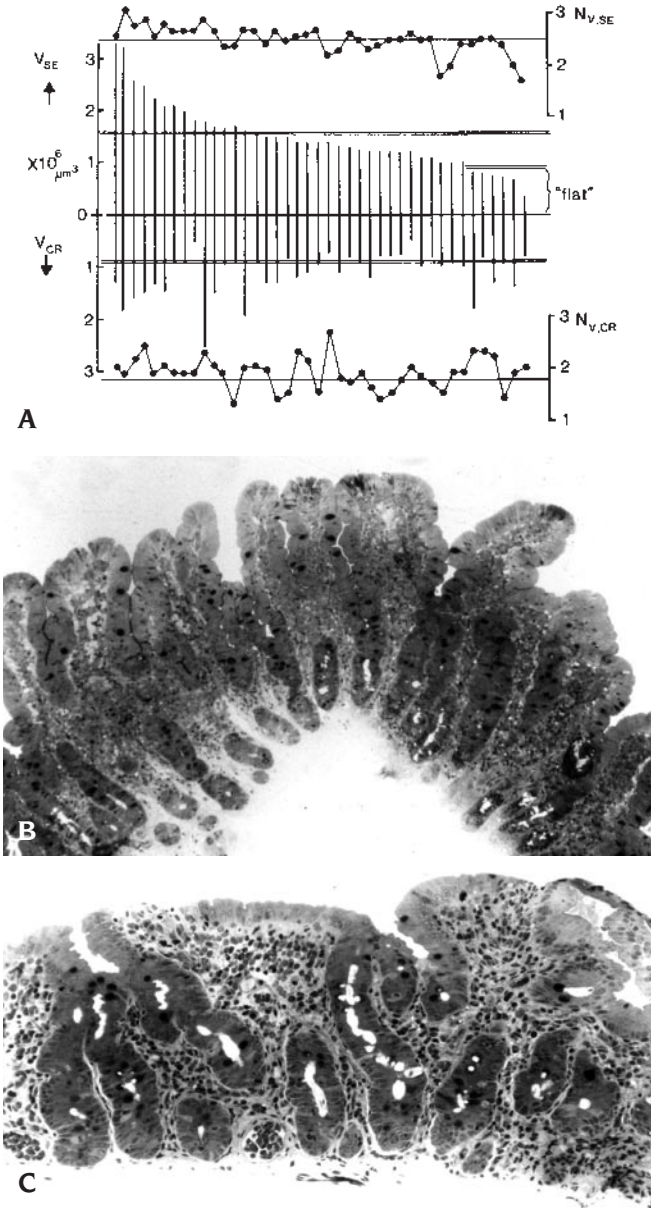

FIGURE 18-6 *A*, A marked range of enteropathy was observed in malnourished Gambian children. Mucosal morphometry for 40 Gambian children with persistent diarrhea and malnutrition displayed in descending order of villous (surface) epithelial volumes (V_{SE}), together with their corresponding crypt epithelial volumes (V_{CR}, left axis). *Horizontal lines* represent lower reference range for control villi and upper reference ranges for flat (celiac sprue) mucosae and control crypts. N_{VSE} refers to the numbers (log transformed) of intraepithelial lymphocytes in surface epithelium (upper) and N_{VCR} crypt epithelium (lower, right axis), and *horizontal lines* represent upper reference ranges. The final specimen in the series is from a child with kwashiorkor. Adapted from Sullivan PB et al[74] and reproduced with permission from Lippincott Williams & Wilkins. *B*, Mucosal biopsy from a child with marasmus. The villi are well preserved, but there is crypt hyperplasia and an increased inflammatory infiltrate. Dark areas at the tips of villi are fat globules. Specimen fixed in formaldehyde and stained with toluidine blue; ×100 original magnification. Reproduced with permission from Dr. P. B. Sullivan. *C*, Atrophic mucosa with intense inflammatory infiltrate and loss of surface epithelial cells in a child with marasmic kwashiorkor. Specimen fixed in formaldehyde and stained with toluidine blue; ×100 original magnification. Reproduced with permission from Dr. P. B. Sullivan.

Given the long-term persistence of mucosal abnormalities following marasmus, it is not surprising that significant enteropathy also occurs in mildly to moderately malnourished children living in the community. Histologic evidence of environmental enteropathy was found in infants with mostly mild to moderate malnutrition living in a Brazilian slum when compared with eight hospital controls from middle-class families.[76] Twenty-nine (73%) of the slum-dwellers had varying degrees of villous atrophy and increased inflammatory infiltrate in the lamina propria, with severe lesions in some children. Mucosal biopsies from stillborn fetuses in Southern India[77] and African neonates[30] had normal appearances with fingerlike villi. Therefore, environmental enteropathy appears to be an acquired lesion, the timing of its onset coinciding with weaning and possibly bacterial colonization of the upper gut (see below).

Immunohistochemistry

A recent study of Gambian children with nutritional status ranging from normal to severely underweight used immunohistochemical techniques to characterize the mucosal inflammatory response.[78] Although most children had diarrhea, stool pathogens were infrequent. However, giardiasis may have been underestimated based on stool microscopy alone. All children were HIV antibody negative. Age-matched children living in the United Kingdom investigated for vomiting or possible enteropathy but shown to have no gastroenterologic disorder were used as controls.

All of the Gambian children, regardless of nutritional status, had increased mucosal permeability, crypt hyperplastic villous atrophy, and increased intraepithelial lymphocytes—the latter with an increased proportion of γδ cells and within the range characteristic of celiac disease (Figure

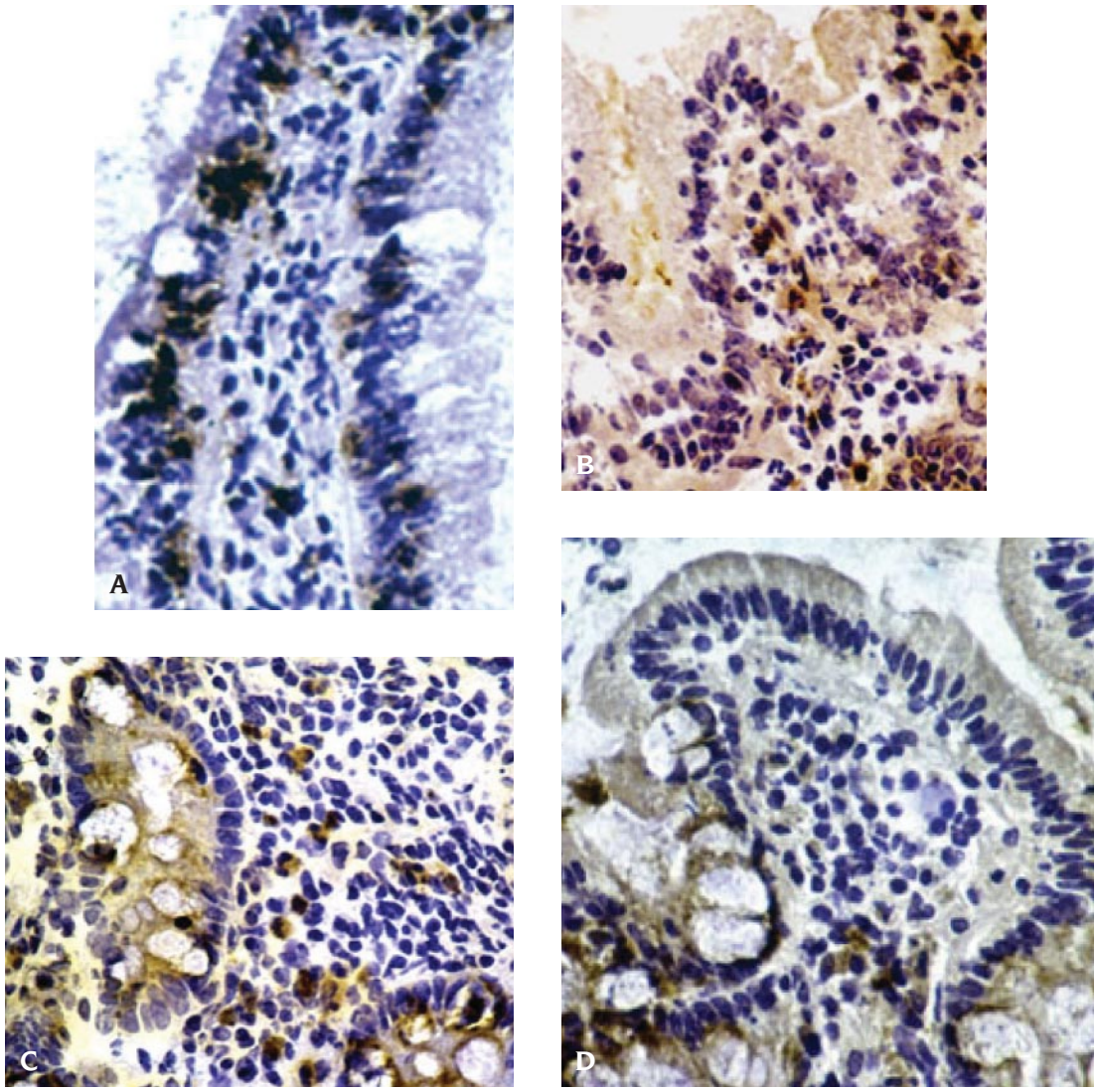

FIGURE 18-7 Immunohistochemistry of mucosal specimens from Gambian children.[78] Reproduced with permission from Lippincott Williams & Wilkins. *A*, High density of γδ intraepithelial lymphocytes despite normal villous architecture in a marasmic child. *B*, Tumor necrosis factor-α immunoreactive cells within the lamina propria in a marasmic child. *C*, Transforming growth factor (TGF)-β+ cells in the lamina propria of a child with failure to thrive but not marasmus. TGF-β expression is also seen in the epithelium. *D*, Contrasting reduction of mucosal and epithelial TGF-β+ cell density in a marasmic child.

18-7A). There was no correlation between permeability, morphometric indices of small bowel architecture, or number of intraepithelial lymphocytes and nutritional status.

Although a wide variation was observed, compared with the UK controls, the median density of cells in the lamina propria in the Gambian children was 4 to 5 times higher for CD3+ and 15 to 30 times higher for CD25+ cells. Activation of the epithelium was evidenced by increased expression of perforin by cytotoxic lymphocytes and human leukocyte antigen (HLA)-DR by crypt cells. The numbers of B cells were increased two- to threefold compared with the UK controls, with an even greater increase in mature B cells. The density of mucosal cytokine-immunoreactive cells was greater in Gambian than in UK children for both proinflammatory (interferon [IFN]-γ and tumor necrosis factor-α) (Figure 18-7B) and putative regulatory (interleukin-10, transforming growth factor [TGF]-β) cytokines (Figure 18-7C). However, the density of TGF-β–producing cells fell as nutritional status worsened, whereas that of proinflammatory cytokine-producing cells remained unchanged (Figure 18-7D). These findings suggest a chronic cell-mediated enteropathy, similar to that in celiac disease, that did not appear to be caused by specific gut pathogens.

The presence of an enteropathy in Gambian children of different nutritional states, with a shift toward greater proinflammatory responses in the most malnourished, suggests that the enteropathy of severe malnutrition may be a continuum of that seen in "tropical" or "environmental" enteropathy.

These findings in Gambian children are broadly similar to the findings of a study of Zambian and black and white South African adults investigated for dyspepsia but without other systemic or gastrointestinal illness.[79] Living conditions for the Zambians were considered to be worse than those for the South Africans. Mean body mass index and serum albumin was significantly lower in the Zambians than in the South Africans, but none were overtly malnourished. In mucosal biopsies, compared with the South Africans, the Zambians had significantly decreased villous height, increased crypt depth, and increased crypt mitotic count. Increased mucosal T-cell activation in the Zambians was evidenced by increased numbers of cells expressing CD69 and HLA-DR.

Intestinal Permeability: Severe Malnutrition

Markedly reduced absorption of D-xylose, consistent with reduced mucosal surface area, was observed in malnourished South African,[80] Indian,[25] Ethiopian,[22] Guatemalan,[34] and Brazilian[26] children. Absorption improved with clinical recovery.

Differential absorption of different-sized sugar molecules to assess simultaneously mucosal surface area and leakiness has been used extensively in studies of malnourished children in both community and hospital settings. Several studies of Gambian children have shown that decreased surface area and increased leakiness are associated with worsening nutritional status. Behrens and colleagues reported a mean (± 2 SD) urinary lactulose-to-mannitol (L:M) ratio of 1.3 (0.2–13) in repeated tests done in children with marasmus compared with 0.42 (0.2–1.4) in well children living in an urban environment.[81] L:M ratios were even higher in 15 children with chronic diarrhea (2.85 [0.2–10.4]). Ratios improved with weight gain and recovery from diarrhea. In the malnourished children with persistent diarrhea reported by Sullivan and colleagues, the mean (± SD) L:M ratio on admission was 0.66 (± 0.36).[82] Mannitol absorption improved slowly but progressively during treatment, suggesting some increase in mucosal surface area, but a marked increased recovery of lactulose after treatment for 3 to 4 weeks suggested persistence of abnormal mucosal leakiness.

Brewster and colleagues studied 149 Malawian children with kwashiorkor on admission and during inpatient rehabilitation, of whom one-third were likely to have had HIV infection.[83] Lactulose-to-rhamnose ratios were much higher on admission (geometric mean 0.17 [95% CI 0.15–0.20]) than those in hospital controls (0.07 [0.06–0.09]) because of decreased rhamnose absorption in the cases. Abnormal permeability was associated with oliguria, sepsis, diarrhea, wasting, young age, and death during admission. In logistic regression analysis, diarrhea and death were associated independently with both decreased absorption and increased leakiness, whereas wasting was associated with decreased absorption only. The association between increased permeability and death suggested that sepsis may have been caused by translocated bacteria from the gut. In survivors, permeability improved little despite clinical recovery and, 3 to 4 weeks later, remained higher than that of local controls, suggesting impaired intestinal cell renewal after enteric infection and malnutrition.

Permeability of the mucosa to nondegraded proteins, assessed by permeability of jejunal explants to horseradish peroxidase in Algerian children with marasmus, marasmic kwashiorkor, and kwashiorkor, was markedly increased on admission.[72] Permeability was lower during clinical recovery but remained abnormal. This finding is consistent with the increased serum antibodies to several food proteins in malnutrition, but whether immune responses to food antigens are involved in the pathogenesis of enteropathy remains unclear.[84]

Intestinal Permeability: Community Studies

In keeping with histologic evidence of enteropathy, noninvasive tests of mucosal permeability are frequently abnormal in children living in the community. D-Xylose absorption was markedly decreased in infants living in a Brazilian slum, most of whom were moderately underweight.[76] Lunn reported that the mean (SD) L:M ratio in infants living in Gambian villages was 0.38 (0.30) compared with 0.12 (0.09) in matched UK control infants.[85] Tests were repeated frequently during the first year of life in 119 infants and correlated with growth. By UK standards, L:M ratios in the Gambian infants were abnormal in 76% of tests. In regression analysis, abnormal L:M ratios accounted for about 40% of growth faltering for both weight and length gains.

A recent study of gut permeability in older children and adults in The Gambia showed that mannitol recovery was always at least half of expected UK values and did not improve with age.[86] However, lactulose recovery improved progressively to fall into the UK range from the age of 10 years. L:M ratios showed within-subject correlation over time, suggesting long-term persistence of enteropathy within individuals. A significant correlation between both L:M ratio and lactulose recovery and height for age z-score was present during both childhood and adult life, suggesting that enteropathy may adversely affect growth both in childhood and during puberty.

LARGE INTESTINE

Sigmoidoscopy in South African children with kwashiorkor showed increased vascularity of the rectal mucosa.[87] On microscopy, there was mild atrophy of epithelial cells, which had a flattened surface and displaced nuclei. Goblet cells were numerous in crypts but reduced on the luminal surface. Polymorphonuclear leukocytes were noticeable in the surface epithelium, and the number of plasma cells increased throughout the lamina propria. The numbers of lymphocytes and macrophages appeared normal. Mucosal histology returned to normal in most cases after 3 to 4 weeks of treatment, although the plasma cell infiltration persisted. In a study of 16 moderately to severely underweight Brazilian infants, colitis was present in 10, and, of these, only 6 had an enteropathogen isolated from the stools.[73] As with inflammation in the small bowel, colitis appears to be a feature of PEM even in the absence of gut infection and is likely to contribute to diarrhea.

GASTROINTESTINAL FLORA

Increased numbers of a wide variety of bacteria in gastric juice have been reported in malnourished Indonesian,[88] Brazilian,[89] and Bangladeshi children.[61] Large numbers of bacteria were found in 13 children with marasmic kwashiorkor living in poor areas of Guatemala City, but 3 of 4 normal controls also had high numbers of streptococci in gastric juice.[56] Bacterial overgrowth was associated with increased gastric pH in underweight Brazilian children with chronic diarrhea (57% had pH > 4), but, interestingly, hypochlorhydria was equally common in better nourished breastfed controls who did not have increased gastric microbial contamination.[89] Similarly, gram-negative bacterial colonization of gastric juice (> 100 colony-forming units/mL) was associated with reduced gastric acid output and increased pH in the Bangladeshi series, but colonization was not observed in any of 20 controls despite hypochlorhydria in many. [61] This suggests that other factors, in addition to gastric pH, determine susceptibility to bacterial colonization of the stomach. In malnourished children, the numbers of microorganisms fell with clinical recovery.[56,89]

In addition to bacterial contamination, large numbers of *Candida* sp (up to 10^9/mL) in gastric juice were found in malnourished Australian aboriginal and Indonesian children[90] and in malnourished Guatemalan children with diarrhea.[56] Whether yeasts contribute to the gut changes in malnutrition remains unclear.

Increased bacterial colonization of the small bowel has been reported frequently in malnourished children, and, as in gastric juice, a wide variety of organisms have been isolated. However, several authors have noted that similar bacterial colonization also occurs in children living in the same environment as malnourished children. In a series of hospitalized, underweight Brazilian children,[73] 11 of 16 had > 10^4 bacterial colonies/mL in jejunal aspirates, including enteropathogenic strains of *Escherichia coli*, *Proteus*, *Enterobacter*, *Pseudomonas*, and *Klebsiella*. Bacterial colonization was associated with a mucus-fibrinoid pseudomembrane over the luminal surface but not with other mucosal abnormalities. In the later study of mostly moderately underweight infants living in a Brazilian slum, colonization of jejunal juice with colonic flora varied from 10^2 to 10^9 colonies/mL, with 5 of 40 children having > 10^4/mL.[76] In the Guatemalan series,[56] apart from greater numbers of *Enterobacteriaceae* in the cases, bacterial colonization of the small bowel was similar in cases and controls. Between 10^3 and 10^7 bacteria/mL, mainly streptococci, were present in three of four controls. In Gambian children with a range of nutritional deficiency, 22 of 25 had > 10^5/mL facultative anaerobes in jejunal juice, with some children having counts > 10^{10}/mL.[91] Most children were colonized with three to four types of organisms, mainly *E. coli,* bacteroides, and enterococci, and counts were higher in those with chronic diarrhea. Although no controls were tested in this study of hospitalized children, the findings can be compared with those of a later study carried out in 37 young Gambian children living in rural villages.[62] About half of these infants had > 10^5 organisms/mL in jejunal juice.

Omoike and Abiodun, working in Benin City, Nigeria, reported mean bacterial counts ranging between 10^3 and 10^9/mL among 30 malnourished children.[92] A wide range of organisms was identified, including *Enterobacteriaceae*, *Bacteroides*, and *Candida*. In contrast to the previous studies, bacterial counts were significantly lower in 11 well-nourished hospital controls, in 2 of whom duodenal juice was sterile, who lived in the same socioeconomic environment. In underweight Australian aboriginal children with chronic diarrhea, mean small intestinal bacterial counts were 5×10^6/mL in those receiving antibiotics and 2×10^6/mL in those not receiving antibiotics compared with 2×10^3/mL in Caucasian controls.[93] Bacteria were of the oral and fecal type, but anaerobes were rarely isolated. In the Indonesian series, the mean microbial count was 7.8×10^7/mL, consisting mainly of gram-positive cocci, enterobacteria, and streptococci, with gram-negative organisms also identified in many children.[88] In the series of Australian and Indonesian children, there was marked contamination of intestinal aspirates with *Candida* species (10^4 to 10^8/mL) compared with Caucasian controls.[90] In the studies in Australia and Indonesia, less microbial contamination of the gut in the controls may have been explained by better living conditions in this group.

Applying the new molecular methods[94] to complement the findings of existing studies and better define the intestinal microflora in both malnourished and healthy children in developing countries is a priority. The marked increased bacterial contamination of the upper gut in malnourished children is likely to contribute to malabsorption, for example, through the deconjugation of bile salts. In addition, loss of immune tolerance to intestinal bacteria is implicated in the pathogenesis of the T cell–mediated enteropathy of inflammatory bowel disease.[95] More research is needed on the role of bacterial contamination of the gut in causing environmental enteropathy and the enteropathy in severely malnourished children.

MICRONUTRIENT DEFICIENCY

The role of two key micronutrients, vitamin A and zinc, in childhood malnutrition, morbidity, and mortality has been reviewed.[96,97] Both are an essential part of the nutritional rehabilitation of severely malnourished children.[58]

Vitamin A

Although there is good evidence that vitamin A supplementation reduces child mortality in some situations, its effect on specific infections, including diarrhea, is less clear.[96,98] Based on vitamin A's role in maintaining mucosal integrity and an observation in Gambian infants that mucosal integrity is least impaired at times of the year when dietary vitamin A is abundant,[99] randomized intervention studies were done in 144 hospitalized and 80 rural infants in India.[100] Infants living in the community had significantly lower L:M ratios after vitamin A supplementation (16,700 IU weekly for 8 weeks) than those receiving placebo, although the differences were small. Infants hospitalized with diarrhea or respiratory infections received 200,000 IU of vitamin A, either at admission or discharge, or placebo. The mean L:M ratio fell in all groups but was significantly lower at 10 and 30 days following discharge in the treated groups. Data on the absorption of the individual sugars were not reported.

Vitamin A supplementation during pregnancy and at delivery of HIV-positive South African mothers did not affect L:M ratios in non–HIV-infected infants.[101] However, among infants who themselves acquired HIV infection, those of supplemented mothers maintained significantly lower L:M ratios over the first 14 weeks of life compared with those of unsupplemented mothers. This effect was due to increased absorption of lactulose in the infants of the control mothers, whereas mannitol absorption in the two groups was similar. The authors concluded that the effect of vitamin A in reducing mucosal permeability may help to counter growth faltering in HIV-infected infants.

Zinc

Like vitamin A, zinc is considered essential for normal immune function and protection against infections.[102] Zinc supplementation prevents episodes of diarrhea and reduces the duration and severity of acute and persistent diarrhea, with some evidence of a greater beneficial effect in malnourished children.[97,103]

Roy and colleagues studied the effects of zinc supplementation (5 mg/kg/d elemental zinc for 2 weeks) on intestinal integrity in Bangladeshi children with acute and persistent diarrhea.[104] Many children had low serum vitamin A, and all received vitamin A supplements. Although mannitol absorption remained unchanged, supplementation reduced lactulose absorption in both conditions. The effects were greatest in the most undernourished children and those with hypozincemia at recruitment.

In a randomized community study of 110 Gambian children aged 0.5 to 2.3 years, zinc supplementation (70 mg twice weekly for 1.25 years) resulted in a small increase in mid–upper arm circumference but no difference in weight gain.[105] Although the mean L:M ratios were not affected by the supplement, lactulose absorption was significantly decreased in the supplemented group.

Supplementation with both vitamin A and zinc appears to have beneficial effects on the integrity of the intestinal mucosa in children. However, further studies are needed to determine the clinical importance of these effects.

CONCLUSIONS

Malnutrition remains a public health problem of enormous importance. In economically poor countries, growth faltering is the norm, and underweight is the leading risk factor for morbidity and mortality. Severe malnutrition is common in children admitted to hospital, and many of these children have severe abnormalities of the gastrointestinal system, including a severe enteropathy. Despite detailed WHO management guidelines, case fatality often remains high, and translocation of bacteria from the gut through a leaky mucosa may contribute to deaths from sepsis. The persistence of the enteropathy in many of the survivors is likely to contribute significantly to their poor longer-term outcome. However, the greatest impact on child survival will be achieved by targeting mild to moderate malnutrition in children living in the community. Both histologic studies of intestinal mucosa and the measurement of mucosal permeability reveal that significant enteropathy, sufficient to impair growth, is common in apparently normal children living in the community. This environmental enteropathy may be a continuum of that seen in severely malnourished children.

Specific interventions to prevent or ameliorate enteropathy in children living in the community and to heal the gut in malnourished children are an urgent priority. Initial research suggesting that the enteropathy in Gambian children with a range of nutritional status is mediated by T cells needs to be confirmed in other locations.[78] However, this exciting finding may present new opportunities for specific interventions. In keeping with approaches to other T cell–mediated enteropathies, interventions to prevent or modify the gut bacterial overgrowth that is common in children in developing countries should be explored.

Interventions to prevent growth faltering in "normal" children living in the community will have the greatest impact on child survival. A group of leading international

experts met in Washington, DC, in 1971 under the auspices of the Committee on International Nutrition Programs and addressed a specific question: "Are there now sufficient data to justify efforts to ameliorate or prevent subclinical malabsorption as one approach to the global problem of malnutrition?"[106] We now know more about the global burden of malnutrition but are only beginning to understand the cause(s) of environmental enteropathy. The tantalizing question as to whether specific interventions to prevent or improve gut function in children living in poor circumstances in economically poor countries would improve growth and decrease morbidity and mortality remains unanswered.

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CHAPTER 19

OBESITY

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At the beginning of the twenty-first century, poverty and undernutrition have worsened in some countries, whereas overnutrition and obesity have reached epidemic levels in many others.[1,2] Currently, the increasing trend in obesity makes it the most important nutritional problem globally, and the associated medical problems account for substantial morbidity, mortality, and health care costs. The notion of "nutrition transition" has been developed to describe the unique changes in diet and energy balance that accompany patterns of economic and technological development.[3,4] Understanding the mechanisms causing obesity and associated medical problems in developed countries is essential to reverse the worldwide trend.

DEFINITION

Any definition of obesity is useful only if it predicts medical disability or complications. Because most medical complications of obesity are associated with body fat and not muscle mass, measures of obesity represent an attempt to estimate the adipose compartment. At present, there is no precise clinically practical method to measure body fat, so most methods rely on measurements of body weight as a surrogate for adiposity. Such methods are imperfect because they may misclassify a patient with an unusual proportion of fat to lean body mass, but they are inexpensive and practical for use in the clinical setting and in epidemiologic studies.

Traditionally in the United States, obesity has been defined as weight for height above the 90th percentile on the National Center for Health Statistics (NCHS) growth charts or excess weight above 120% of the median for weight given the child's age, height, and gender. More recently, the body mass index (BMI), defined as the weight of the child in kilograms divided by the height in meters squared (kg/m^2), has been established as a useful standard measure of adiposity. Although BMI does not directly measure body fat, it is typically used to evaluate adiposity in adults and has been recognized as a useful predictor of adiposity in children and adolescents, which, in turn, also predicts risks for present or future medical complications of obesity.[5] BMI in children is correlated not only with other predictors of body fat but also with blood pressure,[6,7] lipid levels,[8,9] and insulin levels.[10]

Like height and weight, BMI is not constant during childhood and adolescence, and it differs by gender. BMI growth charts are a useful way to track an individual against established standards for clinical purposes. BMI also depends on pubertal stage, reflecting disproportionate gains in fat-free compared with fat mass.[11] In addition, there is some evidence that BMI varies with ethnicity.[12] Therefore, research studies involving BMI in children should consider not only age and gender but also pubertal stage and ethnicity.

In 2000, the Centers for Disease Control and Prevention (CDC) established new growth charts using data from the NCHS in collaboration with the National Center for Chronic Disease Prevention and Health Promotion.[13] These growth charts do not include data from the past decade because of the sharp rise in BMI during that period. Recognizing that the BMI of children and adolescents tends to predict obesity and related complications in adulthood, the CDC has also suggested specific nomenclature for the pediatric age group: subjects above the 85th percentile are considered "at risk for overweight" and those above the 95th percentile are considered "overweight."[5] Use of the term "overweight" rather than "obese" reflects the fact that the weight status of the adolescent may still improve before he or she reaches adulthood; thus, the overweight adolescent may not face the medical risks conferred by the term "obesity" in adulthood.

Most industrialized countries and countries in economic transition are experiencing a trend toward increasing obesity but at different rates, so creating definitions appropriate for international use is challenging but important. Using data from six large data sets in various countries, an International Obesity Task Force (IOTF) agreed on standard cutoff points to identify degrees of overweight among children and adolescents in both developed and developing countries.[14] Like the CDC charts, these data provide age- and gender-specific cutoff points for children aged 2 to 18 years. The 85th percentile on the IOTF standard charts, which defines children and adolescents "at risk for overweight," also corresponds to a BMI of 25 kg/m^2 by age 18, the adult definition of overweight. The 95th percentile on the standard chart, defining children and adolescents as overweight, corresponds to about 30 kg/m^2 by age 18 years, the standard adult definition of obesity.

EPIDEMIOLOGY

Relevance and Significance

Presently, 1 in 5 children in the United States is at risk for overweight, and 14% of children and adolescents in the United States are overweight (above the 85th and 95th percentiles for age and gender, respectively, based on the new CDC standards). Since the 1960s, the prevalence of obesity in children and adolescents has tripled.[15] Similar but more gradual trends are seen worldwide. Determining the specific causes of this rapid increase in rates of obesity is clearly essential, yet remarkably complex. Both genetic and environmental factors have been shown to contribute significantly to this problem. In general, genetic factors explain a large part of the variation of body weight within a given population in a common environment, whereas environmental factors tend to explain changes in obesity over time in that population. The study of Pima Indians provides an important example of the interaction between environmental and genetic factors.[16] The Pima Indians who live in the southwestern United States are predisposed to obesity and diabetes, and these traits assort in patterns indicating genetic inheritance. The genetically similar Pima living as subsistence farmers in Mexico are substantially less obese. Genetic factors clearly explain a large part of the obesity among Pima Indians in this country, whereas environmental factors explain the dramatic difference in rates of obesity between the two Pima populations.

Epidemiologists have used cohort studies and case-control designs to determine which environmental factors may contribute to obesity. Such studies have pointed to dietary trends, sedentary lifestyle, decreases in structured physical activity, psychosocial stressors, and cultural trends as likely contributors to the obesity epidemic.[3] A number of dietary factors have been proposed to play important roles. These include the easy availability, high caloric content, and strong marketing techniques of the fast-food industry; general trends toward consumption of foods that are highly processed and contain high carbohydrates and/or total calories (including sugary beverages); and decreased consumption of fiber and low-density foods. Other factors include decreases in structured physical activity, particularly for children, and decreasing lifestyle activity (occupations and transportation require less movement than in the past) and increasing sedentary activities (particularly television viewing and computer use). However, it is important to note that, to date, no single factor among these has been shown to play a pivotal role in the increasing prevalence of obesity.

Obesity and Related Complications

A large body of evidence supports an association between obesity and important risk factors for cardiac disease and type 2 diabetes. Hyperinsulinemia, dyslipidemia, obesity, and hypertension often cluster together and are termed the "metabolic syndrome," "syndrome X," or "insulin resistance syndrome."[17,18] More recently, these findings have also been shown to be closely associated with nonalcoholic fatty liver disease (NAFLD), such that fatty infiltration of the liver is now often considered part of the metabolic syndrome.[19]

The mechanisms underlying the association between these endocrine abnormalities and disease affecting diverse organ systems are the subject of ongoing research. There is support for the concept that an increased ratio of visceral to subcutaneous adipose tissue, perhaps acting through adipocyte-derived hormones such as resistin and leptin and through substrates such as circulating fats, leads to insulin resistance and high circulating levels of insulin.[20,21] Many adults with obesity display all of the elements of the metabolic syndrome, but there are some striking exceptions even among those with severe obesity. For example, a multicenter study using clamp techniques in a group of obese adults showed that 26% of participants aged 18 to 85 years with a BMI > 25 kg/m^2 and 60% of those with a BMI > 35 kg/m^2 were insulin resistant. The frequency of hyperinsulinemia was 41% in participants with a BMI > 25 kg/m^2 and 77% in participants with a BMI > 35 kg/m^2.[22]

Similar clustering patterns have been found in children. Studies in children have shown a relationship among fasting insulin and lipids,[23,24] blood pressure,[25–28] weight,[29] and BMI.[30–32] As in adults, body fat distribution is also correlated with cardiovascular risk factors.[33,34] Berenson and colleagues showed that blood pressure, lipid levels, and BMI were positively correlated with aortic and coronary atherosclerosis at autopsy in both children and adults (2–34 years), suggesting that the metabolic syndrome starts before adulthood.[35] Using data from the Bogalusa study of cardiovascular risk factors, Tershakovec and colleagues showed that the expression of the hypercholesterolemia in children precedes the expression of increased body fat and that insulin and blood pressure subsequently rise as the children grow older and body fat increases.[36,37]

Although there are many similarities between the findings of the metabolic syndrome in children and adults, it is important to recognize that children have a different hormonal milieu than adults, especially during puberty. All children become more insulin resistant at the time of puberty compared with either before or after puberty.[38] Increased body fat and BMI correlate strongly with fasting insulin levels and insulin resistance and have been proposed as potential mediators of the pubertal changes in insulin resistance.[39–42] However, insulin resistance can also occur during puberty in the absence of changes in BMI, coinciding with a period of rapid growth during puberty.[40]

In addition to hyperinsulinemia associated with the metabolic syndrome, frank type 2 diabetes is becoming increasingly common in children. The Third National Health and Nutrition Examination Survey (NHANES III) estimated a prevalence rate of 0.13% for type 2 diabetes and of 1.76% for impaired glucose tolerance among a representative sample of US adolescents.[43] Obesity increases the risk for diabetes substantially: in a study of obese adolescents, 4% had silent type 2 diabetes and 25% had impaired glucose tolerance.[44] The prevalence of type 2 diabetes is particularly high in children of non-European origins.[43] It has been estimated to be 3.6% among adolescent North American Indians[45] and 5.9% among Pima Indian adolescents.[46] In Ohio, type 2 diabetes accounted for 33% of all cases of diabetes among African American and Cau-

casian adolescents, representing a 10-fold increase in the incidence of type 2 diabetes this past decade in Cincinnati.[47] Risk factors for type 2 diabetes include obesity, a family history of diabetes, female gender, acanthosis nigricans, and nonwhite ethnicity.

NAFLD represents a spectrum of liver disease associated with obesity in children and adults.[48,49] Microscopic analysis of fatty liver disease reveals either accumulation of fat alone (steatosis) or fat accompanied by inflammation and fibrosis (steatohepatitis); the latter findings are generally termed nonalcoholic steatohepatitis (NASH). Up to 75% of adults with obesity have NAFLD,[19] and these rates are even higher in patients with severe obesity. Only a minority of patients with steatosis develop progressive liver disease, but the incidence of severe NASH is increasing in parallel with increasing rates of obesity. Obesity and diabetes are the strongest predictors of fibrosis on biopsy, and fibrosis and ballooning degeneration are the strongest predictors of disease progression.[50] NASH has become one of the most common causes of cirrhosis and liver failure and is the third leading indication for liver transplant in adults. Strauss and colleagues estimated that 10% of obese adolescents living in the United States may have NAFLD,[49] whereas other authors have estimated rates up to 25% among children and adolescents evaluated in obesity programs.[48,50–52] Although rare, several cases of cirrhosis associated with NASH in obese children have been described.[19,53,54]

Liver biopsies are considered the gold standard to evaluate NAFLD and are typically performed when liver enzymes are elevated and when the history and serologic testing exclude other common causes of aminotransferase elevation, such as viral hepatitis and alcohol-related hepatitis. Because liver biopsy is costly and is associated with appreciable morbidity, many gastroenterologists biopsy the liver in patients with suspected NAFLD only when alanine transaminase (ALT) levels are persistently at least twice the upper limits of normal.[55] NAFLD is frequently associated with mild (two- to threefold) elevations in aminotransferases, particularly ALT, but these measures are not good predictors of disease severity.[53,54,56] Liver imaging is a more sensitive way to detect fatty liver but also does not predict disease severity. A strong correlation has been shown between ALT > 30 U/L and fatty liver on ultrasonography among obese children,[57] and in a recent study of obese children and hepatic magnetic resonance imaging, all subjects (n = 7/7) with a low fat fraction (≤ 18%) had normal ALT values, but 92% (n = 12/13) of the subjects with a high fat fraction (> 18%) had elevated ALT values.[58]

Although NAFLD is strongly associated with obesity, it appears that this relationship may depend on insulin resistance rather than on the obesity itself. The prevalence of elevated ALT increases with BMI, but this relationship is independently associated with measures of insulin resistance and not with degree of obesity.[50,59] For example, increased insulin levels are associated with elevated ALT levels in obese children,[60] obese adults,[59,61] and lean adults with evidence of insulin resistance.[62] Indeed, animals and humans with lipodystrophy exhibit severe insulin resistance and steatohepatitis despite a paucity of subcutaneous fat.

Epidemiologic observations thus suggest that the pathogenesis of NAFLD involves insulin resistance. Recent laboratory investigations suggest that inflammatory cytokines may, in turn, be important determinants of the observed insulin resistance. Tumor necrosis factor-α (TNF-α) is known to mediate insulin resistance through Jun N-terminal kinase pathways, and animals without TNF-α activity are protected from insulin resistance.[63] Furthermore, treatment of ob/ob(–/–) mice with anti–TNF-α antibodies reverses both steatosis and steatohepatitis, suggesting that TNF-α may be an important stimulator of fat deposition and hepatic inflammation.[64] Clamp studies have suggested that insulin resistance in muscle and liver increases the delivery of free fatty acids to the liver and contributes to the development of steatosis. Identifying the molecular mechanisms underlying steatohepatitis and explaining its association with insulin signaling dysfunction will be helpful in developing specific treatments for NAFLD.

No good treatments for NAFLD have been established to date, but investigators have explored several possible targets. Weight loss is usually recommended,[19,65] but the efficacy of this approach is difficult to measure with intervention studies because of the difficulty achieving significant and sustained weight loss in a study population. Nonetheless, the association between obesity and NAFLD provides some justification for this approach. In addition, there is some evidence that obesity can potentiate other insults to the liver, such as alcohol and hepatitis C virus infection, suggesting that weight control and resolution of steatosis may make the liver less susceptible to such insults.[66] Gastric weight loss surgery is the most reliable way to achieve long-term weight control, and one study suggests that this approach is effective in reducing hepatic steatosis.[67] It remains unclear, however, how this type of rapid weight loss affects the inflammatory component of NAFLD. Indeed, rapid weight loss owing to caloric restriction can itself cause steatohepatitis, raising the question as to whether weight loss surgery might exacerbate steatohepatitis in some cases. Other treatments have been explored that attempt to reduce the production of oxidized lipids that are thought to induce inflammation; these include antioxidants such as vitamin E[68,69] and the lipid-lowering agent atorvastatin[70]; both showed some promise in small uncontrolled trials.[68–70] Most such trials are limited by small sample size, nonrandomized design, and/or a lack of histologic outcomes. Currently, large randomized trials are in progress to evaluate the effects of insulin-sensitizing medications (thiazolidinediones and metformin) in the treatment of fatty liver disease.

Other consequences of obesity seen in childhood are sleep apnea, cholelithiasis, pseudotumor cerebri, gastroesophageal reflux disease, polycystic ovary disease, and orthopedic problems, including Blount disease and slipped capital femoral epiphysis (Table 19-1). Sleep apnea can cause significant hypoxia, heart strain, and reduced daytime functioning and is probably underdiagnosed in pediatric populations. Screening for these and other medical problems is discussed in the medical assess-

TABLE 19-1 PREVALENCE OF DISEASES ASSOCIATED WITH OBESITY IN CHILDREN

DISEASE	PREVALENCE (%)	STUDY POPULATION
ENDOCRINE		
Type 2 diabetes	0.13	Community, 10–19 yr; n = 2,867[204]
Fasting blood sugar > 110 mg/dL	1.76	
Impaired glucose tolerance	21–25	Obesity clinic, 4–18 yr; n = 167[42]
Polycystic ovaries	45 of oligomenorrheic girls 9 of girls with regular menses	Community, all ninth grade girls (n = 2,249)[205]
GASTROINTESTINAL		
Gallstones	0.6	Pediatric inpatients[175]
Fatty liver (elevated aminotransferases)	10	Community, obese, 12–18 yr (n = 332)[206]
Fatty liver (elevated aminotransferases)	20	Obesity clinic, 2–18 yr (n = 72)[207]
GERD	22	Community, 14–17 yr (n = 449)[208]
Constipation	25	Obesity clinic, 2–18 yr (n = 80)[209]
Encopresis	15	
ORTHOPEDIC		
SCFE	0.01 (50–6% of patients are obese)	Community[210]
Blount disease	Prevalence not well established	
RESPIRATORY		
Sleep apnea	1.6 (4.5-fold higher risk if obese)	Community; children[211]
Sleep apnea	5	Obesity clinic[212]
Asthma	8.9 nonobese 14.9 obese	Community[213]

GERD = gastroesophageal reflux disease; SCFE = slipped capital femoral epiphysis.

ment section below. In addition to these complications, obesity in adults is associated with debilitating or life-threatening degenerative problems (axial arthritis and cardiovascular and cerebrovascular disease),[71,72] as well as with increased risk of certain neoplasias (breast, ovarian, prostate, and colon cancers).[73]

TRACKING

"Tracking" describes the risk for a disease state persisting over time. In the case of obesity, there is a moderate risk of childhood obesity persisting into adulthood, and that risk increases if the child stays overweight as he grows older (Figure 19-1). Moreover, the child's risk for obesity in adulthood also depends on the weight status of his parents. Whitaker and colleagues showed that the rate of obesity in adulthood ranged from 8% for children aged 1 and 2 years old without obese parents to 79% for adolescents aged 10 to 14 years old with at least one obese parent.[74] Before 3 years of age, the primary predictor of obesity in adulthood was the parents' obesity status, and the child's obesity status was not an important indicator of the risk of adult obesity. In contrast, after 7 years of age, the child's own obesity status became the more important predictor of his risk for obesity in adulthood.

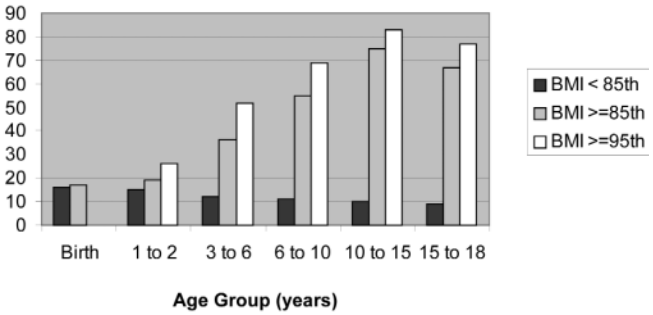

FIGURE 19-1 Percentage of children who will become obese adults. Adapted from Whitaker WC et al.[74] BMI = body mass index.

Obesity in childhood thus confers a higher risk of obesity in adulthood. Moreover, adults who were obese in childhood have a greater risk of morbidity and mortality, independent of their BMI in adulthood, family history of cardiovascular diseases or cancer, and smoking.[75,76] In the Harvard Growth Study, overweight adolescents were shown to have an increased risk for developing obesity-related medical problems in adulthood, including cardiovascular disease and diabetes, compared with adults with a more recent onset of obesity.[75] Likewise, Sinaiko and colleagues showed that weight gain during childhood and adolescence predicts cardiovascular risk in young adults.[30]

HERITABLE FACTORS

Studies of twins and adoptees provide useful estimates of the role of heritable factors in determining an individual's body weight (see Bouchard's 1997 summary of relevant articles on heritability[77]). Adoption studies tend to generate the lowest heritability estimates (30%), whereas twin studies provide the highest heritability estimates (70%). The variability in these estimates of heritability depends in part on definitions of obesity: more severe obesity tends to have a greater heritability factor than lesser variations in BMI.[78] Observations of a genetic contribution to obesity gave rise to the "thrifty genotype hypothesis," which posits that genes predisposing the individual to energy conservation were preserved as a survival characteristic in former times of famine but become a liability in environments with plentiful food and low required physical activity.[79] Possible mechanisms through which genetic polymorphisms can translate to differences in body weight regulation are discussed below.

Fetal Programming

Hales and Barker showed that poor fetal growth is associated with an increased risk for type 2 diabetes and other elements of the metabolic syndrome and proposed that poor nutrition early in life imprints permanent changes in glucose and insulin metabolism.[80] Interestingly, many more recent studies have also shown clear associations between high birth weight and later obesity,[81,82] suggesting that newborns at both ends of the weight spectrum are at risk for obesity-associated disease. This concept of "fetal programming" has now been supported by numerous studies in other human populations and in animals.[83] The hypothesis has also been extended to include the possibility that postgestational influences can participate to create a lifelong metabolic phenotype.[84] For example, a study of adults in Finland showed that the development of type 2 diabetes mellitus was associated with the combination of low birth weight followed by accelerated gain in height and weight during childhood and with high maternal BMI.[85] Similarly, a study of adults in England showed that accelerated weight gain in early childhood added to the effect of low birth weight on the risk of high blood pressure in adulthood.[86] Indeed, populations in transition from conditions of low to high nutrition may be at the greatest risk for such obesity-related complications because of the combination of fetal undernutrition and childhood overnutrition.[87]

A few studies specifically address whether the observed associations between birth weight and future risks for obesity can be directly related to the intrauterine environment rather than to genetics, distinguishing the concept of a "thrifty phenotype" from that of the "thrifty genotype." In a study of the effects of wartime famine in the Netherlands, infants exposed to famine in utero had higher rates of obesity and diabetes in adulthood compared with a genetically similar cohort not exposed to famine, and this effect was largely independent of birth weight.[88] Furthermore, the timing of the famine exposure during gestation appeared to have important effects because fetuses exposed to famine during the first trimester of gestation were more severely affected than those exposed later in gestation. These observations are strongly supported by animal studies, which also demonstrate lasting effects of intrauterine and postpartum nutrition.[89] The mechanisms underlying the observed associations, including the contributions of maternal hyperglycemia, insulinemia, and postnatal growth to the development of later complications, are an important subject for future studies.

BIOLOGY

Regulation of Body Weight

Both animals and humans have a strong tendency to maintain a stable body weight over time owing to a close but sometimes imperfect matching of energy intake with energy expenditure. Animal studies in which energy intake is manipulated reveal powerful influences from homeostatic mechanisms defending body weight.[90] Similarly, the poor long-term results of weight reduction therapies in humans (about 95% of adults regain all weight after dieting) suggest that there are mechanisms that defend a highly individualized "set point" for body weight. When an individual has a heritable or acquired susceptibility to positive energy balance, superimposed on these native homeostatic mechanisms, he or she has a tendency to become obese.

Animal models of obesity have been invaluable in establishing an understanding of the complex mechanisms regulating body weight. Our growing understanding of these pathways is likely to lead to better-targeted interventions, both pharmacologic and behavioral. This is an area of vigorous ongoing research; the major elements of the pathways as we currently understand them are outlined below.

Afferent Pathways. Circulating insulin, which reflects recent nutrient intake and metabolic demands, is an important regulator of nutrient partitioning in peripheral tissues and also communicates to centers regulating appetite in the brain. Peptides (such as cholecystokinin) secreted by the gastrointestinal tract in response to intraluminal nutrients and plasma concentrations of the macronutrients themselves also provide independent signals to the central nervous system, affecting short-term appetite and satiety.[91]

Ghrelin is a peptide secreted by the stomach that is an important short-term mediator of appetite. Its name is derived from its ability to stimulate growth hormone release from the pituitary, but it also stimulates appetite through specific receptors in the ventromedial hypothalamus. Ghrelin is released from the stomach during periods of fasting and is suppressed by nutrient administration.[92] The specific stimulants of ghrelin release are not clear, but volumetric stretching of the stomach wall has no effect. A recent report shows that ghrelin is suppressed in humans who lose weight after gastric bypass surgery but not in the setting of weight loss through caloric restriction.[93] These findings suggest that ghrelin may be the mechanism for the appetite-suppressing effect and high success rates of gastric weight loss surgery but are yet to be confirmed.

Leptin is an important regulator of body fat, first identified in 1994 through studies of the leptin-deficient obese mouse.[94] It is produced primarily in adipose tissue and provides feedback to specific receptors in the ventromedial hypothalamus, an important center for regulation of appetite and energy expenditure. The leptin signal decreases appetite, increases both voluntary and resting energy expenditure, permits fertility,[95] and even activates central "reward" pathways that may, in turn, affect appetitive behavior.[96] The leptin-deficient animal or human therefore has hyperphagia and decreased thermogenesis and physical activity, all of which are reversible by leptin administration.

The central mechanisms through which leptin exerts these diverse effects have been partly elucidated through studies in other animal models. Animals with defects in the leptin receptor (the diabetes mouse and Zucker rat) predictably have phenotypes indistinguishable from leptin deficiency itself.

Central Nervous System. The leptin signal activates a network of regulatory neuropeptides in the central nervous system. The anatomy of this network is the subject of

ongoing research, but many important elements have been described. Some of the pathways are orexigenic (favoring energy intake), whereas others are anorexigenic (inhibiting energy intake). In general, leptin-generated signals tend to inhibit the orexigenic pathways and to stimulate the anorexigenic pathways, thus decreasing appetite. The network also participates in leptin's effects on the reproductive system and energy expenditure (Figure 19-2).

The melanocortin pathway is among the most important links downstream of leptin. Leptin appears to directly increase expression of the pro-opiomelanocortin (POMC) gene, which is cleaved by prohormone convertase to α-melanocyte stimulating hormone (α-MSH),[97] as well as β-endorphin. α-MSH, in turn, stimulates the melanocortin-4 (MC4) receptor,[98] a potent inhibitory influence on the lateral hypothalamus. Meanwhile, leptin also directly inhibits the expression of agouti-related protein, which opposes α-MSH action at the melanocortin receptors.[98] The melanocortin pathway appears to be a particularly important regulator of body weight homeostasis because it exhibits less redundancy than other leptin-related pathways; an interruption in the melanocortin pathway can produce severe obesity, as seen in the agouti yellow mouse.

Leptin also decreases appetite through melanocortin-independent pathways. It inhibits the expression of the orexigenic agent neuropeptide Y, while increasing expression of cocaine- and amphetamine-related transcript (CART). In addition to decreasing appetite, CART has actions on the paraventricular nucleus of the hypothalamus and spinal sympathetic preganglionic neurons, where it affects energy expenditure via the autonomic nervous system (see Figure 19-2).[99]

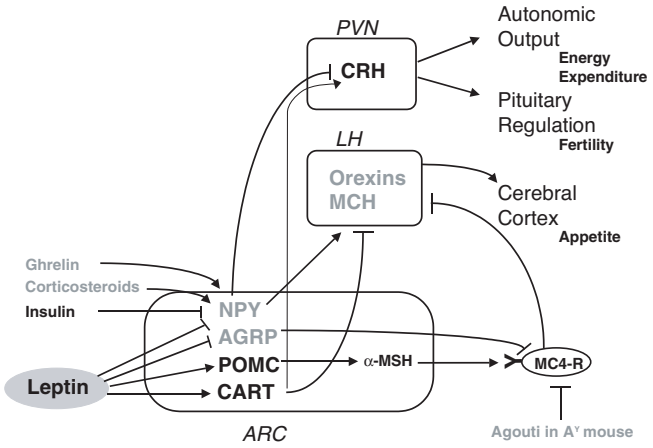

FIGURE 19-2 Central nervous system pathways regulating appetite and energy metabolism. Leptin positively regulates pro-opiomelanocortin (POMC) while negatively regulating agouti-related protein (AGRP)-releasing neurons in the arcuate nucleus (ARC) of the hypothalamus. POMC is a precursor of α-melanocyte-stimulating hormone (α-MSH), which is an antagonist at the MC4 receptor (MC4-R). AGRP and agouti protein are antagonists at MC4-R. The MC4-R pathway negatively regulates appetite, perhaps acting through appetite-stimulating neuropeptides in the lateral hypothalamus (LH), including melanin-concentrating hormone (MCH) and the orexins. Meanwhile, leptin has some actions that are independent of the POMC pathway, including negatively regulating neuropeptide Y (NPY), which is itself a potent appetite stimulant. Ghrelin also appears to act to stimulate appetite through the NPY pathway.[203] NPY also influences autonomic and pituitary output through the paraventricular nucleus (PVN), acting in part through corticotropin-releasing hormone (CRH).

Efferent Pathways. This leptin-responsive network of neuropeptides in the hypothalamus acts on effector pathways in the cerebral cortex, pituitary-adrenal axis, and autonomic nervous system. Most signals regulating appetite and satiety meet in the nucleus tractus solitarius in the medulla, where they are further modulated by afferent signals from the autonomic nervous system. The pituitary-adrenal axis mediates leptin's effects on fertility and likely also affects energy expenditure. Indeed, adrenalectomy prevents the development of obesity in most animal models.[100]

Efferent signals to regulate energy expenditure are integrated in the locus ceruleus, from which the sympathetic nervous system stimulates lipolysis in white adipose tissue and mediates processes that facilitate voluntary energy expenditure and heat generation. Genetically engineered animal models have clarified some elements of these effector pathways. Animals in which uncoupling protein-1 in brown adipose tissue is knocked out have increased body fat, decreased cold tolerance, and decreased thermogenic response to food,[101] but the related peptides, uncoupling protein-2 and -3, which are more widely expressed, are more likely to be relevant to human adiposity.[102] A knockout of the β_3-adrenergic receptor has a similar phenotype, suggesting that elements of the sympathetic nervous system are involved in increasing energy expenditure to match energy intake.[103] Parasympathetic efferent signals through the vagus nerve increase insulin secretion in the pancreatic β cells and may be a mechanism for the hyperinsulinemia in some groups of obese people.[20]

In addition to these autonomic processes, energy expenditure has a "voluntary" component (5–50% of total energy expenditure, depending on the level of exercise)[104] and a component of "fidgeting," or nonexercise activity thermogenesis (NEAT). NEAT has been proposed as a genetically determined system of protection from obesity and may also be mediated by the sympathetic nervous system.[105]

Genetics of Obesity

Exploration of the genetic determinants of body weight can be done in several ways, each offering different insight and limitations. Candidate gene approaches focus on specific genes and pathways that previous studies have shown are likely to be important in producing a phenotype. Each of the neurohormones described above, as well as their receptors and the enzymes responsible for processing, can be considered a candidate gene with potential importance in the regulation of body weight. Association studies use a case-control design to assess the association between variations in genotype and obesity phenotype. This technique lends itself to testing of several polymorphisms in candidate genes within a population; however, it is also prone to substantial false-positive and false-negative results,

depending on the sample size. Such studies are therefore most reliable if similar gene associations can be demonstrated in several different populations. Linkage studies rely on genome-wide scans of large populations to assess the strength of the association between variations in a genomic locus and the phenotype. This technique does not rely on a priori assumptions about the biologic significance of a particular gene and is therefore important in identifying new areas for inquiry. However, it also has relatively low sensitivity and can easily overlook linkages that are common contributors to less extreme phenotypes (eg, common genes predisposing the individual to moderate degrees of obesity). A complete list of published linkages to obesity phenotypes is summarized yearly.[106]

Candidate Genes. Any of the genes encoding a component of the mechanism for regulating body weight homeostasis, including those mentioned above, could be considered a candidate gene for a predisposition or resistance to obesity. Specific mutations in a few of these genes have been shown to cause obesity in rare kindreds. Mutations with strong effects were found in the leptin gene,[107] the leptin receptor gene,[108] the POMC gene,[109] the prohormone convertase gene (*PCSK1*),[110] and the MC4 receptor gene (*MC4R*).[111] The latter is the most common gene in which specific mutations cause obesity, but it is still very rare (27 mutations in 68 individuals published by 2001).[106]

Association studies have analyzed many other candidate genes from the afferent and efferent pathways mentioned above in genetically similar populations (siblings, twins, or kindreds). By 2001, polymorphisms linked to 58 candidate genes had been shown to have some association with obesity phenotypes,[106] including ghrelin,[112] peroxisome proliferation-activated receptor-γ,[113] uncoupling proteins,[114] and the β_3-adrenoreceptor genes.[115]

Linkage studies in large populations have identified many chromosomal loci with associations to a variety of obesity-related phenotypes, including BMI, leptin levels, fat distribution, and hyperlipidemia.[36] Such loci have been identified as of the 2001 gene map update,[106] some of which appear to represent the chromosomal regions of previously identified candidate genes such as the leptin or MC4 receptors. For many other regions or quantitative trait loci, the biologic mechanisms for the apparent linkage with obesity phenotypes remain unclear.

Mendelian Disorders. A number of human genetic syndromes displaying mendelian patterns of transmission and whose phenotype includes obesity have been identified and catalogued in the Online Mendelian Inheritance in Man (OMIM) database.[116] Twenty-five of these syndromes have been mapped to one or more chromosomal locations. The most common syndrome with severe obesity is Prader-Willi syndrome (short stature, hyperphagia, hypogonadotropic hypogonadism, cognitive defecits), which is mapped to chromosome 15q 11-13, a region containing several candidate genes for which a mechanistic explanation for the Prader-Willi phenotype is being sought.[106] The Bardet-Biedl syndrome requires a mutation at one of six loci plus an additional mutation in a second locus[117] and includes polydactyly and retinopathy. Alström syndrome (obesity, retinopathy, and deafness; no cognitive deficits)[118] and Cohen syndrome (hypotonia, retinopathy, and cognitive deficits)[119] have been mapped to one location each, but no obvious candidate genes have been identified as yet.[106] Each of these syndromes has characteristic findings as outlined in Table 19-2 and in the OMIM database[116] and can usually be distinguished from common obesity by a careful medical history and physical examination (see Table 19-2).

EVALUATION

Medical Assessment

The initial step for the assessment of the overweight child is to exclude potential associated syndromes or endocrinopathies and to diagnose possible associated complications, as summarized in Table 19-1. Several syndromes associated with obesity should be considered, including the mendelian syndromes mentioned above. Obesity may also accompany the more common, easily recognizable syndromes of trisomy 21 and Turner. In most cases, these syndromes can be distinguished on the basis of their unique features (listed in Table 19-2 and detailed in some excellent recent reviews[120–122]), and specific laboratory testing is valuable for confirmation but not for screening. The assessment of medical conditions related to overweight (see Table 19-1) has also been summarized elsewhere.[123]

The value of screening laboratory testing has been debated[124] but can be useful to establish whether there is dyslipidemia, steatohepatitis, or evidence of glucose intolerance, particularly because specific treatments for some of these disorders are increasingly considered.[67,68] Blood testing should be done in a fasting state if practicable. Thyroid stimulating hormone, hemoglobin A_{1C}, total cholesterol, very-low-density lipoprotein, low-density lipoprotein, high-density lipoprotein, aspartate aminotransferase, and alanine aminotransferase have been recommended to screen for possible hypothyroidism, diabetes, dyslipidemia, and steatohepatitis, respectively. Fasting glucose and insulin levels will provide information on carbohydrate metabolism and insulin resistance and may predict a risk for diabetes. Specific guidelines to screen for type 2 diabetes in overweight children have been developed (Table 19-3) but are also controversial because of the large number of adolescents fitting the screening criteria (currently about 2.5 million in the United States) and the relatively low yield of the suggested screening tests.[125]

Further laboratory testing may be useful in selected cases but can be expensive, and some tests are not readily available. Sleep studies can and should be performed if there are strong clinical symptoms of sleep apnea, and radiographic evaluation is necessary when slipped capital femoral epiphysis or Blount disease is suspected. Indirect calorimetry can be used to predict the energy deficit necessary for weight loss.[126] This might be useful when poor compliance or an eating disorder is suspected and at times may be useful to provide concrete caloric goals to support dietary changes. Bone age may be

TABLE 19-2 CHARACTERISTICS OF THE MAJOR SYNDROMES ASSOCIATED WITH OBESITY

SYNDROME	COGNITIVE DEFICIT	OBESITY	FEATURES
Albright[122]	Mild	Variable (general) Early onset	Neuroendocrine anomalies Normal or short Skin hyperpigmentation/vitiligo Polydactyly Bone fibrous dysplasia Precocious puberty
Alström[214]	None	Moderate (central) Onset age 2–5 yr	Retinitis pigmentosa Deafness Neuroendocrine anomalies Normal or short stature Normal or hypogonadism
Bardet-Biedl[121]	Moderate	Moderate (central) Onset age 1–2 yr	Normal or short stature Hypotonia Compulsive behavior Retinitis Heart anomalies Polydactyly Renal dysfunction Hypogonadism
Carpenter[215]	Mild	Central	Acrocephaly Polydactyly Syndactyly Short stature Flat nasal bridge High arched palate Heart anomalies Hypogonadism
Cohen[120]	Mild	Variable (central) Midchildhood	Short or tall stature Hypotonia Microcephaly Retinochoroidal dystrophy Short philtrum Low hairline Heart anomalies Normal or hypogonadism
POMC mutation[109] (autosomal dominant)	None	Early onset	Red hair ACTH deficiency Hyperphagia
Prader-Willi[216]	Mild to moderate	Moderate to severe (generalized) Onset 1–3 yr	Short stature Hypotonia Almond-shaped eyes V-shaped mouth Neuroendocrine anomalies Compulsive behavior High arched palate Hypogonadism

ACTH = adrenocorticotropic hormone; POMC = pro-opiomelanocortin.

helpful in supporting the diagnosis of an endocrinopathy. Consultations with specialists in sleep disorders, neurology, otorhinolaryngology, pulmonology/allergy, endocrinology, genetics, ophthalmology, and surgery may be necessary to manage specific complications.

The medical history should include assessment of concomitant medical diseases for their potential to contribute to weight gain (such as a history of significant head trauma or hypothalamic dysfunction) and to identify barriers to treatment (factors limiting mobility or ability to be physically active). A variety of drugs used for the treatment of psychiatric disease, epilepsy, diabetes, and migraines are associated with weight gain. Identifying these as a likely trigger of weight gain may prompt consideration of alternate drugs (Table 19-4).

Nutritional Assessment

Nutritional evaluation should include, at a minimum, anthropometric measurements and a history of the onset of obesity, as well as of weight loss attempts. Periods of adiposity rebound and potential triggers for excess weight gain should be identified. A family history of obesity and related medical problems should be evaluated to help establish the genetic factors underlying the weight disorder and the potential future medical risks. Assessing dietary intake by recalled food frequency helps to identify diet

TABLE 19–3 AMERICAN DIABETES ASSOCIATION GUIDELINES FOR THE ASSESSMENT OF TYPE 2 DIABETES IN CHILDREN WITH OVERWEIGHT OR OBESITY

Children and adolescents should undergo specific testing for diabetes if they are

Overweight, as defined by
1. BMI > 85th percentile for age and sex, or
2. Weight for height > 85th percentile, or
3. Weight > 120% of ideal (50th percentile) for height,

and have at least two of the following risk factors:
1. Have a family history of type 2 diabetes in first- and second-degree relatives
2. Belong to one of the following specific race/ethnic groups
   - American Indians
   - African Americans
   - Hispanic Americans
   - Asians/South Pacific Islanders
3. Have one or more of the following signs of insulin resistance or conditions associated with insulin resistance:
   - Acanthosis nigricans
   - Hypertension
   - Dyslipidemia
   - Polycystic ovary syndrome

Testing should consist of either
1. Fasting plasma glucose (8-h fast) or
2. Oral glucose tolerance test (blood glucose level 2 h postchallenge)

Further study is called for to determine the predictive value of hemoglobin A_{1C} and fasting insulin levels in determining risks for type 2 diabetes in children.

Adapted from the American Diabetes Association.[47]

composition but, in most cases, does not provide a good estimate of energy intake because underreporting of food intake by obese subjects is well described.[127] In particular, estimating the proportion of each macronutrient and fiber in the diet may help identify targets for dietary change if the diet is particularly skewed toward fat or energy-dense foods. In addition, the diet recall may be useful to assess the diet for deficiencies in micronutrients, particularly calcium and vitamins A and E, β-carotene, folic acid, and other B vitamins; excessive energy intake does not always mean adequate intake of micronutrients.

To identify possible areas for intervention, the amount and type of dairy products, fruits, vegetables, and legumes should be evaluated, and particular attention should be given to the consumption of sugary beverages, including juices and soda. Excessive consumption of sugary beverages has been associated with excess weight gain and increased rate of obesity[128] and is an important early target for dietary change. Similarly, the frequency of use of fast-food restaurants and of other restaurant meals should be examined because these meals often contain excessive caloric content and set appropriate portion standards. The diet history should also explore usual meal patterns, with attention to whether there is regular skipping of meals, binge pattern of eating, and the social context of meals, particularly if there are routine family dinners. Each of these factors may become a target for intervention to establish regular meal-based eating patterns.

Finally, the potential role of exercise and sedentary behaviors should be evaluated, with particular attention to determining the frequency of television and computer use. Several studies have suggested specific causal effects of television viewing on obesity,[129] and the American Academy of Pediatrics recommends limiting television viewing to 1 or 2 hours per day.[130] Assessment and encouragement of lifestyle exercise, in the form of outdoor play or regular walking (eg, walking to school), are as important as assessing structured exercise (eg, participation in sports programs).

Behavioral Assessment

The psychosocial complications of obesity are often subjective and difficult to measure in a standard fashion but undoubtedly represent one of the greatest burdens of obesity in children and adolescents. In many cases, psychosocial issues are best understood as consequences of the disease, brought on by feelings of discouragement and criticism by family, peers, or self. In other instances, the psychological issues precede or exacerbate the obesity. Regardless of the causality, it is important to acknowledge and assess these behavioral and psychological issues in each individual to best target treatment.

Depressive symptoms,[131,132] anxiety,[133,134] binge-eating disorder,[135–138] decreased self-esteem,[139–141] and problems with social interactions[142,143] have generally been found more frequently in obese children and adolescents than in their lean peers. Negative psychosocial associations also persist into early adulthood, when obesity is associated with lower educational attainment and household income and lower rates of marriage, independent of baseline education and aptitude.[144,145] Many of these problems can be attributed to the widespread and culturally entrenched bias against obese individuals in our society.[145,146]

Optimal assessment of the psychosocial issues contributing to and stemming from obesity in a particular patient has not been established and varies greatly between centers and providers. Whether or not standardized instruments are used, some effort should be made to assess mood, motivation, school and social performance, self-image, and eating attitudes and behaviors. It is also important to assess

TABLE 19-4 SELECTED DRUGS ASSOCIATED WITH WEIGHT GAIN

DRUGS WITH WEIGHT GAIN POTENTIAL	ALTERNATIVES WITH LESS POTENTIAL FOR WEIGHT GAIN
Atypical antipsychotics[217,218]	
Clozapine ++++ (gain 4–12 kg)	Quetiapine
Olanzapine ++++	Ziprasidone
Risperidone ++	
Mood stablizers[219,220]	
Divalproex sodium +++	Lamotrigine (weight neutral)
Lithium +++ (10 kg over 6–10 yr)	Adjunctive topiramate (6% of weight lost at 1 yr)
Tricyclic antidepressants[219,221]	
Amitriptyline ++	Desipramine
Imipramine ++	Nortriptyline
Monoamine oxidase inhibitors[219,222]	
Phenelzine > isocarboxazide	Tranylcypromine
Selective serotonin reuptake inhibitors[223]	
Paroxetine ++ (3.6% gain at 6 mo; 25% of patients gained more than 7% of body weight)	Fluoxetine
Sertraline +	
Atypical antidepressants[224]	Buproprion (weight loss)
Anticonvulsants[225]	
Valproate +++ 10–60% of patients gain weight; average gain 8–20 kg[226]	Zonisamide
	Topiramate (weight loss)[227]; 50% lose > 5 lb
Carbamazepine ++ 15 kg/3 mo	
Gabapentin ++ (23% gained > 10% of weight)	
Antidiabetic agents	
Insulin +++ (average 4 kg over 10 yr)[228]	Metformin (2–3 kg weight loss over 6 mo)[229]
Thiazolidinediones ++ (2–5 kg gain)[229]	

+ = mild weight gain potential; ++++ = very strong weight gain potential.

these issues in parents or close caretakers of the patient because factors such as motivation, mood disorders, and eating disorders in a parent will have a substantial impact on the child's attitudes, behaviors, and ability to respond to treatment.[143] The family's financial resources and level of cognitive stimulation in the household also predict the development of obesity[147] and may also affect the family's ability to respond to treatment. A "stages of change" model may be helpful in establishing the readiness of the patient and family for making lifestyle changes (Table 19-5). If the patient or family is in an early stage of change (precontemplation or contemplation), efforts should be focused on helping them forward into the next stage, perhaps using motivational interviewing techniques.

TREATMENT

Nutritional Therapy

Long-term studies of nutrition and exercise interventions for prevention of pediatric obesity are sparse and generally inconclusive. However, studies with short and moderate lengths of follow-up have shown benefits from a variety of "lifestyle" interventions, including nutrition education (in 3- to 9-year-old schoolchildren),[148] exercise, and measures to decrease sedentary activity.[149] All of these interventions have some effects in specific settings, although similar interventions in different settings failed to show an effect.[150–152] These studies are the subject of several recent reviews.[153–155] Most such studies target a school-based population and do not include the family in the intervention. The interpretation of such studies is hampered by high attrition rates, lack of standardization in definitions of obesity and treatment techniques, and limited generalizability.

There is better information available about treatment interventions for preadolescents and adolescents with established obesity, but the majority of studies still do not provide long-term data, and many issues, such as optimal macronutrient composition of diets and strategies to change food preference, have not been adequately studied. A few studies have suggested that structured exercise increases weight loss compared to diet alone,[156,157] and treatments focusing on increasing lifestyle exercise and reducing sedentary behaviors have also shown beneficial effects.[158–160] In general, regimens that combine hypocaloric diets with exercise and behavior modification have been particularly effective.[159–161] Epstein and colleagues have shown a medically significant long-term effect of an 8-month family-based behavioral intervention for families with children 6 to 11 years old. The improvement persisted 10 years after the intervention was completed.[162] The design of this study supports the conclusion that the behavioral intervention was a critical component of the success.

Typically, dietary recommendations for adults and children with obesity have consisted of reduction in dietary fat and energy intake as a balanced, hypocaloric diet. Recommendations have been developed by the American Academy of Nutrition as summarized in the handbook *Pediatric Nutrition*[163] and are similar to those supported by other governmental agencies, as described in a recent review.[164] The goal of the intervention is to reach a healthy weight without affecting linear growth. Specific recommendations include limiting beverages and foods with high caloric density and

TABLE 19-5 STAGES OF CHANGE

Precontemplation
Unaware of, denies, or minimizes the problem
Needs: encouragement to re-evaluate current behavior; encouragement of self-exploration, NOT action; provide information, personalizing the risks
Contemplation
Aware of the problem; ambivalent about change
Needs: gentle confrontation, information and rationale for change, clarification of any misinformation
Preparation
Has decided to make change, plans to do so within the next month, or is gathering information
Needs: assistance in identifying and overcoming obstacles, assistance to identify social supports, encouragement to take small initial steps
Action
Plan is in progress; attitudinal and behavioral changes have begun
Needs: tools and techniques to implement goals; positive reinforcement; support to deal with obstacles and losses, focusing on long-term benefits
Maintenance/relapse
Action maintained over 6 months (maintenance) or return to old habits (relapse)
Needs: self-monitoring tools for successful maintenance, feedback, and encouragement; stress management; use of support systems

Adapted from Prochaska J et al.[230]

low nutritional value, including sugary beverages and full-fat or low-fat baked goods and candies, and encouraging whole grains, fruits, and vegetables. Simple behavioral measures such as meal planning and label reading during grocery shopping are also generally encouraged to support the implementation of these nutrition guidelines.

The "traffic-light diet" provides a structured, balanced, hypocaloric diet in a simple format and has been used effectively for preadolescent[157,162] and preschool children.[165] It uses a simple color-coding scheme to categorize foods into categories for free consumption (low-density foods, "green"), moderate consumption (moderate-density and protein-containing foods, "yellow"), and very limited consumption (foods with high caloric density and/or with high sugar or fat content, "red"). The prescribed caloric content of the diet is generally between 900 and 1,300 kcal daily.

The protein-sparing modified fast (PSMF) diet provides high-quality lean protein while strictly limiting total calories. It has been used to treat severe obesity in a variety of settings, including hospitalized patients and school-based interventions. This diet has been effectively used in settings in which short-term weight loss is medically necessary, but there are no data to suggest that the diet reliably improves obesity in the long term. The principles of this diet are described in Table 19-6 and have been reviewed elsewhere, with sample menus.[166,167] Typically, patients start with a hypocaloric diet for 2 weeks before the PSMF diet is started (1,200 kcal/d); the PSMF diet continues for about 12 weeks (600–800 kcal/d) and is followed by a maintenance diet (balanced, 1,200 kcal/d). Of note, Figueroa-Colon and colleagues reported an 11.2 kg weight loss after 10 weeks of a PSMF diet, which was substantially more than that achieved by less restrictive measures, but by 15 months follow-up, the weight loss achieved by the two groups was similar.[168] Potential complications of the diet include protein losses, hypokalemia, inadequate calcium intake, cholelithiasis, and intravascular volume depletion with orthostatic hypotension.

Although the caloric content of a diet has been shown to relate to treatment success at 1 year,[169] to date, there is little evidence to suggest that alterations in specific macronutrients yield long-term weight reduction. A variety of popular diets have arisen around alterations of specific macronutrients, with or without limitations on total caloric intake. Many of these show good short-term weight loss, but whether these diets achieve long-term effects on obesity is unclear because no adequate long-term studies (with 5 or more years of follow-up) of these diets in representative adult or pediatric populations have been published. Freedman and colleagues reviewed the available evidence of benefits and risk associated with popular diets in adults.[170] Diets that specifically limit fat intake, which were popularized by Dean Ornish, were the subject of a recent review of studies with 6 to 18 months follow-up.[171] The authors of that review concluded that fat-restricted diets are no better than calorie-restricted diets in achieving long-term weight loss in overweight or obese people.

Several dietary approaches have focused on limiting carbohydrates, with (as in the "Zone" diet) or without (as in the Atkins diet) restrictions on fat intake. Careful analysis reveals that in the short term, low-carbohydrate diets cause a greater loss of body water than body fat. If the diet

TABLE 19-6 PROTEIN-SPARING MODIFIED FAST DIET

High-protein, hypocaloric diet for 12 wk
600–800 kcal
Protein 2 g/kg/d (maximum 100 g/d; approximately 50% of calories)
13–20 oz/d lean meat or substitute
Fat approximately 30–40% of calories
Carbohydrate 10–20% calories
As low-starch vegetables, may include one fruit
Ad libitum: tea, bouillion, pickles, spices, mustard
2 L of water per day
Supplements
Multivitamin with minerals
Elemental calcium supplement to meet the recommended daily intake
Monitor serum potassium and supplement as needed
Maintenance diet (36 wk)
Balanced macronutrients
1,200 kcal/d

Adapted from Suskind RM et al.[166]

is maintained in the long term, it results in the loss of body fat. There are few long-term data regarding the overall efficacy of these low-carbohydrate diets. High-fat, low-carbohydrate diets such as the Atkins diet are nutritionally inadequate and require supplementation of calcium and water-soluble vitamins.[170]

Caution should be used when considering the results of studies such as these that focus on adult populations because there is compelling evidence that at least some "lifestyle" approaches to obesity are substantially more effective in children than they are in adults.[172] Some dietary approaches might prove to have long-term results in children even if none can be demonstrated for adults. Diets that include a low glycemic index/load approach or higher consumption of calcium are currently under investigation[128,173] and will require long-term study before any useful conclusions can be drawn. A trial of increasing the fiber content of a hypocaloric diet in children yielded no better short-term results than a hypocaloric diet alone.[174] Medical guidance is important because there are ongoing concerns about medical complications, including dyslipidemias arising from the use of some popular diets and from the use of dietary supplements for weight loss, including in children. As many as 80% of children using unsupervised diets from popular magazines had medical problems resulting from these diets.[164]

As rates of obesity rise, obese children represent an increasing proportion of hospital inpatients.[175] Whether or not the child is hospitalized for an obesity-related condition such as gallstones, diabetes, sleep apnea, or orthopedic problems, nutritional needs must be considered. It is particularly important to recognize that the obesity is a chronic problem and will not resolve by attempting weight loss acutely during the inpatient stay. Moreover, acute severe caloric restriction is inappropriate and can lead to metabolic problems, including refeeding syndrome, despite the child's adequate energy stores. Guidelines for nutritional care of the obese adult inpatients have been developed,[176] but this issue has not been examined in children. It may be appropriate to prescribe a modest reduction in caloric intake, guided by indirect calorimetry or by calculations based on adjusted body weights and using a stress factor that is appropriate to the child's condition. The inpatient stay may also present an opportunity for nutritional education and for engaging the patient and family in a therapeutic plan to address the obesity beyond the hospital stay.

Behavior Therapy

As discussed above, some interventions in childhood have demonstrated long-term improvement of obesity with 5[177] or 10 years[162] of follow up; thus, children appear to have a more consistent and durable response to therapy than adults in the same family[172] or adult populations in general. The importance of including behavior therapy in the treatment of obesity in children has been demonstrated, at least in a family-based setting.[178] Although many interventions use combinations of dietary, exercise, and behavioral interventions, it is notable that the few studies with long-term results have had a rigorous and structured behavioral component.[161]

Commonly used techniques include self-monitoring of food intake and weight, modeling, positive reinforcement (praise), contingency management (certain behaviors are paired with predictable, reinforcing responses), and stimulus control (learning to avoid situations that are cues to overeat). Some studies have tested specific elements of these behavioral techniques. These have shown superiority of family-based over patient-focused treatment,[162] of gradual behavioral treatment (eight sessions over 15 weeks) over rapid behavioral treatment (eight sessions over 4 weeks),[179] of positive reinforcement over restrictive or critical approaches,[170] and of frequent (daily) over less frequent (weekly) positive reinforcement.[180] The value of problem-solving techniques has not been consistently shown.[181,182] Thus, there is ample evidence to support the use of behavioral modification techniques in the treatment and possibly the prevention of obesity in children. Behavior therapy should be thought of as a tool to achieve long-term changes in diet and exercise. In contrast, there are few data to support the use of behavior "micromanaging" techniques such as the rate of eating and bite size.

Weight Loss Drugs

Many of the drugs used for the treatment of obesity in adults in the past are characterized by unproven claims, highly variable efficacy, or dangerous side effects. Nonetheless, the incomplete but growing understanding of the mechanisms underlying the homeostatic control of body weight and the increased rigor applied to clinical drug trials hold promise for the development and use of pharmacologic agents to treat this chronic disease. In the short term, drugs can be important for a patient whose medical condition requires acute weight loss. Drugs may also have a role in treating the chronic component of obesity. Modification of the environmental and societal pressures contributing to obesity should be thought of as an important ultimate goal, but the use of drugs to promote weight loss may keep an individual patient engaged in the "lifestyle" component of treatment and might even change some of the consumer pressures that contribute to our obesity-promoting environment. Thus, pharmacotherapy for obesity is not at odds with lifestyle-changing approaches. As increasingly specific drugs are developed, efficacy and safety improve, and pharmacotherapy may ultimately be an essential tool to treat an otherwise refractory and devastating disease. Whether pharmacologic treatment is cost-effective depends in large part on whether it prevents the medical complications of obesity and associated costs of medical care.

Current options for the pharmacologic treatment of obesity are limited but may have some clinical utility. In general, the drugs demonstrate only modest efficacy but minimal side effects. Sibutramine (Meridia) and orlistat (Xenical) are the two drugs approved by the US Food and Drug Administration (FDA) for treating obesity in adults for periods of up to 1 year. Both demonstrate only modest efficacy but also have few side effects. Weight regain typically occurs on discontinuation of the medication. Concurrent efforts to reduce energy intake and increase exercise are important. Long-term studies (up to 5 years) of these drugs in adults are in

progress. Based on current literature, the modest weight loss achieved by the use of currently available drugs in conjunction with reduced calorie diets is probably not adequate to treat individuals with severe life-threatening complications of obesity, but the drugs may be considered to boost weight loss, assist weight loss maintenance, and reinforce lifestyle change. Whether phenotypic or genotypic analysis can be used to select for patients who respond relatively well to these agents is a subject for future study.

Sibutramine is an appetite suppressant that has been the subject of extensive clinical trials and was approved by the FDA for use in adults with obesity in conjunction with a dietary regimen. It is an inhibitor of both norepinephrine and serotonin and also weakly inhibits dopamine reuptake. At doses of 10 or 15 mg daily, combined with a reduced-calorie diet, modest weight loss is achieved in most patients (loss of 5 to 8% of baseline weight compared with 1 to 4% of weight on placebo),[183–185] but the range of weight loss varies greatly. Weight loss is sustained for most patients as long as the drug is continued (observation periods up to 3 years reported)[186] but generally is regained after the drug is discontinued. Side effects include modest increases in blood pressure (2 mm Hg average) and heart rate, dry mouth, constipation, and insomnia. Most side effects are transient and lead to discontinuation of the drug in about 5% of patients.[186] Importantly, no evidence of valvular heart disease such as that associated with fenfluramine treatment has been found in rigorous studies. Metabolic abnormalities associated with obesity, including hyperlipidemia and insulin resistance, tend to improve commensurate with weight loss.

Orlistat acts by inhibiting gastrointestinal lipases, reducing fat digestion and absorption by about 30%. Predictably, its side effects are related to fat malabsorption, consisting of steatorrhea when high-fat meals are taken, and decreases in serum levels of fat-soluble vitamins, primarily vitamin D.[160] Daily administration of a multivitamin is therefore recommended. Modest weight loss (3.2% more than placebo) is generally achieved,[187] and sustained use partially prevents weight regain during a second year of treatment.[188]

The safety and effectiveness of weight loss drugs in adolescents and children have not been established. A randomized trial of sibutramine in adolescents with concurrent behavioral therapy demonstrated significantly more weight loss than placebo (7.8 vs 3.2 kg). Adverse effects were similar to those seen in adults and prompted reduction or discontinuation of medication in more than one-third of subjects.[189] Large multicenter randomized placebo-controlled trials of sibutramine and orlistat in adolescents are in progress and will provide better assessment of the safety and efficacy of these drugs in adolescents.

Metformin improves insulin sensitivity and also promotes modest weight loss in adults.[190] In contrast to other antihyperglycemic agents, metformin does not increase insulin secretion but decreases hepatic glucose production and improves insulin sensitivity in both diabetic and nondiabetic adults.[191] Small open-label trials of metformin have suggested that it may be useful in ameliorating psychotropic drug–induced weight gain in children.[192] A small randomized trial of metformin in adolescents with hyperinsulinemia and a family history of diabetes showed improved glucose tolerance and a modest decrease in BMI.[193] Larger trials of metformin in adolescents are in progress, as is a small trial of ephedrine and caffeine. Careful review of these results will be necessary before pharmacotherapy outside of clinical trials can be recommended in adolescents or children.

Phentermine, diethylproprion, phendimetrazine, and benzphetamine are noradrenergic agents with appetite-suppressant effects but are studied and approved for short-term use only in adults (generally 12 weeks or less).[183] The latter two drugs are considered to have some potential for abuse and are listed in Schedule III of the US Drug Enforcement Agency.[183] The use of any agent with only short-term goals for weight loss in children and adolescents, particularly one with any potential for abuse, is highly questionable.

Dietary "supplements" or herbal medicines are popular, and consumers spend more than 1 billion dollars on these products annually in the United States.[194] However, they are unregulated and relatively untested. The commonly used herbal supplements are listed in Table 19-7. Supplements that could be used with caution in adults include conjugated linoleic acid, ginseng, chromium, hydroxycitric acid, dehydroepiandrosterone, hydroxymethylbutyrate, chitosan, and St. John's wort, but there is little evidence of the effectiveness of these drugs. Substances that have questionable safety and should be discouraged include Ephedra (or *ma huang*), horsetail, herbal laxatives, and some forms of caffeine and fiber.[195,196] To the best of our knowledge, there are no well-designed studies of dietary supplements and weight loss in children, and no supplements for weight loss can be recommended or even considered with caution.

Weight Loss Surgery

Over the past 15 years, surgically induced weight loss has emerged as an important option for adults with severe obesity. In contrast to poor long-term success rates for nonsurgical treatments of obesity, surgical approaches generally produce durable and substantial weight loss. Over 80% of patients lose at least half of their excess body weight during the first year.[197] Weight generally stabilizes 12 to 24 months after surgery, and 10 to 20% of patients regain a significant portion of the lost weight. If a patient maintains weight loss for 5 years, there is an excellent likelihood that the weight loss will persist for at least 14 years.[198] Studies have shown improvement or resolution of many of the medical complications of obesity, including diabetes mellitus, hypercholesterolemia, and obstructive sleep apnea.[199]

The jejunoileal bypass was an early surgical procedure for weight loss and caused global malabsorption. It caused frequent and unacceptable side effects, including intractable diarrhea, nutrient deficiencies, kidney stones, and hepatic failure. The two most common operations performed today are the Roux-en-Y gastric bypass and vertical banded gastroplasty (Figure 19-3), both of which reduce the gastric capacity to restrict caloric intake. In contrast to the jejunoileal bypass, these operations do not cause sig-

TABLE 19-7 DIETARY SUPPLEMENTS FOR WEIGHT LOSS[194–196]

DIETARY SUPPLEMENT	OTHER NAMES	MECHANISM	EFFECTIVENESS	SAFETY
Ephedra alkaloids	*Ma huang* Norepinephrine	Thermogenic	Yes, only in combination with caffeine	Unsafe (hypertension, palpitation, tachycardia, stroke, seizures, death)
Caffeine	Guarana (*Paullinia cupana*) Yerba maté (*Ilex paraguayansis*)	Thermogenic	No, when used alone	High doses or combinations may be unsafe (hypertenstion, tachycardia, nausea, dizziness)
Chromium	Chromium picolinate	↑ Insulin sensitivity	Uncertain	Uncertain
Ginseng	Korean ginseng (*Panax ginseng*)	↑ Insulin sensitivity	Uncertain	Uncertain
	American ginseng (*Panax quinquefolux*) Siberian ginseng (*Eleutherococcus senticosus*)	Thermogenic ↑ Lipolysis		May interfere with anticoagulant effect of warfarin
Fiber	Guar gum Psyllium Flaxseed Glucomannan	Malabsorption ↑ Insulin sensitivity	Unlikely	Generally safe, but some forms may have risk of gastrointestinal obstruction
Hydroxycitric acid	Malabar tamarind (*Garcinia cambogia*)	↓ De novo fatty acid synthesis	Unlikely	Uncertain
Dehydroepiandrosterone	Adrenal steroid hormone	↓ Fat synthesis	Uncertain	Uncertain Metabolites may stimulate breast and prostate tissue
Chitosan	Chitin (crustacean shells)	Blocks dietary fat absorption	Uncertain	Uncertain
Horsetail	*Equisetum* sp	Diuretic	Uncertain	Unsafe (may be K^+-wasting)
Senna Cascara	*Cassia* sp *Rhamnus pushiana*	Laxatives	Uncertain	Unsafe for treatment of obesity
St. John's wort	"Herbal phen-fen" *Hypericum perforatum*	Antidepressant	Unlikely	Uncertain Phototoxicity; drug interactions with many psychoactive drugs

nificant malabsorption, and their safety profile is substantially better. The mechanism through which these operations cause weight loss is not fully understood, although recent studies suggest that it may suppress gastric production of ghrelin, thereby reducing appetite.[93] About 10% of patients have important complications of these procedures, which include anastomotic strictures, incisional hernias, and gallstone formation requiring cholecystectomy. Anastomotic leaks, staple line disruptions, and dumping syndrome can occur but have been reduced to 1 to 2% each by modifications in surgical technique.[200] Although protein-calorie malabsorption is rare, malabsorption of selected micronutrients, particularly iron and vitamin B_{12}, is common and requires postoperative monitoring and treatment.

Gastric restrictive procedures for weight loss are thus an appropriate treatment option for adults with medically significant obesity, but there is still significant uncertainty regarding optimal patient selection. To date, no psychological or physiologic factors have been defined that will determine which patients are most likely to suffer weight regain after surgery (approximately 20% of patients regain most or all of their lost weight) or to suffer medical or psychological complications of surgery. Similarly, there are limited data on the outcomes of weight loss surgery in adolescent patients, but a few series have been published,[201,202] and these suggest that short- and long-term outcomes and complications in adolescents are probably similar to those seen in adults. Weight loss surgery may therefore be appropriate in selected severely obese adolescents. Given the limited data available on outcomes in this age group, these procedures should probably be limited to patients who have exhausted other management approaches and have significant medical complications of their obesity. To optimize long-term outcomes, any concomitant psychiatric disorders should be carefully assessed and under good control before surgery, and measures should be taken to ensure long-term follow-up for medical, surgical, and nutritional issues, ideally in the setting of a multidisciplinary obesity treatment center with substantial experience in surgical treatment of obesity.

Future Directions

With the exception of surgery, current treatments for established obesity have disappointing long-term outcomes. However, research is likely to lead to advances in several important arenas. Perhaps most importantly, public recognition of the obesity problem should lead to public education and public programs designed to prevent obesity during childhood. Such population-wide approaches are more likely to be effective than treatment approaches targeting individual patients with resource-intensive and weakly effective therapies. However, the identification of which preventive strategies will be most effective is important before they can be implemented widely. Second, improved

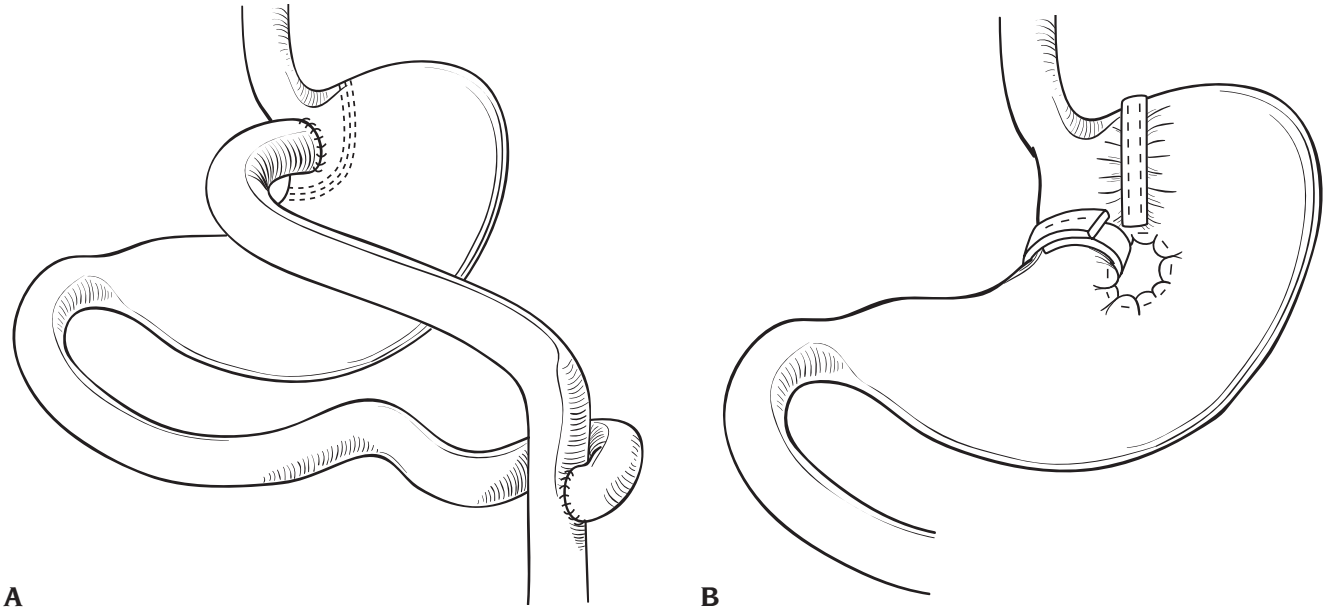

FIGURE 19-3 Gastric restrictive surgery for weight loss. *A*,Vertical banded gastroplasty; *B*, Roux-en-Y gastric bypass.

understanding of the genetic determinants and neural pathways underlying the homeostatic control of body weight is likely to lead to pharmacologic interventions that are more specific and therefore safer and more effective. Third, rigorously scientific and detailed analysis of specific environmental factors contributing to obesity, including issues of diet composition and meal patterns, may lead to more focused dietary and behavioral interventions to treat obesity. Finally, education of the public and health care community to recognize and reverse the commonly held bias against obese individuals should help to minimize the stigma and therefore much of the psychological burden associated with the disease.

USEFUL WEB SITES

Nutrition and Exercise

An excellent rating guide to other nutrition Web sites, with links: <http://navigator.tufts.edu/>

A site with nutritional and exercise material: <http://www.brightfutures.org/>

Promoting Better Health for Young People Through Physical Activity and Sports: <http://www.cdc.gov/nccdphp/dash/physicalactivity/promoting_health/index.htm >

Tips for parents (in English and Spanish): Available at: <www.niddk.nih.gov/health/nutrit/nutrit.htm>

Anthropometric Measurements

New growth charts (2000): <www.cdc.gov/growthcharts>

Nutritional Supplements and Alternative Therapies

National Institutes of Health: <http://nccam.nih.gov/>

US Department of Agriculture: <http://www.nal.usda.gov/fnic/pubs/bibs/gen/dietsupp.html>

Peer-reviewed journal that analyzes the claims of alternative medicine: <http://www.quackwatch.com/04ConsumerEducation/sram.html>

Genetics

The Human Obesity Gene Map: <http://www.obesity.chair.ulaval.ca/genes.html>

Online Mendelian Inheritance in Man (OMIM): <http://www3.ncbi.nlm.nih.gov/omim>

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CHAPTER 20

MUNCHAUSEN SYNDROME BY PROXY: FACTITIOUS DISORDER BY PROXY

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The syndrome now classified as a "factitious disorder by proxy"[1] (FDP) has historically been known as "Munchausen syndrome by proxy." Originally described in 1951 by Richard Asher,[2] the syndrome entails deliberate falsification of one's medical history and symptoms for the purpose of justifying extensive medical evaluations, invasive procedures, prolonged medical observations, and treatment. The objective of the behavior is to assume the role of being seriously ill.[1] Asher named the remarkable constellation of behaviors and objectives "Munchausen syndrome" after the legendary eighteenth century German baron who was renowned for his outrageous and utterly fabricated stories.

Following Asher's description of the syndrome, those adults and children meeting the criteria were increasingly recognized and reported.[3,4] In 1977, British pediatrician Roy Meadow expanded on the existing conceptualization with his reporting of two cases of Munchausen by proxy, entailing deliberate parental falsification of a child's medical circumstances.[5] Thus, the condition's relevance to general pediatricians and pediatric subspecialists expanded into the realms of child maltreatment.

In cases of FDP, rather than falsifying their own medical circumstances, adult caregivers deliberately feign or exaggerate the symptoms of another. The victim is typically a preschool-age child and often preverbal; the perpetrators are usually parents, most frequently mothers.[1] The perpetrator's motivation, which is a need to occupy a sick role, distinguishes FDP from malingering, in which secondary gains, either financial or legal, are typically relevant.[1] Although secondary gains and malingering may coexist alongside FDP, the motivations vary dramatically, with other motivations being secondary to those of FDP.[6] The perpetrator's behavior is not better explained by another mental disorder, such as a delusional disorder (somatic type) or a shared psychotic disorder ("folie à deux"). With FDP, a child's medical or psychiatric history and illness are intentionally fabricated, exacerbated, or induced by the perpetrator for the purpose of achieving a sick role vicariously or "by proxy." In some cases, hospitalization is an objective, which can become a way of life.[7]

FEATURES

The majority of cases of FDP involve gastrointestinal (GI), genitourinary, or central nervous system symptoms.[8,9] The type and severity of the symptoms produced depend primarily on the intellectual and medical sophistication of the adult. Feigned psychiatric symptoms are more rare.[1] Cases involving psychiatric FDP are typically much more complicated to uncover than their medical counterparts.[6]

Perpetrators often possess some level of nursing, medical, or other health professional training or experience, which allows them some familiarity with medical environments and equipment.[1,8,9] In these cases, it is important to recognize that a child's health is potentially jeopardized, thus justifying the term "perpetrator" as it applies to the adult in question. FDP is a psychiatric condition that applies to the perpetrator rather than to the pediatric patient. For the child, issues of medical and emotional abuse or maltreatment apply.

The typical presentation of FDP is complex, and assessment poses medical, psychiatric, and ethical challenges. Some warning signs of FDP are provided in Table 20-1. The parent is often very articulate, with educational and socioeconomic advantages. She or he usually appears to have a profound attachment to the child and is possibly eager to appear to be an "ideal" parent. The nonperpetrating parent often appears uninvolved, if not invisible, or may be physically absent.[1,8,9] The children have frequently been repeatedly admitted to multiple institutions during their early childhood with various complex conditions.[10] As they grow older, the children may actually collaborate in the production of symptoms.[1] Having spent childhood in an environment in which sickness was viewed as normal, if not desirable, a child's need for parental approval and attention may motivate such behavioral and emotional distortions. The urge to protect a parent may also play a part in this collusion. Children of FDP perpetrators typically have been conditioned to perceive themselves in a sick or even disabled role.[5] They are at risk of developing self-mutilation behaviors and factitious disorders later in life and eventually becoming FDP perpetrators as parents.[6,11,12]

Despite the FDP perpetrator's apparent devoted concern, she or he often manifests an incongruent disregard for the severity of the medical implications of the child's symptoms.[9,11] Parents with FDP frequently present with significant comorbid histories of depression, somatoform disorders, and personality disorders (particularly narcissistic and borderline). They may report having suffered childhood abuse.[1,8,9,13–16] Such reports must be interpreted cautiously because they may also be fabrications. Confirmation would necessitate documentation, which is rarely available, regardless of FDP.

SPECIFIC MANIFESTATIONS

FDP involves many health risks, including death. Cases often involve multiple signs and symptoms, thus complicating their assessment. GI and other manifestations of FDP are listed in Table 20-2.

With younger children, especially infants, seizures, cyanosis, and apnea-like symptoms are common; patterns involving diarrhea, vomiting, inability to walk, and limb paralysis seem more likely to emerge in toddlers.[7] Cases involving apnea, cyanosis, and seizures carry the greatest risks of mortality.[17,18]

Presentations may be further complicated by comorbid conditions such as failure to thrive or developmental disabilities.[19–21] Commonly encountered FDP-generated GI symptoms include factitious gastrointestinal hemorrhages, fabricated reports of emesis, and deliberately contaminated central venous lines. Examples of specific GI diagnoses commonly noted in children who have subsequently been recognized as victims of FDP are listed in Table 20-3,[22] along with examples of mechanisms of deception.

Children often present with symptoms that are incongruent with their general health; experienced specialists respond with comments such as "I have never seen a case quite like this one." The FDP parent's response to expressions of professional concern is typically one of obsequious vigilance, ostensibly on the child's behalf. The parent may praise and sympathize with a perplexed physician or appear eager to assist equally perplexed nurses or technicians. Parents with FDP frequently refute any improvement in their child's health and appear ready to imply that the physician or professional teams are remiss in having failed to recognize the severity of their child's circumstances. When faced with professional reluctance to escalate medical management, FDP parents are apt to transfer to another care provider. Discharges against medical advice among children with FDP parents are more frequent than they are among the general pediatric population.[9,18,19]

Symptoms provided by an FDP parent far exceed some embellishment or even falsification of a child's medical history by an overanxious parent in search of additional medical attention for their child. At the core of FDP, the perpetrator is engaged in such behavior as intentionally contaminating specimens, sabotaging a central line, poisoning food and beverages, withholding food, surreptitiously injecting insulin, pumping air into a gastrostomy tube, and possibly smothering or suffocating a child.[12,16,17,19] The purposefulness of the behavior is consistent. The objective is to attain a sick role (by proxy) by means of the child's (induced) medical status. Rosenberg used the phrase "disorder of empathy" in describing the mothers with FDP.[15]

TABLE 20-1 WARNING SIGNS OF FACTITIOUS DISORDER BY PROXY

Persistent recurrent illnesses that are unexplained or prolonged
Clinical signs are incongruent with a child's general health status
Signs or symptoms are extraordinarily rare, prompting such comments as "I've never seen anything quite like this before" from experienced clinicians
Repeated hospitalizations and evaluations have failed to provide a conclusive diagnosis or etiology
Noteworthy signs or symptoms do not recur when perpetrator is absent
Perpetrator is often hypervigilant, insisting on participating in procedures or bringing food or medicine from home, often refusing to leave the hospital
Perpetrator appears comfortable or pleased about being around medical environments, sometimes forming unusually close relations with staff
Perpetrator welcomes even invasive or painful diagnostic or surgical procedures
Perpetrator's concern for prognosis is incongruent with severity of symptoms
Clinical symptoms do not respond to treatment as anticipated
Families in which sudden deaths have occurred during childhood
Child's nonperpetrating parent is absent or rarely evident during treatment
Perpetrator has had some medical or nursing training or describes experiences with a similar condition in the past
Perpetrator's anxiety increases with child's medical improvement
Prior medical records that could confirm or preclude diagnostic impressions are reported missing by the perpetrator
Perpetrator becomes defensive or hostile if the information she/he provides is questioned or proven inaccurate

The medical professional's primary responsibility is to protect the well-being and rights of the child. A failure to diagnose or sustain an assessment of FDP raises issues of child abuse and a risk of fatality. Although FDP parents are unlikely to intend to murder, misjudgments may result in fatal interventions in pursuit of medical attention.[9] Additionally, medically abused children may lack the physical stamina or resilience to endure continued maltreatment. Tragically, the literature reflects a heightened sudden death rate among older siblings of children with FDP parents.

RESPONSE AND TREATMENT

The obscure motivation behind FDP behavior makes recognition and response particularly difficult. Recognition of FDP requires astute clinical skills and some common sense, along with considerable willingness to undertake a particularly unpleasant task. When a child's illness is sufficiently extraordinary as to bewilder the most experienced professional, and all reasonable measures taken have proven ineffective, close consideration of a child's environment is indicated. When a child's symptoms are evident only in the presence of the perpetrator, medical suspicions should be alerted. Especially when the perpetrator's anxiety level over the child's symptoms appears considerably less than that of the staff, physicians need to be particularly mindful of FDP. At such times, all staff should be reminded to document specific empiric observations rather than

reports of symptoms given by the perpetrator or the child. Self-reports and parental reports should be noted as such.

An FDP assessment necessarily entails acknowledgment by the medical professional(s) of having (albeit unknowingly) possibly contributed to behavior that endangered a child. Professional resistance to consider FDP is often further complicated by a reluctance to "pathologize," either psychiatrically or criminally, a person who has a child who is possibly genuinely ill. Professionals may also be reluctant to acknowledge the gullibility of the staff in their previous interactions with the perpetrator. Turmoil often already surrounds the child's treatment, and this element often intensifies on the articulation of suspicions of FDP. The staff may find itself "split" as regards the suspected FDP. The perpetrator's capacity to endear herself or himself to staff can potentially confound a timely response, in which a child's well-being is at stake and likely to suffer needlessly. Therefore, when FDP is suspected, the swiftest possible response to confirm or preclude the diagnosis is crucial.[6,16–18]

Even following admission, symptoms often continue to be fabricated.[15] Therefore, when FDP is suspected, all biologic specimens should be protected. The reliability of all apparent findings should be established (eg, is the blood actually that of the child?). Biologic specimens taken when the child is symptomatic should be saved for further investigation (eg, toxicologic). In cases in which a child has diarrhea, the possibility of laxative abuse must be evaluated. Phenophtalein is a frequently used laxative in cases of FDP, and its presence in the stool can be detected by the appearance of a pink color when alkanized to pH 8.5.[21] Stool levels of magnesium and sulfate may also be useful in detecting the abuse of laxatives.[21] All laboratory findings must be carefully documented.

Covert videotaping in hospital rooms has been used, but the practice remains highly controversial for ethical and legal reasons. Covert videotaping should not be used without first obtaining carefully considered legal advice.[23–25] Once FDP is detected, existing interdisciplinary support systems should be applied. Resources such as a hospital's child protection team, or its equivalent, are useful venues for discussions of the assessment and treatment plan. Ideally, such a team comprises professionals from various disciplines with expertise in areas related to child maltreatment.

Premature confrontation of FDP perpetrators or challenges made without sufficient documentation or staff cohesion can result in a detrimental crisis that is best avoided. Physicians and other medical staff must be well organized and solidly prepared to address the unpleasant tasks at hand.

When confronted with the medical and legal implications, the perpetrator typically will disclaim awareness of the medical problem's origin or any element of fabrication. The perpetrator's response to implied or overt accusations is often dramatic. Denial and projection of blame are frequent defensive maneuvers. Lawsuits are sometimes threatened in response to such inferences. Immediate discharges from medical care against medical advice are frequent consequences of confrontation.[8,11,18,25,26] The continued insistence on denying the fabrication of symptoms may reach a near-delusional level. Some perpetrators, however, may be sufficiently familiar with the existing literature to respond to accusations of FDP with spontaneous admissions, along with allusions to their behavior as a "cry for help" on their own part.[7,23,27] The legal incentives for such behavior are both clear and ominous. Dynamically, such admissions carry with them little or no indication of the perpetrator's capacity to distinguish between his or her own needs and the well-being of the child. Clinically, such admissions are not to be mistaken for sufficient evidence of

TABLE 20-2 SIGNS AND SYMPTOMS OF FACTITIOUS DISORDER (MUNCHAUSEN SYNDROME) BY PROXY

GASTROINTESTINAL
Abdominal pain (unexplained)
Anorexia
Diarrhea
Biochemical derangement
Failure to thrive
Hematemesis
Rectal bleeding
Vomiting (sometimes feculent)
Recurrent central line infections
OTHER
Allergies
Apnea, recurrent
Ataxia
Cardiorespiratory arrest
Coma, recurrent
Dehydration: hypernatremia
Diabetes or hypoglycemia
Mental status changes
Near-miss sudden infant death syndrome
Respiratory depression
Sepsis (often polymicrobial); joint and soft tissue infections
Upper respiratory tract bleeding
Urinary tract infection

TABLE 20-3 GASTROINTESINTAL DIAGNOSES FREQUENTLY CONFOUNDED BY FACTITIOUS DISORDER BY PROXY

PRESENTING DIAGNOSIS	METHOD OF FABRICATION
Colitis	Laxatives[8,17,21]
Cystic fibrosis	Altered, contaminated sweat tests and fecal fat analysis[21]
Diarrhea (intractable)	Laxatives Phenophtalein poisoning; salt poisoning[8,13,21]
Failure to thrive	Withholding of food, fluids[8,21]
Gastrointestinal hemorrhages (otherwise unexplained)	Patient's blood withdrawn from Broviac catheter; exogenous sources of blood (usually the perpetrators); warfarin poisoning[12,21,26]
Rectovesical fistula	Altered urine specimens[5]
Seizures/apnea secondary to gastroesophageal reflux	Asphyxiation (manual) Phenothiazine poisoning Salt poisoning Imipramine poisoning[8,14,21] Perpetrator's fabricated report
Vomiting (with or without altered sensorium)	Emetic poisoning Salt poisoning, injecting air into a gastrostomy[11,18] tube[8,12,25]

diminished risk of continued harm to the child or the presence of empathy on the part of the perpetrator.

After the disclosure of medical suspicions of FDP, the secondary gains associated with the condition are no longer reliably available to the perpetrators. The abrupt loss of medical attention and sympathetic regard has been associated with precipitous relocations to new areas in which the FDP patterns may be repeated for a new "audience" of health professionals. When relocation is not an option, the loss of such gains has also been associated with the perpetrator avoiding medical attention altogether, thus placing the child at continued risk from failure to seek appropriate care.[27,28] The rates of recidivism among perpetrators of FDP are noteworthy, even among relatively mild forms of the condition.[6,14]

CHILD ABUSE AND PROTECTION CONSIDERATIONS

The diagnosis of FDP is an Axis I condition according to the multiaxial classifications found in the American Psychiatric Association's *Diagnostic and Statistical Manual, Fourth Edition-Text Revision* (*DSM-IV-TR*).[1] Axis I conditions are those clinical disorders that are generally considered the primary focus of mental health treatment.

Along with the psychiatric implications of FDP, the realities of having inflicted deliberate harm on a child remain a criminal consideration. Although FDP represents a significant Axis I mental disorder, it also entails intentional harm inflicted by a caregiver on a child and thus meets the criteria for child maltreatment established by the laws established by the 1974 Child Abuse and Prevention Act, which applies to all 50 states.[29–31] Accordingly, mentally competent adults must be held accountable for their behavior, particularly behavior that entails medical harm to a child. The primary goal of both short- and long-term treatment must first address the protection of the child from further harm. The existing literature strongly documents the continued vulnerability of children who remain with FDP parents following an established diagnosis and Child Protective Services (CPS) involvement.[9]

Physicians are strongly encouraged to use existing expertise for support, collaboration, and guidance in such difficult decisions. Existing child protection teams and clinical social workers within the medial or CPS systems are the most likely sources of information on local or regional resources and protocols. The importance of careful collection and documentation of data pertaining to FDP cannot be underestimated. Perpetrators' fabrications are often so compelling and so complex that CPS agencies and courts rely heavily on the documentation provided by physicians.[15,17,24] An overview of ideal management recommendations is provided in Table 20-4.

Sadly, the recidivism rate found with cases of FDP, along with the absence of a recognized or effective treatment modality other than removal from the perpetrator, further underscores the necessity of placing the child elsewhere throughout any evaluation and intervention. The existing literature strongly documents children's continued vulnerability to harm when left in the care of FDP perpetrators, even following an established diagnosis and CPS involvement.[6,9,13,26,30,31] Thus, the disruption of an already traumatized child's family life necessarily remains a primary element of intervention. Such decisions are always difficult, but they are made even more poignant in cases involving a child who has reason to perceive himself or herself as medically fragile and a perpetrator who continues to insist on his or her innocence and parental devotion. Degrees of risk and safety must be carefully assessed. The severity of medical and emotional consequences must be carefully considered. The presence of comorbid psychiatric conditions on the perpetrator's part needs careful consideration, along with histories of prior episodes of relocation following extensive medical treatment elsewhere.[11,31,32]

Ongoing clinical considerations for the perpetrator entail attention to both the FDP and the other comorbid Axis I psychiatric conditions, including depression, substance abuse (including prescriptions), and Axis II personality disorders. According to the *DSM-IV-TR*, the personality disorders generally reflect inflexible, maladaptive patterns of perceiving and relating to the world.[1] Patterns of behavior, inner experiences, and impulse control that define Axis II conditions far exceed personality traits and are associated with significant distress and impaired levels of functioning.[1] Axis II conditions are typically associated with problematic ego functions such as impulse control and frustration tolerance. Object relations and object constancy may be problematic, making fulfilling the role of a parent challenging. The specific Axis II conditions most frequently associated with perpetrators of FDP are borderline and narcissistic personality disorders.[8,9,14,15,27] Characteristic and distorted defense mechanisms are generally relevant to Axis II personality disorders. For example, such defenses as denial, splitting, and projection are characteristically overused.[1] Long-term psychotherapy is gen-

TABLE 20-4 MANAGEMENT RECOMMENDATIONS FOLLOWING THE ASSESSMENT OF FACTITIOUS DISORDER BY PROXY (FDP)

Removal of the child from the care of the perpetrator and placement in a situation in which his/her safety is ensured. Placement with relatives is not necessarily a sufficient assurance of safety. Therapeutic foster care is indicated.
Separation of the child from the perpetrator should continue at least until the perpetrator has received a full psychiatric evaluation and a comprehensive social history has been obtained.
Primary pediatric medical care for the child should be coordinated by a practitioner specifically knowledgeable about FDP, who is also familiar with the case.
Comprehensive medical and psychosocial evaluations of siblings.
Long-term psychotherapy for both the perpetrator and the child provided by clinicians familiar with FDP. The perpetrator's partner should be included in therapy, as should siblings.
Ongoing monitoring of the child's medical, developmental, and psychosocial progress should be coordinated between providers of medical and psychosocial care, as well as coordinators of foster care arrangements.
If family reunification is eventually indicated, supervision should continue, with arrangements made through the courts for monitoring to continue regardless of relocation.

erally considered indicated for the Axis II conditions, but the literature generally lacks conclusive material about the successful clinical models suited to FDP in conjunction with Axis II conditions.[29,32] In fact, the literature suggests that fabrications of illness persist indefinitely.[12,14,33,34]

In cases involving court-mandated therapy for perpetrators, the capacity to deceive even experienced clinicians is well documented.[6,12,14,34] Adherence to psychotherapy is often superficial and frequently terminated prematurely by the perpetrator. For such reasons, treatment plans (particularly those entailing reunification) may necessitate having contingencies in place when treatment can be circumvented by the perpetrator.

The longer-term clinical implications for the child of an FDP perpetrator necessitate well-organized collaborative care efforts on the part of physicians, mental health professionals, and CPS and legal professionals. For physicians who continue to provide medical care to the child, ongoing issues of trust and realistic expectations will be crucial. Particularly in cases that necessitate removal from the family of origin and placement with others, physicians are urged to be mindful of issues of bereavement, loss, and stigma associated with such experiences, especially during childhood. Clearly, aspects of medical expertise must be combined with considerable compassion and exquisite sensitivity to children who have sustained such bewildering medical and emotional trauma.

Further research is clearly needed to explore the long-term implications for the children of FDP perpetrators. Longitudinal data are needed to clarify lifetime patterns of responses to determine whether the condition predicts replication in subsequent generations and how and whether children remember and resolve such trauma perpetrated by caregivers.

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CHAPTER 21

DISORDERS OF THE ORAL CAVITY

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The oral cavity is the most accessible part of the gastrointestinal tract, and lesions of the oral mucosa may be important manifestations of systemic disease. Such lesions may be a direct diagnostic marker of a disorder elsewhere in the gastrointestinal tract (eg, oral lesions of Crohn disease) or may be a manifestation of a systemic disturbance, such as malabsorption or immunodeficiency. This chapter considers the more common disorders that arise in the mouths of children and discusses the impact of gastrointestinal disease on the mouth.

ORAL DISEASE OF CHILDHOOD

A wide range of congenital disorders can give rise to defects of tooth structure, form, and number (Table 21-1). This chapter focuses on the more common primary disorders of tooth structure.

Primary Disorders of Enamel Formation

Amelogenesis Imperfecta. Amelogenesis imperfecta comprises a group of disorders characterized by defects of enamel, inherited in an autosomal dominant or recessive pattern.[1,2] Some are inherited in an X-linked recessive pattern.[3] The classification of this disorder is complex, but three basic types of defect occur:

- *Hypocalcified type.* This is the most common form of amelogenesis imperfecta. The enamel of newly erupted teeth is of normal thickness but is soft and rapidly lost through attrition. This disorder may be inherited in an autosomal dominant or recessive manner.
- *Hypomaturation type.* The enamel is of normal thickness but has a mottled, brown-yellow or white appearance. The enamel is not as soft as that of the hypocalcified type but can still chip off. This type of amelogenesis imperfecta includes an autosomal recessive pigmented form and an X-linked recessive form.
- *Hypoplastic type.* The enamel is of reduced thickness in some or all areas of the crowns of the teeth. There are thus variable degrees of pitting, grooving, and roughness of the enamel surface. This group includes autosomal dominant, smooth, and rough or pitted forms, as well as an autosomal recessive form in which there is almost total absence and an X-linked recessive form.

Occasionally, amelogenesis imperfecta may be a feature of other congenital disorders (eg, in amelocerebrohypohidrotic, enamel-renal, trichodento-osseous, and amelo-onychohypohidrotic syndromes).[4]

Children with amelogenesis imperfecta require evaluation and management by specialists in pediatric dentistry,[5,6] together with other relevant specialists (eg, in clinical genetics).

Primary Disorders of Dentine Formation

Dentine Dysplasia. In this autosomal dominant disorder, the crowns of the deciduous and/or permanent teeth are of a generally normal shape but have an amber or opalescent appearance owing to abnormal dentinal structure. In dentine dysplasia type I (radicular dentine dysplasia), there is obliteration of the pulp chambers, short or absent roots, and resultant tooth mobility and migration. In type II disease (coronal dentine dysplasia, pulpal dysplasia), the disease is not as severe as that of type I and tends to affect the deciduous dentition more than the permanent dentition, and the root structure may be unaffected. A third type of dentinal dysplasia may be accompanied by sclerotic bones.[1,4]

Dentinogenesis Imperfecta. This is probably the most well known of the primary dentinal disorders, probably as a consequence of its potentially profound clinical presentation, there being early loss of the overlying enamel.[1] At least three types of disease are known:

- *Dentinogenesis imperfecta Shields type II (opalescent dentine).* In this, the deciduous and permanent teeth have a blue-gray or translucent amber appearance, and there is

TABLE 21-1 DENTAL ANOMALIES IN CHILDHOOD

ANOMALIES OF TOOTH STRUCTURE
Enamel
- Amelogenesis imperfecta
- Hypocalcification
- Hypoplasia/hypomaturation
- Others

Dentine
- Dentinogenesis imperfecta
- Dentine dysplasia
- Regional odontodysplasia
- Others

Cementum
- Hypophosphatasia
- Others

ANOMALIES OF TOOTH NUMBER
Hyperdontia
- Supernumerary teeth
- Supplemental teeth
- Others (supernumerary teeth in Apert syndrome, Gardner syndrome, cleidocranial dysplasia, Down syndrome, Crouzon anemia, orofaciodigital syndrome, Hallerman-Streiff syndrome)

Hypodontia
- Ectodermal dysplasia
- Idiopathic hypodontia
- Others (in chondroectodermal dysplasia, achondroplasia, Rieger syndrome, incontinentia pigmenti [Bloch-Sulzberger syndrome], Seckel syndrome)

ANOMALIES OF TOOTH SIZE
Microdontia
Macrodontia
Connation (fusion or germination)

ANOMALIES OF TOOTH SHAPE
Dilaceration
Dens in dente
Dens evaginatus
Taurodontism

ANOMALIES OF ERUPTION (see Table 21-3)

ANOMALIES OF TOOTH COLOR (see Table 21- 4)

a tendency for the enamel to shear off the underlying dentine. The crowns are bulbous and have a pronounced cervical constriction. The roots are short and rounded, and the pulp chambers may become rapidly obliterated.

- *Dentinogenesis imperfecta Shields type III (brandywine dentine)*. The dentinal defects are similar to those of type II, but there are also "shell teeth," characterized by an abnormally large pulp chamber. This type of dentinogenesis imperfecta is named after the residents of an area of southern Maryland, in whom it was first observed.
- *Osteogenesis imperfecta with dentinogenesis imperfecta*. Dentinogenesis imperfecta can be a feature of type IB, IIIB, or IVB osteogenesis imperfecta, Ehlers-Danlos syndrome type II, Goldblatt's syndrome, Schimke immuno-osseous dysplasia and skeletal dysplasia, and rootless teeth.[7]

Defects of Tooth Shape and Size

Abnormal tooth shape may arise as a consequence of primary disease of the enamel or dentine (see above) but may be acquired local or systemic disease. Early periapical infection of a deciduous tooth can result in abnormal crown formation of the permanent tooth ("Turner's tooth").[8]

Congenital syphilis affects the teeth in which calcification occurs in the first year of life—hence typically the permanent incisors and first molars. The incisors have a screwdriver shape, a notching of the incisal edge, and/or a depression on the labial surface of the crown. The first molars may be bud-shaped and reduced to the size of the adjacent second permanent molar. The normal mesiodistal convexity of the crown may be reduced, and there may be enamel hypoplasia.[9–13]

Other defects of tooth size and shape are detailed in Table 21-2 and in the relevant sections of this text. Other defects of eruption are listed in Table 21-3.

Ectodermal Dysplasia

The ectodermal dysplasias (EDs) comprise a large group of genetically determined disorders, clinically characterized by alterations of two or more ectodermally derived structures, giving rise to a wide variety of defects of the skin, nail, hair, or sweat glands.[14]

There are multiple congenitally missing primary teeth, coronoid primary incisors, with moderately to severely taurodontic second primary molars. Supernumerary cusps may also occur. The permanent teeth are always reduced in number (hypodontia), and the crown shape of any present teeth is usually abnormal; in particular, the permanent incisal crowns are often conical or pointed, whereas the permanent molar crowns have a reduced diameter. The absence of teeth results in an underdevelopment of the alveolar processes and hence a reduction in the lower third of the face height and lip protuberance, the latter causing dry, cracked, and fissured lips.[14–17]

ED typically encompasses a spectrum of ectodermal abnormalities, and those patients with anhidrotic forms may be liable to heat intolerance, which may give rise to episodes of hyperthermia, eventually leading to cerebral damage. Indeed, death owing to hyperthermia is possible but uncommon.[18] Sparse blonde hair, including a reduced density of eyebrow and eyelash hair, is common in ED. The nails may also appear dystrophic and brittle. The periocular skin may show a fine wrinkling with hyperpigmentation. As the salivary glands are ectodermally derived, patients may have varying degrees of salivary gland aplasia, xerostomia, and an increased liability to dental caries.[19]

Early pediatric dentistry affords the child the opportunity to develop normal forms of speech, chewing, and swallowing; normal facial support; improved temporomandibular joint function; and improved self-esteem.[20,21] When a child with ED reaches his or her early teens, orthodontic treatment may be indicated, together with definitive restorative dental care—possibly including endosseous implants.[22]

Other Causes of Hypodontia

Hypodontia can be a feature of a number of other disorders (see Table 21-1).[8,23,24] In addition, it may arise as an isolated anomaly affecting one or more teeth. Commonly missing teeth are the permanent upper lateral incisors, third molars, and second premolars. Hypodontia may be a feature of cleft palate.

TABLE 21-2 DISORDERS OF TOOTH SHAPE AND SIZE

DISORDER	COMMENTS
Dilaceration	A bend in the root or crown of a tooth. Usually affects the permanent incisors. Arises as a consequence of childhood trauma to teeth.
Connation (double teeth)	Teeth joined together. More common in the deciduous than in permanent dentition. May represent fusion or partial development (germination) of teeth.
Macrodontia	Abnormally enlarged teeth. Uncommon but usually affects all of the dentition.
Microdontia	Abnormally small teeth. Uncommon.
Taurodontism	Not a true defect of tooth shape. A radiologic feature characterized by an enlarged pulp chamber, long crown, and short roots.
Prominent tubercles or cusps	A variety of defects may occur.
Enamel cleft	Small cleft in the enamel crown in the cervical region.
Disorders of tooth number and eruption	
Reduced tooth number	

Hyperdontia

Additional teeth are usually smaller than the normal dentition and are termed supernumerary, although when in the midline (usually of the maxillary teeth), they are termed mesiodens.[23,24] Occasionally, supernumerary teeth do not erupt but manifest as a malocclusion. Additional teeth of normal shape and size are termed supplemental and are much less common than supernumerary teeth (see Table 21-1).

Both supernumerary and supplemental teeth can cause delayed or failed eruption of adjacent teeth and, when unerupted, may cause resorption of adjacent roots.

Delayed Eruption of Teeth

The eruption of teeth is usually due to local factors such as malocclusion but may rarely reflect systemic (including gastrointestinal) disease.[8,25]

Acquired Disorders of Teeth

Dental Caries (Decay) and Sequelae. Dental decay is caused by destruction of the dental hard tissues by metabolic acids of dental plaque.[26–31] It initially manifests as areas of white decalcification of enamel, with later destruction of the enamel and dentine. Early decay is asymptomatic, although later disease, when there is involvement of the dentine, gives rise to short-term localized pain in response to hot, cold, and sweet foods. Unchecked decay eventually results in severe pulpal inflammation and death and possibly inflammation of the apical areas of the periodontium (periapical periodontitis).

Periapical periodontitis will give rise to local long-standing pain, usually in response to hot and cold foods and particularly with occlusal pressure. The affected tooth may be slightly elevated, and there may be formation of a sinus that drains from the gingivae at the level of the apex of the tooth. Periapical infection can give rise to periapical abscess formation ("gum boil") and cellulitis, the latter occasionally causing pyrexia and malaise, as well as facial swelling. Other possible sequelae of a periapical abscess include periapical (radicular) cyst formation or, rarely, discharge of a sinus onto the skin.

Dental caries is liable to arise in children who maintain poor oral hygiene and/or have a diet comprising frequent sweet foods. The decay tends to arise in the fissures of premolars and molars, although it can arise interdentally between any teeth.

Factors that predispose individuals to plaque accumulation (eg, malocclusion, enamel anomalies, inability to achieve effective tooth cleaning, frequent sticky foods) increase the liability to dental caries. In addition, xerostomia owing to salivary gland disease or local radiotherapy greatly increases the risk of caries development.

Rampant Caries. Rampant caries is characterized by almost immediate decay of erupting teeth. It typically affects the upper incisors, although almost all of the deciduous dentition can be affected. Rampant caries is due to the frequent use of sugary drinks in bottles or soother.[32] Rarely, rampant caries may be a feature of children with salivary gland aplasia (eg, as in some forms of ED).[19] Of interest, rampant caries can be a feature of methamphetamine and/or cocaine abuse.[33,34]

Other Causes of Loss of Dental Hard Tissue

Dental Erosion. Erosion is the consequence of dietary acidic destruction of enamel. The erosion is usually due to the frequent use of low-sugar (but acidic) soft drinks[35–37] and is thus more likely in adolescents than in young children. Erosion can be a feature of children and adults who

TABLE 21-3 DEFECTS OF DENTAL ERUPTION

NEONATAL TEETH
Ellis-van Creveld syndrome
Hallerman-Streiff syndrome
Pachyonychia congenita
PREMATURE ERUPTION
Precocious puberty
Hyperthyroidism
Hemifacial hypertrophy
Sotos syndrome
Sturge-Weber syndrome
DELAYED ERUPTION
Local causes
Hyperdontia
Small skeletal base
Ankylosis of deciduous teeth
Systemic causes
Albright hereditary osteodystrophy
Cleidocranial dysplasia
Down syndrome
Hypothyroidism
Hypopituitarism
Gardner syndrome
Goltz syndrome
Incontinentia pigmenti

suck and eat large amounts of citrus fruits.[38] It has been observed that some,[39] but not all,[40] examined groups of athletes may be at risk of dental erosion, presumably through the frequent use of diet-type soft drinks. Almost any tooth surface can be affected, but, typically, the palatal aspects of the upper anterior teeth become thinned and smooth.

Although much less common than diet-related disease, erosion can be caused by gastroesopharyngeal reflux disease.[41–46] The posterior teeth and the palatal aspects of the upper anterior teeth of the permanent and, rarely, the deciduous dentitions can be affected. Erosion may also arise in bulimia nervosa (see later also).[47–49]

Attrition. Attrition is the loss of dental hard tissues as a consequence of mastication. Attrition is a normal feature of the late deciduous dentition and is particularly noticeable on the incisors and canines. Severe attrition of the permanent dentition in childhood is uncommon but may arise with severe malocclusion and may be a feature of Rett syndrome.[50]

Abrasion. Abrasion is the loss of dental hard tissues owing to frictional damage by foreign hard substances, typically a toothbrush. It is uncommon in children. Abrasion typically gives rise to concavities within the dentine (and to a lesser extent the enamel) of the cervical margins of teeth.

Dental Discoloration and Staining

The teeth can be extrinsically or, more rarely, intrinsically stained (Table 21-4).

Most discoloration of teeth in childhood is due to dental caries. Enamel decalcified by dental decay can become stained with foodstuffs, and caries that arrests takes on a black appearance. Teeth that become nonvital, typically owing to trauma, may take on a darkened color because of the pigments associated with pulpal necrosis, whereas internal resorption of the dentine can produce pink spots on the crowns of affected teeth.[8]

Systemic tetracycline therapy in the years of crown development can give rise to gray, yellow, or brown pigmentation of the teeth. The degree and color of tooth discoloration will depend on the dose, duration, and type of tetracycline prescribed. Fluorosis causes variable degrees of tooth discoloration, which are detailed later. Other, albeit uncommon, causes of intrinsic pigmentation of the teeth include congenital erythropoietic porphyria,[51,52] hemolytic disease of the newborn, and hyperbilirubinemia owing to rare disease such as biliary atresia. Chlorhexidine gluconate mouthrinse and gel cause brown extrinsic staining of the teeth. The stain can be easily removed by professional dental cleaning and can be prevented by application of chlorhexidine immediately following regular tooth cleaning because the chlorhexidine stains the pellicle that forms on teeth within a few minutes after tooth cleaning.[53]

TABLE 21-4 ABNORMAL TOOTH COLOR

Extrinsic discoloration
Food stuffs
Drugs (eg, chlorhexidine)
Poor plaque control
Intrinsic discoloration
Caries
Trauma
Restorative materials
Internal resorption
Fluorosis
Tetracyclines
Congenital disorders of dentine and enamel structure
Erythropoietic porphyria
Severe neonatal (or early childhood) jaundice

GINGIVAL AND PERIODONTAL DISEASE IN CHILDHOOD

Most gingival disease in childhood is plaque-related gingival inflammation (gingivitis); however, a wide range of congenital disorders can give rise to gingival and periodontal manifestations. Gingival and periodontal disease in childhood most commonly manifests as swelling (Table 21-5), accelerated periodontal destruction (Table 21-6), and/or ulceration (Table 21-7).

Nonspecific Gingivitis

Acute. Acute nonspecific gingivitis is due to inflammation secondary to local accumulation of plaque. Signs of acute gingivitis develop within 7 to 10 days of plaque accumulation and initially arise on the interdental papillae before spreading to the adjacent free gingival margins. Rarely, there may be involvement of the free and attached gingivae. Acute gingivitis initially manifests as redness and swelling of the affected gingivae; later there is gingival bleeding that may arise with tooth cleaning and eventually will occur during eating. Patients with severe acute gingivitis may have oral malodor and complain of dysgeusia and awaking from sleep to find blood on the pillow owing to drooling of bloody saliva. Unlike acute necrotizing ulcerative gingivitis (ANUG), there is no tissue destruction.[54–57]

Chronic. Chronic nonspecific gingivitis arises as a sequela to long-standing mild acute gingivitis. The gingivae become variably enlarged and fibrous, and there is often some associated acute gingivitis.

Management of Nonspecific Gingivitis. Improvement in oral hygiene is the mainstay of treatment of both acute and chronic nonspecific gingivitis. Surgical reduction of any hyperplastic tissue (eg, by gingivectomy) may be required for the treatment of long-standing chronic gingivitis.

Acute Necrotizing Ulcerative Gingivitis

ANUG (acute ulcerative gingivitis, Vincent gingivitis) is a common disorder of adulthood but can arise in children, particularly those who are malnourished or immunocompromised, for example, with human immunodeficiency virus (HIV) disease. ANUG is characterized by notable, painful, necrotic gingival ulceration and edema with bleeding and malodor. The ulceration typically commences

TABLE 21-5 CAUSES OF GINGIVAL SWELLING IN CHILDHOOD

GENERALIZED ENLARGEMENT
LOCAL CAUSES OF LOCALIZED ENLARGEMENT
Chronic gingivitis
Hyperplastic gingivitis owing to mouth breathing
LOCALIZED ENLARGEMENT
Abscesses
Fibrous epulides
Exostoses
Eruption cysts
SYSTEMIC CAUSES OF GENERALIZED ENLARGEMENT
Congenital disease
Hereditary gingival fibromatosis
Mucopolysaccharidoses
Mucolipidoses
Hypoplasminogenemia
Lipoid proteinosis
Infantile systemic hyalinosis
Acquired disease
Drugs
Phenytoin
Cyclosporine
Calcium channel
Blockers
Amlodipine
Diltiazem
Felodipine
Isradipine
Lacidipine
Lercanidipine
Nicardipine
Nifedipine
Nimodipine
Nisoldipine
Verapamil
Others
Crohn disease
Leukemia (acute myeloid)
Scurvy
SYSTEMIC CAUSES OF LOCALIZED ENLARGEMENT
Heck disease
Tuberous sclerosis
Cowden disease
Fibrous epulis
Giant cell epulis
Pyogenic granuloma
Papilloma
Crohn disease, orofacial granulomatosis and related conditions
Kaposi sarcoma and other (rare) neoplasms

interdentally but, in severe disease, may extend to cause ulceration of all marginal areas. The tissue destruction results in irreversible flattening of the interdental papillae. Involvement can be localized or generalized.

Patients may complain of an abnormal, sometimes metallic, taste (dysgeusia) and oral malodor. Periodontal destruction is rare, although in severe malnourishment, an ANUG-like disorder (termed noma) can cause ulceration and destruction of the periodontium and adjacent tissues and sometimes perforation of the facial tissues, fistula formation, and eventual orofacial disfigurement. In severe ANUG, there may be cervical lymphadenopathy, pyrexia, and, rarely, malaise.

Poor oral hygiene is the most common cause of ANUG, although viral lymphopenia (particularly upper respiratory virus infections) and, less commonly, immunodeficiency associated with undiagnosed or poorly controlled diabetes mellitus, leukemia, HIV disease, profound malnutrition, and other severe immunocompromised states can be contributing etiologic factors.

There is unlikely to be a specific causative micoorganism, but ANUG is typically associated with *Borrelia vincentii*, fusiform bacteria, *Treponema denticola*, and other gingival spirochetes.

For the management of ANUG, oral hygiene must be improved. Where possible, supragingival deposits and tissue debris should be removed immediately, but subgingival cleaning may be possible until there has been some resolution of the acute disease. Chlorhexidine gel and/or sodium perborate mouthrinses may be helpful, although significant evidence that these topical agents are effective is lacking. Systemic metronidazole (typically 200 mg three times daily for 3 days) or phenoxymethylpenicillin (250 mg four times daily for 5 days) is indicated when the disease is severe and/or there is lymphadenopathy or pyrexia. Appropriate referral (eg, to endocrinology or infectious diseases specialists) may also be warranted.[55,56]

TABLE 21-6 SYSTEMIC CAUSES OF ENHANCED GINGIVAL/PERIODONTAL DESTRUCTION

PRIMARY IMMUNODEFICIENCIES
Reduced neutrophil number
Cyclic neutropenia
Benign familial neutropenia
Other primary neutropenias
Defective neutrophil function
Hyperimmunoglobulinemia E
Kartagener syndrome
Chronic granulomatous disease
Chédiak-Higashi syndrome
Acatalasia
Leukocyte adhesion deficiency
Actin dysfunction syndrome
Other immunodeficiencies
Fanconi anemia
Down syndrome
Severe combined immunodeficiency
OTHER CONGENITAL DISORDERS
Hypophosphatasia
Ehlers-Danlos syndrome type VIII
Acro-osteolysis (Hajdu-Cheney syndrome)
Type Ib glycogen storage disease
Oxalosis
Dyskeratosis benigna intraepithelialis mucosae et cutis hereditara
Familial dysautonomia (Riley-Day syndrome)
Papillon-Lefèvre syndrome
Haim-Munk syndrome
SECONDARY IMMUNODEFICIENCIES
Malnutrition
Diabetes mellitus
Crohn disease
HIV disease
OTHER ACQUIRED CAUSES
Vitamin C deficiency

HIV = human immunodeficiency virus.

Hereditary Gingival Fibromatosis

Hereditary gingival fibromatosis is usually inherited as an autosomal dominant or recessive disorder, although sporadic disease can occur. There is fibrous enlargement affecting many or all gingivae, particularly the free gingiva about the smooth surfaces of the teeth. The disease manifests in early childhood, sometimes causing delayed or partial eruption of teeth. Gingival fibromatosis can be a feature of a spectrum of rare syndromes that usually includes deafness, learning disability, and hypertrichosis. Similar gingival enlargement may be seen in a number of rare conditions (see Table 21-5).

Surgical reduction by gingivectomy or gingivoplasty is usually required to improve esthetics and permit better oral hygiene maintenance. Surgery is more effective postpuberty, when recurrence is unlikely.[57,58]

Other Congenital Causes of Gingival Enlargement

Genodermatoses. Sturge-Weber syndrome can give rise to hemangioma of the gingiva, usually the maxillary gingiva of one side. There is usually an extensive orofacial hemangioma that roughly follows the distribution of one or more of the divisions of the trigeminal nerve and extends into the parietal and occipital lobes of the brain. The maxillary gingivae are often involved, the affected tissue being enlarged, boggy, and purple or blue colored, often covering the crowns of several teeth. The eruption and form of the involved teeth are variably affected. Because epilepsy is a common accompaniment, there may also be phenytoin-induced gingival enlargement. Sturge-Weber syndrome may also include epilepsy, learning disability, hemiplegia, and glaucoma.[59,60]

Tuberous sclerosis may give rise to multiple fibrous enlargements of the gingivae,[61] whereas neurofibromatosis (usually type 1) may give rise to variable numbers of gingival neurofibromas (as well as occasional pigmentation).[62] Cowden disease causes multiple gingival fibrous swellings.[63]

Orofacial Granulomatosis. Orofacial granulomatosis and Crohn disease in childhood may give rise to localized or generalized gingival enlargement. The swelling is diffuse and salmon pink in color, often with a granular surface, and affects the free and attached gingivae. The other oral manifestations of orofacial granulomatosis are discussed later.

Drug-Induced Gingival Enlargement. Gingival enlargement commonly arises with long-term phenytoin, cyclosporine, and calcium channel blockers.[57,58] The gingival enlargement usually commences interdentally and affects both the labial and the lingual/palatal aspects. The enlargement is most likely in patients who do not maintain good plaque control and in those receiving high-dose regimens. The enlargement associated with phenytoin is fibrous in quality, whereas that associated with cyclosporine and calcium channel blockers is softer and more erythematous. Surgical reduction followed by the maintainance of good plaque control is the mainstay of treatment of drug-induced gingival enlargement.

TABLE 21-7 CAUSES OF GINGIVAL AND ORAL MUCOSAL ULCERATION IN CHILDHOOD

TRAUMA (physical, chemical, radiation, thermal)
APHTHAE AND ASSOCIATED SYNDROMES
Recurrent aphthous stomatitis
Behçet disease
Others
INFECTIONS
Primary or recurrent herpes simplex virus infection
Varicella-zoster virus
Epstein-Barr virus
Cytomegalovirus
Coxsackievirus
Echovirus
Acute necrotizing ulcerative gingivitis
Treponema pallidum (may signify sexual abuse)
Mycobacterium tuberculosis
Gram-negative infections (rare)
Atypical mycobacteria
Chronic mucocutaneous candidiasis
DERMATOSES
Lichen planus
Mucous membrane pemphigoid
Pemphigus vulgaris
Dermatitis herpetiformis
Erythema multiforme
HEMATOLOGIC DISORDERS
Neutropenia(s)
Leukemia(s)
Hematinic deficiencies
Others
GASTROINTESTINAL DISORDERS
Crohn disease and related disorders
Ulcerative colitis
DRUGS
Cytotoxics and others
MALIGNANCY
Rare (eg, Kaposi sarcoma, non-Hodgkin lymphoma [in HIV disease])
OTHER
Lipoid proteinosis
Hypoplasminogenemia

HIV = human immunodeficiency virus.

Periodontal Disease in Childhood. Periodontitis—loss of periodontal attachment—is uncommon in childhood but, when present, is usually associated with poor plaque control. Periodontitis manifests as increased tooth mobility and migration and is usually accompanied by features of acute and/or chronic gingivitis. Some loss of alveolar bone may be observed radiographically.

Aggressive periodontitis—periodontal destruction in excess of that expected irrespective of the levels of plaque—is likewise uncommon in childhood and usually reflects an underlying primary defect of phagocyte number or function, deficiency of cathepsin C (as in Papillon-Lefèvre syndrome), a structural defect of cementum (eg, hypophosphatasia) or connective tissue of the periodontium (eg, Ehlers-Danlos syndrome type VIII), or HIV disease (see Table 21-6)

The management of periodontitis of childhood is principally directed toward reducing the infection with *Actinobacillus actinomycetemcomitans* and other periodon-

topathic bacteria by thorough subgingival mechanical cleaning, subgingival antimicrobial agents, intermittent use of systemic tetracyclines (typically doxycycline), and, when indicated, periodontal surgery and perhaps orthodontic movement of malaligned teeth.

Oral Mucosal Disease in Childhood

Oral mucosal disease in childhood principally encompasses lesions that manifest as ulcers, white or red patches, or pigmentation.[8]

Oral Mucosal Ulceration in Childhood. The causes of oral and gingival ulceration in childhood are summarized in Table 21-7.

Traumatic Ulceration. Traumatic ulceration in childhood tends to be due to physical trauma, for example, injury from a toothbrush or orthodontic appliance or accidents in the home or during play. The ulcers are usually solitary, arise at the site of trauma, and heal within about a week of removal of the cause. Traumatic ulceration of the gingivae, lips, or labial frena may be suggestive of physical abuse, particularly when accompanied by facial bruising, laceration, and bite marks.[8,64–66]

Radiotherapy to the mouth or chemotherapy can give rise to oral mucositis. The mucosa becomes red, painful, necrotic, and ulcerated. The cause of mucositis remains unclear; it may simply reflect damage to the basal cells of the epithelium or infection with gram-negative bacteria.[67] There is no specific treatment for oral mucositis, although the use of ice pops during radiotherapy or chemotherapy may lessen the severity of the ulceration, as may good plaque control.[67] Combination topical antifungal/antibacterial regimens (eg, polymyxin, tobramycin, and amphotericin) would seem to be of limited benefit in the treatment of oral mucositis.[68,69]

Infectious Causes of Mouth Ulcers in Childhood. Viral infections most commonly cause mouth ulcers in childhood. Details of these and other possible infections giving rise to oral mucosal ulceration are summarized in Table 21-7.

Recurrent Aphthous Stomatitis. The most common form of nontraumatic ulceration affecting the oral mucosa is recurrent aphthous stomatitis (RAS).[70,71] This condition is characterized by the presence of one or more oral ulcers, which heal within days or sometimes weeks, only to reappear at regular intervals. The associated constitutional effects may vary from minor discomfort from the ulcers themselves to, more rarely, a severely incapacitating illness caused by persistent oral ulceration. The overall prevalence of the condition is 20 to 30%, and it has been estimated that 30% of affected individuals have their first attack of ulceration by the age of 14 years, with 10% having ulcers before the age of 10 years. An associated family history has been demonstrated in 24 to 46% of cases, with a very high incidence in patients whose parents both suffered from the condition.[70] Furthermore, in a study carried out on twins, a 90% concordance between identical twins was found, in contrast to a 57% concordance between nonidentical twins.[72]

RAS may be subdivided into three distinct groups, largely on the basis of the clinical appearance and history of the individual lesions. All three types of RAS may be seen in children and are referred to as minor aphthous ulceration, major aphthous ulceration, and herpetiform ulceration.

Minor aphthous ulcers account for 80% of the total and are most common in patients between 10 and 40 years of age.[70] Ulcers of this type characteristically affect the nonkeratinized oral mucosa of the lips, cheeks, vestibule, and margins of the tongue. The hard palate, gingivae, and dorsum of the tongue are typically unaffected. The appearance of a painful ulcer is frequently preceded by a prodromal phase of 1 to 3 days, during which the patient may complain of a burning or pricking sensation accompanied by a degree of paresthesia at the site of future ulceration. The ulcers, which may occur alone or in groups of three or four during a single episode, usually last between 10 and 14 days but reach a maximum size at 4 to 5 days. Healing is complete, with no residual scarring, but recurrent episodes of ulceration tend to occur regularly at 1- to 4-month intervals. Individual ulcers are shallow, surrounded by an area of reddened mucosa, and vary in shape depending on the site.[73]

In major aphthous ulceration, the ulcers are much larger and more longer lasting than those seen in minor aphthous ulceration. They may appear singly or up to three or four at a time, are very painful, and give rise to extensive tissue destruction. Both keratinized and nonkeratinized oral mucosa may be affected, including the dorsum of the tongue and the oropharynx. Herpetiform ulcers occur with a frequency similar to that of major aphthous ulcers and may also affect the keratinized and nonkeratinized oral mucosa, typically of the floor of the mouth, lateral borders, and ventral surface of the tongue.[70–74] At the outset of an episode of ulceration, numerous discrete ulcers, approximately 1 to 2 mm in diameter, appear. These may later coalesce to form a single, large, painful lesion with a serpiginous outline. The ulcers usually heal within 10 days without mucosal scarring, although recurrent episodes of ulceration may supervene within days. Repeated attacks of this type may give rise to severe dysphagia.

There are no distinguishing histopathologic features. Microscopy reveals an appearance similar to that seen in cases of traumatic ulceration of the oral mucosa.[73] The epithelium shows no diagnostic features, and the underlying connective tissue is infiltrated with inflammatory cells, predominantly lymphocytes and plasma cells.

The precise etiology of RAS is not known. Although there are occasional family patterns of involvement, inheritance does not follow any mendelian patterns, and no particular human leukocyte antigen (HLA) haplotype is associated with RAS.[70,71] An infectious etiology also seems unlikely because patients do not have a raised frequency of past or present herpetic infections and associations with *Helicobacter pylori* have not been proven.[75–80] There are tenuous associations between RAS and psychological

stress, and no consistent pattern has been demonstrated between episodes of RAS and the menstrual cycle. No significant immunologic defects have been consistently detected in patients with RAS.[70,71]

Associations between RAS and gastrointestinal disease are tenuous. Certainly, a small number of patients with undiagnosed, or poorly managed, gluten-sensitive enteropathy (GSE) may have oral signs. Usually, up to 66% have superficial oral ulcers similar to those of RAS[81]; however, patients with RAS do not have a significantly increased likelihood of having clinical, serologic, or small bowel features of GSE, and the introduction of a gluten-free diet does not cause resolution of RAS.[82–89] Up to 20% of patients with RAS may have a hematinic deficiency, usually iron.[71,90] An underlying cause for these deficiencies is rarely found, and replacement therapy infrequently produces any cessation of RAS.[91,92]

A systematic review of the management of RAS is available (Table 21-8).[93] The investigation of patients with possible RAS should be focused on excluding other causes of acute bouts of ulceration (see Table 21-7), in particular the exclusion of an underlying hematologic or gastrointestinal disorder.[70,71]

All patients should be advised to use an oral hygiene procedure that is as atraumatic as possible, and all dental appliances should fit well and not damage tissue. The correction of any hematinic deficiency is of limited benefit unless the cause is corrected.[92] Topical corticosteroids remain the mainstay of RAS treatment in most countries, although there are few well-controlled studies of their precise efficacy.[93] A wide range of different topical corticosteroids may reduce symptoms.

Benzydamine hydrochloride mouthwash is of no more benefit on ulcer healing than placebo[94]; nevertheless, it (or lidocaine gel) can produce transient relief of pain.

Chlorhexidine used as a 0.2% w/w mouthrinse or 1% gel can reduce the duration of ulcers and increase the number of ulcer-free days.[95–98] Topical tetracyclines (eg, chlortetracycline, and tetracycline) may reduce healing times and/or reduce the associated pain of RAS,[99–102] but they may cause dysgeusia, oral candidiasis, and a burning-like sensation of the pharynx and are not suitable for young children, who might ingest them, with resultant tooth staining.

A variety of other topical agents have been suggested to be of some benefit in the management of RAS, but the supportive data are quite sparse. There have been several studies of the efficacy of amlexanox in the management of RAS, including one detailed randomized controlled study, suggesting that the 5% paste may significantly reduce the pain and time of healing of ulceration of RAS. [103–105]

Systemic immunosuppression is rarely warranted in view of the limited efficacy of topical agents and the sometimes profound pain and/or long-standing ulceration. However, although a variety of such agents have been proposed to be clinically useful, there is little supportive evidence.[93]

Thalidomide remains the most effective agent for the management of RAS, producing a remission in almost 50% of treated patients in one randomized controlled trial.[106] Open and double-blind studies of patients with HIV-related oral ulceration and in non–HIV-related RAS and several case studies confirm that thalidomide is of some clinical benefit.[106–114] Thalidomide gives rise to mild adverse side effects (particularly somnolence) in up to 75% of treated patients, and polyneuropathy can arise in about 5%. Clearly, the risk of teratogenicity also limits the clinical application of thalidomide in the management of RAS.

TABLE 21-8 TREATMENT OF RECURRENT APHTHOUS STOMATITIS

ANTIMICROBIAL
Chlorhexidine gluconate mouthrinse
ANALGESIA
Benzydamine hydrochloride mouthrinse/spray (provides symptomatic relief)
TOPICAL CORTICOSTEROIDS
Triamcinolone acetonide (0.1%) in Orabase*
Betamethasone mouthrinse
Flucinonide cream/ointment
Fluticasone cream/spray/inhaler
SYSTEMIC THERAPIES†
Systemic corticosteroids
Corticosteroid-sparing agents

*This is the only corticosteroid licensed for local oral application.
†Rarely warranted.

Behçet Disease. Behçet disease is clinically characterized by recurrent oral and genital ulceration together with a spectrum of cutaneous, ocular, neurologic, and other systemic manifestations. Although very uncommon in childhood, almost all affected children will have RAS-like oral ulceration.[115] The ulcers are clinically and histologically indistinguishable from those seen in RAS, and all three types of RAS may be seen in this closely related condition. The local management of oral ulceration in this condition is similar to that for RAS; however, as with adults, systemic therapies, including thalidomide, are often required.[116]

White Patches of the Oral Mucosa in Childhood. The causes of white patches of the oral mucosa in childhood are summarized, reflecting a wide spectrum of possible pathologies (Table 21-9).[81,117] In general, it is possible to consider white patches as being adherent and nonadherent, solitary and multiple. Common causes of oral white patch in children include material alba (food debris) and acute pseudomembranous candidiasis, although a variety of other disorders can manifest as white lesions of the mouth.

Infectious Causes of Oral Mucosal White Patches. ***Acute* Pseudomembranous *Candidiasis*.** The most common fungal infections seen in the oral cavity in children are caused by *Candida albicans*, which is commensal in the mouths of up to 70% of the general population.[118] Candidiasis may present in a variety of clinical forms but is, in all cases, an opportunistic infection caused by a change in the local or systemic host response. Although *C. albicans* remains the most common fungal commensal of the

mouth, there is arising frequency of non-*albicans Candida* species in the oral cavity, particularly in patients with HIV disease or poorly controlled diabetes mellitus or those receiving iatrogenic immunosuppression. In addition, these patient groups may be liable to carry and transmit azole-resistant fungal infections.[119–121]

Acute pseudomembranous candidiasis (thrush) may occur in the newborn but more typically occurs in children receiving broad-spectrum antibiotics or systemic corticosteroids or in those with some primary or secondary immunodeficiency states (eg, HIV disease). It manifests clinically as asymptomatic soft, creamy yellow areas raised above the surrounding mucosa, which leave a red bleeding surface when wiped off. The lesions may be multiple or a confluent mass and may affect all mucosal surfaces, particularly the soft and hard palate, tongue, and vestibule.

Diagnosis is usually based on the clinical picture and history, although, rarely, it can be confirmed by taking a smear of the material; a Gram or periodic acid–Schiff stain of the preparation reveals the typical branching hyphae of *Candida*. Depending on the severity and the underlying cause of the candidiasis, topical and/or systemic antifungal agents may be required, although the principal aim of management must be to find the underlying cause.[117,118]

Chronic Mucocutaneous Candidiasis. Chronic mucocutaneous candidiasis (CMC) is a group of rare immunodeficiencies characterized clinically by recurrent and/or persistent candidal infection of the skin and mucosae. At least four types of CMC have been described: diffuse CMC, sporadic CMC, candidiasis endocrinopathy syndrome, and late-onset CMC. Children with CMC can have widespread and/or recurrent oral pseudomembranous candidiasis, angular cheilitis, and chronic hyperplastic candidiasis. Children with hypoparathyroidism as part of candidiasis endocrinopathy syndrome may have enamel hypoplasia. Antifungal therapy is often difficult in CMC in view of the potential for the azole resistance of *Candida* to develop. Detailed discussions of CMC can be found elsewhere.[122,123]

TABLE 21-9 ORAL MUCOSAL WHITE PATCHES IN CHILDHOOD

NONADHERENT
Pseudomembranous candidosis (thrush)
Other mycoses
Food debris
Furred tongue
Drug-associated necrotic debris (eg, aspirin, cocaine)
ADHERENT
Solitary
Papillomas (warts—these are rarely of sexual origin)
White sponge nevus
Geographic tongue (erythema migrans—red and white lesions)
Oral (idiopathic) leukoplakia
Frictional keratosis (eg, cheek biting)
Keratosis owing to smokeless tobacco
Carcinoma (very rare)
Multiple
Traumatic keratosis
Lichen planus
Oral hairy leukoplakia
Chronic mucocutaneous candidiasis
Others

Other Types of Candidal Infection. ***Acute Atrophic Candidiasis.*** Acute atrophic candidiasis is very occasionally seen in children and is the result of candidal overgrowth in patients being treated with broad-spectrum antibiotics or with immunosuppressive drugs. The mucosa is typically sore, inflamed, and sensitive to hot and spicy foods. Therapy with topical nystatin (eg, pastilles) or amphotericin may be warranted, but signs and symptoms usually resolve on cessation of the causative treatment.[117,118]

Chronic Atrophic Candidiasis. Chronic atrophic candidiasis, which has also been termed denture-associated candidiasis, is characterized by a red, inflamed mucosa and is precisely limited to the area covered by a well-fitting (usually upper) denture. The condition is often seen in children who are wearing a removable orthodontic appliance, with the area of inflammation confined to the mucosa covered by the acrylic base plate. Diagnosis is usually easily made from the clinical picture. Microbial culture for fungal infection is not warranted unless the child is immunocompromised, when non-*albicans Candida* species are most likely to occur. Treatment usually simply requires improving the hygiene of the appliance together with the application of antifungal gel (eg, miconazole) to the fitting surface of the appliance, as well as the administration of topical nystatin or amphotericin; systemic antifungal agents are rarely warranted.[117,118]

Angular Cheilitis. Angular cheilitis (stomatitis) presents as reddened folds at the corners of the mouth. Occasionally, there is ulceration. The lesions are usually colonized by *C. albicans* and/or *Staphylococcus aureus*. Although most commonly associated with a reduction in vertical face height in adult denture wearers, angular cheilitis in children may reflect the presence of an iron deficiency, neutropenia, malnutrition, or cell-mediated immunodeficiency, particularly HIV disease. Angular (and median) cheilitis may accompany the labial enlargement of Crohn disease and orofacial granulomatosis. The diagnosis is usually based on the clinical picture and history; microbiologic studies are usually neither warranted nor helpful. Miconazole gel is usually of some benefit in the treatment of angular cheilitis, although, of course, the underlying cause must be identified and corrected.[117,118]

Oral Hairy Leukoplakia. Oral hairy leukoplakia manifests as an adherent asymptomatic bilateral white patch on the lateral borders and dorsum of the tongue and sometimes the floor of the mouth. Caused by Epstein-Barr virus, this lesion almost always arises in immunosuppressed patients, typically those with HIV disease and individuals receiving long-term corticosteroids (including inhalers) or other immunosuppressants. Although caused by Epstein-Barr virus, it does not warrant any antiherpes intervention. The signs often wax and wane and may resolve if the immunosuppression lessens. This lesion is not potentially malignant.[124,125]

Potentially Malignant and Malignant Disease of the Mouth in Childhood

Oral squamous cell carcinoma is the most common malignant disease of the mouth. Although rare in children, it can still arise and may manifest as a solitary white patch (leukoplakia), speckled area, or ulcer. Any adherent white patch that does not appear to be due to trauma should be examined histopathologically to exclude the, albeit rare, possibility of malignancy.[126]

Human Papillomavirus Infection. Human papillomavirus manifests as warts or cauliflower-like white squamous papillomas. The lips, palate, and gums are the most commonly affected sites. The lesions are usually solitary and small, although in immunocompromised children (eg, those with HIV disease and iatrogenic immunosuppression), they can be multiple.[127] Heck disease (focal epithelial hyperplasia) presents as multiple white papular lesions. Most human papillomavirus infection of the mouth in children is not sexually associated. The lesions can be removed surgically or with cryotherapy, although topical interferon-β may be an alternative therapy.[128,129]

Lichen Planus. Oral lichen planus is common, affecting 1 to 2% of most populations. It typically arises in middle to late life and has a slight female predominance; nevertheless, disease can occasionally arise in children and young adults. Oral lichen planus gives rise to white patches that typically arise bilaterally on the buccal mucosa, dorsum of the tongue, and/or labial and buccal aspects of the gingival. The white lesions are generally asymptomatic, although patients occasionally report a roughness or dryness of the affected mucosal surfaces. Erosions and/or ulceration can arise within the white patches, giving rise to erosive and ulcerative lichen planus, respectively; these lesions can be notably painful, with symptoms being profound with hot, spicy, or acidic foods.[130]

Although lichen planus can be occasionally caused by drug therapy (eg, β-blockers, sulfonylureas[131]), oral lichen planus in children tends to be idiopathic. Rarely, oral lichen planus will be a complication of chronic graft-versus-host disease.

Unlike most other white patches of the oral mucosa, lichen planus does not give rise to solitary lesions; however, it is often advantageous to confirm the clinical diagnosis by histopathologic examination of lesional tissue.

Oral lichen planus only warrants treatment when lesions are erosive, ulcerative, or bullous, when topical corticosteroids are the mainstays of therapy (see Table 21-8).[130,132] Although there are no data, topical tacrolimus may be of benefit for the treatment of symptomatic oral lichen planus in children recalcitrant to topical corticosteroids.[133] Systemic immunosuppressive therapy is rarely warranted for the treatment of oral lichen planus in children or adults. The white lesions of oral lichen planus rarely resolve. It remains unclear if oral lichen planus has a malignant potential; however, in view of the related controversy,[130,134] careful lifelong clinical follow-up is advisable.

White Sponge Nevus. White sponge nevus is a rare autosomal dominant disorder of otherwise well children and adults. It gives rise to bilateral adherent white or gray thickened patches. Similar lesions may occur on the anal or vaginal mucosa. It is generally asymptomatic but may require histopathologic examination to exclude lichen planus.[135]

Oral Mucosal Pigmentation. Oral mucosal pigmentation in childhood is usually racial in origin, although a number of local and systemic disorders may give rise to various pigmented areas in the mouth.[8] Children may have localized nevi, which manifest as localized areas of hypermelanotic or blue pigmentation. Malignant melanoma is rare in the mouths of children[136]; however, when there is any doubt as to the cause of hypermelanotic lesions, histopathologic examination of lesional tissue should always be undertaken.

Amalgam tattoos are more common in adults but may arise in children, presenting as areas of blue macules or, less commonly, papules on the gingivae, floor of the mouth, or buccal mucosa. These lesions are harmless and asymptomatic.

Kaposi sarcoma is the most common oral malignancy of HIV in childhood. It manifests as a blue, red, or purple macule, papule, nodule, or ulcer, usually of the hard palate and/or gingivae. These tumors can be locally destructive and often reflect more widespread systemic involvement of the human herpesvirus 8–associated tumor; however, they may regress with effective highly active antiretroviral therapy (HAART). Similar, but less extensive, Kaposi sarcoma can occur in the mouths of children receiving long-term iatrogenic immunosuppressive therapy.[137]

Addisonian pigmentation manifests as diffuse hypermelanotic pigmentation of the buccal mucosa. More extensive pigmentation can occur but is uncommon. Other causes of oral mucosal pigmentation are indicated in Table 21-10.

Other Oral Mucosal Disorders in Childhood

Ankyloglossia (Tongue-tie). Ankylogossia is common, manifesting as an exaggerated lingual frenum that may limit the ability to protrude the tongue. It does not usually cause difficulties with speech development, although it can limit the ability to clean food debris and plaque from the teeth, gums, and vestibules of the mouth. Ankyloglossia requires simple surgical care.[8]

Abnormal Labial Frenum. The frenum of the upper or, less commonly, lower lip can be exaggerated, giving rise to a diastema (space) between the related central incisors. Children may have difficulty maintining good plaque control at this site. Surgical reduction, together with orthodontic care, will correct this common problem.[8]

Salivary Gland Disease in Childhood

Other than mumps, salivary gland disease in childhood is uncommon. Salivary gland disease may manifest as localized swelling (Table 21-11) and/or xerostomia (Table 21-12).[138]

TABLE 21-10 ORAL MUCOSAL AND GINGIVAL PIGMENTATION IN CHILDHOOD

LOCALIZED
Amalgam tattoo
Nevus
Freckle (ephelis)
Melanotic macules
Kaposi sarcoma (eg, in HIV disease)
Peutz-Jeghers syndrome
Malignant melanoma (rare in childhood)
Laugier-Hunziker syndrome
Complex of myxomas, spotty pigmentation, and endocrine overactivity
GENERALIZED
Racial
Addison disease
Drugs (eg, minocycline)
Albright syndrome
Central cyanosis
Neurofibromatosis
Hemochromatosis
Incontinentia pigementi
Chronic hepatic disease (affects gingivae mainly)

HIV = human immunodeficiency virus.

Mumps (Epidemic Parotitis). Mumps is an acute generalized paramyxovirus infection of children and young adults. Mumps typically affects the major salivary glands, although involvement of other structures can occur, including the pancreas, testis, ovaries, brain, breast, liver, joints, and heart.[139]

Mumps is transmitted via the droplet route and has an incubation time of approximately 14 to 18 days. Patients manifest with initial pyrexia, chills, and facial pain. The parotid glands are typically bilaterally enlarged, although this may initially be unilateral. There is often swelling of the submandibular glands together with lymphadenopathy, giving rise to profound facial and neck swelling. Rarely, sublingual swelling may be so profound as to cause elevation of the tongue, dysphagia, and dysarthria. The salivary swelling tends to diminish after approximately 4 to 5 days and may precede more complicated aspects of illness.

Orchitis may develop approximately 4 to 5 days after the onset of parotitis. Typically, only one testicle is affected, and, occasionally, there can be bilateral involvement. Orchitis tends to arise in postpubertal boys and rarely gives rise to serious, long-standing disease.

TABLE 21-11 SALIVARY GLAND SWELLING IN CHILDHOOD

Mumps
Recurrent parotitis of childhood
Sjögren syndrome and related disorders
Acute suppurative sialadenitis
Duct obstruction (uncommon in children)
Sarcoidosis
Cystic fibrosis
Sialosis (rare—eg, with bulimia nervosa)
HIV disease
Hepatitis C virus disease
Mucoceles

HIV = human immunodeficiency virus.

Mumps can give rise to a lymphocytic or viral meningitis. This again commences a few days after the development of parotitis, although it can occur in the absence of salivary gland disease. Other neurologic manifestations include retrobulbar neuritis and encephalitis. Deafness is possible but rare. Pancreatic infection may give rise to mild upper abdominal pain, but acute and long-term complications are unusual. Likewise, although cardiac, hepatic, and joint infections can occur, they are rare and do not generally cause notable complications.

The diagnosis of mumps is typically based on the clinical picture; however, it may be confirmed by detection of viral-specific immunoglobulins G and A. Viral culture is possible but generally unnecessary because serologic methods are highly sensitive.

There is no specific treatment for mumps; analgesia and appropriate fluid intake are the mainstays of therapy. It has been suggested that corticosteroids may be effective for profound parotitis, but, generally, these are not required unless the patients have other systemic symptoms, such as orchitis. Mumps can generally be prevented with appropriate vaccination (mumps/measles/rubella).

HIV Salivary Gland Disease. Salivary gland disease can arise in 4 to 8% of adults and children with HIV infection. The salivary gland disease of HIV infection manifests as swelling and/or xerostomia and reflects underlying bacterial sialadenitis, intraparotid lymphadenopathy, primary or metastatic non-Hodgkin lymphoma, or Kaposi sarcoma.[138,140]

The specific disorder HIV salivary gland disease (HIV-SGD) gives rise to recurrent and/or persistent major salivary gland enlargement and xerostomia. The parotids are most frequently affected; often there is profound bilateral enlargement. Salivary gland disease tends to arise in late HIV infection, although, occasionally, it can be the first manifestation of HIV disease. It may be associated with HLA-DR5 and is part of a more generalized disorder

TABLE 21-12 CAUSES OF LONG-STANDING XEROSTOMIA IN CHILDHOOD

Sjögren syndrome and related disorders
Sarcoidosis
Cystic fibrosis
HIV disease
Hepatitis C virus disease
Drugs
Anticholinergics
Antihistamines
Tricyclic antidepressants
Serotonin reuptake
Sympathomimetics
Phenothiazines
Occasional cytotoxic drugs
Radiation of the head and neck
(when the salivary glands lie within the field of radiation)
Chronic graft-versus-host disease
Dehydration (hypercalcemia, diabetes mellitus)
Anxiety
Depression
Salivary gland agenesis

HIV = human immunodeficiency virus.

termed diffuse infiltrated lymphocytosis syndrome, which is characterized by CD8+ T-cell infiltration of the lungs, salivary glands, and lacrimal glands.[138,140]

The clinical picture of HIV-SGD mimics that of Sjögren syndrome; however, there are distinct histopathologic and serologic differences between the two disorders. Patients with HIV-SGD generally do not have anti-Ro or anti-La antibodies, but they do have hypergammaglobulinemia.

The diagnosis of HIV-SGD is similar to that of Sjögren syndrome. Fine-needle aspiration biopsy may be particularly useful because it allows rapid exclusion of malignancy.[141]

There is little information regarding the specific management of HIV-SGD. The clinical signs of HIV-SGD are usually nonprogressive; hence, therapy is indicated only if there is notable cosmetic deformity or xerostomia. HAART will, at least in the short term, cause resolution of the swelling of HIV-SGD. Less practical, suggested therapies are repeated aspiration, tetracycline sclerosis, or surgical removal of an enlarged gland.[142] External radiation may cause transient improvement, although there are no data on the effectiveness for affected children.[143] Xerostomia independent of HIV-SGD may arise in HIV infection as a consequence of some nucleoside analog HIV reverse transcriptase inhibitors or protease inhibitors (see below).[144]

Hepatitis C Virus Infection. Although there are no data specific to children, it would be expected that hepatitis C virus (HCV) infection would give rise to HCV-related sialadenitis, which manifests as salivary gland enlargement and xerostomia. The histopathologic features of HCV-associated sialadenitis are similar to those of Sjögren syndrome, although the two disorders are etiologically distinct.[138]

Suppurative Sialadenitis (Suppurative Parotitis). Acute suppurative sialadenitis is an uncommon disorder characterized by painful swelling, usually of parotid glands (suppurative parotitis), purulent discharge from the duct of the affected gland, associated dysgeusia, and cervical lymphadenopathy. When disease is severe, there may be accompanying pyrexia, malaise, and a risk of abscess formation and parapharyngeal space infection, including Ludwig angina.

Acute suppurative sialadenitis can arise in childhood (prematurity being a possible risk factor), and sialadenitis can occur in newborns.[145] The highest incidence of childhood disease seems to arise in children aged 3 to 6 years of age.[146] Aseptic sialadenitis has been observed in preterm children receiving long-term orogastric tube feeding. Immunodeficiency and concurrent illness may predispose children to suppurative parotitis.

The causative organism of acute suppurative sialadenitis is often not found. Although facultative anaerobes, particularly *S. aureus* and *Streptococcus viridans*,[147,148] have frequently been reported to be of etiologic significance, a wide range of other bacteria have been implicated.

The diagnosis of acute suppurative sialadenitis is based on the history and clinical picture. Microbiologic culture of pus, under both aerobic and anaerobic conditions, may reveal likely causative agents, although specific relevant tests may be useful if a particular infection seems likely. Additional investigations such as sialography and scintigraphy are rarely warranted, although ultrasonography can be useful, particularly because abscess formation is likely, as can magnetic resonance imaging.

Effective hydration and antibiotics are the mainstays of therapy of uncomplicated acute suppurative sialadenitis.[149] Typically employed antibiotic therapies are antistaphylococcal penicillins (eg, flucloxacillin, amoxicillin, or amoxicillin-clavulanate), cephalosporins, or clindamycin, although the precise choice of antibiotic will often depend on any likely causative organism that is identified. Other alternatives may include flurithromycin. Intraductal injection of antibiotics is unlikely to be of practical benefit. Surgical drainage should be considered if there is a lack of clinical improvement after 3 to 5 days of antibiotic therapy, any unlikely facial nerve involvement, any involvement of deep fascial spaces, or abscess formation within the parenchyma of the gland.[149] Superficial parotidectomy may be required if disease becomes recurrent or chronic.[149,150]

Recurrent Parotitis of Childhood. Recurrent parotitis of childhood (juvenile recurrent parotitis) gives rise to recurrent parotid inflammation, usually associated with nonobstructive sialectasia of the parotid gland. Recurrent parotitis can arise at any age, but the usual age at onset is 3 to 6 years. The disease is characterized by localized pain and swelling, which may last up to 14 days. Fever and overlying erythema are common, and occasionally white mucopus can be expressed from the parotid duct. Recurrent parotitis of childhood tends to be unilateral rather than bilateral. The number of attacks varies from 1 to 5 per year, but some patients may have up to 20 episodes of swelling per year. The frequency of recurrence tends to peak between 5 and 7 years of age, and up to 90% of patients have resolution of disease by puberty.

Sialography and ultrasonography reveal sialectasia. This feature can also be observed in nonaffected glands of the opposite side.

The precise etiology of recurrent parotitis of childhood is unknown. There is no evidence that viral infection underlies this disorder. Analgesia is the mainstay of therapy. Antibiotics do not shorten attacks. In general, the disease tends to resolve, and there is no need for profound surgical intervention.[151]

Long-standing Xerostomia (Dry Mouth). Long-standing xerostomia gives rise to a range of disorders of the oral hard and soft tissues (Table 21-13). Xerostomia can give rise to dysarthria and dysphagia. The oral dryness leads to retention of food on the teeth, mucosa, and gingiva and thus increases the frequency of caries (particularly cervical disease) and acute gingivitis. There is an increased liability to candidal infection, notably acute pseudomembranous candidiasis, and median rhomboid glossitis, chronic atrophic candidiasis (denture-associated stomatitis), and angular cheilitis. Long-standing xerostomia increases the liability to acute suppurative parotitis (see above). The poor salivary output can lead to dysgeusia and loss of taste; many affected persons report that most foodstuffs taste "cardboard-like."[138]

TABLE 21-13 CLINICAL FEATURES OF LONG-STANDING XEROSTOMIA

SYMPTOMS
Oral dryness
Dysarthria
Dysphagia
Loss of taste (often blunting of taste of all foods)
SIGNS
Dryness of the oral mucosa
Variable lack of saliva
Depapillation, redness, and crenation of the dorsum of tongue (scrotal tongue)
Loss of upper denture retention
Increased liability to gingivitis
Increased liability to dental decay (eg, cervical caries)
Increased liability to bacterial sialadenitis (usually of the parotid glands)

Xerostomia is more common in adults than in children; however, children are clearly more likely to manifest the features of salivary gland agenesis than adults and can also be liable to the common causes of acquired salivary gland dysfunction disease.

Salivary Gland Agenesis. Agenesis of one or more of the major salivary glands is extremely uncommon. There can be variation in the number of absent salivary glands and hence varying severity of the associated xerostomia. Lack of saliva predisposes the patient to dental caries, gingival inflammation, candidiasis, and acute suppurative sialadenitis, although, in children, rampant dental caries may be the only initial sign of underlying salivary agenesis.

The precise incidence of major salivary gland agenesis is difficult to establish owing to the asymptomatic nature of many affected individuals. Familial clustering of salivary gland agenesis has occasionally been reported. Salivary gland aplasia may occur in isolation or be associated with other ectodermal defects, in particular lacrimal apparatus abnormalities. Associations with hypohidrotic ED and lacrimal-auriclodentodigital and ectodactyly-ED syndromes have been reported.[152]

Radiotherapy-Associated Salivary Gland Dysfunction. Brachytherapy of head and neck malignancies can cause profound xerostomia and salivary gland acinar destruction when the radiotherapy is directed through the major salivary glands. The degree of xerostomia clearly reflects the duration and dose of radiotherapy. The xerostomia is irreversible and thus can greatly affect the quality of life of affected children.[138] In adults, orally administered pilocarpine may lessen the severity of radiotherapy-induced xerostomia; however, there are no studies of the effectiveness of this cholinergic agent in children with radiotherapy-associated xerostomia. As a consequence, the treatment of long-term oral dryness in children is not specifically directed to enhancing cholinergic stimulation of the salivary glands.[138]

Sjögren Syndrome. Sjögren syndrome is characterized by xerostomia and xerophthalmia owing to profound lymphocytic infiltrate into the salivary and lacrimal glands.[153] Sjögren syndrome can be classified as primary disease, of which there are only symptoms and signs affecting the eye and mouth, and secondary Sjögren syndrome, in which there is xerostomia, xerophthalmia, and associated connective tissue disorder, most frequently rheumatoid arthritis or systemic lupus erythematosus.

Although possibly the second most common connective tissue disorder in adults, Sjögren syndrome is an uncommon disorder of childhood. The etiology of Sjögren syndrome remains unknown. A viral etiology—human retrovirus 5—was proposed but now seems unlikely; nevertheless, a viral basis cannot be excluded because the salivary features of human T lymphotropic virus 1, HCV, and HIV infection mimic those of Sjögren syndrome. To date, there is no evidence of a strong genetic basis for Sjögren syndrome. The pathogenesis of Sjögren syndrome is discussed in detail elsewhere.[154] The investigation of Sjögren syndrome centers on a series of clinical, radiologic, and immunologic tests,[154] of which the histopathologic examination of labial gland tissue and the detection of serum anti-Ro and/or anti-La antibodies are cardinal. The management of the oral complications of Sjögren syndrome is similar to that outlined in Table 21-14.[138] In addition, pilocarpine and other similar agents (eg, cevimeline) may enhance salivary flow. At present, no immunologically based approach has been successful for the treatment of Sjögren syndrome other than perhaps hydroxychloroquine. All children with confirmed Sjögren syndrome will require lifelong specialist follow-up to ensure the early detection of possible non-Hodgkin lymphoma, particularly mucosa-associated lymphoid tissue lymphoma.

MINOR SALIVARY GLAND DISEASE OF CHILDHOOD: MUCOCELES

Mucoceles are common, presenting as single blue or translucent sessile swellings on the lower lip. The swelling may rupture to release a viscid salty mucus. Mucoceles occur in both genders and in all age groups, and the peak age of incidence is between 10 and 29 years. They are rare in infants, although they can occur in neonates. The lateral

TABLE 21-14 MANAGEMENT OF LONG-STANDING XEROSTOMIA

THERAPY	COMMENTS
SALIVARY SUBSTITUTES	
Nonsynthetic agents	Sips of water; convenient but of limited benefit. Soft drinks should be avoided in view of the risk of caries or dental erosion.
Synthetic agents	A variety of sprays, mouthrinses, and gels are available; no one agent is better than another; benefit can be transient.
SALIVARY STIMULANTS (SALOGOGUES)	
Nonspecific	Nonsucrose confectionary can be of benefit, but there may still be a risk of dental erosion. Sorbitol-containing pastilles may be helpful.
Specific	Pilocarpine (and possibly cevimeline) may be of application, but there are no detailed studies of their application in children with long-standing xerostomia.
Oral hygiene care and dietary advice	Minimized risk of caries and gingivitis
Fluoride supplements	Reduces risk of caries

aspect of the lower lip is the most common site of recurrence of mucoceles, but other common sites include the floor of the mouth and ventrum of the tongue. The majority of mucoceles are extravasation type, in which duct damage causes pooling of mucus in the adjacent connective tissue. Retention mucoceles, the less common variant, arise after partial or complete obstruction of the excretory duct (eg, a sialolith), leading to retention of glandular secretions and dilation of the duct. Extravasation mucoceles may be more frequent in young patients, whereas retention mucoceles may occur most often in middle to late life.

Some mucoceles may resolve spontaneously, but large, recurrent, or unsightly mucoceles often require surgical excision or are removed by laser or cryotherapy. Other therapies of less well-proven efficacy include intralesional corticosteroid injections and γ-linolenic acid (oil of evening primrose).[155]

ORAL ASPECTS OF GASTROINTESTINAL DISEASE OF CHILDHOOD

Dietary and Related Disease

Dental caries is the most common diet-related disease of the mouth.[27] The clinical aspects of dental caries are detailed previously and are also reviewed elsewhere.[28–31] Although the prevalence of dental caries may be falling in some communities (particularly in areas of water fluoridation), erosion of the dentition in childhood, as a consequence of the increasing consumption of low-sugar, low-pH carbonated drinks. The details of dental erosion are discussed above and are reviewed elsewhere.[156,157]

Malnutrition. Malnutrition gives rise to gingival and oral mucosal disease but seems to have little impact on tooth structure or eruption.[158] Profound malnutrition in childhood, particularly in areas of political unrest and economic poverty, such as some African states,[159–161] gives rise to severe ANUG (see previously) and later necrotic ulceration and loss of orofacial skin and muscles, when the descriptive term noma or cancrum oris is applied.

Vitamin C Deficiency. Profound vitamin C deficiency may give rise to gingival enlargement. In addition, the gingivae are friable and bleed easily, often spontaneously.[162] Although appropriate vitamin C supplements will resolve these gingival lesions, there is no evidence that vitamin C supplementation is of clinical benefit in the management of plaque-related gingival or periodontal disease.[163]

Hematinic Deficiencies. Anemia secondary to any hematinic deficiencies gives rise to superficial ulceration of the nonkeratinized (mobile) oral mucosa. Deficiencies of iron, vitamin B_{12}, and folate also predispose the patient to angular stomatitis (cheilitis) and glossitis, the latter manifesting as a sore, erythematous, and smooth tongue.[8] Vitamin B_{12} deficiency rarely gives rise to linear erythematous patches of the dorsum of the tongue, sometimes termed Moeller glossitis. Vitamin B_{12} deficiency, sometimes in the absence of anemia, may cause lingual dysesthesia. It has been suggested that deficiencies of vitamin B_1, B_2, and B_6 give rise to angular stomatitis and/or oral dysesthesia; however, much of these data is unsubstantiated.[164] Longstanding iron deficiency in CMC may give rise to a glossitis and postcricoid webbing akin to Paterson–Brown Kelly (Plummer-Vinson) syndrome in late childhood.[123]

Zinc Deficiency. Zinc deficiency in acrodermatitis enteropathica may cause oral mucosal ulceration and angular stomatitis, but, in general, zinc deficiency does not give rise to oral disease. A link between erythema migrans (geographic tongue) and zinc deficiency is controversial; certainly, there are no data showing that children with erythema migrans are zinc deficient or benefit from zinc supplementation.[165]

Hypocalcemia. The hypocalcemia of GSE may give rise to hypocalcification of the deciduous and permanent dentitions, manifesting as areas of whiteness and brown staining of enamel.[166,167] In mild hypocalcemia, the crown shape is not affected, although when there is severe hypocalcemia, there may be pitting of the enamel.[168,169] The hypocalcified enamel is particularly liable to dental caries. Hypocalcemia secondary to autoimmune hypoparathyroidism in candidiasis endocrinopathy syndrome may also give rise to enamel hypoplasia, usually of the permanent dentition.[123,170,171]

Fluorosis. Excess fluoride intake, either as a consequence of drinking water with a naturally high fluoride concentration (greater than 0.03–0.04 ppm; eg, in certain areas of Southeast Asia and South and North America)[172] or following excess ingestion of fluoride supplements (eg, in toothpaste or fluoride tablets), will cause some staining of the enamel of developing teeth.[173,174] Fluorosis most commonly affects the permanent dentition, although in areas where there is endemic fluorosis, the deciduous teeth will also be affected. Mild fluorosis presents as chalky white patches of the enamel of otherwise normal teeth, more severe disease manifests with intrinsic brown staining of the enamel, and severe fluorosis gives rise to brown pitting, mottling, and brittleness of the enamel. As expected, the enamel associated with fluorosis is less liable to dental caries than normal enamel, although in severe fluorosis, the pitting and mottling can lead to unusual patterns of dental decay, for example, on the smooth surfaces of teeth. Other causes of intrinsic staining of the teeth in childhood have been discussed.

Anorexia and Bulimia Nervosa. As a consequence of resultant anemia, patients with long-standing anorexia nervosa or bulimia nervosa often develop oral mucosal ulceration.[175] In bulimia nervosa, the trauma of a patient's finger scraping over the palate may cause traumatic ulceration, palatal petechiae, and, rarely, necrotizing sialometaplasia.[138]

The acidic reflux of bulimia nervosa causes erosion of the deciduous and permanent dentition. The erosion particularly affects the posterior teeth and the palatal aspects of the upper anterior teeth; both the deciduous and the permanent dentitions can be affected.[42,176,177] The reflux of the acidic gastric contents may cause painless bilateral enlargement of the parotids without xerostomia, sometimes termed sialosis.[178,179]

Tylosis. Tylosis gives rise to extensive areas of leukoplakias of the oral mucosa. However, in contrast to the esophageal disease of tylosis, the oral lesions are not potentially malignant.[180]

Disease of the Small Bowel and Related Structures

Gluten-Sensitive Enteropathy. A minority of children (probably about 3%) with undiagnosed or poorly controlled GSE can develop superficial oral ulceration, usually of the nonkeratinized oral mucosa,[181,182] although up to 60% of patients with GSE can have some symptoms (see previously). As discussed previously, it has been suggested that RAS may reflect undiagnosed GSE, but supportive data are lacking; for example, few patients with RAS have histopathologic or immunologic features suggestive of GSE and few have resolution of the ulceration of RAS following instigation of a gluten-free diet. Additional oral features of GSE in childhood include glossitis and angular stomatitis secondary to hematinic deficiencies and enamel hypoplasia as a consequence of long-standing hypocalcemia.[167–169,183,184] Children with dermatitis herpetiformis may have the dental defects of GSE.[185] Chronic bullous disease of childhood may also give rise to vesiculobullous disease of the mouth, although it is rarely associated with gastrointestinal lesions.[186–188] Long-standing dapsone therapy for dermatitis herpetiformis can cause methemoglobinemia, which manifests as blue pigmentation of the tongue.

Cystic Fibrosis. Tetracycline staining of the teeth is the most likely oral feature of cystic fibrosis. As discussed previously, the degree of staining depends on the age at which therapy was given, the duration of treatment, and the type of tetracycline provided. The staining can vary in color from yellow to gray and may range from mild banding to profound discoloration of the complete enamel surface.[189,190] The tetracycline staining does not influence the risk of dental caries, although, interestingly, the areas of tetracycline deposition will fluoresce under ultraviolet light. Minocycline causes profound melanotic hyperpigmentation of the oral mucosa, which can mimic addisonian pigmentation.[191,192] Cystic fibrosis rarely gives rise to other oral lesions. Enlargement of the submandibular, but not the parotid, glands has been reported, and although sialochemical changes do occur, there is no evidence that patients develop profound xerostomia.[193] One report suggested that patients with cystic fibrosis may be liable to calculus formation, but this has never been fully substantiated.[194] Patients may have oral malodor, a predisposition to mouth breathing, and anterior open-bite.[190] Central cyanosis will give rise to blueness of the oral mucosa and gingiva. Some pancreatic supplements may cause oral mucosal ulceration, and a low-fat, high-carbohydrate diet potentially increases the liability to dental caries. Vitamin K deficiency may predispose the child to spontaneous gingival bleeding.

Peutz-Jeghers Syndrome. Peutz-Jeghers syndrome rarely gives rise to oral features per se. There is, however, notable circumoral melanosis manifesting as discrete brown to bluish black macules. Almost all patients have lesions on the lips, particularly the lower lip.[195,196]

Large Bowel Disease

Ulcerative Colitis. There are few detailed descriptions of the oral manifestations of ulcerative colitis in childhood. Oral pyostomatitis vegetans is probably more likely in ulcerative colitis than in Crohn disease and manifests as multiple small, ragged, superficial pustules and ulcers or fissures of the reflected mucosa of the lips (usually upper), soft palate, and buccal mucosa. The course of the oral pyostomatitis vegetans may follow that of the bowel disease.[197–207] Other oral manifestations of ulcerative colitis include superficial ulceration related to hematinic deficiency. There are no reports of oral pyodermia gangrenosum in children with ulcerative colitis, although oral pemphigus vulgaris developed in one child with inflammatory bowel disease.[208] Children will be liable to corticosteroid-induced acute pseudomembranous candidiasis and immunosuppression-related oral hairy leukoplakia (see previously).

Gardner Syndrome. Gardner syndrome commonly affects the mouth, with up to 69% of patients, usually by adolescence, having clinical or radiologic evidence of oral lesions.[209–215] Multiple odontomes and/or supernumerary teeth are common, causing delayed or failed eruption of the permanent teeth, resulting in malocclusion and dentigerous cyst formation. Osteomas occur on the mandible and maxilla.

Crohn Disease and Orofacial Granulomatosis. A detailed review of the oral features of Crohn disease and the differentiation of Crohn disease from orofacial granulomatosis may be found elsewhere.[216–218] For the purposes of this chapter, the two disorders are regarded as almost synonymous. In Crohn disease, oral lesions can predate the more common ileal disease.

Crohn disease may give rise to persistent and/or recurrent lip swelling of one (typically the lower) or both lips. The persistent enlargement may give rise to angular stomatitis and median fissuring, both features presumably being exacerbated by any accompanying iron or vitamin B_{12} deficiency. In early disease, the epithelium of the swelling lip may tear, giving rise to a ragged appearance. The buccal mucosa can become swollen, giving rise to a cobblestoned appearance.[218–223]

Affected children may develop ragged, deep ulcers, particularly of the labial vestibules, although similar lesions can occur on other mobile oral mucosal surfaces. The ulcers may have a rolled margin and/or be associated with mucosal tags. Crohn disease may also give rise to superficial oral mucosal ulceration secondary to hematinic deficiencies. Pyostomatitis vegetans and epidermolysis bullosa acquisita are rare possible oral features of Crohn disease in childhood.

Oral mucosal tags rarely occur in the mouth, and fistula formation in the mouth is possible but has rarely been reported in childhood Crohn disease.

The gingivae, particularly the attached tissues, can become enlarged. This swelling is unrelated to plaque-related gingival disease. There have been reports of periodontal destruction in a small number of patients with Crohn disease, the exact cause of which remains unclear but is unrelated to the gingival features of Crohn disease.[224]

Some child patients with Crohn disease may have a fissured tongue, and, rarely, patients may develop a lower motoneuron palsy of the facial nerve. The term Melkersson-Rosenthal syndrome is sometimes applied to the combination of orofacial swelling, facial nerve palsy, fissured tongue, and mucosal swelling.[225]

Taste abnormalities have been described in adults with Crohn disease, but it is unclear if this is likely in children.

Other potential oral features of Crohn disease include acute pseudomembranous candidiasis secondary to prednisolone therapy and Epstein-Barr virus–induced oral hairy leukoplakia in long-standing immunosuppressive therapy.[124]

Orofacial granulomatosis may, in some instances, represent an intolerance to food additives; hence, appropriate patch testing and elimination diets may occasionally be helpful in the management of some affected patients.[226–228] However, immunosuppressive regimens similar to those of ileal Crohn disease are probably the mainstays of treatment of orofacial granulomatosis. Systemic thalidomide can cause resolution of all of the oral features of orofacial granulomatosis,[229] but relapse may occur on cessation of therapy.

Hepatic Disease

Kernicterus can cause intrinsic yellow staining of the teeth,[8,230,231] although in erythropoietic porphyria, the teeth can be stained orange or red.[51,52] The hyperbilirubinemia and hyperbiliverdinemia of biliary atresia may give rise to yellow or green pigmentation of the gingival margins.[232,233] Severe primary biliary cirrhosis may give rise to spontaneous gingival bleeding. In addition, primary biliary cirrhosis may be associated with secondary Sjögren syndrome with resultant xerostomia.[234]

Penicillamine therapy in primary biliary cirrhosis may cause a lichenoid drug reaction in the mouth that is clinically identical to idiopathic lichen planus. Long-term cyclosporine therapy following liver transplant commonly gives rise to gingival enlargement. The degree of enlargement does not always correlate with cyclosporine dose or plasma levels and may not relate to the patient's oral hygiene status.[235] Median rhomboid glossitis and oral hairy leukoplakia can also arise with long-term cyclosporine (and probably tacrolimus therapy). Aspects of viral hepatitis are discussed above.

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CHAPTER 22

CONGENITAL ANOMALIES

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Congenital anomalies of the mouth and esophagus are relatively common. The majority of these anomalies are readily apparent at birth or, in many cases, can be appreciated on prenatal ultrasonography. Increasing knowledge of the embryologic events that result in the normal development of these structures has led to the identification of various genes and gene products that help to orchestrate these events. With that, there has been a rapid advancement in identifying various genetic mutations that result in abnormalities of development and subsequent syndromic and nonsyndromic presentations.

These malformations are associated with various clinical presentations. Whereas some allow patients to be asymptomatic, others can cause difficulties in feeding or articulation or life-threatening respiratory difficulties. More complex malformations often require multidisciplinary teams, including surgeons (general, otolaryngologic, orthodontic), gastroenterologists, speech pathologists, and geneticists.

FACIAL CLEFTS (CLEFT LIPS AND PALATES)

Oral clefts are among the most common of all birth defects, second only to clubfoot. Cleft lip with or without cleft palate (CL[P]) occurs with an incidence of 1 in 500 to 1 in 2,500 in different populations based on ethnic group, geographic location, and socioeconomic conditions.[1] The highest incidence is among Native Americans (3.6 in 1,000 live births), whereas among blacks, it is less (0.3 in 1,000 live births). Whites have an incidence of 1 in 1,000 live births. Defects are unilateral in 80%.[2]

Isolated cleft palate (CP) occurs in approximately 1 in 2,000 live births, and there is little to no racial preponderance.[2] CL(P) is more common in boys, whereas CP is seen more commonly in girls. The cause is likely multifactorial disruption of embryologic morphogenesis.[2] Higher birth order may also be a risk factor for CL(P) and CP. However, studies are not conclusive and may be confounded by other factors, such as advanced maternal or paternal age or increased exposure to teratogens, which, in themselves, may be risk factors.[3]

The risk of having subsequent children with clefts is different for those with CL(P) from those with CP. When both parents are unaffected and have an affected child, the risk of recurrence is 4.4% for CL(P) and 2.5% for CP. If one parent is affected, the risk is increased to 15.8% for CL(P) and 14.9% for CP. If two children are affected and the parents are unaffected, the risk for a third child is 9% for CL(P) and 1% for CP.[2] Concordance among monozygotic twins ranges between 40 and 60%, whereas it is 5% among dizygotic twins. The lack of 100% concordance rates among monozygotic twins argues against genetic events alone being responsible for the clefting phenotype.[4]

Cleft lip is a unilateral or bilateral gap in the upper lip and jaw, which form during the third to seventh week of embryologic development.[1] The incisive foramen divides the hard palate into a primary and secondary palate. The primary palate lies anterior to the incisive foramen and includes the bony premaxilla, mucoperiostal covering, and incisor teeth. The secondary palate is posterior to the incisive foramen and is composed of the horizontal plates of the maxilla and palatine bone. The remaining dentition arrives from the secondary palate. Primary palate formation begins at 4 to 5 weeks gestation with the fusion of the paired median nasal prominences. This marks the separation of the oral and nasal cavities. Ultimately, the median nasal prominences give rise to the dental arch, incisor teeth, and philtrum of the upper lip. Formation of the secondary palate (hard and soft) begins at approximately the seventh week of gestation. The posterior maxillary prominences form palatal shelves, which rotate inferiorly and medially to fuse with the vomer in the midline. Anterior to posterior palatal closure occurs in a zipper-like fashion. At 9 weeks gestation, the hard palate fuses with the septum to complete the separation of the oral and nasal cavities. The soft palate is composed of five paired muscles: tensor veli palatini, levator veli palatini, palatoglossus, palatopharyngeus, and musculus uvulae. Midline approximation of the soft palatal musculature marks the completion of palatogenesis at approximately 12 weeks gestation.[2]

The multifactorial inheritance model is currently the most widely accepted theory of nonsyndromic clefts. In this model, the risk of developing a given anomaly is determined by the presence of either genetic or environmental liabilities. Each liability occurs in a normal distribution within the population. The accumulation of multiple small liabilities eventually reaches a threshold, beyond which a defect occurs. Variable penetrance of the phenotype for many genes results in nonmendelian inheritance patterns.

An estimated 300 syndromes include CL(P) in their phenotype; however, syndromic clefts account for only 30% of CL(P).[1] The proportion of patients with CP who

are syndromic versus nonsyndromic remains unresolved, with estimates varying widely, between 15 and 80%.[1] Approximately 25% of syndromic clefts are associated with Stickler syndrome, whereas another 15% are associated with velocardiofacial syndrome.[2] The most common malformations in association with clefts are found in the central nervous system and the skeletal system, followed by the urogenital and cardiovascular systems.[5] Various syndromes associated with CP (Table 22-1) and CL(P) (Table 22-2) have been described.

Defects in the *PVRL1* gene (chromosome 11q23) result in abnormal formation of nectin 1, a cell-cell adhesion molecule expressed in the developing face and palate that is essential for fusion of the medial edge epithelia. A 50% reduction in the amount of nectin 1 appears to be a risk factor for nonsyndromic CL(P) in patients on Margarita Island and Venezuela.[1] A similar gene, *OFC3*, on chromosome 19q13 has also been implicated in nonsyndromic CL(P) based on genetic linkage studies. Other candidate genes include *OFC1* (6p24.3), *OFC2* (2p13), *TP63* (3q27), *MSX1*(4p16.1), *TGFA* (2p13), *TBX22*,[1] *PGD1* (1p36),[6] methylenetetrahydrofolate reductase (1q36),[6] and *TGFalpha* (2p13).[6]

The role of teratogens in the formation of clefts has been supported by studies suggesting causation associated with maternal exposure to corticosteroids, phenytoin, valproic acid,[4] thalidomide,[4] alcohol,[4] cigarettes,[4] dioxin,[4] or retinoic acid; maternal diabetes mellitus; maternal hormone imbalance; and maternal vitamin deficiency. However, there is no consensus that any particular teratogen or environmental factor is implicated in most clefts.[1] Folic acid may have a protective effect to reduce the risk of clefting.[6]

TABLE 22-1 CLEFT PALATE OR BIFID UVULA WITHOUT CLEFT LIP

SYNDROME	OMIM NUMBER*
Catel-Manzke	302380
Cerebrocostomandibular	117650
Deletion 4q	
Dubowitz	223370
Duplication 3q	
Duplication 10q	
Escobar	265000
Femoral hypoplasia–unusual facies	134780
Fibrochondrogenesis	228520
Hay-Wells syndrome of ectodermal dysplasia	106260
Hydrolethalus	236680
Kabuki make-up	147920
Kniest dysplasia	156550
Marden-Walker	248700
Meckel-Gruber	249000
Nager	154400
Orofaciodigital	311200
Otopalatodigital, type I	311300
Otopalatodigital, type II	304120
Popliteal pterygium	119500
Retinoic acid embryopathy	243440
Short-rib polydactyly, type II	263520
Velocardiofacial	192430
Spondyloepiphyseal dysplasia congenita	183900
Stickler	108300
Treacher Collins	154500
Van der Woude	119300

Adapted from Jones K.[55]
*Searching the OMIM (Online Mendelian Inheritance in Man) can be done at <http://www3.ncbi.nlm.nih.gov/omim/>.

TABLE 22-2 SYNDROMES WITH CLEFT LIP WITH OR WITHOUT CLEFT PALATE

SYNDROME	OMIM NUMBER*
Deletion 4p	
Ectrodactyly–ectodermal dysplasia–clefting	604292
Fryns	229850
Hay-Wells syndrome of ectodermal dysplasia	106260
Holoprosencephaly sequence	157170
Miller	247200
Mohr	252100
Orofaciodigital	311200
Popliteal pterygium	119500
Rapp-Hodgkin ectodermal dysplasia	129400
Roberts	268300
Short-rib polydactyly, type II	263520
Trisomy 13	
Van der Woude	119300

Adapted from Jones K.[55]
*Searching the OMIM (Online Mendelian Inheritance in Man) can be done at <http://www3.ncbi.nlm.nih.gov/omim/>.

Prenatal diagnosis allows for early parental counseling. Current technology can detect CL(P) at gestational week 15 because the soft tissues of the fetal face become distinct to transabdominal ultrasonography.[5] During the second trimester, ultrasonography detects less than 20% of cases of isolated CL(P) and far fewer cases of isolated CP.[5] However, syndromic CL(P) is detected at 38%, perhaps because a more detailed scan is undertaken given the associated anomalies, or because these clefts are larger and more readily visualized. Optimum timing for diagnosis is regarded as 20 to 22 weeks gestation. The ability to see the defect is influenced by the position of the fetus, position of an overlying hand or umbilical cord, maternal obesity, multiple pregnancies, oligohydramnios, and the experience of the technician. The use of transvaginal ultrasonography and three-dimensional ultrasonography also increases the sensitivity and specificity of the test.[2]

Initial evaluation of a patient with CP should include prenatal care, birth history, teratogen exposure, and a family history of clefting or syndromes. A multidisciplinary team is often helpful in assessing the family's medical and psychosocial needs. The cleft team should consist of a surgeon, otologist, audiologist, dentist (orthodontist or oral surgeon), social worker, geneticist, pediatrician, nutritionist, and speech pathologist. Breastfeeding is possible in some patients with a short or narrow cleft. Infants with larger clefts can rarely generate adequate suction for traditional breast- or bottle feeding. Various specialized nipples have been created to facilitate feeding. Feeding typically takes longer, and frequent burping may be required in these infants because they often swallow large amounts of air. Infants should be weighed on a weekly basis initially to ensure adequate intake.[2]

Palatal clefting disrupts all layers of the normal palate architecture, including mucosa, muscle, and bone. The muscles of the soft palate must wrap anteriorly and insert

on the cleft margin or the posterior palate. Aberrant tensor veli palatini insertion results in eustachian tube dysfunction, so nearly all CP patients will have chronic otitis media requiring myringotomy tube placement. Abnormal insertion of the levator veli palatini results in loss of normal velopharyngeal competence.[2]

CP may be classified as primary or secondary, complete or incomplete, unilateral or bilateral, or submucous. Primary CP results in incomplete closure of the hard palate anterior to the incisive foramen, whereas secondary CP results in a midline defect posterior to the incisive foramen. Secondary clefts appear to be distinct genetic entities, unrelated to cleft lip but often associated with Pierre Robin sequence (PRS). Complete CP involves the primary secondary and soft palate and is usually associated with cleft lip. Submucous CP results from inadequate development of the muscles of the soft palate without disruption of the mucosa. They can characteristically include a bifid uvula, dehiscence of the central palatal musculature (may be palpable or result in bluish discoloration in the midline, termed a zona pellucida), and loss of the posterior nasal spine.[2]

Presurgical orthopedic techniques are used to modify the shape of the cleft deformity before definitive cleft repair. These increase the ease of the primary repair, normalize facial growth, and prevent alveolar collapse. Active techniques include finger massage, lip taping and strapping, and oral prosthetics. Passive techniques are aimed at inhibiting tongue protrusion between the palatal shelves by using oral obturators. Although these techniques have been shown to effectively narrow the distance between alveolar segments, no differences in esthetic outcome, need for revision surgery, or improvement in feeding have been prospectively demonstrated.[2]

Palatoplasty aims to separate the oral and nasal cavities and restore velopharyngeal competence. An aggressive approach must be balanced with the risk of maxillary growth disturbance.[2]

Although 90% of patients with a cleft lip have repair between 3 and 6 months of age,[7] the timing of CP repair is controversial. Proponents of early CP repair (3–6 months) believe that early velopharyngeal competence is critical to normal speech development. Proponents of late palatal repair (2–15 years) believe that the risk of iatrogenic disruption of palatal growth and midfacial hypoplasia outweighs the risk of speech abnormalities. Clefts delayed for more than 2 years generally require obturation to overcome velopharyngeal incompetence and allow normal speech development. Oral obturators placed prior to 2 years are often poorly tolerated. The lack of clear evidence supporting early versus late repair has led to a compromise in which most surgeons perform repair from 12 to 24 months.[2] Recently, experience with neonatal cleft lip and palate repair has been described as safe, although long-term follow-up is not yet available.[8] The risks of repair include bleeding, infection, wound breakdown, palatal fistula, inhibition of maxillary growth, and velopharyngeal incompetence.[2] A description of the various surgical techniques used in palatal repair is beyond the scope of this text.

The emergence of the deciduous teeth is disrupted by the clefting process. The mean emergence age of the cleft side upper deciduous lateral incisor is delayed by 8 months when an alveolar cleft is present and by 13 months when an alveolar and palatal cleft is present. However, if early orthopedic plates are used, the lower incisors emerge earlier than normal. The emergence of the deciduous primary molar is also delayed in patients with clefts.[7] Also, children with cleft lip and/or palate were found to be at risk for dental caries, with the highest incidence found in the teeth adjacent to the oral cleft.

Special consideration should be given to advising the parent to provide breast milk to infants with CP. This is based on a study by Paradise and colleagues, who evaluated 315 infants with CP. Freedom from effusion in one or both ears at one or more visits was found in 2.7% of those fed cow's milk or soy formula exclusively and 32% of those fed with breast milk exclusively or in part for varying periods.[9] General growth patterns of children with clefts do not differ significantly from those without clefts.[7]

PIERRE ROBIN SEQUENCE

Pierre Robin, a French stomatologist, described the association of micrognathia and glossoptosis (posterior displacement of the tongue into the pharynx) in 1923 and added CP in a 1934 report. This triad is now known as the Pierre Robin sequence, with the word sequence being used to reflect the series of events leading to the clinical phenotype. The significant respiratory symptoms associated with PRS distinguish it from simple CP. An estimate on the incidence varies from 1 in 2,000 to 1 in 30,000.[10] Mortality ranges from 2.2 to 26%, with the cause of death typically related to obstructive apnea and failure to thrive.[11]

The initiating event appears to be mandibular deficiency. In early development, the retroposition of the mandible results in maintaining the tongue in a high position within the nasopharynx. Tongue position prevents the medial growth and fusion of the palatal shelves, and a resultant U-shaped cleft occurs.[10] The degree of the defects results in marked clinical heterogeneity.

The nature of the mandibular hypoplasia is heterogeneous and includes positional malformations in which the mandible has normal growth potential, but external factors, such as oligohydramnios, multiple births, or uterine anomalies, prevent full development; intrinsic mandibular hypoplasia in which there is reduced growth potential, such as is seen with genetic syndromes; neurologic or neuromuscular abnormalities in which abnormal mandibular movement prevents tongue descent, as seen with myotonic dystrophy and arthrogryposis; and connective tissue disorders.[10]

Approximately 20 to 40% of affected individuals are classified as having nonsyndromic PRS. These patients have normal growth potential and development if airway and feeding problems are prevented.[10] The majority of cases are therefore associated with various recognized syndromes (Table 22-3), the most common of which include Stickler syndrome (34%), velocardiofacial syndrome

(11%), fetal alcohol syndrome (10%), and Treacher Collins syndrome (5%).[12]

At birth, patients with PRS have marked anteroposterior mandibular deficiency. The base of the nose is often flattened, and a palatal cleft is present. The possibility for mandibular catch-up growth is related to the etiology of the PRS. When mandibular deficiency is due to positioning, the micrognathia will likely self-correct. However, syndromic PRS often involves altered mandibular growth potential, and correction is less likely.[10] Hearing levels in patients with PRS revealed 83% to have bilateral conductive hearing loss, whereas 60% of CP patients (with or without cleft lip) had hearing loss. All patients with hearing loss had middle ear effusion.[13]

Airway obstruction is multifactorial, involving both anatomic and neuromuscular components. Neuromuscular impairment of the genioglossus and other pharyngeal muscles predisposes PRS patients to airway collapse. Mechanical obstruction is the result of the retroposition of the mandible and diminished anterior traction on the tongue. The airway obstruction may lead to associated cor pulmonale, failure to thrive, and cerebral impairment owing to hypoxia.[10] A study by Sher and colleagues using nasopharyngoscopy found that 59% of the obstruction was the result of posterior movement of the tongue contacting the posterior pharyngeal wall.[14]

If the airway compromise is due to glossoptosis, then positioning has been the mainstay of treatment. Patients are placed in the prone position so as to rely on gravity to displace the tongue anteriorly. However, this maneuver does not allow for easy observation of signs of respiratory distress should airway obstruction occur. For this reason, some have advocated the use of a weighted wire to bring the tongue forward.[10]

The use of a nasopharygeal airway has been described, with good results. The tube should have an internal diameter of 3.0 to 3.5 mm and can be advanced to 8 mm until good air movement is observed. Typically, a single tube is sufficient. Gavage feedings through a nasogastric tube are recommended when this tube is in place.[10] A study of 22 neonates treated with nasopharyngeal airway and nasogastric feeding demonstrated this approach to be safe and allowed for improved growth, development, and parental bonding.[15]

The use of a tongue to lip adhesion or glossopexy was also designed to relieve abnormal tongue positioning. In this procedure, the tongue is sutured in place in a more anterior position. Although this can alleviate upper airway obstruction, there is a significant failure rate. In addition, complications include tongue laceration, wound infection, dehiscence, injury to the Wharton duct, and scar formation of the lip, chin, and mouth.[10] A study by LeBlanc and colleagues suggests that this procedure does not adversely affect speech when compared with affected patients who did not undergo glossopexy.[16]

The use of mandibular distraction osteogenesis has been described as a definitive structural resolution of the micrognathia with correction of the hard and soft tissues. The technique is technically demanding and requires good compliance from the parents. Tracheostomy would be a final alternative for those patients who do not respond to nonsurgical measures. Other procedures described include subperiosteal release of the floor of the mouth and hyomandibulopexy.[10]

Feeding difficulties have been reported to be due to poor tongue mobility or poor muscle coordination during swallowing. This leads to poor suck and poor bolus propagation. A recent study revealed over 50% of patients to have temporary feeding problems that resolved by 1 year of life, whereas almost 25% have chronic feeding problems. Overall, 40% required some form of enteral tube feeding.[11] Electromyography during bottle feeding revealed incoordination between oral and pharyngeal phases of swallowing. Esophageal motility studies revealed increased lower esophageal sphincter mean resting pressure and incomplete or asynchronous lower esophageal sphincter relaxation; simultaneous contractions, multipeaked waves, and very-high-amplitude waves along the esophageal body; and increased mean upper esophageal sphincter resting pressure and asynchronous upper esophageal sphincter relaxation.[17]

A separate study by Abadie and colleagues evaluated 66 neonates with isolated PRS. These authors hypothesized that PRS is the result of brainstem dysfunction that causes poor mandibular growth owing to impaired oral motility in utero. They evaluated the patients with esophageal manometry, laryngoscopy, and Holter-electrocardiography recording. They also graded the degree of clefting, glossoptosis, and retrognathia. They found that 98% had feeding difficulty within the first week of life and 81% had problems for the first 3 months of life. Transient solid food dysphagia was present in 18 to 60%, depending on the severity of their PRS. Manometric abnormalities were present, as previously described. Acute life-threatening events were present in 30% overall, with higher proportions seen in more severely affected individuals. Vagal overactivity was demonstrated in 59%.[18]

Early failure to thrive is seen relatively commonly and is often multifactorial. Factors implicated in poor weight gain include feeding difficulties, syndrome-related hypoxemia, respiratory insufficiency, increased caloric demand,

TABLE 22-3 SYNDROMES ASSOCIATED WITH PIERRE ROBIN SEQUENCE

SYNDROME	OMIM NUMBER*
Beckwith-Wiedemann	130650
Cerebrocostomandibular	117650
Distal arthrogryposis	301830
Femoral-facial syndrome (bilateral femoral dysgenesis)	134780
Fetal alcohol	
Larsen	150250
Miller-Dieker lissencephaly	247200
Spondyloepiphyseal dysplasia congenita	183900
Stickler syndrome	108300
Treacher Collins	154500
Trisomy 18	
Velocardiofacial	192430

Adapted from St-Hilaire H and Buchbinder D.[10]
*Searching the OMIM (Online Mendelian Inheritance in Man) can be done at <http://www3.ncbi.nlm.nih.gov/omim/>.

prematurity, and related operations. In addition, gastroesophageal reflux and respiratory infections may contribute. Full catch-up growth in height and weight typically occurs during early childhood.[11]

A mild variant of the PRS has been described in which there is mild retrognathia and a high arched palate. These patients were noted to share manometric abnormalities with classic PRS and presented with early feeding resistance.[19]

PSEUDOPALATAL CLEFTS

Some patients have marked lateral hard palate swellings. These are typically associated with a high arched palate and median furrow. Careful examination reveals an intact palate, despite the misleading appearance of a cleft. Such pseudopalatal clefts are common in patients with Apert syndrome and have been described in Crouzon disease as well. No treatment is indicated.[20]

EPULIS

The first case of congenital epulis was reported in 1871. This rare benign soft tissue tumor occurs eight times more frequently in females than males and three times more often in the maxilla than in the mandible. The tumor is most commonly at the lateral alveolar ridge, where the lateral incisor or canine teeth erupt. The tumors are multiple in 10%. The histogenesis remains controversial, with proposed origins as odontogenic, fibroblastic, histiocytic, myogenic, and neurogenic. Congenital lesions can present as masses protruding from the mouth and can prevent nutrition and partially restrict respiration. Clinically, one may see occasional spontaneous regression and lack of postnatal tumor growth.[21]

EPIGNATHUS TERATOMAS

Epignathus teratomas are rare congenital malformations giving rise to oropharyngeal tumors. They are classified as mature teratoma. The estimated incidence for all mature teratomas is 1 in 4,000 live births, and at least 2% are oropharyngeal. The lesions do not appear to be familial in nature; however, there is a female predominance of 3:1 over males.[22] Epignathus teratomas occur more frequently in children of young mothers and can be associated with polyhydramnios owing to swallowing difficulty. Prenatal detection of these tumors has rarely been reported.[22] Placental edema owing to fetal cardiac decompensation based on the vascular nature of the tumors has been described as preeclampsia.[23]

The clinical presentation is based on the size and location of the tumor. Large tumors can result in early neonatal asphyxia. Computed tomography (CT) and magnetic resonance imaging (MRI) allow preoperative assessment of the tumor. Intracranial extension should be suspected in the event of sphenoid dehiscence.[23]

Histologically, they are composed of various tissues of ecto-, endo-, and mesodermal origin. The site of origin appears to be the craniopharyngeal canal. The implantation of the base can be single or multiple. The majority of these tumors have their point of attachment at the base of the skull in the posterior region of the nasopharynx. The tumors can be multiple and are associated with other malformations in 6% of cases. CP is the most common associated anomaly; however, bifid tongues and noses have been described. Differential diagnosis usually includes rhabdomyosarcoma of the tongue, retinoblastoma, nasal glioma, heterotopic thyroid, cystic lymphangioma, nasoethmoid meningoencephalocele, sphenoid meningoencephalocele, and giant epulis.[23]

Treatment consists of early and total surgical resection using an oral approach. Malignant degeneration has never been described in association with epignathus teratomas. Recurrence has not been reported.[23]

RANULA

A cyst-like swelling in the mouth floor, ranula, has been described since the days of Hippocrates. They are unilateral and unilocular and confined to the sublingual space, causing no discomfort. Their origin is typically due to mucus extravasation from the sublingual salivary gland that results in a pseudocyst. This condition is rare in the neonatal period but has been detected antenatally on ultrasonography.[24]

The ranula is characterized by a translucent blue color reflecting the viscous mucus contained within and the vascular congestion of the overlying mucosa. They are typically soft and slow to enlarge. Traumatic rupture is common, but with healing of the roofing mucosa, recurrences develop. Larger cysts may cause tongue displacement and result in difficulty with mastication, swallowing, and speech. Treatment initially can involve marsupialization and packing of the cystic lumen with gauze. Maintaining the packing for at least 10 days promotes fibrosis and sealing of the leaking salivary duct. Recurrences can be treated with a more extensive intraoral excision of the culpable sublingual salivary gland.[25]

NATAL AND NEONATAL TEETH

Natal teeth are those observable in the oral cavity at birth, whereas neonatal teeth are those that erupt during the first month of life. The reported incidence is somewhat varied, but among larger studies, the range is between 1 in 1,118 and 1 in 30,000. Overall, there does not appear to be a gender predilection, although some studies suggest that the incidence may be slightly higher among females.[26]

The etiology of early eruption is unknown, although it has been related to several factors, including superficial position of the germ, infection or malnutrition, febrile states, eruption accelerated by febrile incidents or hormonal stimulation, hereditary transmission of an autosomal dominant gene, osteoblastic activity within the germ area related to the remodeling phenomenon, and hypovitaminosis. At times, premature eruption is described in which an immature rootless tooth exfoliates within a short time. This phenomenon, in distinction to early eruption, has been designated "expulsive Capdepont follicle," may result from trauma to the alveolar margin at delivery, and

is associated with gingival inflammation. Natal and neonatal teeth owing to early eruption have been related to various syndromes, including Hallermann-Streiff, Ellis-van Creveld, craniofacial dysostosis, multiple steatocystoma, congenital pachyonychia, and Sotos.[26]

Clinically, the teeth may be conical or may be normal in size and shape and opaque yellow-brown in color. They can be classified as mature or immature based on their structure and development. Histologically, most of the crowns are covered with hypoplastic enamel of varying degree and severity. In addition, they often have absence of root formation, ample and vascularized pulp, and irregular dentin formation and lack cementum formation. These teeth can be differentiated from cysts of the dental lamina and Bohn nodules by radiographic examination.[26]

Radiographic verification of the relationship of the tooth and adjacent structures, nearby teeth, and the presence or absence of a germ in the primary tooth area allows one to determine if the tooth belongs to the normal dentition. Indeed, most natal and neonatal teeth are primary teeth of the normal dentition (95%) and not supernumerary teeth. The teeth are usually in the region of the lower incisors (85%) and double in 61% of the cases.[26]

Treatment depends in part on the tooth's implantation, degree of mobility, difficulty sucking or breastfeeding, possibility of traumatic injury, and whether the tooth is part of the normal dentition or supernumerary. If the tooth is part of the normal dentition and well implanted, it should be left in the arch to avoid loss of space and collapse of the developing mandibular arch, which could lead to future malocclusion. Removal should be considered only if there is difficulty in feeding or they are highly mobile, with risk of aspiration. However, it should be noted that, to date, there are no reports of aspiration of a natal or neonatal tooth. If indicated, extraction is relatively easy and can be accomplished with forceps or even the fingers; however, some experts caution that they should not be removed prior to day 10 of life owing to risk of hemorrhage. This risk, however, is lessened if vitamin K is administered prior to extraction, as is typically performed as part of immediate neonatal care.[26]

TONGUE LESIONS

Tongue development begins at 3 to 4 weeks gestation from the first three to four brachial arches.[27] Specifically, the tongue arises from four swellings (median tongue bud, two lateral tongue buds, and hypobranchial eminence), which merge to form the tongue.[27] Pediatric tongue lesions represent 2.4% of all pediatric oral and maxillofacial tumors. Most lesions are benign and include various local neoplastic solid tumors, cysts, polyps, benign neoplasms, and diffuse hypertrophy. Anterior lesions do not typically obstruct the aerodigestive tract and are typically asymptomatic. Posterior lesions may present with acute respiratory distress or dysphagia. Excision of these lesions may hamper function owing to injury to superficial lingual nerves. Diffuse lesions can present with chronic protrusion, respiratory distress, dysphagia, dysarthria, or salivation. Surgical repair aims at preserving motility, taste, and cosmetic appearance.[28]

Osseous christomas are lesions composed of normal bone mass within the soft tissue. When present in the tongue, they are typically posterior near the foramen cecum. Patients' ages range between 5 and 73 years, with an increased frequency in the third and fourth decades. A female predominance has been observed. The origin is thought to be ossified remnants of brachial arches. They may appear as densely calcified masses on plain film and noncontrast CT. Differential diagnosis includes extraskeletal osteosarcoma and chondrosarcoma, which are both less likely in a pediatric population. Treatment is via surgical excision. No recurrences or malignant transformations have been reported.[28]

Hamartomas represent benign tumor-like proliferation of a tissue in its usual anatomic location. Fewer than 15 cases of lingual hamartomas have been reported, although some may have been mischaracterized as mesenchymomas. Lingual hamartomas occur in more than 50% of the orofaciodigital syndromes.[28] Airway obstruction can be a problem with large lesions. Treatment is local resection.

Lingual teratomas occur at the foramen cecum, where the embryologic tongue buds converge. Grossly, they are typically encapsulated, cystic, solid, or multiloculated masses that may contain hair, skin, cartilage, or mucous membrane tissue. The cause is unknown but thought to be secondary to entrapment of embryologic epithelial cells along the lines of closure for the first and second branchial arch or the differentiation of multipotential cells sequestered during closure of the anterior neuropore.[28]

Aglossia is likely due to a lack of development of the lateral lingual swellings of the mandibular arch. This is an extremely rare anomaly, with only a few reports existing among living children.[27] This typically occurs in association with other malformations and has been associated with aglossia-adactylia syndrome and Goldenhar syndrome, in which one can see partial aglossia. In surviving patients, swallowing may improve after several months.[27]

Microglossia is a not so rare malformation, often associated with other congenital syndromes (Table 22-4).[27] Clinical difficulties depend on the degree of microglossia and associated findings. Patients may have some difficulty with articulation.

TABLE 22-4 SYNDROMES ASSOCIATED WITH MICROGLOSSIA

SYNDROME	OMIM NUMBER*
Aglossia-adactylia (oromandibular limb hypogenesis)	103300
Distal arthrogryposis, type II (Freeman-Sheldon)	193700
Faciocardiomelic dysplasia	227270
Hydrolethalus	236680
Myopathy, congenital nonprogressive, with Möbius sequence and Robin sequence	254940
Pierre Robin sequence	261800

Adapted from Emmanouil-Nikoloussi E and Kerameos-Foroglou C.[27]
*Searching the OMIM (Online Mendelian Inheritance in Man) can be done at <http://www3.ncbi.nlm.nih.gov/omim/>.

Tongue hemihypertrophy or hemiatrophy is usually associated with auricular, mandibular, and maxillary hypoplasia. Affected patients have less developed musculature of the soft palate and tongue on the affected side. Parotic gland aplasia or hypoplasia may also be an associated anomaly. Parry-Romberg syndrome and congenital hemifacial hyperplasia are syndromes associated with this anomaly.[27]

Macroglossia is defined as a resting tongue that protrudes beyond the teeth or alveolar ridge. Sequelae owing to macroglossia include articulation errors, particularly in pronouncing consonants requiring the tongue tip to approximate the alveolar ridge or roof of the mouth (ie, s, z, sh, t, d, and n). One may also develop an anterior open bite, prognathism, increased ramus to body angle, and flattening of the alveolar ridge. Deglutition issues may also arise and result in failure to thrive owing to inadequate intake. Airway obstruction may be a further complication and may lead to pulmonary hypertension and cor pulmonale. Acute respiratory distress owing to sudden respiratory obstruction has also been described.[29]

Lymphangioma is the most common etiology of macroglossia in children. It can be apparent at birth 60% of the time, with 95% becoming symptomatic by 2 years of life. The lymphangioma shares a common embryologic origin with cystic hygroma because both arise from lymphatic tissue rests derived from the primitive jugular sac. They typically involve the anterior two-thirds of the tongue. There is a coincident cystic hygroma in 7%. Grossly, it appears as a nodular swelling on the dorsum of the tongue or a water-filled blister. Discoloration of the tongue is due to blue-red vascular blebs deep within the vesicles. Increasing size can result from inflammation or trauma with hemorrhage into the lymphatic spaces. Recurrence after resection is common and arises from residual unremoved tissue.[29]

There are multiple syndromes associated with macroglossia (Table 22-5). Neonatal hypothyroidism, cretinism, has been associated with macroglossia. Here the tongue is enlarged owing to myocyte hypertrophy and myxedematous tissue deposition. The tongue is smooth and symmetrically enlarged. Treatment involves controlling the underlying endocrine condition, typically by the use of exogenous thyroid hormone.[29]

Beckwith-Wiedemann syndrome, described in 1964, also causes macroglossia. This is thought to have autosomal dominant inheritance with variable penetrance and occurs at an incidence of 1 in 13,500 live births.[30] Associated features include exomphalos, gigantism, facial flame nevus, ear lobe anomalies, mild microcephaly, prominent occiput, maxillary hypoplasia, and short orbital floor. Macroglossia is present in 95% of affected individuals. Hypoglycemia is also a prominent feature owing to pancreatic cell hyperplasia.

Hemangiomas, congenital vascular malformations, may present as macroglossia. Histologically, one sees endothelium-lined vascular spaces. Therapy includes systemic and intralesional steroids and laser excision.[29] Rhabdomyosarcoma of the tongue causes macroglossia and accounts for 20% of head and neck rhabdomyosarcomas. Chemotherapy affords a 70% 3-year survival rate.[29] Neurofibromatosis may be associated with macroglossia when affected individuals develop neurofibromas in the tongue. They are typically unilateral and slow growing. Early surgical excision is recommended prior to spread to the floor of the mouth, thus allowing for total excision.[29] Pseudomacroglossia can be seen in those instances in which there is a small mandible (eg, Down syndrome, PRS).[29]

Treatment of macroglossia with surgical excision is based on the effects on feeding, dentition, speech, and airway compromise. Initially, the patient may achieve benefit from nursing in the prone position or feeding through a nasogastric tube. Management involves a multidisciplinary team of an otolaryngologist, a speech therapist, and an orthodontist. Goals include the restoration of the size and shape of the tongue, preservation of function, and correction of dental arch anomalies. Typically, surgery is performed by 4 to 7 months to avoid maxillofacial deformities and speech defects.[29]

Long tongue has rarely been described in which affected persons have an extremely lengthy tongue with extreme mobility. This has been documented in Ehlers-Danlos syndrome.[27] There do not appear to be any clinical manifestations.

Accessory tongue is a very rare malformation in which the tongue is attached to the tonsil or a process arising from one side of the base of the tongue.[27]

Cleft of bifid tongue has been described with Goldenhar syndrome, orofaciodigital syndrome types I and II, CP lateral synechiae syndrome, and focal dermal hypoplasia.[27]

Lingual thyroid occurs when thyroid gland elements persist in the area of the foramen cecum. This is typically along the midline, immediately posterior to the foramen cecum and resting on a broad base. The color varies from red to purple.[27] The lingual thyroid mass usually increases in size as the child ages owing to the effect of thyroid-stimulating hormone on this marginally functioning thyroid. Common presenting symptoms are dysphagia, dysphonia, dyspnea, and, occasionally, pain. A thyroid scan is required to determine the amount of active thyroid tissue because this may be the patient's only functioning thyroid tissue. Management considerations include functional,

TABLE 22-5 SYNDROMES ASSOCIATED WITH MACROGLOSSIA

SYNDROME	OMIM NUMBER*
4p+	
Beckwith-Wiedemann	130650
Generalized gangliosidosis (GM_1)	230500
Mannosidosis, αB	248500
Mucopolysaccharidosis I (Hurler)	252800
Mucopolysaccharidosis II (Hunter)	309900
Mucopolysaccharidosis VI, A and B	253200
Neurofibromatosis	162200
Pycnodysostosis or osteopetrosis	265800
Simpson-Golabi-Behmel	312870
X-linked α-thalassemia/mental retardation	301040

Adapted from Weiss L and White J.[29]
*Searching the OMIM (Online Mendelian Inheritance in Man) can be done at <http://www3.ncbi.nlm.nih.gov/omim/>.

metabolic, and cosmetic factors. Euthyroid, asymptomatic patients can be followed carefully over time. Patients with abnormalities in their thyroid function can be managed with hormone therapy; otherwise, surgical resection is warranted. There is an increased frequency of thyroid carcinoma in lingual thyroid tissue.[20]

Other syndromes with associated tongue anomalies include Melkersson-Rosenthal syndrome, in which one-third of affected individuals have a folded tongue; Coffin-Lowry syndrome, in which patients have been observed to have a deep central lingual groove and thickened lips; Riley-Day syndrome, in which one observes decreased numbers of fungiform and circumvallate papillae; and Klippel-Trénaunay-Weber syndrome, in which there may be angiomatosis of the tongue.[27]

Ankyloglossia, tongue-tie, is a congenital anomaly characterized by an abnormally short lingual frenulum, which may restrict tongue tip mobility. Incidence figures reported in the literature vary from 0.02 to 4.8%, and there is a male-to-female ratio of 3 to 1.[31] Ankyloglossia occurs most frequently as an isolated anatomic variation. An increased prevalence has been noted among children of mothers who abused cocaine.[31] It may also be associated with various syndromes, including Opitz syndrome, orofaciodigital syndrome, and X-linked CP. The long-term outcome of ankyloglossia is unknown because there are no long-term studies; however, some authors postulate that the short frenulum can elongate spontaneously, with progressive stretching and thinning with use.[31] This might account for the perception that this disorder is more common among children than adults. Sequelae from ankyloglossia are debated; some feel that it is rarely symptomatic, whereas others state that it results in infant feeding difficulties, speech disorders, and mechanical and social difficulties.[31] Surveys report that the majority of lactation consultants feel that ankyloglossia can hamper breastfeeding and result in sore nipples, poor latching on and sucking mechanics, poor infant weight gain, and early weaning.[31] This is, in part, supported by a recent prospective study in which 36 infants with ankyloglossia were compared with normal controls. Although both groups were able to successfully breastfeed for at least 2 months, the affected group reported more frequent nipple pain and difficulty latching on (25% vs 3% for controls).[32] There does not appear to be a problem with affected infants' ability to bottle feed, although this should not be used as an argument to avoid breastfeeding attempts.[31]

Speech sounds that may be affected by tongue tip mobility include lingual sounds and sibilants such as t, d, z, s, th, n, and l. Although ankyloglossia can be a cause of articulation problems and effortful speech, it is not a cause of speech delay.[31]

Additional problems reported by older children and adults include difficulty in intraoral toilet (lip licking and sweeping away oral debris), cuts under the tongue, creation of a diasthesis between the lower central incisors, and poorly fitting dentures. Social difficulties may also occur, including playing a wind instrument, licking ice cream, and "French kissing."[31]

Diagnosis is based on physical examination in which the frenulum is abnormally short and inserts onto the tongue at or near the tongue tip. The tongue may appear notched or heart-shaped on protrusion. Protrusion is limited and may not extend beyond the lower lip. Hazelbaker, a lactation consultant, devised an assessment tool for lingual frenulum function to be used on neonates. Based on a scoring of seven lingual movements from 0 to 2, low scorers are recommended to undergo frenotomy.[33] However, despite this and other more complex measuring scales, there appears to be no way to predict, based on examination, those children who are likely to have problems related to ankyloglossia.[31]

The timing of surgical correction is controversial. Some feel that given the rare incidence of complications, it is warranted to wait until such complications develop, whereas others feel that prophylactic treatment is warranted, especially in light of the minimal surgical risks.[31] Additional considerations with respect to timing include the fact that delayed repair beyond 1 year of age often requires general surgery, whereas younger children tolerate the procedure in a clinic setting. Frenotomy, clipping of the frenulum, is rapid and easy and is best suited for infants. The discomfort is brief and minor and may not warrant anesthesia. In children over age 1 year, frenuloplasty is preferred. Here the frenulum is released via sequential cuts, as is done with frenotomy, and then the resultant wound is closed with a suture. As with frenotomy, antibiotics are not required, and rare postoperative pain can be managed with acetaminophen. Improvements in breastfeeding are often immediate, given that that was the source of the feeding problem. Similarly, articulation improves in 75% of patients postoperatively among patients with ankyloglossia-related problems. Complications of repair include infection, excessive bleeding, recurrent ankyloglossia owing to scarring, new speech disorder, and glossoptosis.[31]

CYSTIC HYGROMA

Cystic hygromas are congenital malformations of the lymphatic system. They are characterized by single or multiple fluid-filled lesions occurring at sites of lymphatic-venous connections. They typically develop between the late first trimester and early second trimester. The incidence of cystic hygroma is unknown; however, rates as high as 1 in 100 have been reported.[34]

The lymphatic system develops at the end of the fifth week as endothelium grows out from the venous system. Six lymphatic sacs, two jugular sacs draining the head, neck, and arms; two iliac sacs draining the legs and lower trunk; and two sacs draining the gut (the retroperitoneal sac and the cisterna chili), develop in close proximity to the body's large veins. Through centrifugal extension and branching, the lymphatic vessels arise from these sacs. The right and left thoracic ducts connect with the venous system at the junction of the internal jugular and subclavian veins at the end of the sixth week of gestation.[34]

The anatomic distribution and severity of lymphatic vessel anomalies vary with the underlying disorder. They

range in size from that of a small pouch to giant extensions along the length of the body. They tend to infiltrate tissue planes, including the tongue and the floor of the mouth. This can lead to life-threatening airway compromise. Owing to their large size and tissue involvement, endotracheal intubation may be difficult, and tracheotomy is necessary to secure the airway. They are either smooth or irregular in contour, the latter suggesting a multilocular fluid collection. Hygroma spaces are lined by endothelial cells and contain serous lymphatic fluid. They are typically located in the posterior neck. Numerous syndromes have been associated with cystic hygromas (Table 22-6), as well as exposure to alcohol, aminopterin, and trimethadone.[34]

Prenatal diagnosis can ensure that the appropriate surgical personnel are in the delivery room and thus offer the best chance for a good outcome.[35] Indeed, operating while the patient remains on placental support and ex utero intrapartum treatment have been described.[36,37] Before excision is attempted, the extent of the lesion and its relationship to surrounding structures must be considered. For superficial lesions, ultrasonography with or without Doppler may help define the lesion. For more complex lesions, CT and MRI have proven useful. Complete excision is the treatment of choice. However, because their extension can be marked and their involvement of vital structures and nerves is common, removal may not be possible. Postoperative complications of recurrence, wound seroma, infection, and nerve damage occur in 30% or more of cases. If the lesion is only partially resected, recurrence rates approach 100%.[34] Nonsurgical treatment with either bleomycin or OK-432 (lyophilized incubation mixture of group A *Streptococcus pyogenes*) has shown some efficacy.[34]

ESOPHAGEAL DUPLICATION

Congenital duplications may arise along the length of the gastrointestinal system. Although midgut duplications are the most common, foregut duplications (esophagus, stomach, and parts 1 and 2 of the duodenum) account for approximately one-third.[38] Among the foregut duplications, esophageal duplications are the most common. The duplications may appear as cysts, diverticulae, and tubular malformations, all of which are thought to have a similar embryologic origin. Gastric mucosa is frequently observed within the wall of the duplication irrespective of their site of origin.[39]

Esophageal duplications are often identified on chest radiograph and barium esophagogram as posterior mediastinal masses.[39] Duplication cysts may be difficult to distinguish from bronchogenic cysts, which can also cause external compression of the esophagus. Vertebral anomalies are concomitantly found in approximately 50% of cases.[38] Many of these are associated with intraspinal abnormalities. This association is best explained embryologically by the split notochord syndrome. The notochord, present from the third week of gestation, may split, allowing endodermal gut to herniate through the gap, resulting in a cyst or fistula. The cyst may interfere with anterior fusion of the vertebral mesoderm, accounting for the vertebral anomalies.[38]

The most common presenting symptom of an esophageal duplication cyst within neonates is respiratory distress owing to the enlarging cyst pressing on the adjacent lungs and airways. Among older children, dysphagia is a more common complaint. Smaller cysts may remain asymptomatic for years and be noted incidentally on chest radiography. Older children may develop massive gastrointestinal or bronchial hemorrhage and spinal meningitis because the wall of the duplication erodes owing to production of acid from the gastric lining of the duplication.

Diagnosis is usually accomplished radiographically as a mass on a chest radiograph or as a compressing mass on contrast esophagogram. Communicating lesions can also be noted to fill with contrast. Chest CT or MRI is helpful in further defining the lesion.

Management is best accomplished via surgical excision of the duplication. However, excision is not always possible, particularly if the esophagus and duplication share a common wall for any distance. Excision of the bulk of the duplication with stripping of the mucosa on the esophageal wall is an option for those cysts that are unresectable. Recently, resection via minimal access surgery has been described.[40]

ESOPHAGEAL STENOSIS

Congenital esophageal stenosis is defined as an intrinsic stenosis caused by a congenital malformation of the esophageal wall that is not necessarily present at birth. The etiology is classified as tracheobronchial rest (TBR),

TABLE 22-6 SYNDROMES ASSOCIATED WITH CYSTIC HYGROMA

SYNDROME	OMIM NUMBER*
Achondrogenesis type II	200610
Achondroplasia	100800
Beckwith-Wiedemann	130650
Cornelia de Lange	122470
Cowden disease	158350
Cumming	211890
Districhiasis-lymphedema	153400
Fraser	219000
Fryns	229850
Hereditary lymphedema	153100
Multiple pterygium	253290
Noonan	163950
Oculodental digital dysplasia	164200
Opitz-Frias	145410
Pena-Shokeir	208150
Polysplenia	208530
Proteus	176920
Roberts	268300
Thrombocytopenia absent radii	274000
Trisomy 13	
Trisomy 18	
Trisomy 21	
Turner	
Williams	194050
Zellweger	214100

Adapted from Gallagher P et al.[34]
*Searching the OMIM (Online Mendelian Inheritance in Man) can be done at <http://www3.ncbi.nlm.nih.gov/omim/>.

membranous diaphragm (MD), and segmental hypertrophy of the muscularis and diffuse fibrosis of the submucosa. The stenosis owing to TBR is the most common and MD is the least common.[41] The overall incidence of esophageal stenosis is estimated at 1 in 25,000 to 50,000 live births, with the incidence of other congenital anomalies associated with congenital esophageal stenosis ranging from 17 to 33%.[42] Symptoms vary with the location and severity of the stenosis. High esophageal lesions typically present with respiratory symptoms, whereas lower lesions present with vomiting. The majority present with the introduction of solids and signs and symptoms of dysphagia.[41]

Esophagograms are helpful in making the diagnosis, and confirmation by endoscopy is diagnostic. When strictures are identified, congenital lesions must be differentiated from acid-related strictures and from compression of the esophagus from external structures such as vascular rings. The majority of cases attributable to TBR are in the distal portions of the esophagus, whereas fibromuscular stenosis (FMS) and MD occur more commonly in the middle third. FMS is classically 1 to 4 cm in length, has a smooth wall with an hourglass configuration, is located at the junction of the middle and lower thirds of the esophagus, and results in only partial obstruction of the esophageal lumen. TBR is typically found within 3 cm of the gastric cardia and often results in high-grade obstruction. MD is more common in the midesophagus.[41]

Segmental stenosis attributable to TBR and FMS can be associated with esophageal atresia and tracheoesophageal fistula (TEF) and accounts for up to one-third of reported cases. TBR, like esophageal atresia, is due to abnormal separation of the foregut into the trachea and esophagus, which occurs at day 25 of gestation. This accounts for their frequent association. MD is likely a form of "partial" esophageal atresia,[41] although case reports of complete obstruction of the esophagus by an intraluminal mucosal diaphragm have been described.[43]

Treatment of congenital esophageal stenosis is typically via excision with end-to-end reanastomosis. If this is in proximity to the lower esophageal sphincter, an accompanying fundoplication should be performed. Some controversy, however, exists as to whether some of these lesions, particularly those with FMS, can be treated by dilatation. Dilatations are not always successful, and there is a risk of perforation. Esophageal webs (MD) may be more amenable to simple dilatation.[41] There have also been reports of treatment of MD with endoscopic laser division.[44]

ESOPHAGEAL ATRESIA

The first reported case of esophageal atresia was an autopsy finding by Durston in 1670. Shortly thereafter, in 1697, Thomas Gibson provided the first clinical description of a TEF.[45] The first successful staged repair was reported in 1939 by Laven and Ladd.[45] Haight reported the first successful primary anastomosis with fistula ligation in 1941.[45] Prior to these reports, esophageal atresia was a uniformly fatal congenital anomaly.

The exact cause remains unknown. Kluth and colleagues used the scanning electron microscope in chick embryos and proposed that the trachea and esophagus normally develop and separate by formation of cranial ventral and dorsal folds in the foregut. Excessive ventral invagination of the ventral pharyngoesophageal fold is thought to be the underlying defect.[46] Developmental disorders of circulation have also been proposed. Genes of the HOXD group have also been linked to these malformations.[45]

Overall, the incidence of esophageal atresia is 1 in 3,000 to 1 in 4,000 live births, with the highest rate among whites.[47] There is a 0.5 to 2% risk of recurrence among siblings of an affected child. Prolonged maternal use of contraceptive pills and exposure to progesterone and estrogen during pregnancy have been implicated as teratogens. Esophageal atresia with TEF takes a number of forms (Figure 22-1); the most common anomaly, accounting for 85% of cases, comprises a blind-ending esophageal pouch with a fistula from the trachea to the distal esophagus.

Approximately 50 to 70% have associated anomalies, including cardiac (11–49%), genitourinary (24%), gastrointestinal (24%), and skeletal (13%). Approximately 10% of patients are classified within the VACTERL association in which three or more of the following anomalies are found: vertebral, anorectal, cardiac, TEF, and renal and radial limb anomalies. Driver and colleagues reported the associated anomalies in 134 patents with esophageal atresia with or without TEF. Of these, 31 had gastrointestinal anomalies, including anorectal malformation (52%), duodenal atresia (19%), malrotation (13%), jejunoileal atresia (10%), duplication (3%), and hiatal hernia (3%).[48] Mee and colleagues identified 119 infants with congenital cardiac anomalies of 554 patients with esophageal atresia. The most common included atrial septal defect (ASD) (8%), ventricular septal defect (VSD) (28%), tetralogy of Fallot (13%), and patent ductus arteriosus (PDA) (13%).[49] Other associations have been noted.[45] Syndromes associated with esophageal atresia and TEF are listed in Table 22-7.

The proximal blind esophageal pouch is typically hypertrophied and has a good blood supply. It is adherent to the trachea, which often has more muscle than cartilage, and thus results in tracheomalacia. The distal pouch is narrow and small; fistulae typically open into the trachea near the carina. The gastroesophageal sphincter is typically incompetent, and the vagus nerve is often defective, accounting for improper peristalsis.[45]

The diagnosis is suspected if there is evidence of polyhydramnios and a smaller than usual gastric bubble. Together these findings have a positive predictive value of 56%.[50] Prenatal ultrasonography may also demonstrate an anechoic structure in the fetal neck, representing the upper pouch. After birth, newborns are typically mucusy and require frequent suctioning. With feeding, there may be coughing, vomiting, and cyanosis. If a distal fistula is present, one may see progressive abdominal distention because the stomach and intestines fill with air introduced from the trachea. With a delay in diagnosis, the patients may develop pneumonitis.

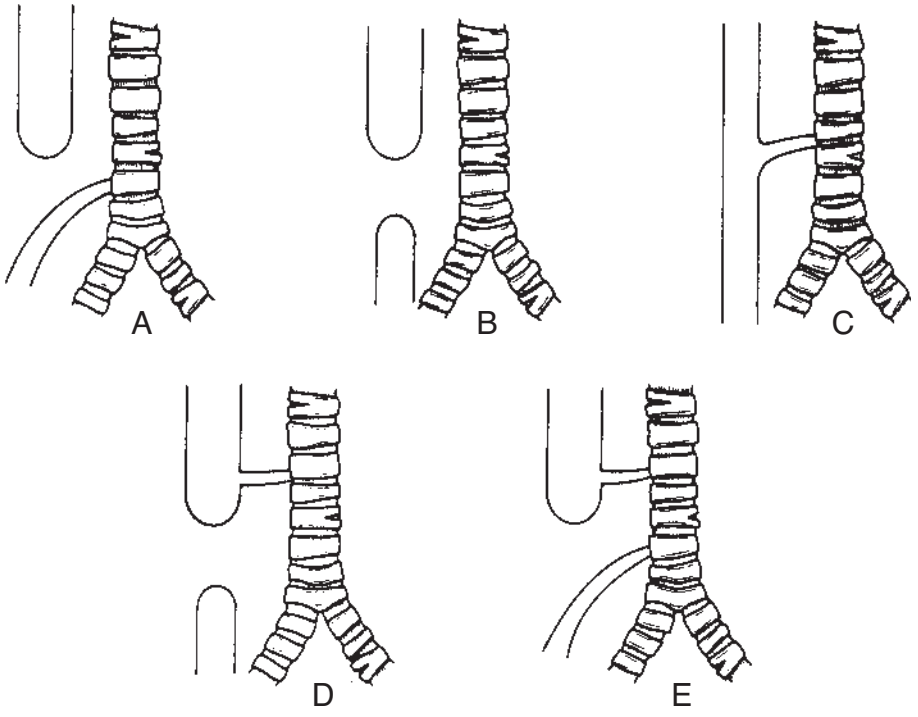

FIGURE 22-1 Types of esophageal atresia with or without fistula. The incidence of each is as follows: *A,* esophageal atresia (EA) with distal tracheoesophageal fistula (TEF), 85%; *B,* EA without TEF, 8%; *C,* isolated TEF, 4%; *D,* EA with proximal TEF, 2%; *E,* EA with distal and proximal TEF, < 1%. By Jean Hyslop, Medical Artist, Royal Hospital for Sick Children, Yorkhill, Glasgow, UK.

The diagnosis is facilitated by the attempted introduction of a nasogastric tube that meets resistance prior to entering the stomach as it coils in the esophagus. A radiograph with the tube in place reveals the coiled esophageal tube. The presence of a TEF is recognized by the presence of intestinal air. The introduction of contrast into the proximal pouch is hazardous owing to the aspiration risk and is typically not warranted. Preoperative bronchoscopy is recommended by some to localize the fistula, exclude an upper pouch fistula, and identify a right-sided aortic arch. Echocardiography is also typically performed preoperatively to assess for associated cardiac defects and determine the laterality of the aortic arch, and thus allow for decision making as to the surgical approach.[45] A right-sided arch occurs in less than 2% of the cases.

The rarer H-type TEF (4%) can be more difficult to diagnose because patients may not be as symptomatic in the immediate newborn period. Patients often present with a history of choking and respiratory difficulty with feeds. They may also have a history of recurrent pneumonia or asthma. Routine esophagography may fail to demonstrate the fistulous connection; therefore, if there is a high index of suspicion, the study should be performed via a nasogastric tube that is slowly withdrawn into the esophagus so that better visualization of the esophageal mucosa can be obtained. Esophagogastroscopy similarly may not be able to visualize the fistula, whereas bronchoscopy is typically the test of choice.

If possible, endotracheal intubation prior to surgey should be avoided because air introduced into the bowel via the TEF can cause abdominal distention and potential perforation.[51] The surgical approach is via a standard extrapleural thoracotomy with division of the fistula and single-layer end-to-end anastomosis using polyglycolic acid sutures. The two ends of the esophagus are typically within 2 cm, allowing anastomosis. Long gap atresias of greater than 2.5 cm pose special problems and may necessitate colonic interposition or pulling the stomach proximately into the chest to allow continuity. The use of drainage tubes or gastrostomy has been abandoned, and early alimentation is practiced.[52] In long gap esophageal atresia with or without TEF in which anastomosis cannot be accomplished despite lengthening myotomies, ligation of the fistula, if present, and gastrostomy with delayed primary repair have been advocated, particularly in small for date or preterm infants. Suctioning of secretions from the proximal pouch or creation of a "spit fistula" is required until subsequent reanastomosis.

Postoperatively, there is a risk of anastomotic leak (10–17%),[52] leading to formation of a salivary fistula, pneumonitis, and/or mediastinitis. Salivary fistula may respond to prolonged parenteral nutrition, ventilatory support, and antibiotics. After the immediate postopera-

TABLE 22-7 SYNDROMES ASSOCIATED WITH ESOPHAGEAL ATRESIA AND TRACHEOESOPHAGEAL FISTULA

SYNDROME	OMIM NUMBER*
CHARGE	214800
Fanconi syndrome	227650
McKusick-Kaufman syndrome	236700
Trisomy 21	
VACTERL	192350

Adapted from Banerjee S.[45]
CHARGE = coloboma of the eye, heart anomaly, choanal atresia, retardation, and genital and ear anomalies; VACTERL = vertebral, anal, cardiac, tracheal, esophageal, renal, and limb anomalies.
*Searching the OMIM (Online Mendelian Inheritance in Man) can be done at <http://www3.ncbi.nlm.nih.gov/omim/>.

tive period, patients are at risk for anastomotic strictures, which may require dilatation, or reanastomosis. If the stricture is resistant to dilatation, it is likely due to concomitant gastroesophageal reflux disease, with acid-related inflammation.

Gastroesophageal reflux is a common problem owing to the impaired esophageal peristalsis. Clinically, the incidence is thought to be between 25 and 40%; however, when pH monitoring is performed, the incidence rises to 70%.[52] Poorly controlled reflux can result in acid-related strictures or life-threatening aspiration pneumonia. Antireflux surgery is considered for those patients with persistent difficulties despite medical therapy. However, fundoplication carries a 25% risk of recurrent reflux, and in these patients with poor esophageal motility difficulty, postoperative dysphagia and food impaction have been observed.[51]

Tracheomalacia or other structural tracheal anomalies can occur in up to 75% of patients.[52] Some may have severe manifestations and require either tracheostomy or aortopexy. Other, less affected individuals have noisy breathing, stridor, or a "barky" cough. Tracheal anomalies appear to occur only in the presence of a TEF and are not seen in isolated esophageal atresia.[52]

Poor outcome is still seen in children with low birth weight (< 1,500 g) and major cardiac anomalies. These risk factors are reflected in the Waterston (Table 22-8) and Spitz (Table 22-9) classification schemes for prediction of outcomes in children with esophageal atresia and TEF. Among the infants in the original Waterston report, group A had a 95% survival, group B had a 68% survival, and 6% of group C survived.[53] With advances in neonatology, reclassification does not include respiratory illness as a separate risk factor. According to the Spitz classification, in the absence of low birth weight and cardiac anomalies (group I), there is a 97% survival, 57% if one risk factor is present (group II), and 22% if both are present (group III).[45]

CONGENITAL LARYNGOTRACHEOESOPHAGEAL CLEFT

The first laryngotracheoesophageal cleft was described by Richter in 1792, who palpated a common cavity at the level of the larynx and hypopharynx in a newborn with feeding difficulties.[54]

The respiratory system develops as a foregut outpouching starting at day 20 of gestation. An abnormality in tracheoesophageal septum formation or progression accounts for a laryngotracheoesophageal cleft involving the tracheal rings. Failure of cricoid fusion may occur following failure of the septum to reach the appropriate level. Absence of cricoid fusion in isolation results in a laryngeal cleft.[54]

Numerous classification schemes have been devised. The most commonly quoted is that devised by Ryan in which type I is a cleft above the cricoid, type II is beyond the cricoid lamina, type III is up to the carina, and type IV is into the mainstem bronchi (Figure 22-2).[54]

Most cases appear to be sporadic; however, a mouse model exists in which a laryngotracheoesophageal cleft is inherited as an autosomal recessive mutation. Laryngotracheoesophageal clefts are seen in up to 50% of patients with Opitz (BBB/G) syndrome. They also are seen with other anomalies, including TEF, anal atresia, malrotation, microgastria, and bronchobiliary fistula. Cardiac anomalies occur in up to one-third of cases and include VSD, PDA, coarctation of the aorta, and transposition of the great vessels. Associations with hypospadia, unilateral lung hypoplasia, and renal agenesis have also been reported.[54]

Neonatal symptoms may be subtle or obvious, with respiratory distress owing to recurrent aspiration pneumonia. One may also see cyanosis, coughing, choking, stridor exacerbated by feeds, sialorrhea, or a weak, toneless, or hoarse cry.

Chest radiography may reveal an aspiration pneumonia, persistent esophageal air, and distended bowel. Nasogastric tubes may be noted to be displaced along the anteroposterior plane on lateral radiography. CT and bronchoscopy can help to define the extent of the laryngotracheoesophageal cleft. The presence of an intact arytenoid fold excludes the diagnosis of a cleft. Swallowing studies with small volumes show simultaneous filling of the esophagus and trachea.[54]

Surgical repair is the definitive treatment. The most common approach is a lateral pharyngotomy through a vertical lateral cervical incision. Care must be taken not to damage the recurrent larygeal nerve on the side opposite the approach. All but the smallest clefts will require tracheostomy, and specialized tubes have been developed to prevent pressure on the posterior wall. Postoperative complications include fistulization, granulation tissue at the repair site, esophageal and subglottic stenosis, nerve injury, and aspiration.[54]

After repair, esophageal dysmotility still places the patient at risk for aspiration. Gastric division with a draining gastrostomy in the proximal segment and a feeding gastrostomy in the distal segment is widely used to protect the esophageal anastomosis. Alternatively, fundoplication with feeding gastrostomy or jejunostomy may suffice. After healing, feeding therapy is often required.[54]

TABLE 22-8 WATERSTON CLASSIFICATION OF INFANTS WITH ESOPHAGEL ATRESIA WITH TRACHEOESOPHAGEAL FISTULA

A	Birth weight over 5.5 pounds and well
B	Birth weight between 4 and 5.5 pounds and well, or higher birth weight with moderate pneumonia and other congenital anomalies
C	Birth weight under 5 pounds or higher birth weight but severe pneumonia and severe congenital anomalies

Adapted from Waterston D et al.[53]

TABLE 22-9 SPITZ CLASSIFICATION OF INFANTS WITH ESOPHAGEAL ATRESIA WITH TRACHEOESOPHAGEAL FISTULA

I	Birth weight greater than or equal to 1.5 kg and no congenital heart disease
II	Birth weight less than 1.5 kg or congenital heart disease
III	Birth weight less than 1.5 kg and congenital heart disease

Adapted from Banerjee S.[45]

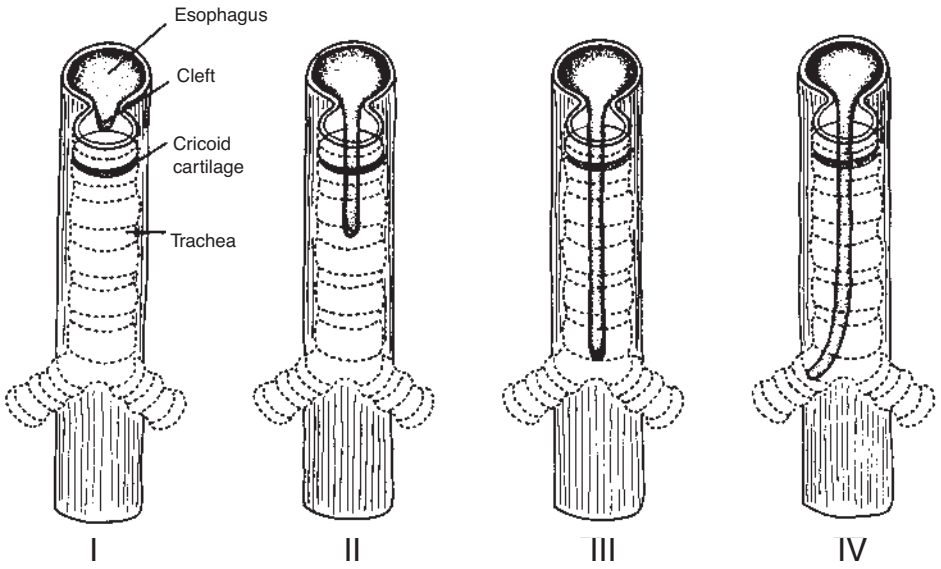

FIGURE 22-2 Types of laryngotracheoesophageal clefts. By Jean Hyslop, Medical Artist, Royal Hospital for Sick Children, Yorkhill, Glasgow, UK.

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CHAPTER 23

DISORDERS OF DEGLUTITION

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The pediatric patient with impaired swallowing poses a number of unique problems for the clinician. In contrast to adults, issues such as the growth and development of the swallowing apparatus, the development of normal oromotor reflexes, the maturation of feeding behavior, the importance of oral feeding in the development of parent-child bonding, the acquisition of adequate nutrition for somatic growth, and the effects of non-nutritive sucking on growth must be considered in the approach to this group of patients. In addition, some groups of patients with impaired swallowing lack the cognitive skills necessary to follow specific therapeutic recommendations (eg, those in the infant age group and children with central nervous system disease), a situation that complicates patient management.

NORMAL DEGLUTITION

The swallowing apparatus transports materials from the oral cavity to the stomach without allowing entry of substances into the airway. To accomplish safe swallowing, there must be precise coordination between the oral and pharyngeal phases of swallowing so that the pharyngeal swallow is initiated at the appropriate moment after the onset of bolus movement. The passage of an oral bolus without aspiration is the result of a complex interaction of cranial nerves and muscles of the oral cavity, pharynx, and proximal esophagus.[1,2]

Deglutition is generally divided into three phases based on functional and anatomic characteristics: oral, pharyngeal, and esophageal.[1,2] The oral stage, which is voluntary, involves a preparatory phase. In the unimpaired child, the oral cavity functions as a sensory and motor organ, changing the physical properties of the food bolus to make it safe to swallow. The oral bolus is modified to allow passage through the pharynx without entry into the larynx or the tracheobronchial tree. Physical properties of the food bolus altered by oral activity include size, shape, volume, pH, temperature, and consistency.[3]

The food bolus then moves into the pharynx, where the respiratory and gastrointestinal tracts interface. Passage of food through this region requires an efficient mechanism to safely direct food into the esophagus. During the pharyngeal phase, the swallow is reflexive and involves a complex sequence of coordinated motions. The pharyngeal phase, which lasts for approximately 1 second, generally consists of the elevation of the entire pharyngeal tube, including the larynx, followed by a descending peristaltic wave. In the adult, this action takes about 100 ms. Food is then injected from the pharynx into the esophagus forcefully, at velocities as high as 100 cm/s.[4,5] Approximately 600 to 900 ms after the onset of the pharyngeal phase, food passes through the upper esophageal sphincter (UES) and enters the esophagus. The cricopharyngeal (CP) muscle, the main component of the UES, relaxes for approximately 500 ms during the swallow to allow passage of the bolus.[6] Normal adults complete the swallow in approximately 1,500 ms[7]; timing data for children are not well described. Following pharyngeal transit, food enters the esophagus and is transported to the stomach via primary peristalsis. Additional discussions of normal and abnormal motility are given in the section on physiology and pathophysiology in Chapter 4, "Motility."

Upper Esophageal Sphincter: Normal Function

The UES, also known as the pharyngoesophageal segment, is a manometrically defined high-pressure zone located in the region distal to the hypopharynx. Composed of striated muscle, the UES is tonically closed at rest and opens during swallowing, vomiting, or belching.[8] The length of the high-pressure zone in adults is from 2.5 to 4.5 cm, averaging about 3 cm.[8] The length of the CP muscle is about 1 cm; this muscle, therefore, although the main contributor to the UES, is not the only determinant of the high-pressure zone.[8–10] The relative contributions of the inferior pharyngeal constrictor muscle and the muscle fibers of the proximal esophagus to the upper sphincter remain controversial.[11,12]

The structure and function of the UES have been summarized by Lang and Shaker.[13] The cricopharyngeus is structurally and biochemically distinct from the pharyngeal and esophageal musculature. Compared with other striated muscles, the CP muscle is more elastic; it contains large amounts of endomysial connective tissue and sarcolemma, factors that contribute to this elasticity. The length at which the CP muscle reaches its maximal tension is 1.7 times its length; other striated muscles develop maximal tension at resting length. The arrangement of the muscle fibers (parallel and series) and fiber composition may account for the length tension properties. The cricopharyngeus muscle is composed of variably sized

fibers that, unlike the fibers of other striated muscle, are not oriented in strict parallel fashion. The structure of the UES allows it to maintain constant basal tone and to rapidly relax during swallowing, belching, and vomiting.[13] The muscle fibers of the UES are not circumferential but are attached at the anterior end to the lamina of the cricoid cartilage, which functions as the anterior sphincter wall. Because of this connection, the UES moves in conjunction with the laryngeal structures during deglutition.

The pressure profile of the UES is asymmetric, with higher pressures noted in the anterior and posterior directions.[14] Orientation of the recording device must take this into account when pressures are measured in this region. Sleeve manometry has been used to monitor UES pressure in children and to determine the influence of the state of arousal on sphincter pressure values.[15] Table 23-1 reports normal values for the pharyngoesophageal region of control infants, obtained by using a low-compliance, water-perfused manometry system in which the directional orientation of the catheter is maintained.[16] UES function, including values for UES pressure and relaxation, has also been evaluated in preterm infants greater than 33 weeks gestation.[17]

Neurologic control of the sphincter has been reviewed by Palmer[8] and by Lang and Shaker.[13] The CP muscle is innervated by the vagus nerve via the pharyngoesophageal, superior laryngeal, and recurrent laryngeal branches; the glossopharyngeal nerve; and the sympathetic nervous system via the cranial cervical ganglion. Based on functional studies, it is believed that the major motor nerve of the CP muscle is the pharyngoesophageal nerve. Vagal efferents probably reach the muscle by the pharyngeal plexus, using the pharyngeal branch of the vagi.[8] The superior laryngeal nerve may also contribute to motor control of the CP muscle.

Sensory information from the UES is probably provided by the glossopharyngeal nerve and the sympathetic nervous system. Although not completely understood, neurologic connections include visceral afferents that travel to the nucleus solitarius and from there to the nucleus ambiguus. There is probably little or no contribution by the sympathetic nervous system to CP control.[8,18]

The UES responds in a reflexive manner to a variety of stimuli. Balloon distention of the esophagus results in increased UES pressure, which is probably mediated by stimulation of esophageal intramural mechanoreceptors.[19] Motor responses of the UES to esophageal stimuli have been measured following intraesophageal infusion of graded air and liquids (distilled water and apple juice) in healthy preterm infants.[20] A volume-dependent increase in UES pressure was noted for air and liquids. This suggests that UES reflexes are present in the preterm infant to protect the supraesophageal structures. Earlier studies reported that acidification of the esophagus caused an increase in UES tone, suggesting that the UES functions to protect against aspiration following a reflux event. More recent studies have not confirmed this finding. In adult controls and in patients with reflux esophagitis, spontaneous episodes of reflux were not associated with an increase in UES pressure.[21] Similarly, esophageal acidification did not alter UES pressures in either group of individuals. A modified sleeve sensor manometric catheter for measuring UES pressures was used in the latter studies, which might account for differing results.

TABLE 23-1 PHARYNGOESOPHAGEAL MANOMETRIC MEASUREMENTS IN CONTROL INFANTS*

Resting UES pressure (cm H_2O)	28.9 ± 10	(18.0–44.0)
Pharyngeal peristaltic wave		
Amplitude (cm H_2O)	74.7 ± 19.9	(37.0–102.0)
Velocity (cm/s)	8.5 ± 3.6	(3.2–15.0)
Duration (s)	0.59 ± 0.18	(0.3–0.86)

Reproduced with permission from Sondheimer JM. Upper esophageal sphincter and pharyngeal motor function in infants with and without gastroesophageal reflux. Gastroenterology 1983;85:301–5.
UES = upper esophageal sphincter.
*Numbers are mean ± standard deviation; values in parentheses are ranges.

During deglutition, the function of the pharynx changes from that of an airway to that of a foodway. Simultaneous manometry and videofluorography allow investigators to observe intraluminal bolus movement and measure intraluminal pressures during the act of swallowing.[22,23] During the pharyngeal portion of the swallow, there is velopharyngeal closure, opening of the UES, closure of the laryngeal vestibule, and tongue loading. The bolus is propelled into the esophagus by tongue pulsion and pharyngeal clearance. During swallowing, UES relaxation is associated with upward and anterior motion of the cricoid cartilage, which is pulled in an anterior direction by motion of the hyoid bone and contraction of the thyrohyoid muscle.[24] The response of the UES during swallowing is not stereotypical but may be modified by varying bolus size[24]; as bolus volumes increase, the orad excursion of the UES, the opening of the UES, and the duration of sphincter relaxation all increase. These findings suggest that feedback receptors in the oral cavity and pharynx provide afferent signals for modulating central nervous system impulses that give rise to the oral and pharyngeal phases of swallowing.[24]

Proposed functions of the UES include prevention of esophageal distention during normal breathing[8] and protection of the airway against aspiration following an episode of acid reflux.[25–27] As previously noted, the latter remains controversial in adults. Studies in infants have demonstrated that UES pressure increases in response to intraesophageal acidification, suggesting that in this group of patients, the UES may function as a dynamic barrier to acid reflux and may protect against aspiration.[16] However, there was no difference in resting UES pressures between control infants and infants with gastroesophageal reflux.[16] In some infants with pulmonary disease, the UES failed to respond following esophageal acidification. Others have documented qualitative abnormalities of UES function in infants with reflux disease.[28]

Neurology of Deglutition

Miller provides an excellent review of the neurophysiologic control of swallowing.[29] Swallowing may be evoked by stimulating many different central pathways, including the cortex (the region of the prefontal gyri), the subcortex, and the brainstem. The swallowing center can be activated

by afferent impulses from the cerebral cortex (voluntary swallowing) and from peripheral receptors in the mouth and pharynx (reflex swallowing). The corticobulbar impulses trigger and control the initial phases of swallowing but not the later esophageal stages. The cortex is not essential for the pharyngeal and esophageal phases of swallowing. In the human fetus, swallowing occurs prior to the time when the descending cortical-subcortical pathways have fully innervated the brainstem.[30] Deglutition has been noted to occur in infants, with loss of nervous tissue rostral to the midbrain.[31] Higher pathways, however, are important in allowing the voluntary elicitation of deglutition and in integrating facial and oral movements and other responses to swallowing.[1,29] The neurons important to the pharyngeal and esophageal phases of swallowing are located in different regions of the pons[32,33]and medulla.[1,34,35] Lesions placed in the medulla fractionate the sequence of muscle activity during pharyngeal swallowing, suggesting that interneurons located in this region are important to the pharyngeal and esophageal phases; these core interneurons are termed central pattern generators.[30,36] The deglutition center integrates afferent impulses and coordinates the activity of the motor nuclei of the fifth, seventh, tenth, and twelfth cranial nerves. Other activities, such as respiration, are inhibited during swallowing.[37] Swallowing may be evoked by stimulating the oropharyngeal regions innervated by the pharyngeal branches of the glossopharyngeal nerve (ninth cranial nerve) or by the superior laryngeal and recurrent laryngeal nerves of the vagus (tenth cranial nerve). Sensory fibers from these nerves synapse in the nucleus tractus solitarius. Multiple receptive sites that elicit swallowing are present in the oral cavity and pharynx.[29] Once activated, the sequence of muscle activity during the pharyngeal phase remains the same in spite of altering the duration of this phase; this is termed a time-locked sequence.[38,39]

All phases of deglutition may be modified by sensory feedback, although each to a different degree. Oral phase activity is modulated by peripheral feedback received from the touch and pressure receptors in the oral cavity and from the mandible and temporomandibular joints.[40] Feedback from sensory receptors modifies the duration of the pharyngeal phase, the intensity of muscle activity, and the threshold necessary to evoke a response.[6,41–45] Sensory feedback may have therapeutic implications in the clinical management of the swallowing-impaired individual. For example, maintaining jaw control during deglutition may facilitate a safe swallow by improving feedback signals from the mandible or temporomandibular joints, whereas modifying the size of an oral bolus may favorably alter the motor response of the pharynx.

Prenatal Deglutition

Deglutition in utero occurs at approximately 16 to 17 weeks of gestation, although a pharyngeal swallow has been described in a delivered fetus at a gestational age of 12.5 weeks.[46] It is estimated that the normal fetus, at term, swallows approximately 500 to 1,000 mL of amniotic fluid per day. Based on animal studies, the ovine fetus swallows fluid volumes that are greater on a per-kilogram basis than those swallowed by the adult (100 to 300 mL/kg versus 40 to 60 mL/kg).[47] Fetal deglutition plays an important role in amniotic fluid resorption, helping to recirculate urine and lung fluid volumes to the fetus and maintain normal amniotic fluid volume.[48,49] Fetal swallowing may be influenced by a variety of factors, including neurobehavioral changes (such as hypoxia, hypotension, and plasma osmolality), fetal maturation, and volume of amniotic fluid.[47]

Postnatal Development of Deglutition

Changes in Structure. Most changes in the size and relative location of components of the oral and pharyngeal cavities occur during the postnatal period.[50,51] In general, the central mobile elements of the oropharynx in the infant are large in comparison to their containing chambers. For example, the tongue is large compared to the oral cavity, and the arytenoid mass is nearly mature in size, in contrast to the small-sized vestibule and ventricle of the larynx (Figure 23-1).[51]

In the infant, the tongue lies entirely within the oral cavity, whereas the larynx is positioned high in the neck, resulting in a small oropharynx.[52] Between 2 and 4 years of age, the tongue begins to descend so that by approximately 9 years of age, its posterior third is present in the neck.[52] The larynx also moves in a caudal direction. The larynx descends from the level of the third to the fourth cervical body during the prenatal period, an arrangement that persists during infancy.[53] During childhood, the larynx descends to a level opposite the sixth vertebra and finally to the seventh cervical vertebra level in adulthood. As maturation progresses, the face vertically elongates, and the chambers of the oral cavity and oropharynx enlarge (Figure 23-2).[51,54]

Developmental Changes in Feeding Behavior. The development of normal feeding behavior in the infant and child has been reviewed in detail.[51,54] Briefly, in the normal infant, the oral phase of swallowing is characterized by a pattern known as suckle-feeding. Developmental changes in the relationship between suck and swallow, such as the suck-to-swallow ratio and differing rhythmic patterns, have been described in the preterm and term infant.[55,56] Feeding behavior in preterm infants has been assessed by using recording devices to measure pharyngeal pressure, oxygen saturation, heart rate, and nasal airflow. Sucking pressure, frequency, and duration were noted to mature with increases in postconceptual age. In younger infants, swallowing occurred during pauses in respiration but, after 35 weeks of age, occurred mainly at the end of inspiration.[57] Suckle-feeding is followed by the development of transitional feeding (ages 6–36 months) and eventually mature feeding, characterized by biting and chewing. Maturation of feeding behavior occurs mainly as a result of central nervous system development, with motor activity being directed by higher centers such as the thalamus and cerebral cortex.[54]

Nutritive and Non-nutritive Sucking. The pattern of nutritive sucking is characterized by a series of short bursts

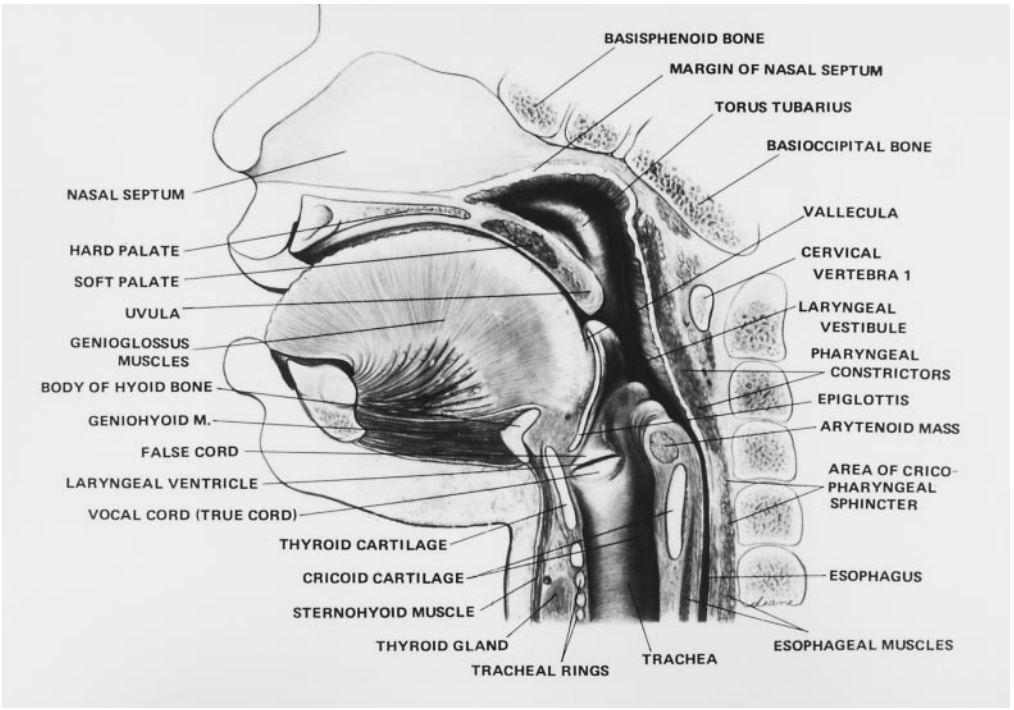

FIGURE 23-1 Drawing of postnatal anatomy of the oral and pharyngeal cavities (see text). Reproduced with permission from Kramer SS. Special swallowing problems in children. Gastrointest Radiol 1985;10:242.

and pauses, occurring at approximately one suck per second.[58] Non-nutritive sucking is defined as rhythmic movements on a nonfeeding nipple. The patterns of non-nutritive and nutritive sucking differ. In non-nutritive sucking, short bursts and pauses occur at a faster frequency.[58] Interestingly, non-nutritive sucking may improve weight gain during gavage feeding in preterm infants. Bernbaum and others studied the nutritional effects of non-nutritive sucking in a group of low birth weight infants receiving formula by an enteral tube and found that, compared with control infants, the group engaging in non-nutritive sucking gained relatively more weight.[59] The mechanism accounting for this weight gain is not clear, although it has been hypothesized that non-nutritive sucking results in more efficient nutrient absorption or a decrease in energy requirements secondary to a lessening of infant activity or restlessness.[60,61]

Non-nutritive sucking may have effects on pulmonary function as well. In preterm infants, non-nutritive sucking is associated with increased transcutaneous oxygen tension and respiratory frequency.[62,63] In contrast, lowered oxygen tension may occur during nutritive sucking, although the mechanism for this effect remains unclear and may not be related to the action of sucking per se.[63]

DISORDERS OF DEGLUTITION IN THE PEDIATRIC PATIENT: CLINICAL OVERVIEW

In the pediatric age group, swallowing disorders rarely present as isolated problems but more often occur in infants and children with multiple impairments. Although accurate epidemiologic data are lacking, underlying conditions that predispose to impaired swallowing in childhood

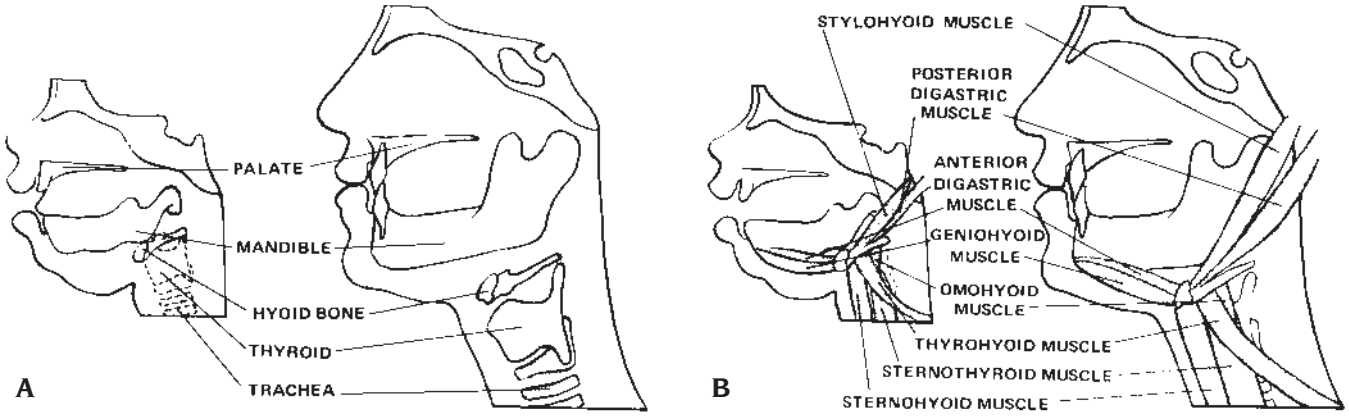

FIGURE 23-2 *A,* Drawing of infant and adult anatomy shows alteration in shape and orientation of the pharynx that accompanies growth and the descent of the larynx. Laryngeal cartilages and hyoid bone are shown in their relationship to the mandible. The airway is depicted (*hatched area*). *B,* Drawing illustrates change in orientation of muscles (*stippled*) that suspend the larynx in the infant and the adult. Reproduced with permission from Kramer SS. Special swallowing problems in children. Gastrointest Radiol 1985;10:242.

include central and peripheral nervous system dysfunction, disease of muscle, and structural anomalies of the oral cavity and pharynx. Structural and motor disorders of the esophagus, which may also present with dysphagia, are discussed elsewhere in the text. Other groups at risk for the development of impaired swallowing and its complications include premature infants with poor coordination of breathing and swallowing, infants with long-term deprivation of oral feeding, and infants with chronic pulmonary disease. The spectrum of pediatric swallowing disorders has been reviewed in detail by others.[51,54,64–69] Table 23-2 provides a broad list of disorders that result in impaired deglutition in the pediatric age group.

Complications of Impaired Deglutition

Respiratory complications of impaired swallowing have been reviewed; they include apnea and bradycardia, choking episodes, chronic noisy breathing, reactive airway disease, chronic or recurrent pneumonia, bronchitis, and atelectasis.[70] Aspiration of oral contents may occur directly, that is, in association with a swallow that does not protect the airway. In addition, some patients may be unable to protect the airway from the aspiration of oral secretions. Aspiration may also occur in individuals with impaired swallowing after an episode of gastroesophageal reflux; also, acid reflux may result in bronchospasm, pneumonia, or apnea.[71–73] Unfortunately, in the swallowing-impaired child (and adult), it may be difficult to detect aspiration based on clinical signs and symptoms alone because "silent aspiration" (aspiration without coughing, gagging, and choking) may occur. The prevalence of this condition remains unknown, and predictors of aspiration associated with impaired swallowing have not been clearly defined.

In the severely affected child with impaired swallowing, poor oral and/or pharyngeal function may lead to decreased energy intake as a consequence of prolonged feeding time and the inability to ingest adequate volumes. As a result, protein-energy malnutrition may develop, with deleterious effects on the immune system and on muscle strength. Repeated pulmonary infections may become more debilitating in the face of worsening nutritional status (Figure 23-3).

Another complication of impaired swallowing is sialorrhea, or excessive drooling, defined as the unintentional loss of saliva and other oral contents from the mouth. Drooling usually occurs in patients with neurologic disease complicated by abnormalities of the oral phase of deglutition. Examples of this relationship include cerebral palsy, peripheral neuromuscular disease, facial paralysis, and severe mental retardation.[74] In children who drool, the primary problem is usually related to oromotor dysfunction and not excessive production of saliva.[75] Clinical complications of drooling include soaking of clothes, offensive odors, macerated skin around the mouth and chin, and, if "posterior" drooling occurs, aspiration. In addition to impaired swallowing, the differential diagnosis of drooling includes dentition problems, sinusitis, and the increased production of saliva by the salivary glands. Excellent reviews of this subject include those by Blasco and colleagues[74] and Bailey.[76]

Therapy for the control of drooling may include orosensory motor treatment to improve oromotor skills, medical therapy using anticholinergic medications such as glycopyrrolate, and surgical therapy to redirect the submandibular ducts.[77–80] Anticholinergic side effects (dry

TABLE 23-2 DIFFERENTIAL DIAGNOSES OF DYSPHAGIA IN PEDIATRIC PATIENTS

PREMATURITY
UPPER AIRWAY-FOODWAY ANOMALIES
Nasal and nasopharyngeal
Choanal atresia and stenosis
Nasal and sinus infections
Septal deflections
Tumors
Oral cavity and oropharynx
Defects of lips and alveolar processes
Cleft lip and/or cleft palate
Hypopharyngeal stenosis and webs
Craniofacial syndromes (eg, Pierre Robin, Crouzon, Treacher Collins, Goldenhar)
Laryngeal
Laryngeal stenosis and webs
Laryngeal clefts
Laryngeal paralysis
Laryngomalacia
CONGENITAL DEFECTS OF THE LARYNX, TRACHEA, AND ESOPHAGUS
Laryngotracheoesophageal cleft
Tracheoesophageal fistula/esophageal atresia
Esophageal strictures and webs
Vascular anomalies
Aberrant right subclavian artery (dysphagia lusorum)
Double aortic arch
Right aortic arch with left ligamentum
ACQUIRED ANATOMIC DEFECTS
Trauma
External trauma
Intubation and endoscopy
NEUROLOGIC DEFECTS
Central nervous system disease
Head trauma
Hypoxic brain damage
Cortical atrophy, microcephaly, anencephaly
Infections (eg, meningitis, brain abscess)
Myelomeningocele
Chiari malformation
Peripheral nervous system disease
Traumatic
Congenital
Neuromuscular disease
Myotonic muscular dystrophy
Myasthenia gravis
Guillain-Barré syndrome
Poliomyelitis (bulbar paralysis)
Miscellaneous
Achalasia
Cricopharyngeal achalasia
Esophageal spasm
Esophagitis
Dysautonomia
Paralysis of esophagus (atony)
Tracheoesophageal fistula/esophageal atresia–associated nerve defects
Aberrant cervical thymus
Conversion dysphagia

Adapted from Weiss MH. Dysphagia in infants and children. Otolaryngol Clin North Am 1988;21:727–735; and Cohen SR. Difficulty with swallowing. In: Bluestone CD, Stool SF, editors. Pediatric otolaryngology. Philadelphia: W.B. Saunders; 1983.

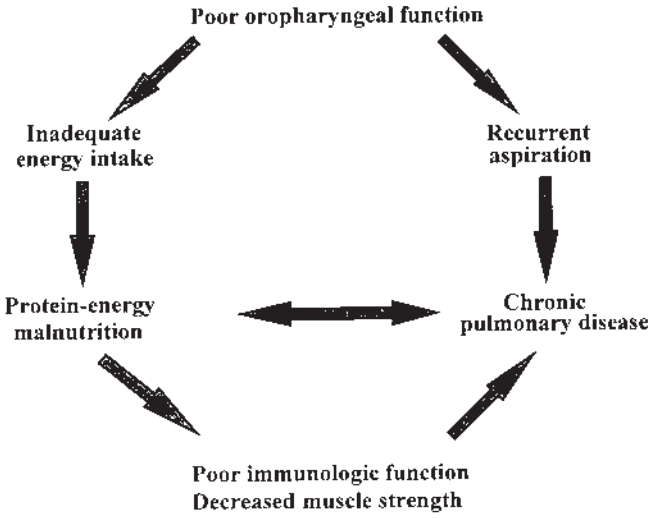

FIGURE 23-3 Clinical sequelae of impaired deglutition.

mouth, thick secretions, urinary retention, flushing) may occur, but these are usually controlled by titrating the dose of medication. Surgical therapy that redirects the flow of saliva posteriorally may increase the flow of liquid to an already compromised swallow and potentially increase the risk of aspiration.

Deglutition in the Preterm Infant

An important clinical issue to consider in the preterm infant is the relationship between deglutition and breathing. Although premature infants are able to suckle-feed at approximately 34 weeks of gestation, successful oral feeding requires the coordination of swallowing and breathing. Poor integration of these activities may result in respiratory difficulties such as aspiration. Wilson and others evaluated the coordination of breathing and swallowing in preterm infants and found that deglutition occurred during both inspiration and expiration and resulted in an interruption of airflow.[81] They concluded that preterm infants are unable to breathe and swallow simultaneously. Shivpuri and coworkers studied the effects of oral feeding on respiratory response in preterm infants and found that tidal volume and respiratory frequency decreased during feeding by continuous sucking, resulting in a decrease of minute ventilation and partial pressure of oxygen.[82] The reader is directed to a review of oromotor function in the neonate.[83]

Critical Period of Learning

A critical period of development refers to a segment of time during maturation when a specific stimulus must be applied to produce a particular action. Inadequate oral stimulation during a critical period may result in difficulty in re-establishing successful oral feeding at a later date. The concept of a critical or sensitive period pertaining to feeding behavior has been reviewed by Illingworth and Lister,[84] and cases of infants and children developing resistance to oral feeding after long-term deprivation of oral stimulation have been reported.[85,86] Successful treatment of this problem has been accomplished by a multidisciplinary feeding team using a behavioral approach.[86]

ISOLATED CP DYSFUNCTION AND CP ACHALASIA

CP dysfunction is usually part of a more global disorder of deglutition also involving the oral phase.[10] CP function may be altered by conditions that affect the central nervous system or cranial nerve function or by conditions that locally involve the function of the muscle or movement of the larynx. Achalasia, meaning failure to relax, does not always accurately describe the type of CP dysfunction present. Nonrelaxation of the sphincter resulting from primary CP disease differs from nonopening of the sphincter secondary to weak forces of propulsion in the proximal pharynx.[34]

Isolated CP dysfunction or achalasia is a rare disorder in infants and children.[87–89] Most patients with CP achalasia present at birth with feeding difficulties, although some may present as late as 6 months of age. Drug-induced dysfunction of the UES has also been reported.[90]

The diagnosis of isolated CP dysfunction is difficult when based solely on radiographic studies. A horizontal bar in the proximal esophagus, representing the CP muscle, may be seen in up to 5% of adults undergoing radiographic examinations for all indications and may also be a normal radiologic sign in infants.[91,92] In some patients with a prominent bar seen on radiographic study, manometric studies have demonstrated normal relaxation and decreased UES pressure.[37,93] Alternatively, manometric studies may be normal in patients with clinical or radiologic evidence of CP dysfunction.[37,94,95] Improvement of CP achalasia may occur spontaneously or after dilatation.[95,96] In some cases, CP myotomy may be required after careful assessment of the patient[97]; surgery is usually contraindicated in patients with gastroesophageal reflux or poor pharyngeal peristalsis.

CLINICAL ASSESSMENT

In clinical practice, disorders of swallowing are often considered in the general context of a feeding disorder. Feeding is a complex process that involves a number of phases in addition to the act of swallowing, including the recognition of hunger (appetite), the acquisition of food, and the ability to bring the food to the mouth. The causes of feeding disorders have been extensively reviewed.[98] The classification of feeding disorders includes the broad categories of abnormalities of structure and function, neurologic disorders, and behavioral feeding disorders.[99]

In the spectrum of feeding disorders, food refusal is a common complaint, but a precise definition of food refusal is not well established. Food refusal may be defined as the developmentally inappropriate intake of food (quality or quantity) for more than 8 weeks (R Wachtel, oral communication, March 1998). Associated symptoms may include behavioral problems such as unusual behaviors at mealtime, an abnormal feeding pattern, and mealtimes that are stressful for the family and child. Food refusal may be secondary to a variety of conditions, including impaired swallowing, mucosal disease of the gastrointestinal tract (eg, reflux esophagitis), behavioral difficulties, and chronic disease (eg, renal, cardiac, and endocrine disease).

Successful evaluation and management of the pediatric patient with impaired swallowing and/or a feeding disorder usually require a multidisciplinary approach. Members of a pediatric "dysphagia" or "feeding" team may include a pediatrician, pediatric gastroenterologist, developmental pediatrician, speech-language pathologist, occupational therapist, and pediatric dietitian. The availability of a pediatric radiologist and an otolaryngologist with experience in the field of pediatric swallowing disorders is essential to assist in the diagnostic evaluation.

The American Gastroenterological Association has published a medical position statement on the management of oropharyngeal dysphagia,[100] and an excellent technical review on the management of oropharyngeal dysphagia accompanies this article.[101] Although concerned mainly with the evaluation and management of adults with dysphagia, the clinical objectives of this statement are applicable to children. The main objectives include the following: (1) determine whether oropharyngeal dysphagia is present and, if so, attempt to identify the etiology; (2) identify the structural etiologies of oropharyngeal dysphagia; (3) determine the functional integrity of the swallow; (4) evaluate the risk of aspiration; and (5) determine if the pattern of dysphagia is amenable to therapy. An algorithm for the evaluation and management of the pediatric patient with possible impaired swallowing is shown in Figure 23-4.

Feeding History

The diagnostic approach to the pediatric patient with impaired swallowing begins with the feeding history, but obtaining an accurate feeding history may be difficult for a number of reasons.[102] First, pediatric patients with severe impairment of swallowing frequently include many with limited cognitive abilities, making direct communication with the patient difficult. As a result, the feeding history must be obtained from individuals directly involved in caring for the child, such as a parent or feeding specialist (eg, a speech-language pathologist or an occupational therapist). Second, severely handicapped children with impaired swallowing may aspirate without coughing, a phenomenon known as silent aspiration (a similar condition has been described in adults).[103–105] Consequently, it is difficult to accurately predict which food substances are swallowed without aspiration solely on the basis of feeding history or clinical examination.

Areas covered in the feeding history include caretakers involved; location or setting for feeding (the nature of which may differ depending on the location, eg, school versus home); method of feeding (eg, type of feeding utensils used); position of the head, neck, and body during feeding; volume of food offered and volume of food tolerated per swallow; presence or absence of chewing; amount of time required to feed; history of dysphagia or odynophagia; pres-

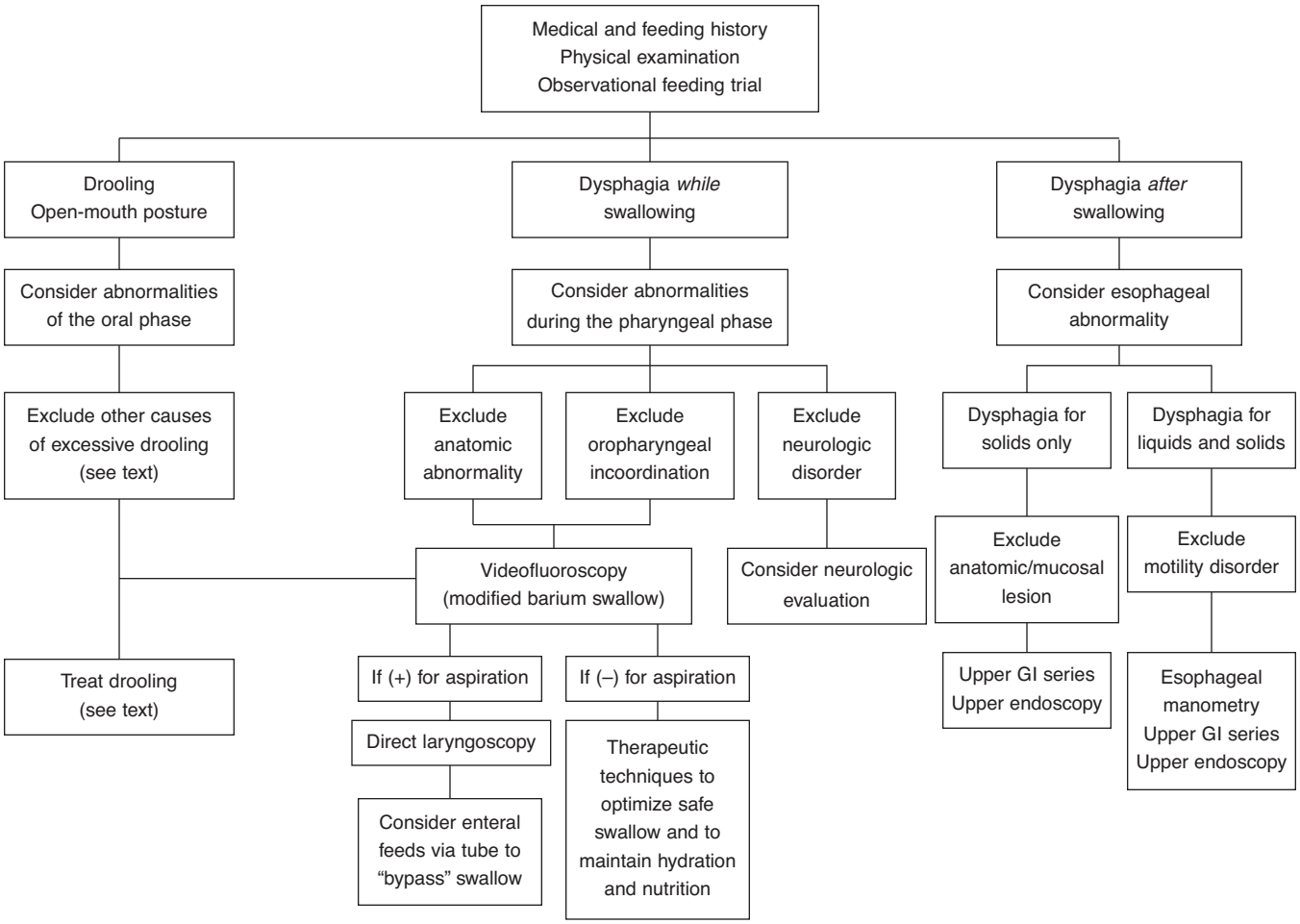

FIGURE 23-4 Algorithm for evaluation and management of the pediatric patient with impaired swallowing. GI = gastrointestinal.

ence or absence of drooling (suggestive of oral phase abnormalities); and history of gagging, choking, or coughing associated with feeding. Determining whether these symptoms occur before, during, or after the swallow helps localize the affected phase.[103] Symptoms that occur prior to the swallow suggest abnormalities of oral control; those that occur during the swallow may indicate pharyngeal phase dysfunction, and gagging and choking just after completion of the swallow probably represent abnormalities of pharyngeal clearance secondary to pharyngeal muscle weakness and/or incoordination or dysfunction of the UES.

In addition to a feeding history, a complete nutritional assessment is essential. Clinical goals should include determining the patient's current nutritional status, estimating energy and protein requirements for establishing optimal growth, and outlining a plan for providing the route and type of feeding. Consultation with a pediatric dietitian will aid in planning a comprehensive nutritional program.

Physical Examination

Physical examination should include the structures of the face, oral cavity, and oropharynx. If structural abnormalities are found and/or are suspected in the pharynx, consultation with an otolaryngologist is indicated. Careful attention should be paid to (a) the presence of an intact soft and hard palate, (b) whether the tongue is midline, (c) the size of the tongue relative to the size of the oral cavity (eg, macroglossia), and (d) the size of the mandible (eg, Pierre Robin syndrome). Head control and head and neck position, particularly when feeding, are also important to note during the examination. It is extremely difficult to swallow with a hyperextended neck, a factor that may be important in the patient with neuromuscular disease (eg, cerebral palsy).

The presence or absence of a gag reflex should be noted, including the existence of a "hyperactive" gag reflex. Lack of a gag reflex is a contraindication to oral feeding, whereas a hyperactive gag may result in significant feeding difficulties. "Hypersensitivity" may involve just the face or oral cavity or may be more pervasive. In infants, oral hypersensitivity is suggested by an aversion to nipple-feeding. In older children, irritability with oral activities such as toothbrushing may suggest hypersensitivity. Children with generalized hypersensitivity may become irritable with any type of sensory stimulation (touch, sound, etc). Issues related to hypersensitivity may be seen in children with developmental disabilities such as autism and cerebral palsy.

Observational Feeding Trial

The diagnostic yield of an observational feeding trial is greatly enhanced if the trial is performed in collaboration with a feeding therapist. During the initial part of the feeding trial, oromotor function is tested by determining the presence or absence of age-appropriate oromotor skills. The acquisition of oral feeding skills and their development have been reviewed in detail by others.[50,106–110] During the feeding trial, the presence of abnormal movements such as jaw thrust, tongue thrust, tonic bite reflex, and jaw clenching is noted. Normal movements seen in the older infant and retained into adulthood include jaw stabilization, chewing, and the ability to lateralize intraoral contents with the tongue. In the impaired patient, normal primitive reflexes or movements (including the phasic bite reflex and suckle-feeding) may extend beyond their expected time of disappearance. During feeding, the positions of the head, neck, and body during swallowing should be noted, as well as abnormal feeding behaviors (such as tongue thrust and averting the mouth) and choking, gagging, or ruminating. A change in voice quality after feeding (such as a "wet," hoarse voice or cry) suggests soiling of the larynx or aspiration.

Diagnostic Tests of Swallowing Function

Specialized tests of deglutition allow broad categorization of swallowing abnormalities. These examinations are mainly descriptive and provide the clinician with limited data regarding specific pathophysiologic mechanisms.

Videofluoroscopy. Videofluoroscopy, or the modified barium swallow, examines swallowing function by visualizing passage of barium-impregnated liquids, pastes, and pureed foods through the oral cavity, pharynx, and esophagus. This is the procedure of choice for evaluating the patient with impaired swallowing[104,110–112] and provides the best means of determining oral, pharyngeal, and esophageal anatomy and function. This study provides objective evidence of oral and pharyngeal incoordination and detects episodes of aspiration, all of which help identify children in whom oral feeding may be contraindicated. Videofluoroscopy is usually performed by a feeding therapist, either a speech-language pathologist or an occupational therapist, in conjunction with a pediatric radiologist. During this procedure, a variety of foods, feeding utensils, and different positions of the head and neck are evaluated to help determine optimal and safe swallowing. Using videofluoroscopy, it is possible to determine whether aspiration occurs prior to, during, or following deglutition.[113] Protection of the airway prior to the swallow is dependent on oropharyngeal coordination and laryngeal elevation. Protection during the pharyngeal phase of swallowing is a result of closure of the laryngeal vestibule secondary to laryngeal elevation, closure of the false vocal cords, and anterior tilting of the arytenoids. Following deglutition, pharyngeal clearance mechanisms help prevent aspiration. The clinical significance of small amounts of aspiration noted on videofluoroscopy deglutition remains unknown. Videofluoroscopy is valuable in the management of swallowing-impaired patients because it aids in determining the bolus characteristics of food that make food safe to swallow (ie, bolus size and consistency).[104] The disadvantages of this procedure include exposure to radiation and lack of quantitative data regarding the function of oral and pharyngeal structures during deglutition. Note that a complete videofluoroscopic examination should include esophagography to evaluate the esophageal phase of swallowing.

Pharyngeal Manometry. Manometry provides quantitative data regarding pharyngeal motor function during de-

glutition, including the amplitude of peristalsis, the speed of propagation of the pharyngeal wave, the response of the UES following deglutition, and the coordination between pharyngeal peristalsis and UES relaxation.[16,28] Recording the response of the pharyngoesophageal region during deglutition is complicated by a number of factors.[114] First, because motor events in the hypopharynx occur at a more rapid rate than in the esophagus, recording equipment with a rapid response time (usually greater than 300 mm Hg/s) is required.[115] Water-perfused catheters with rapid response times are acceptable, but intraluminal pressure transducers provide the most accurate readings. Second, the asymmetric pressure profile of the UES requires that close attention be given to the spatial orientation of the recording device while recording in the UES. Third, there is significant differential axial movement of the recording catheter and oropharyngeal structures during deglutition, which may result in a significant recording artifact.[114,116] A sleeve sensor has been used to monitor UES pressures over time to minimize the effects of catheter and sphincter movement.[46] Manometry does not provide information regarding intraluminal events, such as the movement of fluid in response to recorded pressure changes. The simultaneous recording of videofluoroscopic images and manometric tracings has allowed investigators to correlate motor events with intraluminal movement of substances.[24,117,118]

Ultrasonography. Ultrasonography represents a relatively new diagnostic modality for the evaluation of the swallowing-impaired individual.[119] The motion of structures in the oral cavity such as the tongue and floor of the mouth may be imaged during feeding and deglutition by placing a transducer in the submental region and aiming the beam toward the tongue. This technique has been used to identify feeding movements of oral structures in healthy breastfed and bottle-fed infants.[120] The disadvantages of ultrasonography include poor visualization of the oropharynx (secondary to an acoustic shadow cast by bony structures in the neck) and the lack of standardized measurements.

Nuclear Scintigraphy. Nuclear scintigraphy involves the patient's swallowing a liquid or solid that is labeled with a radiopharmaceutical. Technetium 99m, the radionuclide used in swallowing and esophageal scintigraphic studies, is not absorbed after oral administration and does not become attached to gastrointestinal mucosa. Using a gamma counter and computer processing, regions of interest and selection of time intervals are generated that allow the measurement of transit time and the estimation of intraluminal volumes. Exposure to radiation is less than during analogous fluoroscopic procedures. Problems include poor resolution of the image and poor localization. The technique of nuclear scintigraphy has been reviewed by Cowan.[121] Based on a case report, the radionuclide salivagram has also been used to document aspiration of saliva.[122]

Scintigraphy has been used in adults to assess transit of a liquid bolus through the oropharynx.[123–125] Silver and colleagues attempted to use nuclear scintigraphy to detect and quantify aspiration.[126] Unfortunately, this technique proved to have poor sensitivity for detecting aspiration during swallowing in known aspirators. At present, clinical experience with this technique as a test of swallowing function in children is limited.

Other Tests of Swallowing. Using a stethoscope applied to the neck, the technique of cervical auscultation has been used to study the sounds of swallowing in adults and children.[127,128] Sounds denoting pathologic swallowing have been identified. Cervical auscultation is a noninvasive technique, although the limitations include a lack of standardized measurements and reliance on subjective descriptions of sounds. Recently, workers have performed digital signal processing using an accelerometer placed over the neck to graphically display and quantitatively measure sounds.[129]

A new technique, known as fiberoptic endoscopic evaluation of swallowing safety, allows clinicians to directly observe movements of the anatomic structures involved in the pharyngeal phase of swallowing.[130,131] Using this method, laryngeal penetration (material entering the laryngeal vestibule) and aspiration (material falling below the glottis) can be directly visualized. Episodes can be characterized as occurring prior to and/or following the swallow. Using videotape recording, images obtained during endoscopic evaluation of swallowing provide reproducibility and the ability to closely visualize swallowing events.[132]

A related technique, called fiberoptic evaluation of swallowing with sensory testing (FESST), combines endoscopic evaluation of swallowing with a method that determines laryngopharyngeal sensation.[133] Using the endoscope, air-pulse stimuli are delivered to the pharyngeal mucosa, which is innervated by the superior laryngeal nerves. This allows determination of discrimination thresholds. Laryngopharyngeal sensory capacity is determined by elicitation of the laryngeal adductor reflex, which is a sensorimotor reflex. In adults, FESST is performed at the bedside and has been used as the initial swallowing evaluation for the patient with dysphagia.

TREATMENT

Treatment plans should be developed in the context of a multidisciplinary group. Specific treatment of oral and pharyngeal dysfunction in the neurologically impaired child is not always possible, nor is surgical therapy frequently indicated. Different types of treatment modalities have been discussed in detail by others.[101,134–138] Management techniques involve devising compensatory strategies to minimize swallowing-related complications.[139] Because swallowing abnormalities arise from a diverse group of underlying disorders, management techniques must be individualized. This heterogeneity is also reflected in the fact that patients have differing potentials for recovery. In the patient with acquired brain injury secondary to head trauma, rehabilitation with possible reacquisition of swallowing skills is a major goal; in contrast, compensatory and adaptive maneuvers form the basis for managing the child with severe cerebral palsy.

The management of dysphagia must be considered in the context of the child's level of development and cognitive

TABLE 23-3 IMPAIRED SWALLOWING: MANAGEMENT TECHNIQUES

Alteration of the oral bolus—modify volume, physical properties (eg, consistency, temperature)
Proper intraoral bolus placement
Adjust position of the head, neck, and body during deglutition
Provide jaw control and stabilization during deglutition
Decrease oral hypersensitivity/increase oral hyposensitivity—thermal sensitization/stimulation
Extinguish abnormal feeding behaviors
Swallowing exercises
Tongue resistance/range of motion
Laryngeal adduction
Protection maneuvers—supraglottic swallow procedure
Cricopharyngeal myotomy
Suckle-feeding—valved feeding bottle
Provide alternate means of enteral nutrition
Nasogastric feeding
Gastrostomy tube (surgical or endoscopic)

Adapted from Tuchman DN. Dysfunctional swallowing in the pediatric patient: clinical considerations. Dysphagia 1988;2:203–8.

abilities. The inability of a child to follow directions limits therapeutic maneuvers to passive procedures (eg, bolus modification). Children with intact cognition have the potential to become actively involved with their therapy and learn specific procedures shown to be effective in promoting a safe swallow (eg, the supraglottic swallow procedure).

In general, therapeutic recommendations are based on the patient's ability to swallow safely (ie, the ability of the patient to transfer food from the oral cavity into the esophagus without entry into the larynx or tracheal airway), the patient's nutritional status, the presence of gastroesophageal reflux, and the enjoyment of feeding for parents and the patient.

For a complete discussion of techniques used to facilitate oromotor function in swallowing-impaired infants and children who are receiving some form of either oral feeding or oral stimulation, the reader is referred to Mueller,[109] Morris,[108,140,141] and Ottenbacher and colleagues.[142] Many techniques seek to reduce tactile hypersensitivity, stabilize body position, and optimize the motor response of the oral swallowing mechanism. Modification of the physical characteristics of an oral bolus remains an important part of therapy. The rheologic properties of food have been measured and described.[3] Table 23-3 lists some management options used for children with impaired swallowing. It should be noted that because of a paucity of well-controlled clinical trials, the use of many of these therapeutic maneuvers remains empiric.

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CHAPTER 24

GASTROESOPHAGEAL REFLUX

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Gastroesophageal reflux (GER) is the movement of gastric contents retrograde into the esophagus; in addition to indicating such individual events, the term is also used to signify a benign but symptomatic condition. Gastroesophageal reflux disease (GERD) comprises the objective pathologic sequelae of such retrograde movement of gastric contents, but the term has also been used more recently to denote symptoms affecting quality of life, without regard to objective evidence of disease.[1] GERD now comprises the most common esophageal disorder, as well as one of the most common disorders of any kind affecting infants and children; in adults, it has been documented to be the most costly gastrointestinal disease, estimated to consume more than $9 billion each year in direct costs.[2] The sparser data available in children indicate similar prevalence and cost issues. Therefore, in both children and adults, the promulgation of guidelines for the diagnosis and therapy of various diseases was applied early to GERD, albeit with awareness of the degree to which the current relative dearth of rigorous pediatric data impairs their reliability for children.[3–6]

PRESENTATIONS AND SYMPTOMS

Esophageal versus Extraesophageal

GERD may present as regurgitation, particularly in younger children; as esophageal symptoms, including chest pain, odynophagia, or dysphagia; as respiratory disease; or as diverse other symptoms or signs, including dental and neurologic manifestations.

Age Related

GERD manifests differently in infants than in older children. Although the pathophysiologic underpinnings of this difference are not completely understood, it is likely that the large nutritional demands for growth and the immaturity of neuromuscular and other systems conspire with dietary and postural provocations to generate the infantile forms, whereas the chronicity of the insults plays an increasingly important role in older children and adults.[7] Thus, regurgitation, crying presumed owing to the pain of esophagitis, and malnutrition in the infant transform into symptoms more predominantly centered on the pain and sequelae of esophagitis in the older child. Similarly, the respiratory presentations of GERD may be more centered on the upper airway and apnea in the infant, whereas in the older child, the lower airway and asthma become more important.

Regurgitation

Regurgitation may produce malnutrition or may be associated with pain. If it is unassociated with objective signs of disease, including malnutrition, pain, or respiratory symptoms, it is generally considered benign GER. Regurgitating infants, without other signs of disease, are generally considered "happy spitters" and are managed nonpharmacologically. Regurgitation may persist in toddlers with GERD, and regurgitation into the mouth with reswallowing is sometimes evident in older children and adolescents with GERD.[8] Regurgitation should be differentiated from vomiting, which has a distinct pathogenesis, including retrograde duodenal peristalsis, but, in practice, this differentiation is commonly ignored, and vomiting is used less specifically to encompass regurgitation. Regurgitation in young infants with GERD is often sufficiently projectile to further complicate this distinction.

Pain

Pain has long been considered the primary symptom of esophagitis, represented by crying in young infants, but the correlation of pain with objective manifestations of endoscopic or even histologic esophagitis is incomplete.[9,10] Infant crying is associated with reflux episodes during video and esophageal pH probe monitoring.[11] On investigation, the esophageal pain symptoms may be disclosed to be associated with erosive changes visualized endoscopically, with histologic changes visualized microscopically, or with no evident changes. Recent awareness of differences in visceral sensitivity among individuals with irritable bowel syndrome and dyspepsia has begun to be applied to those with GERD, although, as yet, this concept has not been widely developed or applied to therapeutic strategies.[10] The differential diagnosis of chest pain in children encompasses esophageal motility disorders and eosinophilic esophagitis.[12–14] The differential diagnosis widens in the youngest children, who have the least ability to communicate discriminatory information.[15] Distinctions between odynophagia (painful swallowing) and dysphagia (difficulty swallowing) may be challenging in nonverbal children but can be useful in focusing the differential diagnosis between inflammatory conditions and motor abnormalities of the esophagus.

Other

Esophageal inflammation caused by refluxed acid may result in erosive esophagitis and produce occult blood loss or hematemesis. Such inflammation, if protracted, can also cause strictures that obstruct the esophagus. Barrett esophagus may be asymptomatic until a stricture, or even adenocarcinoma, causes obstructive dysphagia.

Respiratory symptoms and neurologic symptoms may be the presenting signs of GERD (see below). A variety of other symptoms (such as hiccups, sneezing, drooling, or mouthing) have been described.[11,16]

EPIDEMIOLOGY AND NATURAL HISTORY

Epidemiology

The challenges in identifying the epidemiology of GERD are exacerbated by an evolving definition of the disease, unclear demarcation between physiologic and pathologic reflux, lack of a diagnostic gold standard, and a paucity of incidence and prevalence data.[17] For symptoms of GERD, the general population prevalence in infants and children has been estimated to range from 1 to 8%, depending on the symptom and the severity or frequency queried.[18–20] These estimates are further supported by earlier estimates of the proportion of children and adults presenting for evaluation of, or receiving therapy for, GERD.[21] Similarly, 7% of adults experience daily heartburn.

GERD is associated with many other disorders; these associations devolve from provocations of the pathogenic mechanisms of reflux. Thus, neuromuscular disorders can generate GERD by direct effects on upper gastrointestinal tract motility or indirectly by effects on intra-abdominal pressure and posture. Respiratory diseases can affect abdominal-thoracic pressure gradients and so predispose the patient to GERD.

Natural History

Although regurgitation resolves in most symptomatic infants by 12 to 24 months of age, unselected infants with frequent regurgitation may develop feeding problems in the subsequent year of follow-up.[22] By 9 years of age, children with frequent regurgitation during infancy may be more likely to develop persisting reflux symptoms, a phenomenon exacerbated by maternal smoking and maternal reflux symptoms.[23] Children over 1 year of age without neurologic impairment most commonly have "endoscopy-negative GERD," and their esophageal inflammation, even if present, is unlikely to deteriorate during a mean of 28 months of follow-up.[24] However, half of older children with GERD have a chronic relapsing course.[25] Adults with reflux disease are nearly twice as likely as adults without reflux disease to recall having had symptoms of GERD during childhood.[26]

More severe pediatric GERD, the form detected by the earliest investigators, also persists in a minority beyond a year or two of age, but without therapy leads to definite morbidity and mortality.[27] Although strictures, Barrett esophagus, and adenocarcinoma are rare in childhood, it is likely that GERD, beginning in childhood, predisposes the patient to these complications in adulthood.[28]

PATHOPHYSIOLOGY

Understanding of the mechanisms underlying GER has expanded from the primitive conceptualization of the lower esophageal sphincter (LES) as hyptonic to the more complicated and accurate current model (Figure 24-1).[29,30] This current model incorporates dynamic changes at the gastroesophageal junction (GEJ) involving transient LES relaxations (TLESRs) of a sphincter supported actively by the hiatal crura. These motor mechanisms at the GEJ are impacted by more distal motor mechanisms related to gastric volume-pressure relationships promoting TLESRs and reflux and by more proximal motor mechanisms related to esophageal clearance of the refluxed material. Sensory phenomena have been appreciated recently, both for their role in the pain symptoms of reflux (with or without esophageal inflammation) and for their role as the gastric afferent limb to the TLESR. Whether reflux produces esophagitis depends not only on the frequency and duration of the reflux episodes produced by the above mechanisms but also on the balance between the noxiousness of the refluxate and the counteracting esophageal mucosal protective mechanisms. The genetic and environmental factors that modulate all of these pathophysiologic mechanisms and thus underlie the determination of who becomes diseased are currently receiving attention.

Motor Aspects

Tonic LES pressure is maintained above 4 mm Hg, a level adequate to prevent reflux, in most children, even those with GERD. Most reflux occurs when the LES relaxes transiently in a TLESR; the characteristics and provocations of TLESRs have been reviewed.[31,32] The TLESR can be viewed as an "aborted swallow," with afferents in the gastrointestinal tract proximal to the LES, or, more commonly, as a "belch equivalent" venting gastric pressure, with afferents in the stomach, distal to the LES. The TLESR is mediated primarily through vagal pathways via the brainstem. Acting on either the sensory or the motor arm of the arc or on the central pattern generator, nitric oxide and cholecystokinin A seem to play a positive role in TLESR generation because their antagonists reduce the rate of TLESRs; somatostatin, γ-aminobutyric acid B ($GABA_B$), and opiates have the opposite effect. Agents affecting these sites, such as the $GABA_B$ agonist baclofen, have begun to be explored as therapies for GERD, but with ambiguous results. The central role of TLESRs in GERD is challenged by some reports that indicate that TLESR frequency is equivalent in those with and without GERD; the similar frequencies of TLESRs were accompanied by more frequent acid reflux episodes in the GERD patients in contrast to more gaseous or nonacid liquid reflux in the non-GERD patients, differences postulated as attributable to posture or other factors.[33]

The crural diaphragm that surrounds the LES bolsters its tone, particularly during straining; intricate neural connections ensure that TLESRs are physiologically accompanied by coordinated crural relaxation. This fact underlies the important role of hiatal hernia, particularly in more

severe GERD, when the LES pressure can be overcome by gastric pressure, especially during straining (abdominal wall and diaphragm contraction), even in the absence of a TLESR.[34] In adults, hiatal hernia size correlated with esophagitis severity to a greater extent than did manometric LES pressure or esophageal pH probe–monitored (EpHM) esophageal acid exposure, and hiatal hernia size is one risk factor for esophageal adenocarcinoma.[35,36] The etiology of hiatal hernia is unclear, however. It has been proposed either to cause or to be caused by GERD, and both increased abdominal pressure and esophageal traction have been implicated in generating a hiatal hernia.[37] Although hiatal hernia ("partial thoracic stomach") was the earliest identified pathologic correlate of pediatric GERD, a recent retrospective study identified hiatal hernia in only 6% of 718 children with GERD.[38,39] That study found that children with hiatal hernia manifested more delayed esophageal clearance than those without hiatal hernia. Nearly one-fourth of those with hiatal hernia were neurologically impaired, most of whom were treated with surgical therapy initially; nonoperative treatment resulted in an appreciable failure rate (25%) even in neurologically normal patients.[33] Although it is likely that many children do not manifest symptoms with a hiatal hernia, GERD symptoms that are refractory to medical management in patients with hiatal hernia should be treated surgically.[40]

Aspects of gastric function also provoke or impede reflux by affecting the gastric pressure-volume relationship. The volume of gastric material (ingested, secreted by the stomach, or refluxed from the duodenum) is, in turn, modulated by gastric emptying. Gastric accommodation, the stomach's ability to relax to accommodate increased volume, is a further modifying factor.[41]

Increased meal volume increases the frequency of TLESRs in children, consistent with the mechanisms postulated to underlie TLESRs.[42] By slowing gastric emptying, increased meal osmolality increases the rate of reflux episodes when the sphincter does relax.[42] Delayed gastric emptying per se accentuates the volume refluxed per episode in the postprandial period in children and seems to be associated with more severe GERD.[43] Gastric accommodation, measured by barostat or scintigraphically, likely

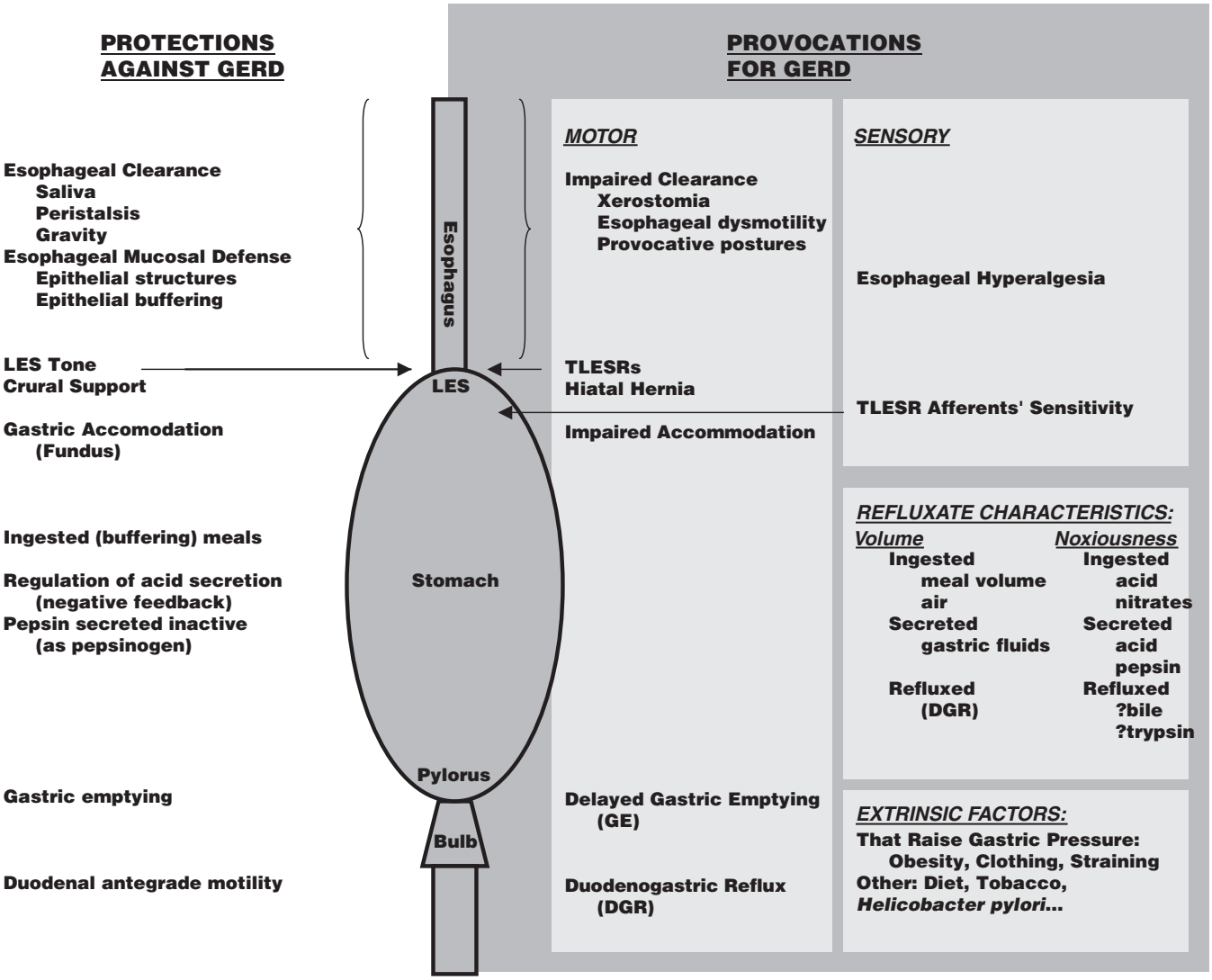

FIGURE 24-1 Cartoon of pathophysiology of GERD. GERD = gastroesophageal reflux disease; LES = lower esophageal sphincter; TLESRs = transient LES relaxations.

impacts the occurrence of TLESRs as a response to a given intragastric volume; the stiffer stomachs (lesser accommodation) of infants than of adults may underlie some of the physiologic reflux of babies.[7] Intragastric pressures are also impacted by extragastrointestinal phenomena: chronically by obesity, tight clothing, or provocative postures; episodically by straining, coughing, or wheezing.[44,45]

Aspects of esophageal motor function determine the clearance from the esophagus of refluxed material.[46] Gravity provides the crude initial component of clearance in upright individuals; infants, who are recumbent for a greater proportion of the day, often lack this component. Esophageal peristalsis clears volumetric reflux not cleared by gravity. Primary peristalsis, peristalsis initiated by swallowing, comprises 90% of all esophageal responses to reflux. Esophageal distention stimulates secondary peristalsis, a backup function that may be particularly important when reflux occurs during sleep.[47] Esophagitis impairs peristaltic function, promoting vicious cycles of worsening esophagitis: 20% of adults with mild and 50% with severe esophagitis manifest failed or hypotensive peristalsis.[48,49] Saliva propelled the length of the esophagus provides a final "wash-down" and neutralizes residual acid. Methods of increasing salivary wash-down of the esophagus have been proposed as therapeutic, and cholinergic prokinetic agents may effect their clearance function by increasing salivation rather than by any change in gastrointestinal motor function.[50,51]

Sensory Aspects

The pain of reflux disease has long been associated with esophageal inflammation in the form of erosive (macroscopic, endoscopic) esophagitis or of histologic (microscopic) esophagitis. Nonerosive reflux disease (dubbed NERD by some) may thus manifest histologic inflammation or regeneration despite the absence of macroscopic changes. Esophageal pain unaccompanied by even microscopic abnormalities suggests mechanisms unassociated with inflammation. Contact of epithelial nerve endings with acid, in the absence of inflammation or regenerative changes, is one potential explanation. Another is that, in some individuals, chest pain is due to visceral hyperalgesia in the esophagus, analogous to the functional pain associated with irritable bowel syndrome or dyspepsia.[52,53]

Although esophageal sensory variations underlie some of the individual differences in the esophageal symptoms of GERD, differences among individuals in the sensitivity of the gastric afferent limb of TLESRs might also produce differences in the frequency of actual reflux episodes from person to person.

Reflux Noxiousness and Esophageal Defense

The components of the refluxate determine its pathogenicity for the esophageal mucosa (and for more proximal sites). An important study in adults found that gastric acid, gastric pepsin, and bile salts transported to the stomach by duodenogastric reflux were important intrinsic components of refluxate, whereas trypsin was seldom found.[54] The study also demonstrated that acid was increased in the esophagus in patients with esophagitis, strictures, and Barrett esophagus; that pepsin was increased (particularly during the night) in patients with strictures and Barrett esophagus; and that bile acids were found in the esophagus of 75% of their patients (particularly during the night) but were at cytotoxic concentrations in only 2% of subjects. Pepsin, when acidified, produces an early, irreversible lesion in the esophageal squamous epithelium, probably by damaging the junctional complex, thus allowing luminal acid greater access to the acid-permeable basolateral membrane and thereby promoting erosive esophagitis.[55] Elevated serum pepsinogen values in neonates with upper gastrointestinal bleeding and esophageal lesions provide further evidence for an important role of pepsin in erosive lesions in children.[56] Because acidity is important in rendering the other components (pepsin, bile salts or trypsin if implicated) pathogenic, therapies directed against acidity are generally effective.

In addition to the intrinsic components, gastric contents are also composed of extrinsic (ingested) material, particularly in the hour or two following a meal. The buffering character of infant milk feeds makes them far less provocative for esophagitis than the acidic liquids consumed by most older children and adults.[7,57] However, adult studies using dual pH probes to analyze the components of refluxate have documented a pocket of acid at the GEJ that escapes the buffering effect of meals, remaining highly acidic (median pH 1.6) compared with the gastric body (median pH 4.7, $p < .001$), and readily traverses the squamocolumnar junction.[58] Of further concern are recent data suggesting that dietary nitrate (commonly derived from green leafy vegetables, particularly when grown with nitrate-based fertilizers) is absorbed, secreted in saliva, reduced by buccal bacteria to nitrite, and further reduced to nitric oxide by acidic gastric juice and ascorbic acid at the GEJ, potentially provoking mutagenesis.[59]

Esophageal defense against noxious refluxate is provided by luminal (pre-epithelial), epithelial, and vascular (postepithelial) factors.[60] Lacking a well-defined surface mucous layer (except possibly in Barrett esophagus) and bicarbonate-secreting surface cells, the luminal defenses of the esophagus are quite limited. The more important epithelial defenses are both structural (apical cell membranes and intercellular junctional structures—both tight junctions and an intercellular glycoprotein material) and functional (acid-buffering mechanisms and two acid-extruding mechanisms—a Na/H exchanger and a Na-dependent Cl/HCO_3 exchanger). The vascular defense is the removal by the blood supply of the excess H+ that enters the epithelium from the lumen.

Genetic

Familial clustering of hiatal hernia, GERD, Barrett esophagus and adenocarcinoma has suggested a genetic predisposition.[61] Genetic linkage analysis identified a locus for a "severe pediatric GERD" phenotype in a group of five kindreds on chromosome 13q14 but excluded the locus in five other kindreds with an "infantile esophagitis" phenotype. It is likely that a disorder as common and as phenotypi-

cally diverse as pediatric GERD will be determined or modified at more than one genetic locus and that responsible genes may affect various aspects of the complex pathophysiology outlined above.

Environmental

Many aspects of the pathophysiology are rather obviously affected by environmental factors, such as dietary factors, habitual postures and activity, or clothing. Increased volume, osmolality, and acidity of the diet are provocative.[42] Supine and seated positions are provocative in infants, compared with the flat prone position, but similar results have not been found in older children and adults.[62] There is some ambiguity regarding whether one lateral position is more provocative than the other; the results are likely affected by meal volume and time postprandial. Increased straining, objectified as rectus abdominis contraction, makes reflux more likely to be propelled from the mouth as regurgitation.[63]

An environmental factor receiving particular attention recently is *Helicobacter pylori*. *H. pylori* gastritis, more common in less developed and less sanitary settings, may progress to atrophic gastritis, thus decreasing the acidity and volume of refluxate.[64] As such, it could be somewhat protective against GERD. Likewise, GERD symptoms and esophagitis may appear after eradication of the organism.[65] *H. pylori* genotypes predisposing the patient to more severe gastric disease may be most protective against GERD.[66]

Special Situations

Nocturnal reflux and reflux in premature infants illustrate some aspects of the pathophysiology of GERD.

In normal children, reflux is uncommon during sleep. Nocturnal reflux does occur in children with GERD, at a time when protective clearance functions, gravitational clearance, swallowing, and salivation are less active.[67,68] Perhaps because of the absence of these protections, nocturnal GERD is associated with increased incidence of complications of GERD: extraesophageal manifestations, Barrett esophagus, and adenocarcinoma. Nocturnal heartburn is associated with sleep disorders, reflux-associated respiratory disorders, and decreased health-related quality of life scores.[69,70]

GER in premature and full-term neonates is particularly challenging because of the dearth of data on which to base management.[71,72] Premature infants have an especially great need for caloric intake for adequate growth contrasted with limited gastric capacity. The relatively large-volume refluxate thus reaches the upper esophageal sphincter (UES) frequently. The response to such refluxate has been described in infants: effective primary and secondary peristalsis and an increase or relaxation in UES pressure.[72] The UES responses can be conceptualized as protecting the airway from refluxate by increased sphincter pressure but relaxing to allow refluxate to escape the esophagus when excessive esophageal pressure is generated by the refluxate. Gravitational provocations to reflux caused by the low torso tone are marked in these young children. Nasogastric tubes, often used to feed premature infants before suck-swallow and gag maturation, may impair refluxate clearance if the tubes are large bore.[73] Reflux is often nonacid at this age because of the very frequent buffered feedings. Chronic lower respiratory disease related to prematurity produces vicious cycles with GERD pathophysiology, to make both worse. Coughing is an uncommon respiratory clearance mechanism in neonates. The limited cross-sectional area of the larynx and upper airway, combined with immature reflexes predisposing the patient to apnea, make upper airway obstruction a particularly hazardous manifestation of GERD at this age: there are suggestions that nonacid reflux may be pathogenic for this manifestation in young infants.[74]

DIAGNOSIS

Historically, diagnostic evaluation of GERD emphasized radiography, then EpHM, and then endoscopy, usually supplemented by histology in children. Nuclear scintigraphy and esophageal impedance measurement provided the ability to evaluate nonacid reflux that EpHM missed. More recently, considerations of cost and invasiveness have motivated less technologic investigations, using validated questionnaires and trials of empiric medical therapy.

Endoscopy

Endoscopy, particularly when supplemented by histology, is the most accurate method of demonstrating esophageal damage by reflux. In a retrospective analysis of 402 neurologically normal children, between 18 months and 25 years of age and without congenital esophageal disease, who were diagnosed to have GERD, erosive esophagitis was reported at endoscopy in more than one-third.[75] The

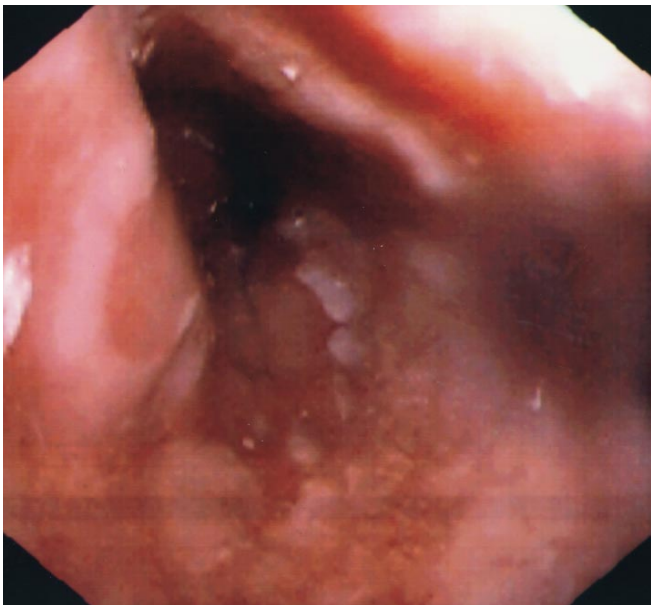

FIGURE 24-2 Endoscopic appearance of Barrett esophagus: salmon-colored patches of columnar mucosa extending proximal to the lower esophageal sphincter as tongue-like projections with surface exudate.

prevalence of erosions increased with age. Strictures were found in 1 to 2%; Barrett esophagus (Figure 24-2) was suspected endoscopically in nearly 3% but was not substantiated histologically by intestinal metaplasia. Optimization and standardization of practice and reporting of endoscopy are unrealized goals in pediatrics; a clinical outcomes research initiative developed in adults is currently being expanded to pediatric practices.[76]

Histology

Microscopic evaluation of biopsy samples from the distal esophagus, but avoiding the most distal area to minimize the false-positive findings at the GEJ, demonstrates abnormalities in many patients who have symptoms but no endoscopically visualized erosions. Infiltration of the epithelium with inflammatory cells, the changes recognizable in esophageal epithelium regardless of orientation of the specimen, received early attention. Neutrophils and eosinophils are not normally present in the epithelium of young children and can be used as indicators of GERD when found even though they are fairly insensitive.[77] Furthermore, eosinophils concentrated above 20/high-power field (or perhaps even above 5/high-power field) are likely to represent eosinophilic esophagitis (see Chapter 25, "Esophagitis"), making them nonspecific for GERD. Intraepithelial lymphocytes ("squiggle cells") are more sensitive than other inflammatory cells but are fairly common; their specificity for GERD remains unclear.

Morphometric histologic parameters require adequate size and proper orientation of the biopsy specimens but seem more reliable for the diagnosis of GERD.[77] They correlate with EpHM documentation of esophageal acid exposure in both adults and infants.[78] The upper limit of normal basal layer thickness and papillary height in infants is 25% and 53%, respectively.

Esophageal pH Probe Monitoring

EpHM (Figure 24-3) has helped to clarify the important role of esophageal acid exposure in GERD. However, many factors contribute to the variability of results in a given patient: technical variability owing to the type of recording equipment or probes, probe placement (methods include radiography, manometry, or regression equations related to patient height), number and placement level of probes (more proximal ones used for airway manifestations of GERD),[79] or duration of recording; patient variability owing to diet, activity, position, and possibly smoke exposure; and scoring systems used (including pH threshold and reflux duration required for an "episode" and the pH data and calculations employed).[80,81] Some scoring systems emphasize early postprandial periods, whereas others emphasize fasting periods. Most analyses place some emphasis on clearance parameters by detailing prolonged acid reflux episodes. Conceptually, one of the most compelling is a method quantifying "area under the curve," such that degrees of acidity, as well as duration of acid exposure, are taken into account; computerized systems will make this scoring system more practical.[80,82]

Although ambulatory and computerized monitoring now simplifies EpHM, the invasiveness and economic and temporal costs of the 24-hour test have prompted more limited and thoughtful use currently. One of the most useful indications is to identify whether acid reflux persists during acid suppression treatment when symptoms have not resolved; therapy can then be augmented or another diagnosis entertained depending on the results. Another

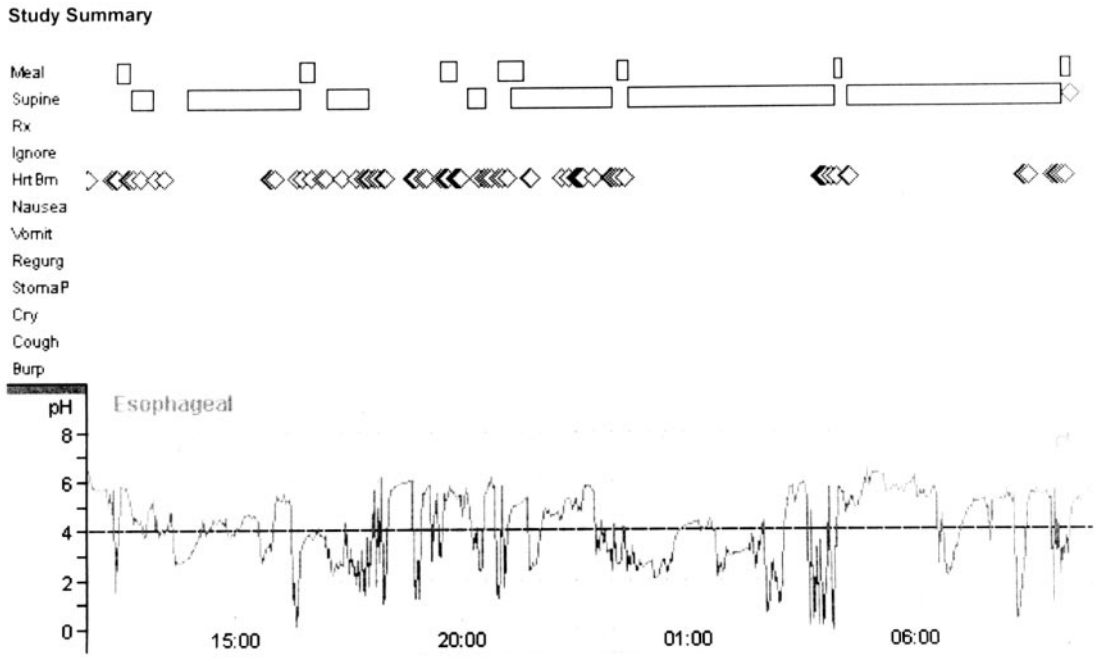

FIGURE 24-3 An intraesophageal pH probe study demonstrating gastroesophageal reflux episodes and relation to meals, position, and symptoms. In this study, reflux index (total time pH < 4) is 29.6%, the longest reflux episode is 64 minutes, and 54 episodes (31.5%) are associated with heartburn. StomaP = stomach pain.

particular use is the temporal correlation of symptoms with acid reflux events.[83]

Radiography

Fluoroscopic evaluation of swallowing and of the upper gastrointestinal tract is often important in the evaluation of the child suspected to have GERD, but radiography is neither sensitive nor specific for diagnosing GERD per se. Fluoroscopy may disclose gastrointestinal obstructions (pyloric stenosis, malrotation with intermittent volvulus) or motor abnormalities (achalasia) that present with symptoms similar to GERD or may demonstrate complications of GERD (strictures—Figure 24-4). Barium esophagography or specialized swallowing studies may be useful in identifying abnormalities of pharyngeal, laryngeal, or upper esophageal function that may prompt aspiration during swallowing and during reflux.

Nuclear Scintigraphy

Scintigraphy, sometimes crudely termed a "milk scan" because of one type of liquid meal used, employs solid or liquid meals labeled with technetium 99m—for its short (6 hour) half-life and limited radiation burden—to assess for delayed gastric emptying, a potential component of the pathophysiology (see Figure 24-1). A second use, based on scintigraphy's ability to demonstrate reflux optimally in the postprandial period (in contrast to EpHM, which is most sensitive during fasting, when gastric acidity is unbuffered), make it potentially complementary to EpHM evaluation.[84] Use in this way, however, requires prolonged restraint of the child and careful, technically demanding analysis and is thus impractical for clinical purposes. The third way in which scintigraphy has been used is to evaluate for aspiration, either with routine upper gastrointestinal scintigraphy or with salivagraphy.[85]

Impedance

Esophageal intraluminal impedance represents another method of demonstrating bolus reflux, without regard for the acidity of the refluxate. Like scintigraphy, it has been shown to be complementary to EpHM: in one study of 50 children monitored with impedance and EpHM simultaneously for about 6 hours, about 15% of impedance-detected episodes were identified by EpHM and about half of EpHM-detected acid episodes were identified by impedance.[86,87] These two tests may be complementary when performed simultaneously, but cumbersome performance and analysis may limit their effective use to research studies.

Miscellaneous Techniques

Esophageal manometry (particularly using Dent "sleeve" components to monitor sphincters) uses an esophageal catheter to demonstrate many of the pathophysiologic components of GERD, such as reduced lower esophageal sphincter tone, frequent TLESRs, and defective esophageal peristalsis. It also can demonstrate volumetric reflux, without regard for acidity, in the form of "common cavity" pressure elevations that are manifest first in the distal esophageal ports and may progress cephalad. However, this technique is cumbersome and of most use for investigative purposes.

Bilirubin monitoring in the esophageal lumen was developed because of the potential pathogenicity of duodenogastric reflux (particularly bile salts) in GERD.[88] However, duodenogastric refluxate does not cause esophagitis except in the presence of acid, so focus on acid reflux remains most useful, and bilirubin monitoring is of most potential interest for research studies.

Surface electrogastrography is a noninvasive technique to detect gastric myoelectrical activity, particularly that generated by the pacemaker interstitial cells of Cajal. It may be useful in identifying gastric myoelectrical abnormalities

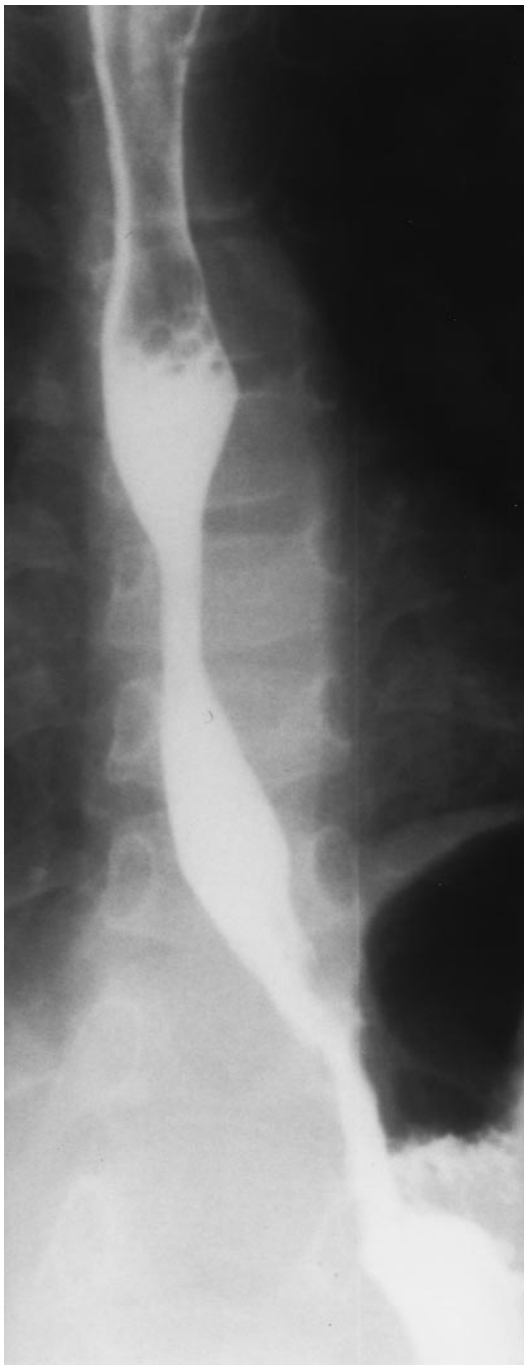

FIGURE 24-4 Upper gastrointestinal series illustrating mid- and distal esophageal strictures in a patient with a history of vomiting and dysphagia for solids.

predisposing some children to GERD, but its use thus far is investigational, and it is predominantly used for functional symptoms such as nausea, anorexia, and dyspepsia.[89]

Respiratory Techniques

Laryngobronchoscopy allows visualization of abnormalities of the upper airway that may be predisposing patients to aspiration during reflux or swallowing (eg, laryngeal clefts) or that may suggest acid reflux–mediated damage to the airway (eg, vocal cord erythema or nodules).[90] Bronchoalveolar lavage may be accomplished concurrently to quantify lipid-laden macrophages (suggesting aspiration of lipid-containing material).[91] Flexible endoscopic evaluation of swallowing with sensory testing uses a small nasopharyngeal endoscope to visualize the larynx during swallowing and to test for appropriate reflexes to tactile stimulation of the larynx.[92] This technique has been minimally evaluated in children but can identify abnormalities that may predispose patients to aspiration. Most of these techniques do not distinguish well between aspiration during swallowing and during reflux. The "sleep study" employs EpHM concurrently with polysomnography (usually including electrocardiography, nasal airflow by thermistor or end-tidal CO_2, and respiratory efforts by chest wall impedance) to identify apneic episodes (particularly obstructive ones) that are temporally associated with acid reflux episodes.

Questionnaires

Questionnaires focus on symptoms and avoid costly and invasive diagnostic testing. They may be used to diagnose GERD or to categorize or stratify the disease. They may assess specific disease symptoms or may focus on quality of life (either generic or disease-specific), such that management strategies may be compared as to efficacy. After treatment or passage of time, they may be used to quantify improvement, stability, or worsening of disease. To be useful, questionnaires must be developed and validated using sophisticated methods to demonstrate the accuracy, reliability, and reproducibility (internal consistency, test–retest consistency, and interobserver consistency). Diagnostic questionnaires are tested for content validity, and questionnaires used for the assessment of changes over time must be shown to be responsive to such changes.

The degree to which reflux is common and manifests as symptoms without objective damage makes GERD questionnaires particularly compelling. Questionnaires to assess GERD in adults[93–96] and infants[97] have been validated, and still others are being developed.

Empiric Trial of Therapy

The availability of powerful acid-suppressing pharmacotherapy has prompted the use of a brief trial of the aforementioned therapy as a diagnostic test in adults, and this practice may reasonably be used in children.[98,99] Children whose symptoms clearly respond, particularly those whose symptoms do not recur after a course of some weeks of such therapy, are thus spared more invasive and costly diagnostic testing. Nonresponding or relapsing symptoms should undergo more definitive testing.

DIFFERENTIAL DIAGNOSIS

The differential diagnosis of GERD in children is large and may be categorized based on the presentation (Tables 24-1 and 24-2). Of particular recent interest is eosinophilic esophagitis, which may overlap with GERD in presentation and in histopathology but which must be treated quite differently (see Chapter 25).

THERAPY

The risks and benefits of therapy are increasingly evaluated and compared in terms of cost-effectiveness, with particular focus on quality of life, especially health-related quality of life. Economic considerations are also increasingly invoked. Cost-effectiveness analysis in adult GERD indicates that proton pump inhibitors (PPIs) are the most cost-effective initial and maintenance medical therapy in most situations, although, in some settings, the costs of drugs make histamine$_2$ receptor antagonists (H_2RAs) preferred.[100,101] The situation is probably different in infants, for whom reflux is predominantly of relatively larger volumes of material that is less acidic, so that, theoretically, lifestyle measures and prokinetic agents could be more cost-effective in this age group, although this expectation has not been realized to date.[7]

Lifestyle Measures (Conservative Therapy)

For infants, lifestyle measures have the clearest benefit.[102] Even telephone teaching of these measures may lead to symptom resolution for a sizeable proportion of infants.[103] The prone position clearly produces less reflux than the supine or seated position, but concerns about provocation of sudden infant death syndrome have significantly limited its use during ages when this syndrome is most common. Thickening of infant feeds reduces regurgitation, decreases crying, and increases sleep time.[104] Lower-volume and lower-osmolality feedings also decrease reflux.[42]

Pharmacologic Therapy

Pharmacotherapeutic agents encompass acid-lowering agents, barrier agents, and prokinetic agents (Table 24-3).

Acid-lowering agents are most useful for symptoms and complications that are acid related, such as heartburn and esophagitis. Acid-lowering agents, listed in order of increasing potency, are acid-neutralizing antacids, H_2RAs, and PPIs. In a review of adult studies without regard to dosing or duration of therapy, PPIs were found to produce more patients free of heartburn, more patients healed, more rapid reduction of heartburn, and more rapid healing than H_2RAs or barrier agents.[105] Such comparisons are unavailable for children; it is likely that similar conclusions would be reached for older children, but it is unlikely that most infants require powerful acid suppression.

"Step-up" and "step-down" strategies signify those that start with H_2RA and "step up" to PPI for those who fail to respond adequately versus those that start with PPI and "step down" to H_2RA when patients have responded adequately to the PPI. Optimal strategies for children are unclear. Recommended H_2RA dosing for children has probably generally been too low but is undergoing re-evaluation.

TABLE 24-1 DIFFERENTIAL DIAGNOSIS OF PEDIATRIC GERD: ESOPHAGEAL PRESENTATIONS

DIAGNOSIS	PRESENTATIONS
VOMITING, REGURGITATION	
GI obstruction	Malrotation/volvulus
	Pyloric stenosis
	Stricture
	Annular pancreas
	Duodenal web/stenosis
	Intestinal duplication
	Bezoar
	Superior mesenteric artery syndrome
	Adhesions
Infections	Viral enteritis
	Parasites
Motility disorders	Achalasia
	Enteritis
	Pseudo-obstruction
Food intolerance	Dietary protein allergy
Nonreflux GI inflammation	Peptic ulcer disease/gastritis
	Eosinophilic gastroenteritis
	Hepatobiliary disorders
	Pancreatitis
Functional GI disorders	Rumination
	Cyclic vomiting syndrome
Extragastrointestinal disorders	Metabolic disorders
	Increased intracranial pressure
	Uremia
	Ureteropelvic junction obstruction
	Infections
	Migraines
	Pregnancy
	Toxins
	Munchausen syndrome
	Adrenal insufficiency
ESOPHAGITIS (PAIN AND IRRITABILITY)	"Colic"
	Food intolerance
	Eosinophilic esophagitis
	Gastritis/peptic ulcer disease
	Infections
	Diffuse esophageal spasm
	Hepatobiliary disorders
	Pancreatitis
	Cardiac pain
	Costochondritis
	Visceral hyperalgesia
	Malingering
Dysphagia	Eosinophilic esophagitis
	Achalasia
	Other esophageal motility disorders
	Globus
	Vascular ring (dysphagia lusorum)
Failure to thrive	Feeding disorder
	Malabsorption
	Metabolic disorder
	Chromosomal anomaly/genetic syndrome
	Underfeeding

GERD = gastroesophageal reflux disease; GI = gastrointestinal.

TABLE 24-2 DIFFERENTIAL DIAGNOSIS OF PEDIATRIC GERD: EXTRAESOPHAGEAL PRESENTATIONS

DIAGNOSIS	PRESENTATIONS
APNEA/ALTE	Prematurity
	Broncholpulmonary disease
	RSV, pertussis
	Sepsis
	Seizure
	Cardiac disease
	Laryngotracheomalacia
	Choanal atresia
	Micrognathia
	Macroglossia
	Foreign body aspiration
	Adenoidal and tonsillar hypertrophy
OTOLARYNGOLOGIC PRESENTATIONS	
Otitis	Infections
Sinusitis	Infections
	Allergies
	Cystic fibrosis
	Immotile cilia syndrome
	Immune deficiency
Stridor, hoarseness	Viral croup
	Subglottic stenosis
	Laryngomalacia
	Tracheomalacia
	Laryngeal cyst
	Vascular ring
WHEEZING, CHRONIC COUGH	Bronchial asthma
	Cystic fibrosis
	Allergies
	Foreign body aspiration
	Immotile cilia syndrome
	Postnasal drip
	Pneumonia
	Bronchitis, bronchiectasis
	Cough tic
DENTAL EROSIONS	Acidic foods and drinks
	Acidic medications (chewable vitamin C tablets, aspirin)
	Bulimia
	Rumination

ALTE = apparent life-threatening event; GERD = gastroesophageal reflux disease; RSV = respiratory syncytial virus.

Nocturnal acid breakthrough for patients on PPIs has been treated with bedtime doses of H_2RA, but there is evidence for tachyphylaxis to daily H_2RAs within a week.[106] When adequate doses of PPIs fail to ameliorate symptoms, considerations include incorrect diagnosis, improper administration (should be given just before a meal and not in the presence of antacids or H_2RAs), or genetic variation in hepatic cytochrome P-450-2C19 that results in more rapid metabolism of PPIs. Studies that compare several different PPIs at different doses should be critically evaluated in light of the fact that, for most PPIs, similar doses seem to have similar efficacies (except that esomeprazole, the S-isomer of omeprazole, is considerably more potent than omeprazole on a weight basis). For children unable to take PPI capsules, granules can be administered orally or into the stomach in weakly acidic material such as apple juice or yogurt or into the intestine dissolved in sodium bicarbonate. Pantoprazole is currently the only PPI available in the United States in intravenous formulation.[107–109]

TABLE 24-3 PHARMACOTHERAPY FOR PEDIATRIC GERD

MEDICATIONS	DOSES	SIDE EFFECTS
Acid neutralization		
Antacids	1 mL/kg/dose, 3–8 times/d	Constipation, seizures, osteomalacia, hypophosphatemia (Al); diarrhea (Mg); fluid retention (Na); milk-alkali syndrome (Ca)
Histamine$_2$ receptor antagonists		
Cimetidine	10–15 mg/kg/dose qid: AC, HS	Headache, confusion, pancytopenia, gynecomastia
Ranitidine	3–5 mg/kg/dose bid-tid: AC, HS	Headache, rash, constipation, diarrhea, malaise, elevated transaminases, dizziness, thrombocytopenia
Famotidine	Pediatric doses not defined	Headache, dizziness, constipation, diarrhea, nausea
Nizatidine	Pediatric doses not defined	Headache, dizziness, constipation, diarrhea, nausea, anemia, urticaria
Proton pump inhibitors		
Omeprazole	0.7–3.3 mg/kg/d, 1–2 divided doses: AC	Headache, rash, diarrhea, nausea, abdominal pain, vitamin B_{12} deficiency
Lansoprazole	1.4 mg/kg/d, 1–2 divided doses: AC	Headache, diarrhea, abdominal pain, nausea
Pantoprazole	Pediatric doses not defined	Headache, diarrhea, abdominal pain, nausea, flatulence
Rabeprazole	Pediatric doses not defined	Headache, diarrhea, abdominal pain, nausea
Esomeprazole	Pediatric doses not defined	Headache, diarrhea, nausea, abdominal pain, flatulence, dry mouth, constipation
Barrier agents		
Sucralfate	40–80 mg/kg/d qid: AC, HS	Vertigo, constipation, dry mouth, aluminum toxicity, decreases absorption of concurrently administered drugs
Sodium alginate	0.2–0.5 mL/kg/dose 3–8 times/d PC	Same as antacids
Prokinetics		
Metoclopramide	0.1 mg/kg/dose qid: AC, HS	Drowsiness, restlessness, dystonia, gynecomastia, galactorrhea
Cisapride*	0.2 mg/kg/dose qid: AC, HS	Diarrhea, cramps, cardiac arrhythmias
Erythromycin	3–5 mg/kg/dose tid-qid: AC, HS	Diarrhea, vomiting, cramps, antibiotic effect, pyloric stenosis
Domperidone	Pediatric doses not defined	Hyperprolactinemia, dry mouth, rash, headache, diarrhea, nervousness
Bethanechol	0.1–0.3 mg/kg/dose tid-qid: AC, HS	Hypotension, bronchospasm, salivation, cramps, blurred vision, bradycardia

AC = ante cibum; GERD = gastroesophageal reflux disease; HS = hour of sleep; PC = post cibum.
*Use in the United States restricted through a limited access program supervised by a pediatric gastroenterologist.

Barrier agents include sucralfate, which complexes with the base of ulcers or erosions, so it is most effective in settings in which the esophageal epithelium is broached. Gaviscon forms a different sort of barrier, floating on top of the gastric contents and potentially reducing reflux in that way.

Prokinetic agents have tremendous theoretical benefit in reflux, particularly in young children, but that theoretical benefit has been challenging to demonstrate objectively, and potential side effects and toxicity have limited their use. Bethanechol is without clear benefit and is currently rarely used. Metoclopramide has a narrow therapeutic range, and extrapyramidal side effects are not uncommon. Cisapride and domperidone are generally unavailable in the United States. Low-dose erythromycin may improve gastric emptying by activating motilin receptors, but the potential effects of widespread use of this antibiotic on antimicrobial resistance are of concern.[110] Baclofen, a $GABA_B$ agonist, is being studied as an antireflux agent because of its inhibition of TLESRs.[111]

Surgical Therapy

Fundoplication, the wrapping of the gastric fundus around the GEJ, has been used for decades to treat refractory GERD. Although it is efficacious in many children, complications are common and distressing. Cost-effectiveness analyses suggest that fundoplication is most likely to be favored in patients with anticipated prolonged duration of requirement for therapy: younger patients with GERD. However, its long-term effectiveness is ambiguous; many individuals treated with fundoplication nonetheless require ongoing pharmacotherapy for symptom relief.

Complications are most common in those most likely to require it, that is, those with chronic neurologic or respiratory disease. Complications include persistence of the symptoms prompting surgery (often owing to a loose wrap) or the side effects of the surgery (often owing to a tight wrap).[112,113] More broadly, persistence of the symptoms may be due to a "bad diagnosis," wherein the symptoms prompting surgery were incorrectly attributed to GERD; a "bad patient," whose challenging physiology makes a successful fundoplication difficult; or a "bad therapy" (incompetent to prevent reflux) performed by a surgeon with deficient or inappropriate technique. Side effects of the surgery, in contrast to ineffective surgery, are usually due to a wrap's excessive tightness: an antegrade obstruction that produces dysphagia or a retrograde obstruction that produces gas-bloat. Other complications may be due to vagal injury, producing nausea and vomiting owing to gastroparesis or diarrhea or hypoglycemic symptoms owing to dumping (rapid gastric emptying).

Gas-bloat is common and a particularly challenging side effect of fundoplication because understanding of its pathophysiology is limited, and the management options are limited and often experimental. Symptoms include inability to belch or vomit when needed, retching, gagging, nausea, early satiety, postprandial fullness, and abdominal distention. The pathophysiology of gas-bloat in an individual patient may include gastric hyperalgesia, abnormal gastric emptying,

impaired accommodation, or impaired vagal function. These various causes require different types of pharmacotherapy.

Methods to prevent complications prior to performing fundoplication include (1) careful elimination of nonreflux causes of the symptoms prompting surgery; (2) awareness that failure of aggressive PPI pharmacotherapy to control symptoms suggests a nonreflux cause; (3) careful tailoring of the operation in those predisposed to complications by neurologic or respiratory disease, delayed gastric emptying or retching, short esophagus (from esophageal atresia, stricture, or large hiatal hernia), or esophageal dysmotility (eg, from esophageal atresia); (4) choice of an experienced or closely mentored surgeon; (5) optimal "tightness" of the fundoplication to prevent reflux but avoid dysphagia (eg, looser in those with defective peristalsis); and (6) use of a venting gastrostomy for those predisposed to problems because of lower gastric compliance or capacity (eg, infants). Some surgeons have used preoperative gastric emptying studies to select patients for concurrent pyloroplasty, but, currently, data argue against this practice because fundoplication itself promotes more rapid gastric emptying.

When complications do occur following fundoplication, the diagnosis and competence of the wrap may be re-evaluated with barium fluoroscopy, endoscopy with histology, and pH probe, using other modalities (eg, esophageal or antroduodenal manometry, gastric emptying scintigraphy) as indicated and treating any other diagnosis if found. Conservative management of complications often includes dietary and feeding modifications. Pharmacotherapy is often required for postfundoplication complications, either directed at the recurrent reflux or directed at the specific side effect of fundoplication if one is present. Fundoplication revision, or dilation of a persistently tight wrap, is sometimes needed.[112,113]

Laparoscopic fundoplication can be as effective as open fundoplication in children and associated with more rapid postoperative recovery but requires a surgeon experienced in performing the technique in these small patients.[114]

Endoscopic therapies aimed at the GEJ in patients with reflux eventually may prove useful enough to be applied to children.[115]

COMPLICATED GERD

Esophageal Complications: Strictures, Barrett Esophagus, and Adenocarcinoma

Chronic reflux may result in scarring of the esophagus, producing a stricture. This scarring appears to depend on acid and perhaps on pepsin.[54] These peptic strictures are generally located in the distal third of the esophagus (see Figure 24-4). Rarely in children, these strictures may be accompanied by Barrett esophagus. They should be distinguished from other types of strictures: caustic (generally more proximal), eosinophilic, postoperative/anastomotic, following radiation therapy or sclerotherapy, or (rarely in children) malignant. Peptic strictures should be biopsied below the stricture to confirm the diagnosis of reflux esophagitis and exclude eosinophilic esophagitis, Barrett esophagus, or malignancy. They are treated with a course of endoscopic dilations at increasing intervals, supplemented by aggressive antireflux therapy. Injection of corticosteroids into the stricture at the time of dilation has been used for resistant strictures. Resection or stricturoplasty may be required for those strictures not amenable to dilation or that perforate during dilation. The relative merits of chronic antireflux pharmacotherapy versus fundoplication continue to be debated. A coexisting large hiatal hernia may encourage surgical treatment.

Barrett esophagus is uncommon in children but has been reported.[116] The intestinal metaplasia of the distal esophagus, manifesting endoscopically as salmon-colored tongues of tissue projecting proximally into the paler pink esophagus (see Figure 24-2), increases with age until about the fifth decade, and the density of goblet cells increases in parallel.[28] In children, associated conditions include neurologic disability, chronic respiratory disease, repaired esophageal atresia, chemotherapy for malignancies, and hiatal hernia.[117] Postulated pathogenesis of Barrett esophagus has included genetic influences, reflux of both acid and pepsin, and possibly a decreased sensitivity to reflux events that results in impaired clearance reflexes.[54,61] The importance of duodenogastric reflux of bile (and possibly trypsin) has been debated.[54,118]

Guidelines for the diagnosis of, therapy for, and surveillance for dysplasia and malignancy in Barrett esophagus have been promulgated for adults and may be applicable to children.[119–122] Hiatal hernia size, length of the Barrett esophagus, and severity of acid reflux are all risk factors for esophageal adenocarcinoma; surgical reduction of hiatal hernia and associated fundoplication thus may be indicated in those with hiatal hernia.[36] Because of the apparent insensitivity to reflux in many patients with Barrett esophagus, it is worthwhile to assess the adequacy of therapy with EpHM and to evaluate periodically via endoscopy for regression or progression of the Barrett epithelium and for dysplasia.[123,124] Successful attempts to ablate the specialized epithelium have been reported in adults. Adenocarcinoma is extremely rare in childhood, but it does occur and should be sought in those with Barrett esophagus.[123]

EXTRAESOPHAGEAL GERD

Respiratory Complications and Interactions

Embryonic relationships between the upper gastrointestinal tract and the airway lead to both luminal and neural connections between the two systems. Consequently, GERD provokes and worsens respiratory diseases, both otolaryngologic and lower respiratory tract disorders, and may do so either by aspiration of refluxate, producing inflammatory changes in the lumen, or by reflexive responses of the airway to refluxed material in the esophagus.[125,126] The respiratory diseases, in turn, may worsen GERD. Epidemiologic evidence suggests that GERD, particularly when reflux occurs recumbent and during sleep, is associated with a several-fold increase in sinusitis, laryngitis, asthma, pneumonia, and bronchiectasis.[70,127,128] Data on otitis media are conflicting.[127,129,130] Laryngotracheoma-

lacia and stridor often coexist with GERD; laryngoscopic changes in the posterior larynx suggest laryngeal irritation by GERD; and chronic hoarseness, chronic cough, and chronic sinusitis have apparently been effectively treated by GERD therapy in open-label trials.[131–133] Dental erosions on the lingual aspects of the posterior teeth also may represent extraesophageal GERD (Figure 24-5).[134–136]

Infantile apnea represents a prototypical upper airway response to reflux in the infant, whereas the bronchospasm of asthma represents a prototypical lower airway response to reflux in the older child.[69,74,125,126,137–142] Probable mechanisms for these phenomena have been reviewed.[143,144] Whether aspiration, esophageal airway reflexes, both, or neither is implicated in the pathophysiology of individual cases is challenging to determine. It is likely that most infants with apnea and most older children with asthma do *not* have reflux-induced respiratory disease. Nonetheless, GERD may play an important role in selected cases, which will be resistant to management if GERD is not considered and treated.[137]

Diagnosis of individual patients suspected of GERD-mediated extraesophageal disease is difficult. The presence of concurrent GERD symptoms makes GERD a more likely factor in respiratory disease. It is important to distinguish aspiration during reflux from aspiration during primary swallowing because the therapeutic approach will be different. The diagnostic methods must be tailored to the particular questions raised by the presentation but may include upper esophageal EpHM,[145] fluoroscopy (particularly specialized upper esophageal swallowing studies, sometimes termed "cookie swallow"),[146] scintigraphy to diagnose aspiration (particularly the salivagram[85]) (Figure 24-6), laryngobronchoscopy (to disclose posterior glottic edema, erythema, and nodules and including bronchoalveolar lavage for lipid-laden macrophages or trypsin to assess aspiration),[91,147–149] and flexible endoscopic evaluation of swallowing with sensory testing.[92] Laryngobronchoscopy may be coordinated with endoscopic and histologic evaluation of the upper gastrointestinal tract.[150] Impedance testing discloses nonacid reflux that may be important in infantile apnea.[74] An empiric trial of aggressive antireflux therapy can also be used.

Therapy for extraesophageal manifestations of GERD has been reviewed.[151–153] When GERD is responsible for extraesophageal presentations, it seems that the therapy must be aggressive in terms of both potency and duration to affect the respiratory symptoms. Twice-daily PPIs maintained for at least 3 months are recommended. Fundoplication surgery seems to provide better results, and, in adults, the best surgical results are obtained in patients with nocturnal asthma, onset of reflux symptoms before pulmonary symptoms, evidence of laryngeal inflammation, and a good response to medical treatment. However, the complication rate for fundoplication is greater in children with respiratory disease than in those without.

Neurologic Presentations and Interactions

Neurologically abnormal children have more GERD, and more complicated GERD, than neurologically normal children. Increased gastric pressure mediated by spasticity, chronic supine posturing impairing refluxate clearance, inability to communicate or move to ameliorate symptoms, and possibly other direct effects of neurologic abnormality all predispose patients to more severe GERD. Thus, erosive GERD, strictures, Barrett esophagus, adenocarcinoma, and chronic respiratory manifestations of GERD are all more common in children with neurologic disability.

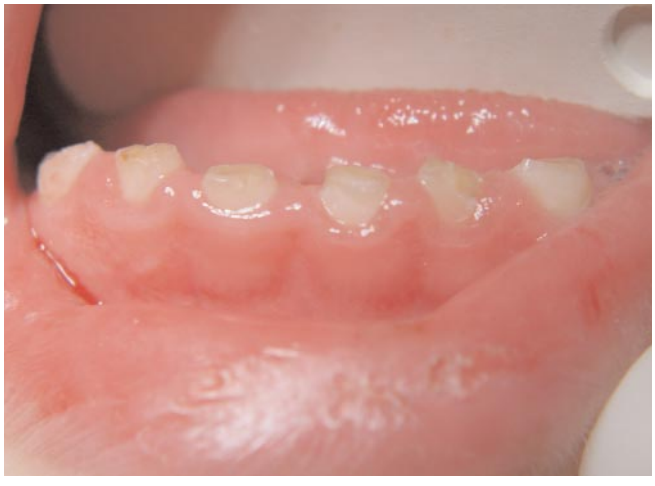

FIGURE 24-5 Dental imaging showing generalized enamel and dentin erosions at the incisal edges in a patient with gastroesophageal reflux disease. Some dental caries are also present.

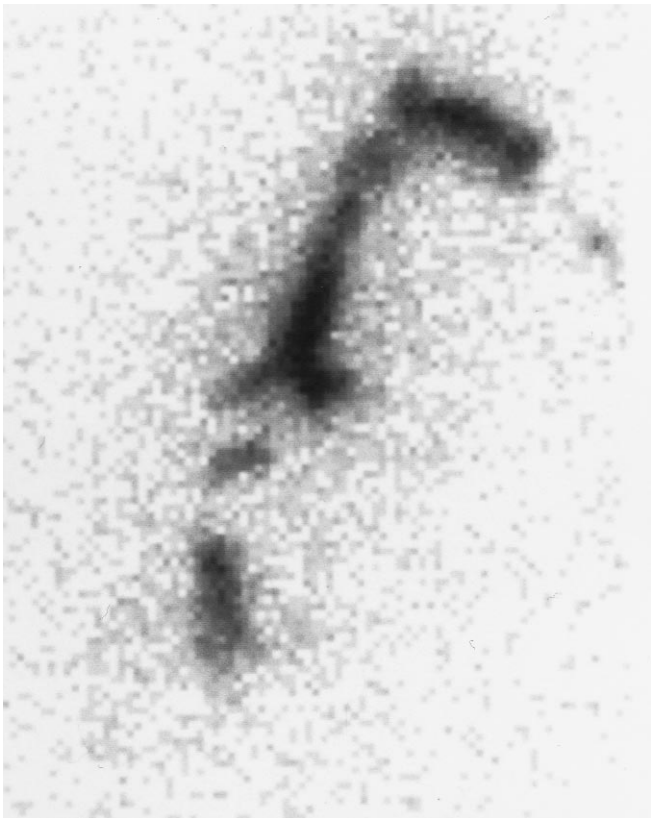

FIGURE 24-6 Salivagram using technetium 99m sulfur colloid–labeled small-volume fluid introduced into the oropharynx produces prompt aspiration extending to the right and left main stem bronchi as seen on posterior imaging of the thorax.

In addition to the impact of neurologic disorders on GERD, GERD may induce symptoms that mimic seizures or neurologic disease. Torso hyperextension ("arching") is a common manifestation of infantile reflux.[11] More unusual and severe manifestations in older children include Sandifer syndrome, a stereotypic torso, and nuchal hyperextension with unclear pathophysiology.[16,154]

GERD, one of the most common and costly disorders afflicting children, presents with a wide array of symptoms, overlaps in presentation with many non-GERD diseases, can be challenging to diagnose, and does not lend itself readily to simple curative therapy. The burgeoning incidence of esophageal adenocarcinoma related to chronic GERD in adults focuses attention on the possible initiation of this cancer in childhood GERD. Further clarifying the pathogenesis of this important disease will have important implications for prevention and treatment.

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CHAPTER 25

ESOPHAGITIS

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Pathologic processes in the pediatric esophagus have received a disproportionately small amount of attention until recently, when appreciation of their pathophysiology and concordant clinical importance has been highlighted. This increase in interest and exposure is probably a phenomenon secondary to a number of important factors, which include improved diagnostic yield from relatively recent technical advances in areas such as infant and pediatric endoscopy; advances in fields such as mucosal immunology, allowing for the realization that etiopathologic mechanisms for esophagitis are more complex than simple luminal chemical damage; and a shift in clinical opinion recognizing esophageal pathology as a major cause of nonspecific ubiquitous symptoms such as infant colic, feeding disorders, and recurrent abdominal pain, among others. A state of knowledge such as this has made pediatric esophagitis, until recently, a relatively underdeveloped area of research and clinical understanding, but this is rapidly changing.[1]

It is now clear, therefore, that esophagitis in infants and children has many responsible etiologic pathways that may have complex interactions and hence requires equally complex diagnostic and therapeutic strategies. Such causative factors are now known to include cow's milk protein (CMP) intolerance or allergy; pH-dependent and -independent gastroesophageal reflux (GER); dysmotility of various causes; and infective, traumatic, and iatrogenic causes, among others. Hence, the term "esophagitis" can be used to describe chemical, infectious, inflammatory, ischemic, immunologic, and degenerative abnormalities.[2] Nevertheless, there remains a minor degree of controversy regarding the definition and significance of esophagitis, as assessed by standard diagnostic techniques, including endoscopy and biopsy.[3,4] This chapter attempts to describe basic etiologies and their interactions, symptomatic presentations, timing and choice of diagnostic measures, current practice of therapeutic interventions, and prognosis of and for esophagitis in infancy and childhood.

EPIDEMIOLOGY

This is a relatively gray area in infancy and childhood. Esophagitis occurs throughout the age spectrum of pediatrics and has even been reported as a cause of prenatal gastrointestinal (GI) bleeding.[5] Neonatal esophagitis is well known as a cause of GI bleeding or anemia.[6,7] It is estimated that the prevalence of reflux esophagitis varies between 2 and 5% of the general population[8]; however, few objective data exist to allow determination of esophagitis distribution in childhood. Some limited data are available regarding GER, and in an early study by Carre, 60% of infants were symptom free by 18 months of age, but 10% developed complications.[9] Such studies would now be considered unethical, and, subsequently, other studies assessing outcome with early therapy have indicated that less than 55% would be symptom free by 10 months of age and 81% by 18 months.[10] In a study of 32 older children (3.5–16 years) with GER, 50% had esophagitis, and less than 50% underwent complete resolution or marked symptom resolution over the ensuing 1 to 8 years.[11] Forty percent came off medication, and 40% required ongoing medication for control of symptoms. Two children required fundoplication. Unfortunately, no wider studies are available, and other information on the incidence and prevalence of esophagitis comes from adult studies. These indicate that 48 to 79% of those with GER have esophagitis, with few having symptoms,[1] which is similar to that quoted for infants.[12]

ETIOLOGY AND PATHOPHYSIOLOGY

The etiologies of esophagitis in infancy and childhood can usefully be divided into the following groups:

1. Chemical: (a) owing to refluxed contents from the stomach and duodenum such as gastric acid, pepsin, bile, and trypsin (b) owing to swallowed substances, either intended, such as medications, or accidental caustic ingestion, such as dishwasher liquid
2. Immunologic: owing to specific responses to specific antigens such as CMP or multiple food intolerance or allergy
3. Infective: associated with organisms as diverse as *Helicobacter pylori* and *Candida*, cryptosporidiosis, herpes simplex, and cytomegalovirus (CMV)
4. Traumatic: secondary to intraluminal trauma (eg, long-term nasogastric tube) or irradiation (eg, as part of bone marrow transplant conditioning)
5. Systemic disease manifestation: associated with conditions such as Crohn disease and chronic granulomatous disease
6. Miscellaneous: such as that associated with passive smoking or that occurring in Munchausen syndrome by proxy
7. Idiopathic: idiopathic eosinophilic esophagitis

The etiopathologic role of each of these situations can therefore be usefully discussed under each heading, bearing in mind that an individual child or infant may, of course, have more than one factor contributing to the esophageal insult at any one time (eg, GER and cow's milk–associated esophagitis).

Chemical

Gastroesophageal Reflux. This is dealt with more comprehensively in Chapter 24, "Gastroesophageal Reflux." Nevertheless, a brief summary of the pathophysiology and etiology of GER is pertinent here.

The natural history of GER is to improve with age; however, one important question arises: why do infants (and subsequently a hard core of older children) have a greater propensity toward GER? Differences in the function of the lower esophageal sphincter (LES) have been investigated[13,14]; this is an area that is tonically contracted at rest, with relaxation occurring consequently on an esophageal peristaltic wave. Inhibitory neurotransmitter production is integral to LES relaxation, and the nonadrenergic, noncholinergic neurotransmitter nitric oxide (NO) has received attention in animal[15] and human studies.[16,17] Vasoactive intestinal polypeptide is another candidate undergoing investigation, and the importance of the ontogeny of neuropeptides in the human fetus and infant is becoming increasingly apparent.[18] Rather than a "weak" LES in infants, it is more likely that a combination of anatomic relationships of the LES precluding effective pressure generation and inappropriate LES relaxation is responsible for infantile GER and its subsequent age-related improvement.[19,20] In adults, 90% of the refluxate is cleared in seconds, and the remainder is neutralized by subsequent swallows.[21] Efficiency of esophageal clearance is therefore vitally important in the genesis of esophagitis.

Although work exists suggesting that acid exposure of the distal esophagus induces dysmotility in pediatric patients,[22] allowing the potential for a "vicious cycle" of LES dysfunction to GER to LES dysmotility to further GER to esophagitis and back to LES dysmotility, it is still not clear how an inflamed esophagus further impairs esophageal tone or motility, but emerging work suggests a role for interleukin (IL)-5 and eotaxin in allergic neurohumoral modification and possible gastroesophageal junction (GEJ) inappropriate relaxation, with an interrelation with mast cell degranulation and histamine release to afferent and then efferent neurons, which control transient lower esophageal sphincter relaxation episodes (TLESRs).[23] Inducible nitric oxide synthetase (iNOS) (which is markedly up-regulated in GI inflammatory conditions such as Crohn disease), is important in relaxation of the LES during TLESRs, which are the single most common mechanism underlying GER, but in one study was not up-regulated in the inflamed pediatric esophagus.[24] However, other researchers suggest an increased release of NO in the inflamed esophagus in children.[25] Other factors that affect clearance are posture-gravity interactions; volume, size, and contents of a meal, for example, breast milk[26,27]; defective peristalsis of the esophagus; gastric emptying; and increased noxiousness of refluxate.

Acid, particularly when combined with pepsin, which is most active below pH 2, is known to cause severe esophagitis in animals and humans.[28–31] Even a 24-week gestation infant in an intensive care setting has the ability to lower intragastric pH below 2.[32] Pepsin plays a critical role in esophagitis owing to acidic refluxate; animal work has shown that in dogs and rabbits, infusion of hydrochloric acid alone caused no damage, but in combination with low concentrations of pepsin at pH less than 2, severe esophagitis resulted.[33,34] Proteolysis may allow deeper penetration of harmful refluxate, and the simple notion that acid causes epithelial damage must therefore be questioned in favor of a more complex interplay of a number of noxious stimuli in the pathogenesis of reflux esophagitis in infants and children.

Furthermore, the role of duodenogastroesophageal reflux (DGER) remains controversial[35,36] and has not, to date, been adequately studied in pediatrics. What is clear from adult studies is that alkaline reflux does not correlate well with bile reflux, the former being attributable to reasons other than DGER, such as saliva, food, oral infection, or an obstructed esophagus.[35] In fact, in one study, bile acid DGER correlated well with acid reflux, and those with the more severe esophagitis had greater exposure to the simultaneous damaging effects of both acid and bile acids.[33] Perfusion studies of the rabbit esophagus show that conjugated bile acids in an acidic environment produce mucosal injury, whereas unconjugated bile acids and trypsin are more harmful at more neutral pH values (pH 5–8); therefore, the latter are less likely to cause reflux-associated damage because this is usually an acidic phenomenon.[36] It is further suggested by animal work that the hydrochloride-pepsin damage may actually be attenuated by the presence in the esophagus of conjugated bile acids, but if damage is done to the squamous epithelium, the un-ionized forms of conjugated bile acids at a low pH may be allowed access to mucosal cells and cause damage by the dissolution of cell membranes and mucosal tight junctions.[37–40]

The histologic appearances typical of luminal chemical-induced esophagitis secondary to GER or GER disease are discussed in the diagnosis section of this chapter.

Chemical Esophagitis owing to Swallowed Substances. The importance of caustic ingestion into the esophagus is dealt with comprehensively in Chapter 27, "Injuries of the Esophagus" (Figure 25-1). Ingested materials are usually household or garden substances and are usually markedly alkaline; the common one was dishwasher fluid, often with a pH of 9 or above. However, fortunately, in most countries, this has been replaced with powder, which is less easy to swallow, and even individually wrapped tablets of powder. Acute perforation, mediastinitis, and subsequent esophageal stricture have frequently been seen. The possibility of nonaccidental injury should not be forgotten in this context. It is notable that the rate of subsequent stricture formation is high, and, more recently, a potentially effective postdilation topical application of an antifibrotic, mitomycin C, has shown promise in preventing restenosis and long-term repeated stricture dilation.[41]

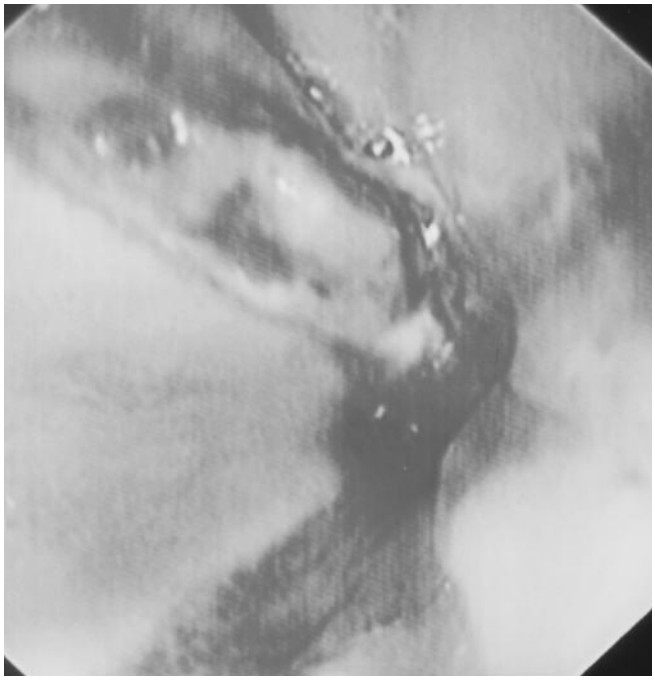


FIGURE 25-1 Example of caustic injury to the esophagus.

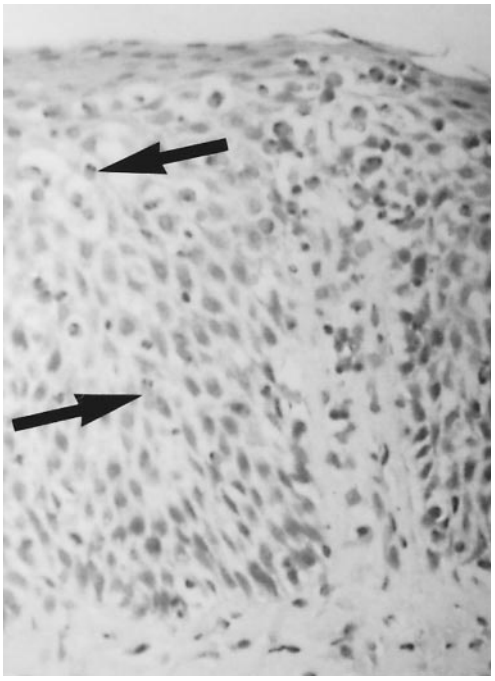


FIGURE 25-2 Esophageal mucosal eosinophilia seen in cow's milk–associated and primary reflux esophagitis and primary eosinophilic esophagitis (hematoxylin and eosin; ×200 original magnification) (eosinophils marked by *arrows*).

Many medications have been associated with esophageal damage and symptoms of esophagitis, and these include tetracyclines (not recommended under the age of 12 years, of course), drugs used in acne therapy, and nonsteroidal anti-inflammatory drugs.[42–45]

Immunologic

Although it is now clear that multiple food antigens may induce esophagitis,[46,47] the most common precipitant is CMP. Standard endoscopic biopsy and histology do not reliably distinguish between primary reflux esophagitis and the emerging clinical entity of cow's milk–associated reflux esophagitis. This variant of cow's milk allergy appears to be a particularly common manifestation in infancy, with symptoms indistinguishable from primary GER but that settle on an exclusion diet.[48] Some differentiation from primary reflux has been suggested on the basis of an esophageal pH testing pattern and a β-lactoglobulin antibody response, although the former has not been substantiated by more than one center.[48,49] There is recent evidence that this esophagitis is becoming a more common presentation of infant food allergy within the developed world and, in fact, may be induced by a variety of antigens in addition to cow's milk.[46,47] Many affected infants have sensitized while exclusively breastfed, and a defect in oral tolerance for low doses has been postulated as the underlying cause.[50,51]

Esophageal mucosal eosinophilia has been described in both suspected cow's milk–associated[46] and primary reflux esophagitis (Figure 25-2),[52] as well as in other conditions, such as idiopathic eosinophilic esophagitis (IEE).[53] However, the density of the eosinophilic infiltrate has been used as a differentiator between allergic esophagitis and IEE, with the latter defined as more than 15 to 20 eosinophils per high-power field (HPF).[54] The clinical significance of eosinophils and their role in the pathogenesis of mucosal injury is poorly understood and the subject of recent debate.[3,4,55] Some have suggested an active role for eosinophils in the inflammatory process of esophagitis and have supported this with the observation of activation of the eosinophils by electron microscopic criteria.[56] In addition to dietary exclusion of cow's milk,[46,48] oral steroids can induce remission of symptoms with decreased mucosal eosinophilia[48,53] suggesting a pathoetiologic role for eosinophils. In addition to eosinophils, intraepithelial T lymphocytes, known as cells with irregular nuclear contours (CINCc), have also been implicated as markers of reflux esophagitis.[57,58] In adults, such cells are of memory phenotype and display activation markers,[59] although little is known of their pediatric equivalents.

A variety of immunohistochemical markers have been used to examine the esophageal mucosa, including eotaxin, a recently described eosinophil-specific chemokine (Figure 25-3),[60] and markers of T-cell lineage and activation. Despite the mild histologic abnormality in CMP-associated esophagitis, an increased expression of eotaxin colocalized with activated T lymphocytes to the basal and papillary epithelium has been shown,[61] distinguishing this from primary reflux esophagitis. The molecular basis of the eotaxin up-regulation in cow's milk protein–sensitive enteropathy (CMPSE) is unknown. However, there is evidence from murine models of asthma that antigen-specific up-regulation of eotaxin expression can be induced by T cells and blocked by anti-CD3 monoclonal antibodies. This suggests the possibility of a distinct mechanism in CMPSE, in which mucosal homing to the esophagus occurs of lymphocytes activated within the small intestine. This may explain the seemingly counterintuitive finding of basal, as opposed to superficial, chemokine

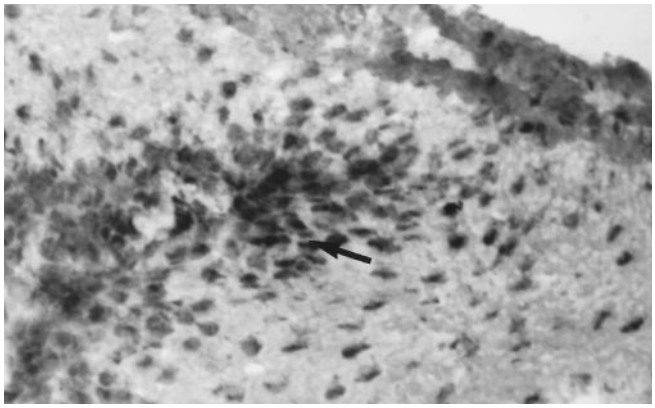


FIGURE 25-3 Eotaxin, a recently described eosinophil-specific chemokine (×200 original magnification) (darker staining area marked by an *arrow*).

expression and the common occurrence of mucosal eosinophilia in this condition. The esophageal motility disturbance of CMPSE-associated esophagitis is thus suggested to occur as a neurologic consequence of the inflammatory infiltration induced from lamina propria vessels into the epithelial compartment.[62] This proposed mechanism contrasts with the current concept of luminally induced inflammation found in primary reflux esophagitis and is consistent with the characteristic delayed onset and chronic nature of cow's milk–associated reflux esophagitis. The up-regulated expression of epithelial human leukocyte antigen (HLA)-DR suggests that the cytokine secretion profile of these cells includes interferon-γ, and thus a mixed T helper (Th)1-Th2 pattern is likely (IL-5 secretion is also likely in view of the frequent mucosal eosinophilia), and it may thus be relevant that small intestinal mucosal lymphocytes in infants with CMPSE show both Th2 skewing and low transforming growth factor (TGF)-β expression.[63] It seems, therefore, that there is a characteristic esophageal mucosal immunohistochemical profile in cow's milk–associated reflux esophagitis. Up-regulation of basal eotaxin expression and focal distribution of T-cell lineage and activation markers suggest a mechanism of mucosal homing of cow's milk–sensitized cells from the small intestine in the pathogenesis of CMPSE-associated esophageal reflux, in a manner distinct from luminally mediated primary reflux esophagitis. It has also been suggested that increased numbers of mucosal mast cells allow a distinction to be made between allergy-induced and reflux-induced esophagitis.[64] Much work is required in this area and is ongoing.

Infective

The majority of infective esophagitis that occurs is in the immunocompromised child and is due to such agents as herpes simplex, CMV, *Candida,* and others. Mucosal damage owing to physical or chemical causes may predispose the patient to opportunistic infection. Oral herpes or *Candida* may offer some clue to etiology, and the older child will often complain of odynophagia or dysphagia. Diagnosis may be made on endoscopy with biopsy, but brushings may offer a greater diagnostic yield.

Viral esophagitis is usually due to herpes simplex, CMV, and, occasionally, varicella zoster.[65–67] Herpes simplex esophagitis can occur in those with normal immune function[68] but is more often seen in those who are immunocompromised; in one series, 10% of post–liver or –kidney transplant recipients had herpes or CMV esophagitis,[69] and it is also commonly seen in pediatric human immunodeficiency virus (HIV) infection.[70] Use of prophylactic acyclovir is conjectural but may be of some benefit.

Diagnosis of herpes esophagitis is often difficult because the characteristic nuclear inclusions and multinucleate giant cells may not be seen in endoscopic biopsies; however, a prominent mononuclear cell infiltrate is described as characteristic (Figure 25-4).[71] It may be that the esophagus is particularly vulnerable in the GI tract owing to affinity of the herpes virus for stratified epithelium. Typically, roundish distinct disseminated lesions with yellowish borders are seen and have been termed "volcano ulcers" (Figure 25-5),[72] although early in the presentation, vesicles may be noted. Although the inflammation can resolve spontaneously in the immunocompetent, in those with poor immune function, acyclovir and a high index of suspicion are recommended.[72] Resistance to acyclovir has been described, in which case, foscarnet is the agent of choice.[73] CMV esophagitis is confirmed by basophilic nuclear inclusions on biopsy of the edge of the ulcers, which are similar in appearance to herpetic ones. CMV is predominantly found in immunocompromised individuals, and treatment is with ganciclovir or foscarnet.[67] Hemorrhage, fistulae, and esophageal perforation in adults with viral esophagitis are described.[74,75] Acute HIV infection can also cause esophagitis.[76]

Candida, the most common infectious cause of esophagitis, has the classic appearance of white plaques on the mucosa, which cannot be washed or brushed off, unlike food or milk residue, and which often extend up to the upper third of the esophagus (Figure 25-6).[77] Oral *Candida* is not predictive of esophageal involvement except in the immunocompromised host, but even in these children, extensive esophageal involvement is seen in the absence of oral candidiasis.[78] Mucositis and a white cell count less than 0.5×10^6/L predisposes patients with leukemia to can-

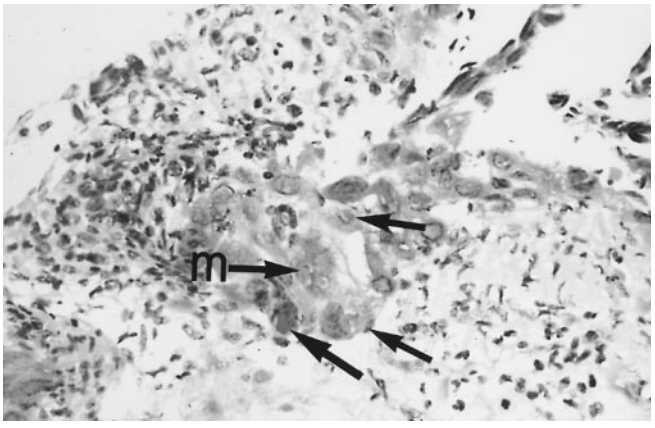


FIGURE 25-4 Herpes esophagitis with nuclear inclusions, multinucleate giant cells, and a prominent mononuclear cell infiltrate (m) (×200 original magnification).

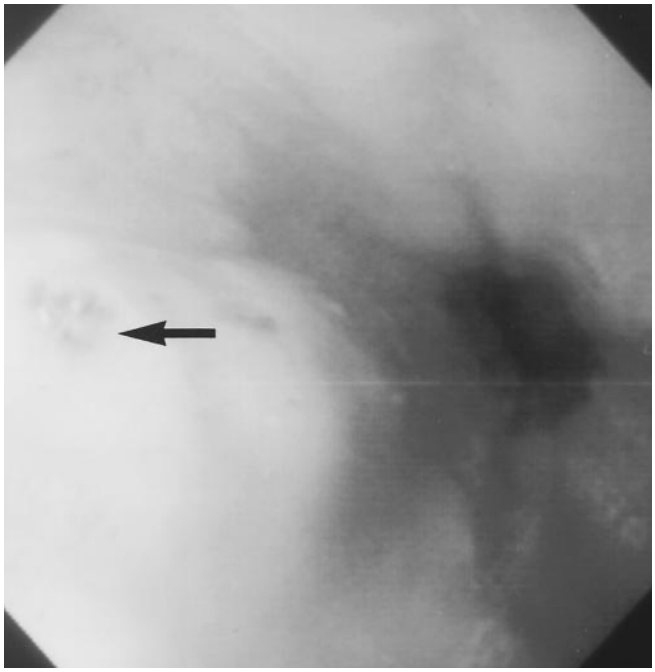


FIGURE 25-5 Macroscopic appearances of herpes esophagitis. Roundish distinct disseminated lesions with yellowish borders are seen and have been termed "volcano ulcers" (*arrow*).

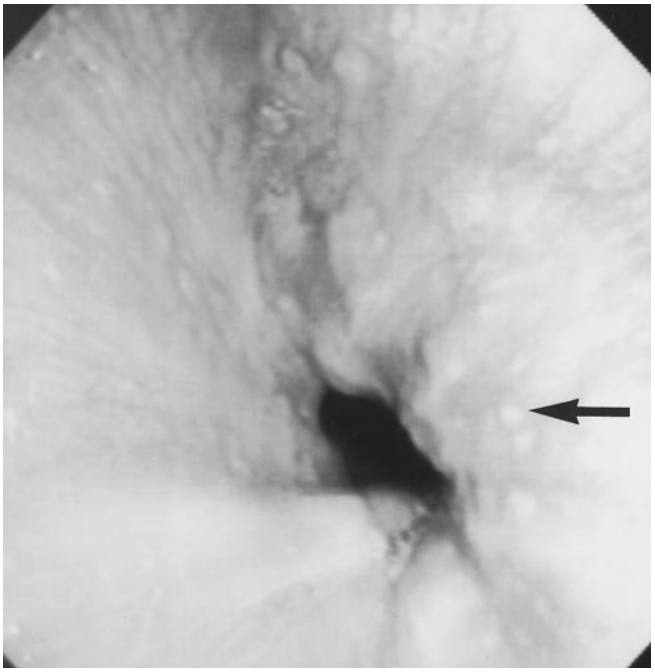


FIGURE 25-7 Candidal esophagitis may have the appearance of white focal lesions on the esophagus, which may be difficult to distinguish from allergic esophagitis.

didal esophagitis.[79] Steroid use (even poor technique with inhaled steroids for asthma) or acquired or congenital immunocompromise may be etiologic and may have the appearance of white focal lesions on the esophageal surface (Figure 25-7). This appearance may be difficult to distinguish from allergic esophagitis. Apart from the macroscopic appearances, diagnosis is confirmed by the presence of hyphae in biopsies (Figure 25-8). Culture is not helpful because coexistent oral *Candida* can confuse the assessment. Complications include fistulae, perforation, painless stricture formation, esophageal dysmotility, transient achalasia,[80] and systemic candidiasis. A 2- to 6-week course of oral nystatin can be effective in those with normal immune function, but it is more convenient to give fluconazole. Fluconazole or liposomal amphotericin is required, and both are effective in the immunocompromised child. Esophageal resection and diversion for necrotizing candidal esophagitis have been successful in a 10 year old.[81]

Eradication of *H. pylori* in adults has been associated with increased acid production and hence more noxious gastroesophageal refluxate. However, there does not seem to be any increased incidence of esophagitis in the presence of or following the eradication of *H. pylori* in children.[82] Because *H. pylori* affects gastric epithelium, it is not surprising that it has been identified on Barrett epithelium in a child, in whom symptoms resolved only with addition of amoxicillin to antireflux therapy.[83] Primary bacterial esophagitis is described in immunocompromised patients.[84]

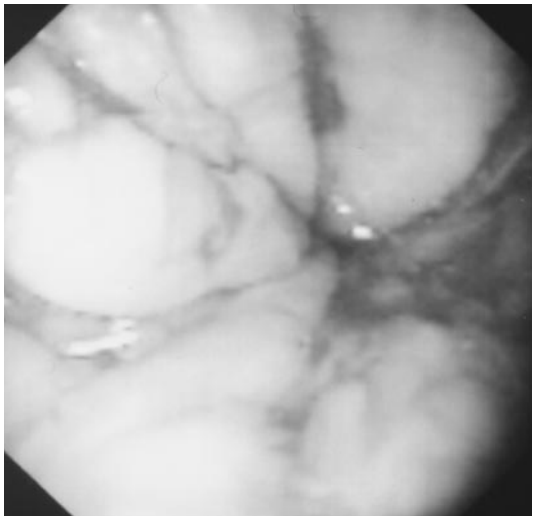


FIGURE 25-6 Candidal esophagitis has the classic appearance of white plaques on the mucosa that cannot be washed or brushed off.

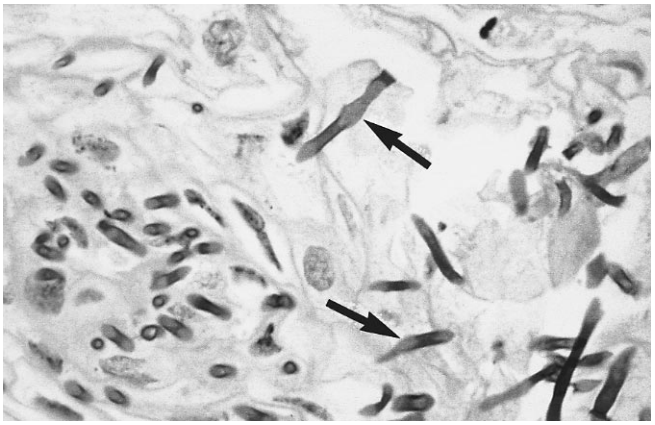


FIGURE 25-8 Candidal hyphae (*arrows*) (×200 original magnification).

Other opportunistic organisms causing esophagitis, such as *Cryptosporidium* and *Acremonium*, have been reported.[85,86]

Traumatic

Trauma causing esophageal pathology could, of course, be accidental, intentional, or iatrogenic. The presence of a nasogastric tube may be associated with abrasive esophagitis, and it has been postulated that the severe esophagitis found in newborn infants in one study, in the absence of other etiologic factors, may have been secondary to enthusiastic upper GI suction at birth.[7] Of particular note was the severity of the esophagitis in the face of relatively minimal symptomatology, such as feeding refusal. Radiation-induced esophageal strictures are described in children receiving mediastinal irradiation (usually greater than 4,000 cGy) and doxorubicin, occurring between 1 and 10 years post-therapy.[87] Radiation-associated esophagitis following bone marrow transplant conditioning is known to occur in the subsequent 1 to 2 weeks but is usually amenable to medical therapy.

Systemic Disease Manifestation

GER occurs more commonly in diverse conditions such as cystic fibrosis, severe combined immunodeficiency, cerebral palsy, raised intracranial pressure, celiac disease, and conditions associated with impaired gastric emptying.[88,89] Certain diseases are, however, associated with esophagitis, which is not via the pathogenetic pathway of reflux. Crohn disease is a prime example, and Crohn lesions in the esophagus are usually distinct rounded ulcers, although diffuse disease may also occur. (Figure 25-9). Endoscopic examination with biopsy of the upper GI tract should be part of the diagnostic workup of a child with suspected Crohn disease.[90] Relapse of the disease may be associated with recurrence of esophageal manifestations.[91] Type 1B glycogen storage disease may present with similar phenotype to Crohn disease, and severe esophageal involvement has been noted in children in this condition.[92] Inflammation and stricturing of the esophagus can occur in chronic granulomatous disease and can involve most of its length, making balloon dilation difficult.[93] Scleroderma and vasculitic conditions such as polyarteritis nodosa have significant esophageal pathology in adults but are very rare in pediatric populations.

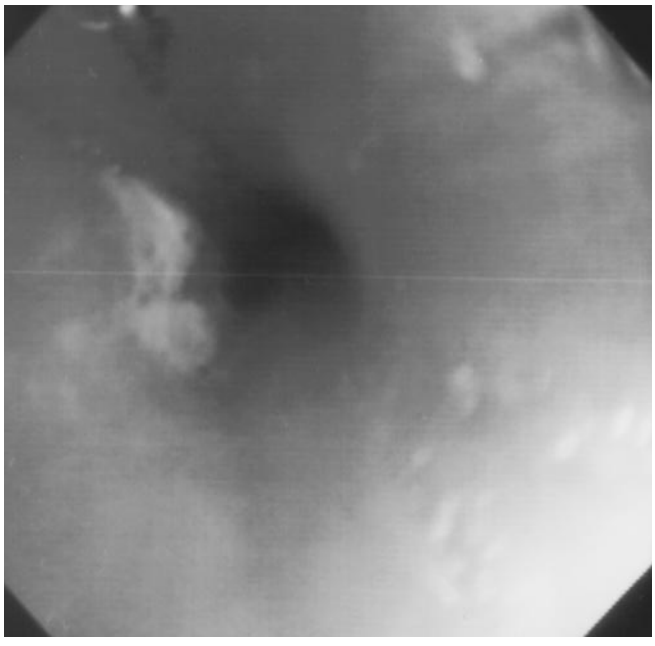


FIGURE 25-9 Distinct round ulcers of Crohn esophagitis.

Miscellaneous

Passive smoking has a strong association with esophagitis in childhood. The reasons behind this are not completely understood, but nicotine is known to relax the LES and may decrease mucosal blood flow. The nicotine levels in swallowed saliva may directly injure the esophagus or render it more susceptible to injury from acid exposure. Also, free radicals present in tobacco smoke may reduce antioxidant defenses.[94]

Munchausen syndrome by proxy (dealt with in more detail in Chapter 20, "Munchausen Syndrome by Proxy: Factitious Disorder by Proxy") can be at the root of esophagitis in children, but this is usually due to the deliberate introduction into the esophagus by the perpetrator of caustic or irritative substances.[95]

Idiopathic

IEE is characterized by a dense eosinophilic infiltrate in the absence of GER, parasitic infection, or other recognized causes of eosinophilic inflammation.[53,96] The importance of IL-5 and eotaxin, both potent eosinophilic chemokines in the mucosal recruitment of the eosinophilic infiltrate, has recently been recognized.[97] It is an unusual entity and may encompass a wide range of symptoms and histology. Dysphagia, odynophagia, and chest pain have been described.[89] It is important to diagnose IEE definitively because it may result in narrowing or stricturing of the proximal or midesophagus. Macroscopically, it appears as either concentric indentations for most of the length of the esophagus, with the suggestion of background mucosal edema and inferred inflammation, or longitudinal furrows (Figure 25-10).[54,98] Indeed, the suggestion of inflammation has been confirmed by one endosonographic study identifying an increase in the diameter of the submucosa and muscularis layers in children with this rare pathology (Figure 25-11).[54] In conjunction with this, it is becoming clear that a distinction can be made between IEE and simple allergic esophagitis on histology. IEE will have more than 20 to 40 eosinophils per HPF, whereas allergic esophagitis will have less than 10 to 15 eosinophils per HPF (Figure 25-12). Interestingly, in contradistinction to reflux esophagitis, the distal esophagus may be spared, and proximal esophageal biopsies are always recommended if IEE or allergic esophagitis is suspected. Allergy testing does not usually help to identify a responsible allergen because IEE, as the name suggests, is idiopathic, but this can sometimes be helpful in allergic esophagitis. Treatment with topical aerosol-delivered steroid (fluticasone propionate 50 to 200 µg sprayed into the back of the throat and then swallowed twice a day), systemic steroids, or even azathioprine may be effective,[53,96] but an elimination diet and dietary

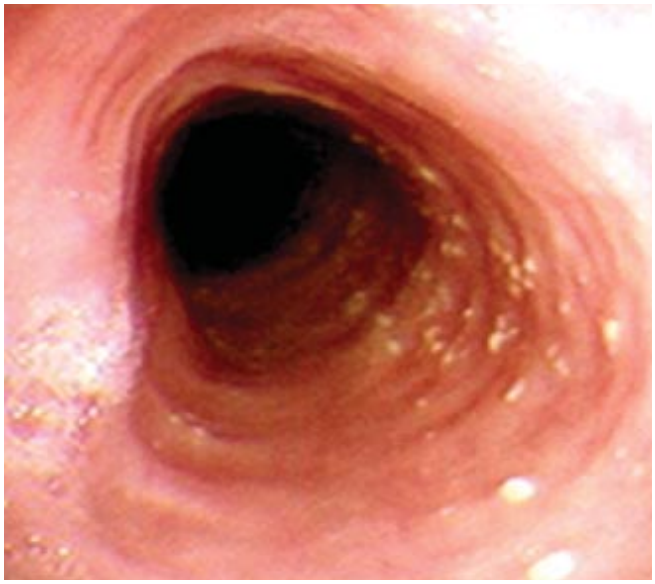


FIGURE 25-10 Macroscopic appearances of idiopathic eosinophilic esophagitis: concentric ring indentations and inference of edema and inflammation.

reintroduction, which are useful in allergic esophagitis, are not usually therapeutic in IEE.

SYMPTOMS

Comparatively little has been validated regarding the appearance, prevalence, or specificity of symptoms of esophagitis.

In the infant, parents and the clinician may have differing opinions on what constitutes excessive crying, irritability, and regurgitation, but parents have often learnt to distinguish what is normal and abnormal crying for their infant. It must be remembered, however, that crying varies with age and often with the time of day, peaking in frequency between 6 weeks and 3 months, with the majority occurring in the evening.[99] Excessive crying or irritability is likely to be associated with pathologic GER or esophagitis over, but not under, 3 months of age,[100] and feed refusal may occur if the infant learns to associate this with pain on swallowing.[101] Excessive crying and irritability causing maternal distress leading to child abuse have been reported in three cases.[102] Visceral hyperalgesia, in which prior painful GER leads to sensory nerve changes, which, in turn, lead to pain with subsequent innocuous stimuli, may be important and may explain in part the lack of a clear correlation of symptoms and esophageal pathology in some cases.[2,102–104] Paroxysmal head posturing, often with torticollis or neck extension, is known as Sandifer-Sutcliffe syndrome (Figure 25-13).[105] Refractory wheezing as an association of GER rather than esophagitis is well recognized and may be secondary to vagal stimulation at the distal esophagus.[106] GER airway–associated sequelae are noted in Table 25-1 and dealt with more fully in Chapter 24.

Anemia is uncommonly due to esophagitis in an infant, but hematemesis merits same-day endoscopy if feasible. Fecal occult blood has been noted in only 15% of infants and toddlers with esophagitis, although all patients with pH study evidence of GER had esophagitis when endoscopy was performed for hematemesis.[103] Even prenatal GI bleeding has been reported secondary to esophagitis.[5]

The term "colic" is defined as paroxysms of irritability, fussing, or crying lasting more than 3 hours per day for 3 days per week and has been estimated to occur in up to 25% of infants (see Chapter 13, "Colic and Gas"). It probably represents a variant of normal infant development. Pediatricians have a difficult decision to make to determine what is abnormal in this situation, but it would be wise to assume a low threshold of suspicion for reflux esophagitis if the irritability is excessive, according to their judgment or that of the parents,[107] bearing in mind that there is a significant overlap between maternal perceptions of "colic" and what is termed "normal infant distress."[108]

In 34 patients with an endoscopic diagnosis of esophagitis (median age of 6.5 months), Ryan and colleagues recognized the following symptoms: repeated regurgitation (100%), excessive crying and irritability (85%), significant sleep disturbance (79%), failure to thrive (41% below 10th percentile for weight/age), and hematemesis (29%). Maternal distress was a common finding in infants less than 6 months. These were compared with a group of 28 infants with no or minimal esophagitis in whom the respective percentages were 100%, 58%, 21%, 11%, and 3%. Hence, regurgitation was not predictive of esophagitis.[102]

Tables 25-1 and 25-2 outline the common symptom characteristics seen in infancy and in older children.

In the group of children between 1 and 5 years who are still not able to verbalize and describe their symptoms accurately, there may be a mixture of both constellations of symptoms, but they may remain nonspecific, with feeding disorders and food refusal, sleeping disorders, and more generalized behavioral problems predominating. In the younger age groups, it is important to realize that there is no clear relationship between symptoms and the severity

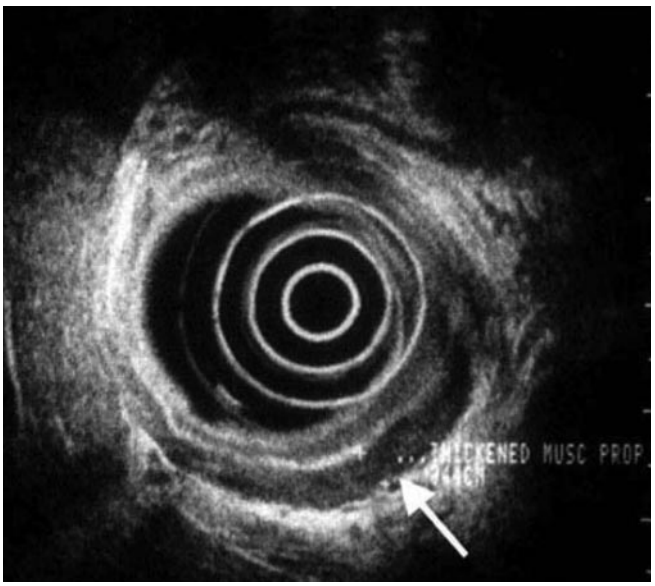


FIGURE 25-11 Endosonographic appearances of idiopathic eosinophilic esophagitis: increase in thickness of submucosa and muscularis layers (*arrow*).

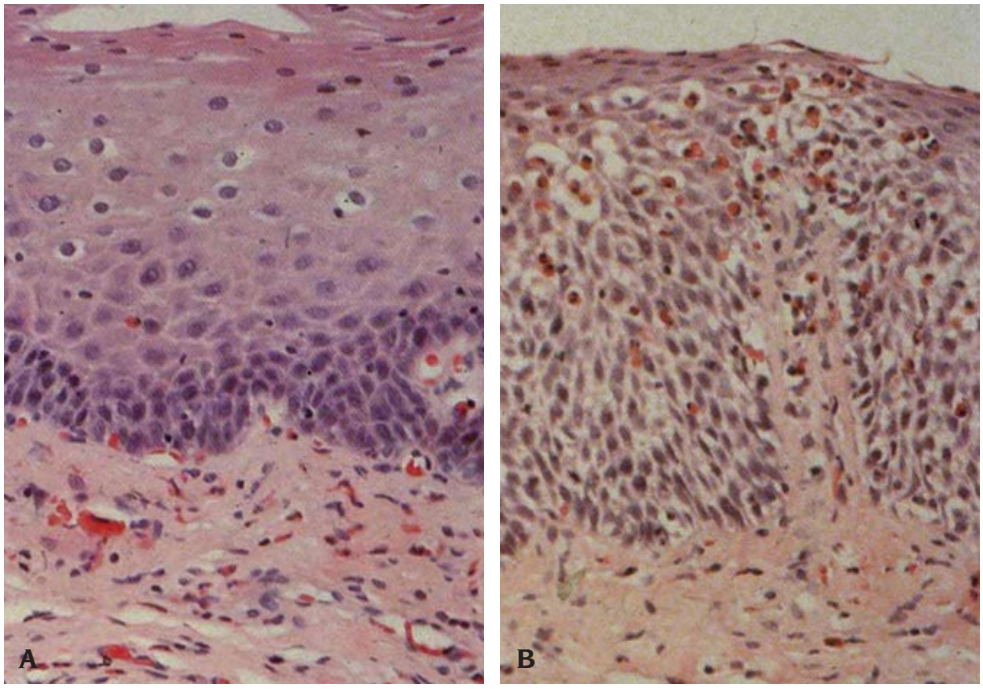


FIGURE 25-12 Density of eosinophilic infiltrate differs between *A*, allergic esophagitis (< 10–15 eosinophils/high-power field) and *B*, idiopathic eosinophilic esophagitis (> 20–40 eosinophils/high-power field).

of the esophagitis. The extent of such symptoms as irritability, crying, failure to thrive, or wheezing does not predict the severity of esophagitis.[1,102,103] It is also important to be aware that GER and associated esophagitis may be secondary phenomena to other pathology outside the GI tract, such as urinary tract infections, raised intracranial pressure, deliberate poisoning, and metabolic conditions.

Older children exhibit symptoms similar to adults and are less of a diagnostic conundrum. Conjecture exists regarding the role of esophagitis in recurrent abdominal pain, and in one series, 38% of such children had esophagitis on endoscopy.[109] However, although this may be true for recurrent epigastric pain, it is not generally thought to account for such a large proportion of classic periumbilical recurrent abdominal pain.

It is reasonable to include endoscopy and esophageal manometry in the diagnostic workup of children with chest pain, as Glassman and colleagues demonstrated esophagitis in 28%, and esophageal spasm and dysmotility in 25% of consecutive children complaining of chest pain.[110] Eleven of 16 children with asthma and chest pain had endoscopic and histologic evidence of esophagitis in a study by Berezin and colleagues, and all 16 had significant GER on pH study.[111] Rarely, hypertrophic osteoarthropathy has been reported with esophagitis in childhood.[112]

DIAGNOSIS

In this context, one is clearly concerned with determining a number of important issues, namely, the presence, severity, extent, etiology, and potential complications of esophagitis. Hence, investigations must be tailored to the question being asked. Indeed, in uncomplicated cases of GER, no investigation may be indicated, and simple therapeutic measures or even a trial of a first-line antireflux medication such as ranitidine may be a first-line diagnostic and therapeutic maneuver.[113,114] Ambulant esophageal pH analysis will give an indication of the nature and severity of acid or alkali reflux, whereas endoscopy with biopsy reveals the nature and severity of the esophagitis and other pathology in the upper GI tract, and investigations such as an upper GI barium series will tell us only about anatomic abnormalities and are clearly an inadequate method for looking at esophagitis.

FIGURE 25-13 Sandifer-Sutcliffe syndrome, which usually manifests as torticollis, in this case as neck hyperextension that resolved on adequate acid suppression.

TABLE 25-1 COMMON SYMPTOMS/ASSOCIATIONS WITH ESOPHAGITIS (USUALLY DUE TO REFLUX) IN INFANTS

GENERAL	SPECIFIC
Excessive crying	Hematemesis/melena/fecal occult blood
Irritability	Anemia
"Colic"	Sandifer syndrome (torticollis)
Feeding refusal	Aspiration
Failure to thrive	Wheezing
Excessive regurgitation	Apnea, stridor
Vomiting	Apparent life-threatening events
	Sudden infant death syndrome

Endoscopy with Biopsy

Endoscopy of the whole upper GI tract (esophagus, stomach, and duodenum) with multiple biopsies is the investigation of choice in evaluation of infants and children with symptoms suggestive of esophagitis.[1,2] This should be performed only by experienced and qualified pediatric endoscopists trained in endoscopy in infants and children. Technology now allows us to perform esophagogastroduodenoscopy in even the smallest infants.[115,116] It is, however, useful only if it will lead to alteration in diagnosis, treatment, or prognosis, and position papers for the North American and European Pediatric Gastroenterology Societies have recently been published.[2,110,113,117,118] Short general anesthetic is preferable to sedation for the procedure for reasons of safety, ease, and success of a complete and comprehensive study.[119]

Macroscopic appearances of the esophagus revealing, for instance, erythema, erosions, or ulceration will guide biopsy acquisition from the areas and lesions most likely to yield highest diagnostic return. A normal endoscopy or an absence of macroscopic lesions does not exclude the presence of histologic esophagitis,[120] and with our increased understanding of the variety of etiologies for esophagitis, biopsies have an enhanced role in altering management; the counterargument to this was previously advanced to defend endoscopy without esophageal biopsy in cases in which no macroscopic lesions existed,[1,3,4] and these authors also suggested that the increase in the cost of endoscopy, when combined with biopsy, may mitigate against the latter in some countries. This is generally held to be an outdated philosophy. No conjecture exists when performing biopsies for detection or surveillance of Barrett esophagus in which four-quadrant biopsies between 2 and 5 cm from the GEJ can be most helpful—the so-called Seattle protocol, using jumbo biopsy forceps.[121]

TABLE 25-2 COMMON SYMPTOMS/ASSOCIATIONS WITH ESOPHAGITIS IN OLDER CHILDREN

Epigastric pain, especially peri-/postprandial and nocturnal
Nausea/regurgitation/vomiting
Anorexia
Food refusal/specific feeding disturbances
Heartburn
"Dyspepsia"/chest pain
Odynophagia
Dysphagia
Early satiety
Hematemesis/melena
Anemia

TABLE 25-3 PROPOSED ENDOSCOPIC CLASSIFICATION OF ESOPHAGITIS

GRADE	FEATURES
0	Normal mucosa
1	Nonconfluent erosions appearing as red patches or striae just above the Z line*
	Erythema or loss of vascular pattern
2	Longitudinal noncircumferent erosions with a hemorrhagic tendency of the mucosa
2a	1 plus bleeding to light touch (friability)
2b	1 plus spontaneous bleeding
3	Circumferent tendency; no strictures
4a	Ulcerations with stricture or metaplasia
4b	Stricture without erosions or ulcerations

Adapted from Savary M and Miller G.[122]
*Z line defined as junction between columnar gastric fungal mucosa and stratified esophageal mucosa.

Classifications and scoring systems are employed in an attempt to semiquantify the appearances suggestive of esophagitis, which helps to remove interobserver error. The most widely used of these are the modified Savary-Miller criteria (Table 25-3, Figure 25-14).[122] The classification of Hetzel and colleagues has also been employed (Table 25-4); however, a criticism of this is that distinction between grades 0 and 1 is relatively subjective.[118] These classification systems have uses other than introducing objectivity, that is, the pretreatment grade of esophagitis is of value in predicting the pattern and severity of acid reflux and healing rates,[123] and improvement to grade 0 or 1 would be the usual aim, in either classification, of treatment. The specific macroscopic appearances of conditions other than GER esophagitis are noted in the relevant sections on pathogenesis above. Hassal suggests that erosions usually found on the tops of esophageal folds are specific for reflux disease, often with a rim of erythema around the white erosions[3]; however, these may mimic, for instance, Crohn disease (see Figure 25-9). Gupta and colleagues suggest that vertical lines in the distal esophageal mucosa are a true endoscopic manifestation of reflux esophagitis in children (Figure 25-15).[124] In severe ulcerated esophagitis, objective proof of recovery following treatment is important, and repeat endoscopy between 3 and 12 weeks later is generally recommended.

Generally, it is held that although the majority of esophagitis is due to reflux, the esophageal appearances themselves do not reliably differentiate between reflux and other causative pathologies. This is perfectly demonstrated in the diagnosis and management of esophagitis in children with cancer, in whom esophagitis is a common occurrence but whose etiology is not predicted accurately by clinical observations (eg, oral candidiasis does not predict for candidal esophagitis) or by macroscopic endoscopic appearances[125]—hence, the requirement for confirmatory biopsy and histology.

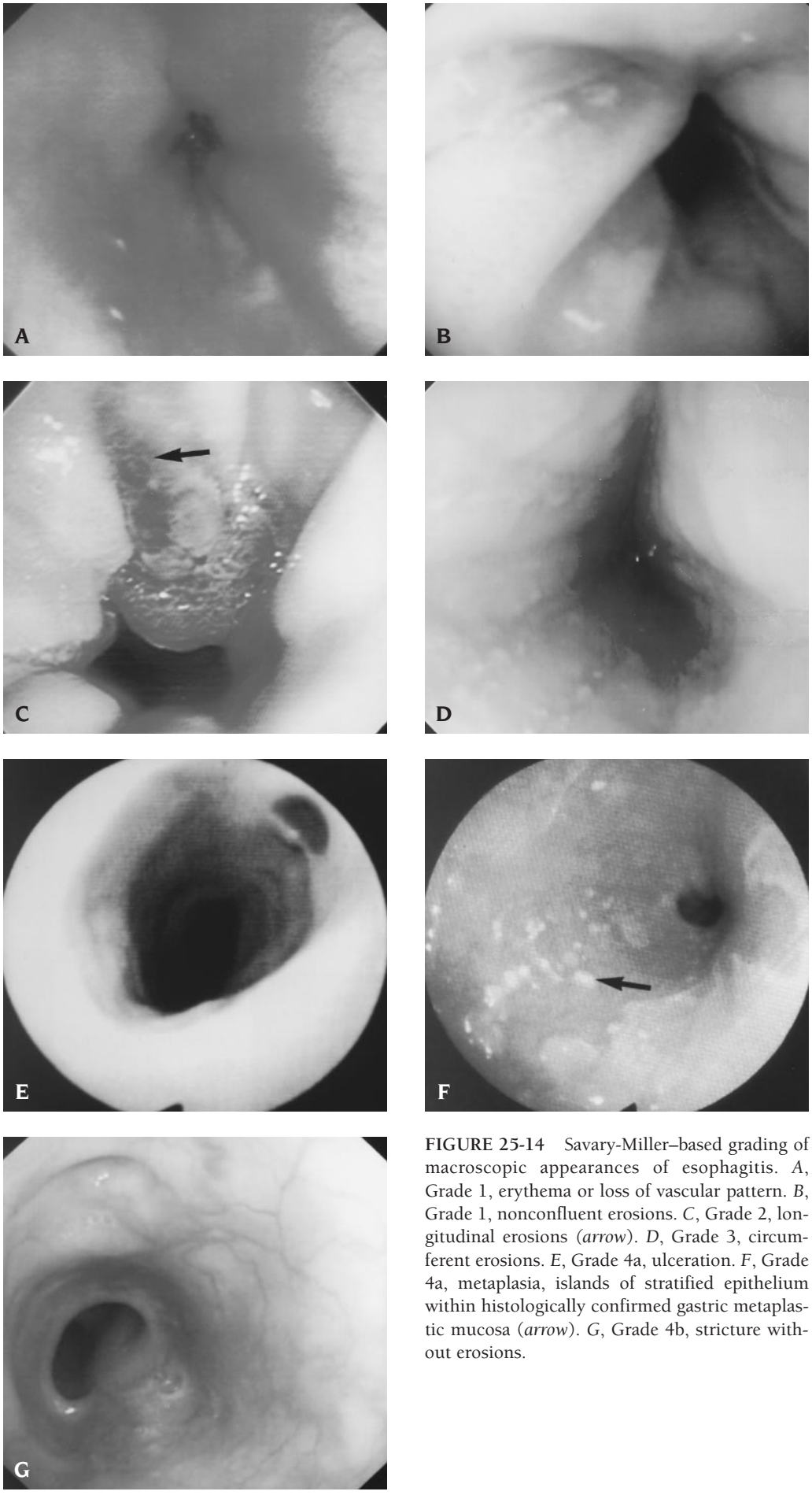


FIGURE 25-14 Savary-Miller–based grading of macroscopic appearances of esophagitis. *A*, Grade 1, erythema or loss of vascular pattern. *B*, Grade 1, nonconfluent erosions. *C*, Grade 2, longitudinal erosions (*arrow*). *D*, Grade 3, circumferent erosions. *E*, Grade 4a, ulceration. *F*, Grade 4a, metaplasia, islands of stratified epithelium within histologically confirmed gastric metaplastic mucosa (*arrow*). *G*, Grade 4b, stricture without erosions.

TABLE 25-4 ENDOSCOPIC CLASSIFICATION OF ESOPHAGITIS

GRADE	FEATURES
0	No mucosal abnormalities
1	Erythema, hyperemia, mucosal friability
2	Superficial erosions affecting < 10% of the distal 5 cm of esophageal squamous mucosa
3	Superficial erosions or ulceration of 10–50% of the mucosal surface of the distal 5 cm of esophageal squamous mucosa
4	Deep peptic ulceration anywhere in the esophagus or confluent erosion of > 50% of the mucosal surface of the distal 5 cm of the esophageal squamous mucosa

Adapted from Hetzel D et al.[118]

HISTOLOGY

A diagrammatic representation of an esophageal cross-section is shown in Figure 25-16. Nowadays, biopsies are endoscopic, but suction biopsies have been assayed in the past and probably yield a deeper, more satisfactory biopsy.[126] When suction biopsies were added to conventional grasp biopsy technique in a study by Hyams and colleagues, the histologic diagnosis of esophagitis was increased from 60 to 83% of cases, although if one takes more biopsies, one would expect a greater diagnostic yield given the patchy nature of childhood esophagitis; hence, this cannot be used to suggest that suction biopsies are superior in pediatric practice.[103] Friesen and colleagues showed no statistically significant difference in predictive value for esophagitis in infants between the two techniques.[127] Correctly oriented endoscopic biopsies (eg, immediate orientation on filter paper or nylon mesh in 10% formalin) are, however, perfectly adequate, and so-called "crocodile" biopsy forceps, which allow the operator to biopsy perpendicular to the esophageal lumen, may be preferable. Large-cup ("jumbo") biopsy forceps are increasingly used and are mandatory for the surveillance of Barrett esophagus because they yield deeper biopsies.[120] The site of biopsy should be above the distal 15% of the esophagus to avoid confusion with normal variance.[103] Biopsies should include epithelium, lamina propria, and muscularis mucosae and be oriented in a perpendicular plane to maximize diagnostic yield, such as evaluating properly the thickness of the basal zone, vascular ingrowth, and the elongation of the stromal papillae. For definitive diagnosis, the presence of two of three of these features is preferable, which will not be possible with poorly oriented tissue.[1,2] In an adult study, failure to use well-defined histologic criteria resulted in only 50% sensitivity for diagnosing esophagitis.[128] The classic histologic findings of GER esophagitis are displayed in Table 25-5

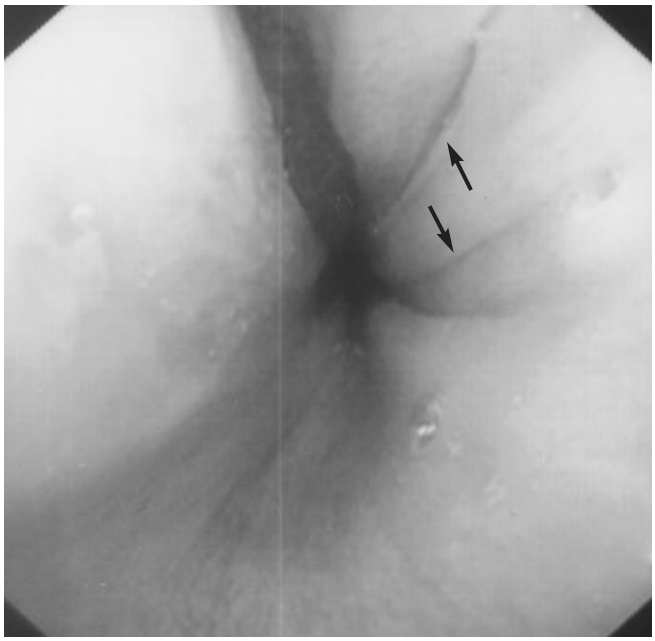
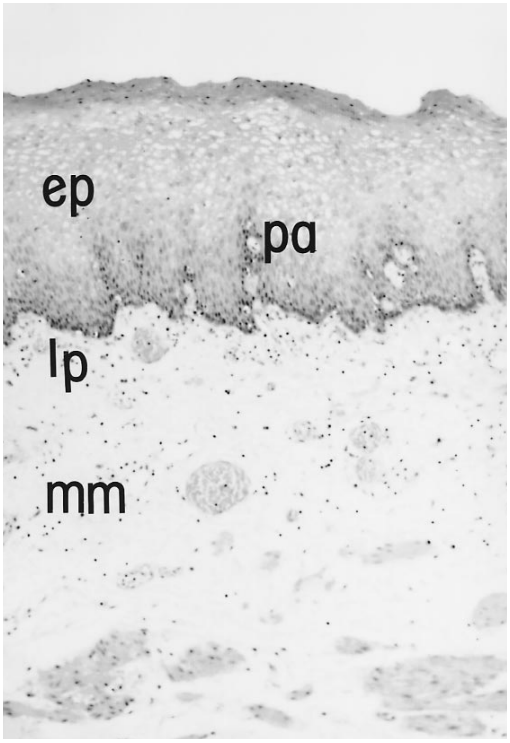


FIGURE 25-15 Vertical lines in the distal esophageal mucosa may be an endoscopic manifestation of reflux esophagitis (*arrows*).



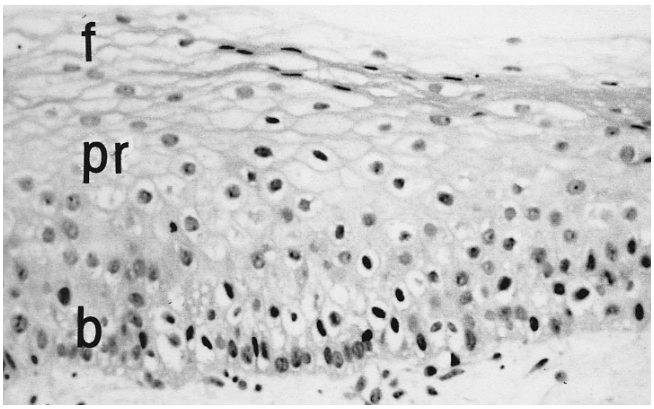


FIGURE 25-16 Diagramatic representation of an esophageal epithelial cross-section. Epithelium (ep) comprises functional (f), prickle (pr), and basal (b) layers. Papillae (pa) are contiguous with lamina propria (lp). Muscularis mucosa (mm) is deep to this but superficial to circular and longitudinal muscle layers and serosa, not shown (hematoxylin and eosin).

(Figure 25-17). Elongation of stromal papillae is a useful indicator of reflux, and basal zone hyperplasia is defined when the papillae are more than 25% of the entire thickness of the epithelium, and if more than 50%, then the papillae are considered to be elongated.[58]

Esophageal mucosal eosinophilia has been described in both suspected cow's milk–associated[46] and primary reflux esophagitis,[52] as well as in other conditions, such as primary eosinophilic esophagitis (see Figures 25-2 and 25-12).[53] The clinical significance of eosinophils and their role in the pathogenesis of mucosal injury are poorly understood and are the subject of recent debate.[3,4,55] Some have suggested an active role for eosinophils in the inflammatory process of esophagitis and have supported this with the observation of resolution of symptoms and eosinophils in the esophagus on dietary exclusion of cow's milk[46,48] or with oral steroids,[46,53] both suggesting a pathoetiologic role for eosinophils. The mucosal density of the eosinophils may be important, as noted above, in distinguishing between allergic esophagitis and IEE. In addition to eosinophils, intraepithelial T lymphocytes, known as CINC or squiggle cells, have also been implicated as markers of reflux esophagitis (Figure 25-18).[57,59] However, the degree of intraluminal esophageal acid exposure did not correlate well with the CINC count in one study in children, and the authors use this fact to question the day-to-day reliability of pH-metry in defining the extent of reflux in children.[58] In adults, such cells are of memory phenotype and display activation markers,[59] although little is known of their pediatric equivalents. The finding of mucosal mast cells may also help to differentiate GER from CMP-associated esophagitis, but there is considerable overlap with the presence of eosinophils.[56] Neutrophils also indicate a degree of inflammation,[12] and actual numbers of eosinophils and/or neutrophils per most involved HPF have been used to indicate the severity of esophagitis.[58] Minimal histologic criteria are simultaneous occurrence of elongated papillae and basal zone hyperplasia. Moderate esophagitis is diagnosed if there is ingrowth of vessels in the papillae, and 1 to 19 eosinophils and/or neutrophils are seen in the most involved HPF. Severe esophagitis is diagnosed if more than 20 eosinophils/neutrophils are seen in the most involved HPF. However, the criteria established by the European Society of Paediatric Gastroenterology Hepatology and Nutrition and displayed in Table 25-5 are probably the most robust to date.[2]

TABLE 23-5 GRADING CRITERIA FOR HISTOLOGIC APPEARANCE OF ESOPHAGUS

GRADE	HISTOLOGIC CRITERIA	CLINICAL DIAGNOSIS
0	Normal	Normal
1a	Basal zone hyperplasia	Reflux
1b	Elongated stromal papillae	Reflux
1c	Vascular ingrowth	Reflux
2	Polymorphs in the epithelium ± lamina propria	Esophagitis
3	Polymorphs with epithelial defect	Esophagitis
4	Ulceration	Esophagitis
5	Aberrant columnar epithelium	Esophagitis

Adapted from Knuff T et al[197] and Leape L.[198]

The important point to realize is that correlation between macroscopic and histologic features is generally poor, partly because the esophagitis may be a patchy lesion but also because histologic esophagitis may exist when the esophagus is macroscopically normal.[119] This is not now merely academic because it does have the potential to direct therapy appropriately, for example, in the case of CMP allergy– or intolerance–associated esophagitis when a cow's milk exclusion diet is associated with a better outcome than use of antacid therapy alone, and it is suggested that up to 40% of cases of esophagitis may have CMP intolerance as an etiologic factor.[46,48,61]

Furthermore, with the advent of more complex diagnostic techniques such as immunohistochemistry and electron microscopy, the esophagus, which is apparently normal both macroscopically and histologically, may still yield diagnostic information. Standard endoscopic biopsy and histology do not reliably distinguish between, for instance, primary reflux esophagitis and the emerging clinical entity of cow's milk–associated reflux esophagitis. Some differentiation from primary reflux has been suggested on the basis of esophageal pH testing pattern and β-lactoglobulin antibody response, although the former has not been substantiated by more than one center.[48,49]

Barrett esophagus and premalignant or malignant esophageal pathology are dealt with in Chapter 24. Cytologic esophageal brushings may be helpful in such situations, as they are in candidal esophagitis.[129]

Immunohistochemistry

A variety of immunohistochemical markers have been used to examine the esophageal mucosa. An increase in Ki-67, a proliferation marker, has been shown in the longer papillae seen in GER, suggesting increased cell turnover (Figure 25-19). Basal focal distribution of CD4 lymphocytes showing expression of the activation markers CD25 and HLA-DR, together with up-regulated epithelial HLA-DR expression, has also been reported.[61] Eotaxin is a recently described eosinophil-specific chemokine,[60] and, despite the mild histologic abnormality in CMP-associated esophagitis, an increased expression of eotaxin colocalized with activated T lymphocytes to the basal and papillary epithelium has been shown, distinguishing this from primary reflux esophagitis (see Figure 25-3).[61] Inhibitory neurotransmitter production is integral to LES relaxation, and the nonadrenergic, noncholinergic neurotransmitter NO has received recent attention in human studies.[16,17] Increased esophageal expression of iNOS has also been noted,[23,25] although in another study, it was not up-regulated in the inflamed pediatric esophagus.[24] Because NO is a powerful smooth muscle relaxant, it is interesting to speculate whether inflammation-induced iNOS may play a role in LES relaxation, leading to more reflux and hence worse inflammation, and so on.

Hence, techniques such as immunohistochemistry will allow better comprehension of the pathophysiology of esophageal pathology in the near future and already allow a diagnostic distinction to be drawn between etiologies.

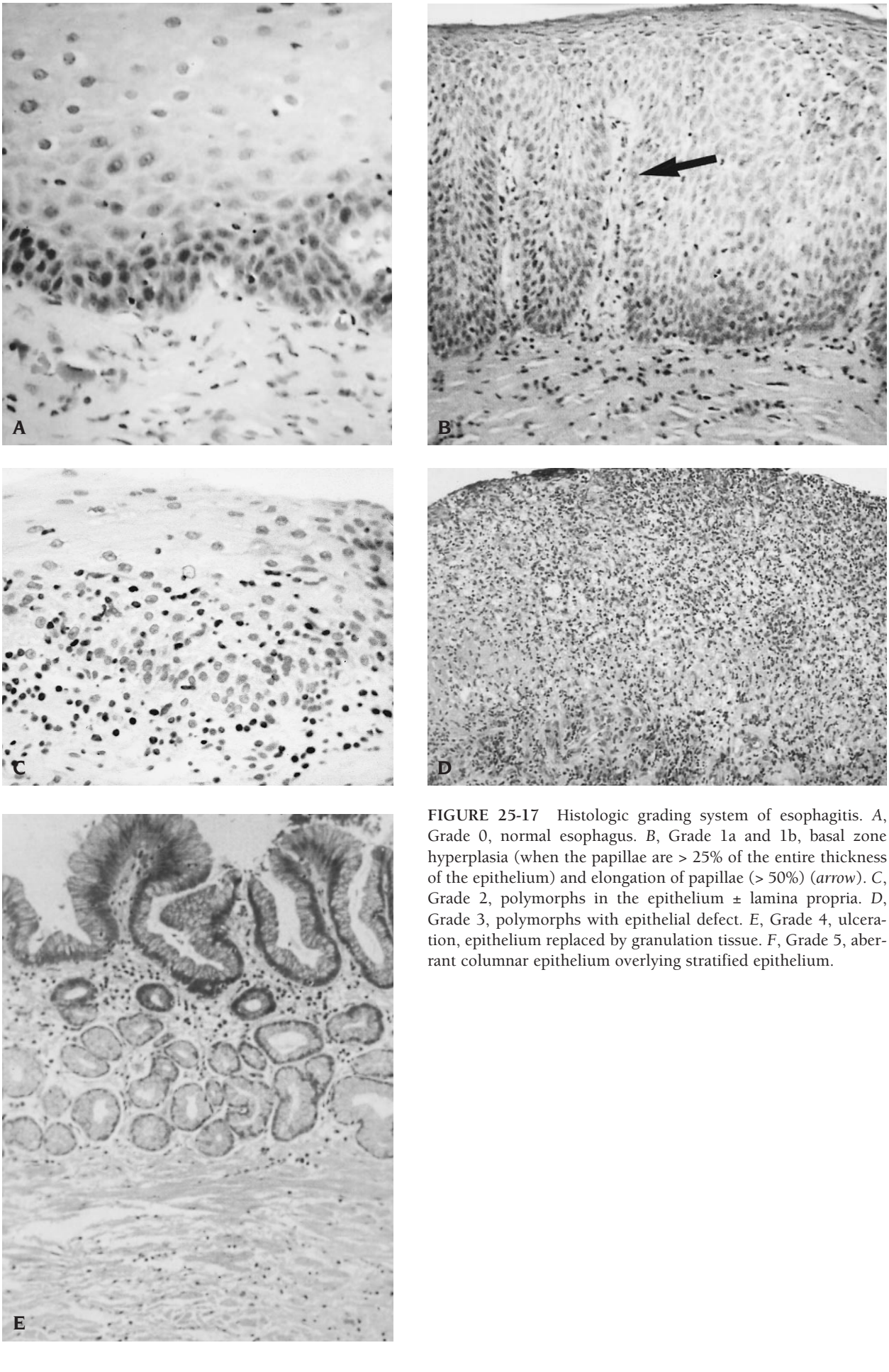


FIGURE 25-17 Histologic grading system of esophagitis. *A*, Grade 0, normal esophagus. *B*, Grade 1a and 1b, basal zone hyperplasia (when the papillae are > 25% of the entire thickness of the epithelium) and elongation of papillae (> 50%) (*arrow*). *C*, Grade 2, polymorphs in the epithelium ± lamina propria. *D*, Grade 3, polymorphs with epithelial defect. *E*, Grade 4, ulceration, epithelium replaced by granulation tissue. *F*, Grade 5, aberrant columnar epithelium overlying stratified epithelium.

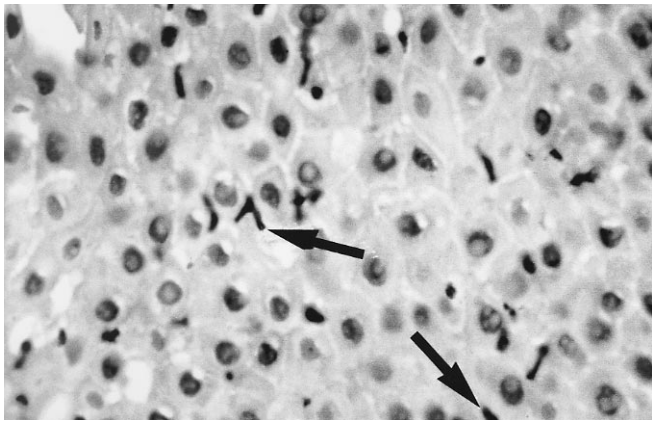


FIGURE 25-18 Intraepithelial T lymphocytes, known as cells with irregular nuclear contours or squiggle cells (*arrows*).

Electron Microscopy

Electron microscopy has demonstrated the ultrastructural changes associated with esophagitis, adding to our comprehension of the lesion. Stratified squamous nonkeratinizing epithelium line the mucosa, and the surface is composed of large flat cells displaying a regular pattern of parallel microridges 200 nm in thickness. Three layers are visible by transmission electron microscopy: (1) the basal layer, composed of polygonal cells with a high nucleus-to-cytoplasm ratio; (2) the intermediate layer, composed of large prickle cells; and (3) the superficial layer, composed of flattened cells. Three grades of ultrastructural changes in esophagitis in children have been identified: grade I, irregular microridges and reduced intercellular junctions; grade II, of the superficial epithelium only, microvilli instead of microridges that, when present, are distorted, and extruding cells with degeneration and interruptions of the cell membrane; lymphocytes and monocytes occupy the large intercellular spaces in the intermediate layer, and the basal layer is thickened; and grade III, microerosive cytopathy, loss of superficial layer microridges with crater-like erosions and abundant cell debris. Degenerating cells are seen in all three layers (Figure 25-20). Reduced numbers of desmosomes and large intercellular spaces containing lymphomonocytes are seen. Ultrastructural damage to nuclei, nucleoli, Golgi complex, and endoplasmic reticulum is seen. Activation of eosinophils by electron microscopic criteria has helped in the distinction of GER and CMP-associated esophagitis.[56] Hence, a more compelling case can be made for biopsy than previously.

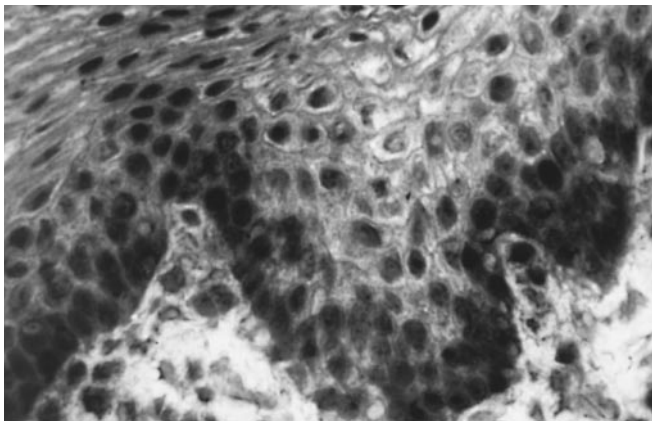


FIGURE 25-19 Increase in Ki-67, a proliferation marker, has been shown in the longer papillae seen in gastroesophageal reflux, suggesting increased cell turnover.

pH Studies

Esophageal pH monitoring has gained general acceptance as the method for assessment of GER in children and until recently has been regarded as the investigation technique of first choice in infants and children with unusual presentations of GER disease, such as apnea and recurrent respiratory disease.[130–132] However, pH measurements cannot detect GER in the pH range of 4.0 to 7.0 owing to the proximity to the normal esophageal pH.[133,134] Consequently, pH-metry misses many episodes of postprandial reflux in young infants because of neutralization of gastric contents by milk formula for 1 to 2 hours after a meal. Therefore, the term acid, neutral, or alkaline GER should be preferred over the blanket term GER. A poor correlation exists between morphometry and histology and classic parameters used in pH studies[3,135]; however, this correlation is improved by analysis of esophageal acid exposure using the parameter area under pH 4.0.[136] Indeed, pH-metry does not detect GER directly but measures the H^+ ion concentration at the sensor site.[137]

Hyams and colleagues found no correlation between any pH parameter (except the acid reflux in the 2 to 4 hours following a clear liquid feed) and the severity of esophagitis or macroscopic appearances suggesting esophagitis and histologic changes, and this has been seen by other groups.[103,119] Ambulant esophageal pH-metry is dealt with in greater detail in Chapter 24.

Currently available techniques for the study of reflux that are pH-independent include ultrasonography,[138] aspiration,[139] scintigraphy,[140] fluoroscopy,[141] bilirubin monitoring,[142] and pH monitoring. However, the disadvantages of these methods include short-term applicability, a high incidence of artefact caused by body movement, and the requirement for unphysiologic, nonambulant body positioning. These methods fall far short of the ideal because they measure only a short time window and do not allow for symptom or event temporal correlation.

Intraluminal Impedance

A new pH-dependent, intraluminal esophageal impedance technique, which relies on the higher conductivity of a liquid bolus compared with esophageal muscular wall or air, has been validated in adults. When used in infants with GER who had simultaneous pH measurement for prolonged periods, intraluminal esophageal impedance showed that 73% of all GER occurs during or in the first 2 hours after feeding. Furthermore, this is pH neutral and, therefore, will be missed by pH-metry. Indeed, 75% of GER extends proximally as far as the pharyngeal space,[143] and this has broad implications for the study of GER-associated respiratory phenomena and symptoms caused by gastrolaryngopharyngeal reflux. Wenzl and colleagues

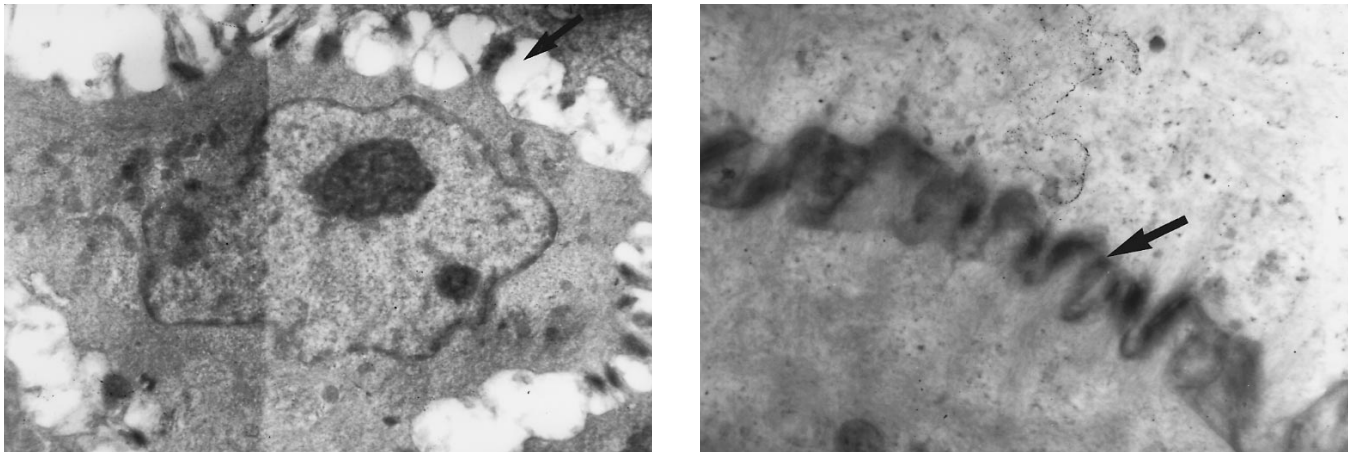


FIGURE 25-20 Transmission electromicrograph of ultrastructural changes in esophagitis in children. Grade I, irregular desmosomes and reduced intercellular junctions (*arrow*).

re-examined the temporal association between infant apnea and reflux in a recent study and found, on the basis of impedance, a marked association between these two phenomena.[143] Approximately one-third of all documented apneas occurred in the 30 seconds before or after an impedance-identified reflux event, of which only 23% were acidic reflux events—hence, the hypothesis that GER may stimulate laryngeal receptors. Conversely, they suggest that forced respiratory effort with increased abdominothoracic pressure, as occurs during episodes of obstructive apnea, can overcome LES pressure and cause a reflux episode.

Multichannel intraluminal impedance measurements have allowed new insight into the physiology and pathophysiology of gastrointestinal function in health and disease.[144] Patterns of antegrade and retrograde bolus movement, length of the swallowed or refluxed bolus, and direction and velocity of bolus movement can be described precisely.[145] Episodes of GER can be characterized by their height in the esophagus and by their duration.[146] This is especially useful in the postprandial period and in clinical situations of gastric hypoacidity.[147] Simultaneous pH monitoring and intraluminal impedance (IMP) allow further categorization of GER episodes.[148] Multichannel IMP measurement is also a valuable tool for describing the process of GER clearance and swallowing, allowing a distinction to be drawn between the protective events of volume clearance and acid clearance (primarily by swallowing of saliva) in the prevention of GER-associated lower esophageal reflux pathology and associated symptoms. Although normative values for various pediatric age groups remain to be defined, this technique already allows time-related associations to be made for GER and symptoms and allows interventional therapeutic studies to be conducted for the first time on a physiologically appropriate basis. With increasing clinical use of the IMP in children, normative data will soon be available. Until then, IMP can be performed in studies with a crossover design so that individual subjects may act as their own controls.

To document symptom association,[149] IMP can be incorporated into other diagnostic systems, for example, manometry[150–152] and sleep studies. Technical effort has now developed a portable recording device for mobile, outpatient impedance studies in all age groups.[153] Semiautomated and soon fully automated software will allow this to emerge from application on a research basis to have clinical day-to-day applicability, and software for IMP has now been developed and improved to aid in detecting characteristic patterns and eliminating artefact, thereby making it more practical for routine clinical use.[154] The importance of independent, long-term assessment of the esophageal and gastric motility is increasingly recognized, especially for pediatric patients. Impedance monitoring is indeed very promising in revolutionizing this aspect of GER investigation. In the next few years, a combination of pH and IMP will be adopted as the new gold standard for investigating reflux events in pediatric settings. Future studies will verify and improve the technique and will broaden our understanding of esophageal motility and its disorders and associated supraesophageal phenomena.

Other techniques, such as IMP, may offer advantages over pH-metry for assessing nonacid or neutral reflux, which is the predominant type in the postprandial 1 to 2 hours when most GER occurs, and a variety of studies now point clearly to the inadequacy of assessment of simple acid exposure of the esophagus in determining the role of reflux in the genesis of GER.[155] More recently, Tasker and colleagues suggested that reflux of gastric juice could be a major cause of glue ear in children by analyzing middle ear effusion fluid at the time of grommet insertion for the presence of pepsin (which can only have come from the stomach by reflux) and found it to be present in 83% of cases. This elegant study further supports the association of GER with tubotympanal disorders and also suggests that nonacid reflux may be just as important as acid reflux.[156]

Manometry

This is useful in limited circumstances in the evaluation of esophagitis per se, although it has a role in the assessment of GER etiology. Berezin and colleagues studied 31 children with mild to moderate esophagitis and concluded that there were no differences compared with 48 normal controls in LES pressure and the amplitude, duration, and velocity of

esophageal contractions.[157] Combined pH/impedance/micromanometry catheters are now available and expose the possibility of a more profound comprehension of all of the issues that contribute to the complex process of a reflux event in an infant or child.

Upper GI Barium Series

Barium studies of the upper GI tract are not helpful in assessment of esophagitis except in detecting the presence or absence of anatomic abnormalities, for example, esophageal strictures, gastric outlet obstruction, and small bowel malrotation, for which they are indispensable. Some authors report specific radiologic abnormalities associated with pathologies such as candidal esophagitis,[125] but the technique of choice is obviously endoscopy in such situations.

Blood Tests

Obviously, hemoglobin estimation will allow anemia to be excluded as a complication of esophagitis. Specific etiologies can be elucidated and distinguished by specific blood tests. Examples include a high anti-β-lactoglobulin in CMP-associated esophagitis; radioallergosorbent tests for specific allergens; low-quartile immunoglobulin (Ig)A, high IgE, high IgG, and specifically high IgG1 and IgG4 subclasses, all suggestive of CMP-associated GER; herpes or CMV serology; and raised inflammatory markers such as C-reactive protein, erythrocyte sedimentation rate, and platelet count suggestive of more widespread GI inflammation such as occurs in Crohn disease.

MANAGEMENT AND PROGNOSIS

Management of esophagitis must, of course, be dictated by its etiology, which further underlines the vital nature of obtaining an accurate diagnosis based on upper endoscopy and histologic assessment.

Because the vast majority of cases of esophagitis in infants and children will be due to GER, then treatment of GER and treatment of GER-related esophagitis will be very closely linked. Treatment of GER is also dealt with in Chapter 24, and the emphasis of this section is toward the rationale for treatment of esophagitis but inevitably touches on anti-GER measures also. Other specific treatments for specific pathologies are also dealt with.

It must be borne in mind that spontaneous resolution of reflux esophagitis may occur. The early studies of Carre indicated in infants that if no active therapy is initiated, approximately 60% will be symptom free by 18 months of age, with the greatest improvement by 8 to 10 months, when the child starts to sit upright; 30% will continue to have symptoms during childhood; approximately 5% develop strictures; and 5% will die of pneumonia or malnutrition.[9] In another, more up-to-date study on the outcome of infant GER esophagitis with accurate recognition and treatment (at that stage, only a histamine$_2$ [H_2] antagonist), 82% had responded satisfactorily to medical management by 18 months of age, with 51% being able to cease treatment with spontaneous improvement by 8 to 10 months, a proportion similar to Carre's.[10,102] With the advent of effective antireflux therapies, one would expect the figure of 18% who required antireflux surgery in the latter study to be much less. Shepherd and colleagues advocate the early use of endoscopy to detect the degree of esophagitis because the constellation and severity of symptoms do not always reflect the degree of esophagitis.[10] It is important to know the degree of esophagitis (see Tables 25-3 and 25-4) because this may allow one to tailor therapy appropriately and, indeed, prognosticate on outcome. One group suggested that more than 7 eosinophils per HPF made the success of treatment with ranitidine and cisapride unlikely, although the confounding factor of allergic esophagitis certainly has importance in this situation.[125] Individualized treatment is the goal, but generalizations may be made based on the severity of the esophagitis and, to an extent, the severity of the symptom constellation. In adult studies, the pretreatment severity of esophagitis is of some help in predicting healing rates on antisecretory therapy.[158,159] It also correlates with the duration and pattern of acid reflux in adults,[160] although a poor correlation exists between morphometry/histology/endoscopic appearances and classic parameters used in pH studies in pediatrics[3,103,120,135]—except, perhaps, area under pH 4.0.[136] Hence, the presence of histologic esophagitis alone may not allow prediction of outcome. It is clear also in adult studies that erosive esophagitis is a chronic problem that has an attendant worse prognosis and will tend to relapse off treatment.[161] This is probably the case in pediatrics also, but this question is very difficult to answer because long-term follow-up studies with treated and untreated patients would be required to provide viable answers.[2]

Figure 25-21 outlines an algorithm for the treatment of GER esophagitis in infants and children. It is generally agreed that for treatment purposes, infants and children with GER esophagitis can be regarded as falling into two main groups: those with normal (grade 0) or mildly erythematous (grade 1) mucosa and only histologic esophagitis and those with esophagitis, which is erosive or worse (grade 2 or more) (see Figure 25-14). The milder group will generally receive so-called simple measures, which are particularly applicable in infancy, such as positioning with the head elevated to 30° in the left lateral Trendelenburg position, which may be effective in up to 25% of infants with simple regurgitation[2]; advice to increase the frequency and decrease the volume of each feed; use of milk-thickening agents (eg, Nestargel, Carobel) or prethickened milks (eg, Enfamil AR); and the use of antacids (eg, Infant Gaviscon), which may be effective in mild, simple GER.[162,163]

For uncomplicated reflux unresponsive to these measures, a case can be made for the use of an H_2 antagonist before further investigation. Unfortunately, cisapride, the noncholinergic prokinetic drug with 5-hydroxytryptamine$_4$ agonist properties that improves pH-metric variables[164] and was the drug of first choice in GER, is no longer widely available owing to concerns, rightly or wrongly, regarding prolongation of the Q–T_c interval and cardiac dysrhythmias in childhood. It is metabolized by the cytochrome P-450 3A4 isoenzyme, as are "azole" antifungal agents and the macrolide antibiotics erythromycin and clar-

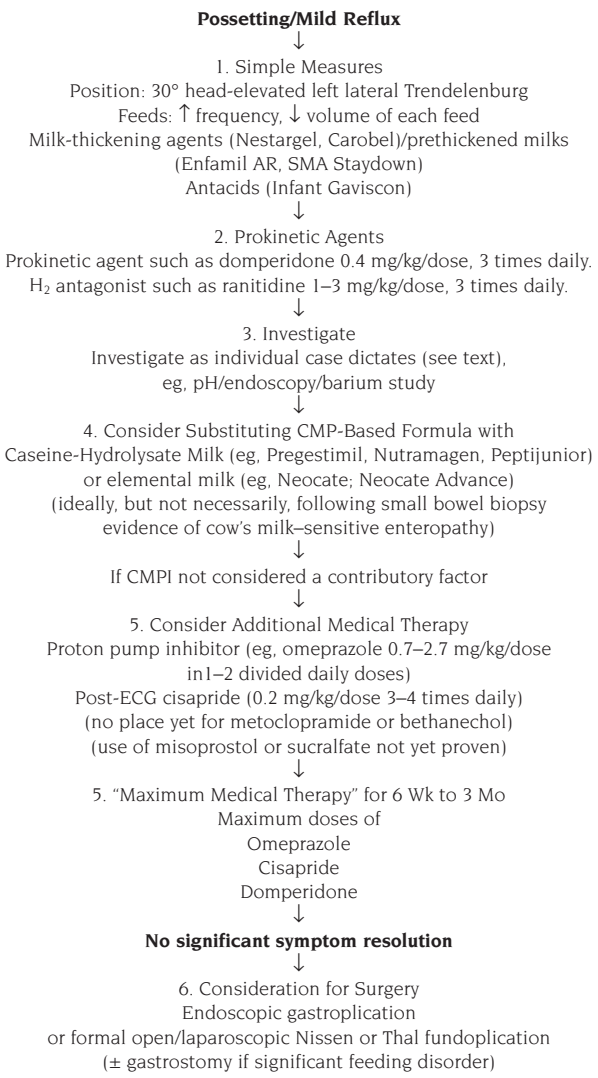


FIGURE 25-21 Gastroesophageal reflux treatment algorithm in infants and young children. CMP = cow's milk protein; CMPI = cow's milk protein intolerance; ECG = electrocardiography.

ithromycin, and combinations of these drugs with cisapride have produced cardiac dysrhythmias and prolonged Q–Tc syndrome in some patients. In a major article purporting to show a prolongation of the Q–T_c interval, there was no statistical difference between the cisapride group and the control group with regard to Q–T_c interval or J–T_c interval. However, 16 of the 35 patients reported have had a prolongation of Q–T_c.[165] There have been recent reports of prolonged Q–Tc syndrome in neonates with cisapride alone,[166] but Levine and colleagues published a report on the use of cisapride in 30 children, 12 of whom were premature neonates, and no effect on the Q–T_c interval was seen.[167] At present, some pediatric gastroenterologists would not recommend the use of cisapride in those infants born prematurely (less than 36 weeks gestation) in the first 3 months of life. In Canada, a survey of use in over 11,000 such neonates who received cisapride revealed three nonfatal arrhythmias, two with 10-fold dosage errors and one with cotreatment with erythromycin.[168] A useful and rational summary of risk and benefit has recently been published.[169] Its active component, termed nor-cisapride, which is purported to have no effect on the Q–T_c interval, may be available soon. Tegaserod may also be available in the near future to treat GER in childhood, but its major market focus has been in adult irritable bowel syndrome.

H_2 blockers can improve esophagitis, but the effect on GER cannot be assessed by pH because they neutralize gastric acid. High-dose ranitidine (6–7 mg/kg/dose 3 times per day) has been shown to be as effective as omeprazole in refractory reflux esophagitis in those children with[170] and without[171] developmental disabilities. A rebound nocturnal acid secretion has been reported.[172]

For the second group of infants and children who have documented erosive esophagitis or whose symptoms are refractory to the use of cisapride and ranitidine (grade 2 or more), the treatment must entail a more aggressive approach.[3] Occasionally, this will pertain to a young child without a proreflux condition, but, more usually, this will be in an older child with a predisposing condition such as neurologic compromise, cystic fibrosis, and repaired esophageal atresia. Medical treatment of the latter has an effect on the duration of reflux and could decrease the rate of subsequent stricture formation.[173] Ongoing trials and recent work with proton pump inhibitors in infants and children suggest that they are a useful therapeutic strategy in refractory reflux esophagitis, producing symptom improvement in all and histologic improvement in 40% in a recent study.[174] It has been proposed as the therapy of choice in children with neurologic compromise.[175] Symptomatic relapse is an issue on cessation of therapy, however, and this may be because the underlying lower esophageal dysmotility is unaltered by omeprazole therapy.[176] Definitive dose-finding studies remain to be carried out, and a higher dose/kg may be required than is observed in adults, for example, 0.7 to 2.0 mg/kg/d of omeprazole.[118,177,178] Lansoprazole has not been studied for GOR/dyspepsia in childhood to date.

Domperidone acts similarly to metoclopramide but has less dystonic reactions or other side effects and may be helpful for a limited period.[179,180]

The role of surgery is as a last-line therapy after maximal medical therapy has failed and significant complications of GER esophagitis remain. Most fundoplications to date have been open Nissen procedures, which involve a full wrap-around of the gastric fundus on the distal esophagus. Thal procedures are performed less commonly and involve an approximately 80% circumferential wrap. Fundoplication probably works because it prevents full LES relaxation and a reduction in the number of transient LES relaxations.[181] A large retrospective (20 years) multicenter study of over 7,000 children showed that so-called good to excellent results were achieved in 96% and 85% of normal and neurologically impaired children, respectively. Mortality was 0.1% and 0.8%, respectively. Recurrent reflux occurred in 7%, gas bloat in 3.6%, and obstruction in 2.6%. Reoperation was required in 3.6% and 11.8%, respectively.[182] Failed fundoplication is reported in those with IEE.[53] Laparoscopic

fundoplication is becoming more widespread. The results now compare favorably with the open procedure,[183] and it is associated with a faster recovery.[184,185]

Of particular interest recently is the advent of endoscopic antireflux procedures whose efficacy is well documented in the adult literature but whose use in pediatrics is still in only a few centers. One such procedure makes use of an Endocinch (C. R. Bard Inc., Murray Hill, NJ, USA) sewing machine attached to the endoscope, which was used to place three pairs of stitches below the GEJ to create three internal plications of the stomach (Figure 25-22).[186,187] Two plications are placed circumferentially 1.5 cm below the GEJ and one is placed 0.5 cm below the GEJ, which differs slightly from the reported adult studies in which various combinations of plications have been employed (eg, two circumferential, two or three longitudinal).

The initial results from a pilot study of 17 children suggest that endoluminal gastroplication is safe and effective in terms of improved quality of life assessed with two validated quality of life scoring tools, in terms of symptom scores, and objectively with improvement of all analyzed pH parameters in 16 of 16 patients and return to normal values in 14 of 16 patients who underwent pH studies subsequent to endoluminal gastroplication (Figure 25-23).[188]

Questions have been asked regarding the way in which the procedure actually improves the degree of reflux. It may be that the plications help to alter the angle of His, addressing the defective LES, and thereby decrease the amount of refluxate entering the esophagus. Swain and colleagues demonstrated an increase in the length and pressure of the LES.[189] However, other groups have not shown similar findings using esophageal manometry. It may be that the angle of the GEJ is improved or that the number of transient LES relaxations is decreased, and it is known that these are a major contributor to GER disease.

The alternative endoluminal techniques that have been promoted are endoluminal delivery of radiofrequency energy (Figure 25-24)[190] and endoluminal injection of inert biopolymers (Figure 25-25)[191]; however, neither of these techniques are reversible and are hence not desirable for application in the pediatric patient. As with any new technique, concerns remain about the learning curve of the endoscopist. However, a previous multicenter study in adults does not show a significant difference in the learning curve of endoscopists in different centers.[191]

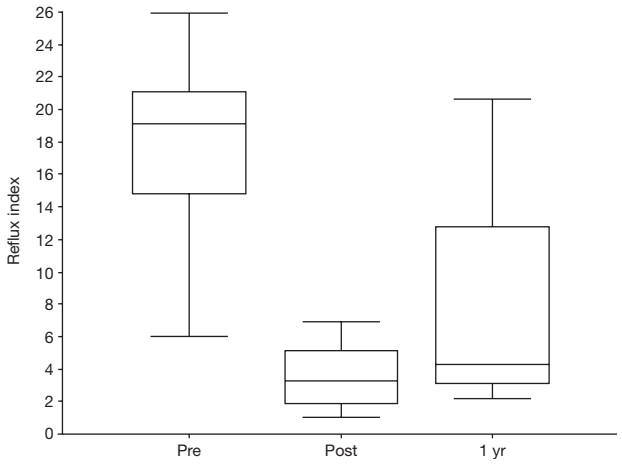


FIGURE 25-23 Reflux index of pH pre–, 6 weeks post–, and 12 months post–endoscopic gastroplication showing medium-term sustained response. (Wilcoxon rank sum test, and data presented as box and whisker plots with interquartile ranges).

Removal of CMP from the diet needs to be complete in a case of CMP-associated esophagitis and may occur even in the breastfed infant whose mother is taking dairy produce in her own diet, in which case, these should be excluded from mother's intake, and breastfeeding can continue. CMP-associated symptoms in exclusively breastfed infants have been reported with a prevalence of 0.37% in a population in which CMP allergy amounts to 1.9%.[192] In those on formula milk, a substitute is required. It is not appropriate to use soy milks because up to 30 to 40% or so of CMP-intolerant infants will also have an intolerance to

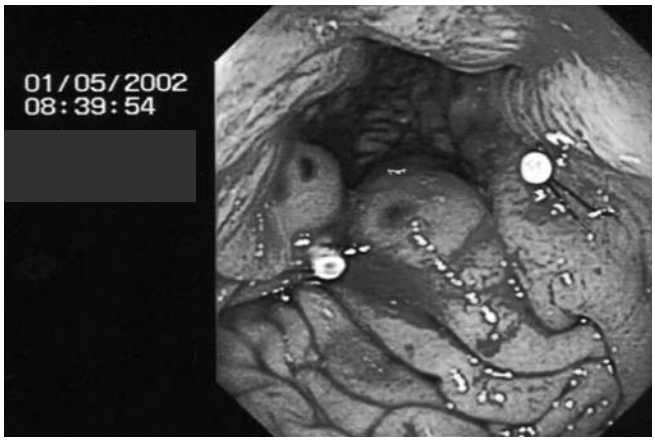


FIGURE 25-22 Endoscopic gastroplication (Endocinch) creating three pairs of sutures (plications): either longitudinally 0.5 cm, 1 cm, and 1.5 cm distal to the gastroesophageal junction (GEJ) and on the lesser curvature; or one each on the greater and lesser curvatures 1.5 cm distal to the GEJ and one on the lesser curvature 0.5 cm distal to the GEJ.

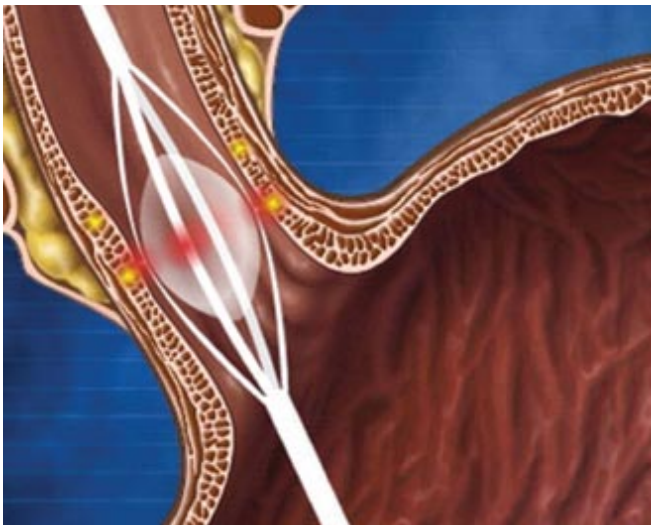


FIGURE 25-24 Delivery of radiofrequency energy to the gastroesophageal junction as an antireflux endoscopic procedure: not performed in children to date.

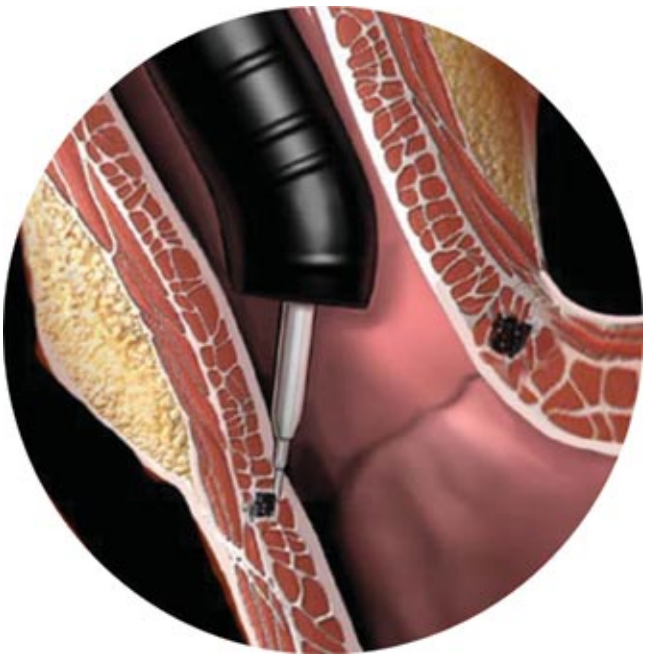


FIGURE 25-25 Injection of inert biopolymer into the gastroesophageal junction to act as an antireflux endoscopic procedure: not performed in children to date.

soy.[48] Classically, an infant will initially improve on the soy formula, and then symptoms similar to those experienced with CMP will ensue some 4 to 6 weeks later. More preferable, and to be recommended as a first-line substitute in this situation, is a casein hydrolysate milk (eg, Pregestimil, Nutramagen, Alimentum) or a whey hydrolysate (eg, Pepti-Junior, Alfa-Ré, Profylac, Hypolac, Nutrilon-Pepti).[193] However, these still contain peptides greater than 15 amino acids in length, which are still capable of precipitating a major histocompatibility complex–mediated immunoreaction. In some instances, it is necessary therefore to go one step further and put the infant exclusively on an elemental milk (eg, Neocate, Neocate Advance, Nutri Junior) containing amino acids, glucose polymer, and long-chain fatty acids. An improvement may be seen within 1 week, but Kelly and colleagues, and other groups, recommend a 6-week trial.[46,194,195] The glucose polymer is used to prevent the osmolality of the milk becoming too high and precipitating osmotic diarrhea. The only drawback of such milks is their unpalatability, but with persistence, infants usually get used to them, especially if introduction occurs early in life. Certain milks may require the addition of calcium to the diet, and involvement of a dietitian is advised. It is normal practice to reintroduce CMP around 12 to 18 months, but some children require dairy exclusion until 3, 4, or more years. Multiple food-associated esophagitis can occur,[47] and in such situations, a few-foods diet starting with hypoallergenic components such as rice, potato, green beans, and chicken with stepwise reintroduction may be necessary. A proportion of these infants are also helped by oral sodium cromoglycate.

IEE has been effectively treated with oral or inhaled corticosteroids,[96] and it is possible that the latter route may be preferable given that multiple courses of systemic corticosteroids are often required.

Infective causes of esophagitis in pediatrics require specific therapies. Viral esophagitis is usually due to herpes simplex, CMV, and, occasionally, varicella zoster.[65–67] Although the inflammation can resolve spontaneously in the immunocompetent, in those with poor immune function, acyclovir and a high index of suspicion are recommended.[72] Use of prophylactic acyclovir is conjectural but may be of some benefit post-transplant. Resistance to acyclovir has been described, in which case, foscarnet is the agent of choice.[73] CMV esophagitis is predominantly found in immunocompromised individuals, and treatment is with ganciclovir or foscarnet.[67] Hemorrhage, fistulae, and esophageal perforation in adults with viral esophagitis have been described.[74,75]

Acute HIV infection can also cause esophagitis, and antiretroviral regimens are needed.[76]

Candida is the most common infectious cause of esophagitis. A 2- to 6-week course of oral nystatin can be effective in those with normal immune function, but it is more convenient to give fluconazole. Fluconazole and liposomal amphotericin are both effective and are necessary in the immunocompromised child. Esophageal resection and diversion for necrotizing candidal esophagitis have been successful in a 10 year old.[81]

Eradication of *H. pylori* is not likely to improve coexistent esophagitis, and, indeed, in adults, eradication has been associated with increased acid production and hence more noxious gastroesophageal refluxate. However, there does not seem to be any increased incidence of esophagitis in the presence of or following the eradication of *H. pylori* in children.[82] Primary bacterial esophagitis is described in immunocompromised patients and requires appropriate antibiotics dictated by sensitivity testing.[84] Other opportunistic organisms causing esophagitis, such as *Cryptosporidium* and *Acremonium*, have been reported and require appropriate therapy.[85,86]

Treatment of caustic esophagitis is initially conservative, with barium swallow at 4 to 6 weeks postingestion, endoscopic assessment, and, if necessary, stricture dilation. The place of steroids in stricture prevention is controversial and not routine in many centers. Recently, the use of an antifibrotic, mitomycin C, applied topically to the mucosa poststricture dilation has been used successfully in patients who have required multiple stricture dilations, with prevention of restenosis.[41] Antibiotic therapy for mediastinitis and judicious use of surgery may be employed; these are dealt with in Chapter 24.

Older children whose esophageal stratified epithelium is exposed to long-term acid may, as with adults, develop gastric metaplasia, eponymously termed Barrett esophagus.[196–198] This increases the risk for esophageal adenocarcinoma 30 to 40 times. Debate surrounds the relative merits and success rates of antireflux surgery or long-term proton pump inhibitor use, and this is dealt with in greater detail in Chapter 24.

Prognostication in infant and childhood esophagitis is wholly dependent on etiology; however, fortunately, the most common causes, reflux and allergy, are relatively self-

limiting, with a natural improvement and recovery by 18 months to 2 years in the vast majority. This is dealt with in greater detail at the beginning of the section on treatment. It is the responsibility of the pediatrician to prevent avoidable complications such as peptic strictures occurring during the period of vulnerability until such an age has been reached. A low threshold for diagnosis and intervention is therefore sensible in this population.

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CHAPTER 26

OTHER MOTOR DISORDERS

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Abnormalities of esophageal function occur frequently and can be primarily confined to the esophagus or can be secondary to systemic illnesses (Tables 26-1, 26-2, and 26-3). By interfering with the normal progression of food transit from the mouth into the stomach or by failing to provide adequate protection from gastric acid, esophageal motor disorders can be debilitating and even life threatening. Because the major esophageal functions are to transport food from the mouth to the stomach and to prevent reflux of gastric contents, the main manifestations of disease in this organ are either feeding difficulties or regurgitation. Chapter 23, "Disorders of Deglutition," and Chapter 24, "Gastroesophageal Reflux," discuss normal esophageal function and development and present a general approach to the patient in whom an esophageal motor disorder is suspected. This chapter provides an in-depth discussion of the clinical manifestations of specific disorders in esophageal motility from the perspective of pathophysiology, diagnosis, and management. Because disorders of the oral and pharyngeal phase of swallowing have been described elsewhere in this volume, the following discussion relates mainly to clinical problems of the esophagus and the esophageal phase of swallowing (see Table 26-1). It first focuses on the disorders that affect the upper esophagus (see Table 26-2) and then on those that affect the rest of the esophageal body and the lower esophageal sphincter (LES) (see Table 26-3).

DISORDERS OF THE CRICOPHARYNGEAL MUSCLE

Problems with the cricopharyngeal muscle usually present like those of the pharyngeal phase and are therefore difficult to differentiate. Normal deglutition depends on precise coordination between relaxation of the upper esophageal sphincter (UES) and the pharyngeal contractions that propel the food bolus through the sphincter into the esophagus. When UES relaxation is incomplete or uncoordinated with respect to pharyngeal activity, the bolus is mishandled.[1]

When there is cricopharyngeal dysfunction, the symptoms usually appear shortly after birth or during the first 2 months of life. Repeated aspirations and choking are usual symptoms and can be life threatening. Evaluation usually includes radiographic studies to assess the anatomy and the coordination of the swallow. Cricopharyngeal dysfunction should be suspected in children with pooling of saliva at the back of the pharynx, holding up of barium at the UES, and a shellfire impression at the cricopharyngeal level.[2]

The usefulness of UES manometry in the diagnosis of cricopharyngeal dysfunction is not clear. Malhi-Chowla and colleagues explored this issue in adults. They found that 80 (17.7%) of 435 of their routine manometries revealed an abnormality of the UES or the pharynx, but these findings changed management in only 6 patients. Therefore, only 1.4% of the total number of UES manometries done resulted in a management change. As a result, the authors argue that unless there is a strong clinical suspicion or an abnormality on a barium swallow that needs further evaluation, routine testing of the UES has a low yield.[3]

Evaluation of motor dysfunction of the pharyngoesophageal junction suggests two main defects of UES motility: abnormalities in the sphincter resting pressure and abnormalities in the UES relaxation.[2,4]

Abnormalities of Resting UES Pressure

Cricopharyngeal Hypertension. The term "cricopharyngeal spasm" was initially introduced after prominent cricopharyngeal impressions were seen by barium swal-

TABLE 26-1 ESOPHAGEAL MOTILITY DISORDERS

Disorders that affect the striated muscle predominantly
Cricopharyngeal dysfunction
Abnormalities of resting tone
Abnormalities in relaxation
Neuromuscular disorders
Neurologic disorders
Muscular disorders
Neuromuscular disorders
Structural lesions
Central nervous system malformations
Disorders that affect the smooth muscle predominantly
Primary esophageal motor disorders
Secondary esophageal motor disorders
Gastrointestinal disorders
Congenital malformations
Collagen vascular diseases
Neuromuscular disorders
Infectious diseases
Exogenous factors
Iatrogenic
Other

TABLE 26-2 ESOPHAGEAL MOTOR DISORDERS THAT AFFECT THE STRIATED MUSCLE PREDOMINANTLY

CRICOPHARYNGEAL DYSFUNCTION
Abnormalities of resting tone
Hypertension
Hypotension
Abnormalities in relaxation
Incomplete relaxation (achalasia)
Premature contraction
Delayed relaxation
NONESOPHAGEAL DISEASES
Neurologic diseases
Disorders of the autonomic nervous system
Familial dysautonomia
Cerebral palsy
Motoneuron disease
Bulbar palsy
Paralysis of the laryngeal nerve
Demyelinating diseases
Multiple sclerosis
Cerebrovascular accidents
Poliomyelitis
Neuromuscular diseases
Myasthenia gravis
Botulism
Muscular diseases
Muscular dystrophies
Myotonic muscular dystrophy
Oculopharyngeal muscular dystrophy
Inflammatory myopathies
Dermatomyositis
Polymyositis
Metabolic
Thyrotoxicosis
Myxedema
Structural lesions
Foreign body
Tumors
Inflammatory disorders
Congenital webs
Extrinsic compression
Drugs
Nitrazepam
Other neuroleptics

low.[4] Later it was found that clinically or radiographically diagnosed "esophageal spasm" did not correlate with elevated UES pressure measured by manometry.[4] It has been reported that a horizontal esophageal bar can be found in up to 5% of adult patients undergoing a barium swallow for all indications[2] and is a frequent radiologic sign in infants.[2] There are, however, reports in which the resting pressure of the UES was found to be elevated in patients with globus sensation in the pharynx, although this finding has not been replicated in all patients.[4]

Cricopharyngeal Hypotension. Because tonic closure of the cricopharyngeus is due to tonic myoneural activity, a variety of myoneural disorders may lead to a decreased pressure in the UES. These include amyotrophic lateral sclerosis, myasthenia gravis, oculopharyngeal muscular dystrophy, dystrophia myotonica, and polymyositis.[5] This hypotension can be diagnosed manometrically but will not be seen by radiography unless the UES weakness is extreme.[4] The clinical significance of UES hypotension is not clearly defined. It is possible that it allows air to enter the gastrointestinal (GI) tract during respiration and also predisposes the patient to tracheobronchial aspiration of the esophageal contents.

ABNORMALITIES OF CRICOPHARYNGEAL RELAXATION

Three types of abnormalities of cricopharyngeal relaxation have been described: incomplete relaxation, premature closure, and delayed relaxation.[4,6] All of these abnormalities may result in dysphagia and will be considered separately.

Incomplete Cricopharyngeal Relaxation. Patients with cricopharyngeal achalasia show incomplete UES relaxation after the majority of swallows.[7] Radiographically, cricopharyngeal achalasia is characterized by a horizontal indentation on the posterior esophageal wall, with the barium passing the muscle very slowly and the cricopharyngeal muscle appearing to relax poorly.[8] At times, there is complete functional obstruction, and no barium passes into the esophagus.[7] Manometric studies have confirmed this incomplete cricopharyngeal relaxation in some patients,[1,7,9,10] whereas in others, the UES has been shown to relax normally.[2,4] The reason for this discrepancy between radiologic and mano-

TABLE 26-3 DISORDERS THAT AFFECT THE SMOOTH MUSCLE PREDOMINANTLY

PRIMARY ESOPHAGEAL MOTILITY DISORDERS
Achalasia
Diffuse esophageal spasm
Nutcracker esophagus
Nonspecific esophageal motility disorders
SECONDARY ESOPHAGEAL MOTILITY DISORDERS
Gastrointestinal problems
Gastroesophageal reflux
Chronic intestinal pseudo-obstruction
Congenital malformations
Tracheoesophageal fistula
Hirschsprung disease
Collagen vascular diseases
Metabolic disorders
Diabetes mellitus
Thyroid problems
Neuromuscular disorders
Muscular dystrophies
Myasthenia gravis
Degenerative disorders
Autonomic nervous system problems
Infectious disorders
Chagas disease
Exogenous factors
Drugs
Silicone breast implants
Food
Caustic ingestions
Other illnesses
Anorexia nervosa
Graft-versus-host disease
Iatrogenic
Endoscopic variceal sclerotherapy
Endoscopic variceal ligation
Surgery

metric findings is not clear, although it may relate to the difficulty in studying this region.[7] In children, most studies have documented abnormal relaxation by cinefluoroscopy,[7,8,11] although manometric abnormalities have also been described.[7,12] Symptoms usually include food regurgitation, choking, nasal reflux, coughing, recurrent aspiration pneumonia, and failure to thrive. Symptoms usually begin at birth or shortly thereafter.[1,7,9,13] It is crucial to evaluate children with recurrent respiratory symptoms for cricopharyngeal achalasia because the diagnosis is often missed, resulting in unnecessary fundoplications because the children are given the diagnosis of reflux.

One of the largest series in children described the findings in 15 children.[12] Because of a lack of suspicion, the diagnosis was usually made at the end of the first year. All patients were diagnosed radiographically, and esophageal motility studies were performed in 5 patients, documenting the lack of cricopharyngeal relaxation in all. Abnormal esophageal motility was also noted. It is interesting to note that 10 of 15 patients had an associated disease: four myelomeningoceles and six congenital anomalies associated with central nervous system (CNS) involvement. Cricopharyngeal achalasia has also been confirmed manometrically in five other studies.[1,7,9,10,14] Dinari and colleagues, however, reported a child with cricopharyngeal achalasia in which there were radiologic abnormalities but normal manometric findings.[2] It has also been reported that cricopharyngeal achalasia may occur in children with Down syndrome[6] or with Chiari malformations.[1,7,9]

The treatment of cricopharyngeal achalasia in adults has ranged from bougienage to surgery.[1,2,4,7,9,15–17] The results with bougienage have been unsatisfactory owing to short-lasting effect,[2,4,7,17] so, for severe cases, cricopharyngeal myotomy has been advocated.[2,7,17] A decrease in cricopharyngeal pressure occurs, and most patients have relief of the dysphagia.[17] Major complications can occur because the protective mechanism of the UES is taken away, and, in fact, deaths from aspiration have been described following myotomy.[7,17] Recently, injections of botulinum toxin in the cricopharyngeal muscle have been successfully used to treat patients who are at high risk for surgery.[15,16,18,19]

There are two recent studies in adults on the use of botulinum toxin in the treatment of cricopharyngeal dysfunction. The first, by Haapaniemi and colleagues, reported on four adults with cricopharyngeal dysfunction from systemic illnesses (CNS lesions, myositis, or neuropathy) who had relief of their dysphagia for 2 weeks to 10 months after injection.[18] Shaw and Searl described their series of 12 patients who had botulinum toxin injection for cricopharyngeal dysfunction owing to CNS lesions or to dysfunction from prior otolaryngologic surgeries. They found that symptom improvement ranged from 0 to 14 months. The complications that have been reported from injection include pharyngeal tears and worsening dysphagia.[19]

Experience with the treatment of cricopharyngeal dysfunction in children is very limited.[7,14] Successful balloon dilatation has been accomplished in cases of cricopharyngeal achalasia[2,7] and incoordination.[2,7,8] Some authors make the observation that, in contrast to adults, even one dilatation in infants and children may be successful in relieving the symptoms.[7] The smallest patient reported with cricopharyngeal achalasia treated with dilatations was 5 months old at the time of the treatment, and she responded well, without any evidence of obstruction 1.5 years after the procedure. The smallest patient with cricopharyngeal incoordination treated with dilatations was 2.5 years and has remained symptom free for 6 months after the dilatation.[2]

In one of the largest pediatric series of 15 patients with cricopharyngeal achalasia, 6 were managed conservatively with nutrition and positioning, 4 with myelomeningocele had shunt revisions, with subsequent improvement in 3, 4 had gastrostomies, and 2 had tracheostomies. Only 2 patients underwent cricopharyngeal myotomy, and only moderate improvement was observed.[12]

There have been case reports of successful cricopharyngeal myotomy performed in children with cricopharyngeal achalasia.[7,10,13,20] Brooks and colleagues described their experiences with five children who had esophagomyotomy (EM) for cricopharyngeal achalasia, two of whom had manometry pre- and postmyotomy.[14] The EM reduced the UES pressures by 29% and 47% in these two patients. Most importantly, however, all five patients were free of symptoms during the follow-up period of 2 to 14 years. Esophagomyotomy has also been reported in children with cricopharyngeal incoordination,[10] with good results reported at short-term follow-up.[7]

Because of the possibility of spontaneous improvement and the good response reported after dilatation in infants and children, a conservative approach should be undertaken, with aggressive nutritional support and dilatations in those patients with severe compromise, reserving surgery for the difficult patients who do not respond to the above-mentioned conservative management.[2,7,12] Even though there is no experience with the use of botulinum toxin for cricopharyngeal disorders in children, it may play a role in the management before more invasive procedures are attempted.

Premature Cricopharyngeal Closure. It had been suggested that premature closure of the cricopharyngeal sphincter following deglutition plays a role in the pathogenesis of Zenker diverticulum.[4] Recent studies, however, have found manometric abnormalities in only some or none of the patients studied. According to manometric data, the universal acceptance of cricopharyngeal myotomy in the management of Zenker diverticulum has little justification.[21]

In a manometric study in children, two patients were found to have delayed pharyngeal contraction with respect to cricopharyngeal relaxation, two were found to have incomplete cricopharyngeal relaxation, and one had both cricopharyngeal incoordination and failure to relax.[10] All had gastroesophageal reflux (GER), and one also had incomplete UES relaxation. In all cases of incomplete relaxation, the barium swallow was reported to be abnormal; all five patients had severe swallowing difficulties, and three had an associated syndrome with mental retardation (Russell-Silver syndrome, 5p- [cri du chat] syndrome, and minimal change myopathy).

Other case reports of cricopharyngeal incoordination in children have been published.[1,2,9] Some authors have described a "transient cricopharyngeal incoordination" that may occur in the newborn period. The clinical features resemble tracheoesophageal fistula or laryngotracheoesophageal cleft, and the diagnosis has been made radiographically.[4,22] This "incoordination" is usually manifest from birth, and even though sucking is normal, infants may choke or even aspirate. The nature of the problem, however, has not been defined manometrically, although there is a case report in which the manometry was normal.[2] Recently, it has been shown that children with Arnold-Chiari malformation type I can have neurogenic dysphagia with pharyngo-UES incoordination preceding other brainstem dysfunction (see discussion below).[1,9] Repeated aspirations and choking can be life threatening. The clinical course is variable, and even though it tends to be progressive, with increasing severity in the respiratory complications and increasing nutritional problems, spontaneous improvement has been described, particularly in those in whom no associated anomalies were found.[2,8] We recently described the natural history of nine newborns who presented with isolated neonatal swallowing dysfunction.[23] We found that most children were able to eat by mouth by the age of 3 years. Four of the nine patients had associated anomalies or syndromes that became evident only through close follow-up. Because it is known that some of these infants "outgrow" their incoordination, careful attention should be paid to the nutritional state of the infants, and feedings by spoon, gavage, or gastrostomy tube may be required for a few years until symptoms disappear.[2,8] It should be mentioned, however, that fatal aspiration has been reported.[22,24] In one infant who died, the autopsy showed marked dilatation of the pharynx above the area of obstruction and hypertrophy of the pharyngeal constrictors. Aganglionosis of the proximal third but not of the distal third of the esophagus was noted, but no further information was provided.[22]

Delayed Cricopharyngeal Relaxation. If reflex cricopharyngeal relaxation is delayed, pharyngeal contents are propelled toward the upper esophagus before the UES is opened. It has been suggested that a delay in cricopharyngeal opening of more than one-third of a second[1] is associated with pulmonary aspiration. In children with familial dysautonomia (Riley-Day syndrome), the cricopharyngeal opening is delayed, but once the sphincter opens, its opening is complete (unlike patients with cricopharyngeal achalasia).[4,25] Careful radiologic studies in 11 patients with Riley-Day syndrome have also demonstrated that they have disordered esophageal motility,[25] although it was suggested that the major cause of the disability was the cricopharyngeal abnormality, and others have suggested that the impaired pharyngeal muscle coordination creates the problem. No manometric studies of these patients exist.

Delayed cricopharyngeal relaxation had not been shown manometrically until Wyllie and colleagues described two children whose drooling, observed with the administration of nitrazepam, proved to be secondary to delayed cricopharyngeal relaxation (Figure 26-1), providing direct evidence that cricopharyngeal incoordination can be secondary to drug administration.[6]

DISEASES THAT AFFECT THE STRIATED MUSCLE PORTION OF THE ESOPHAGUS

Many diseases can produce dysphagia secondary to buccopharyngeal involvement (see Table 26-2). These illnesses can produce abnormalities of the tongue, pharyngeal muscles, cricopharyngeal muscle, upper esophagus, and even, at times, the lower esophageal body. Therefore, the precise genesis of the difficulty in swallowing may be very complex, involving multiple factors rather than a single one.[1,4] Some selected diseases that cause this type of dysphagia with impact in the pediatric age group are described.

Myopathic Diseases

Muscular Dystrophies. Dysphagia is rare in most forms of muscular dystrophy, except for two relatively rare types of muscular dystrophies: myotonic muscular dystrophy and oculopharyngeal dystrophy. The former is the one that can present in the pediatric age group.

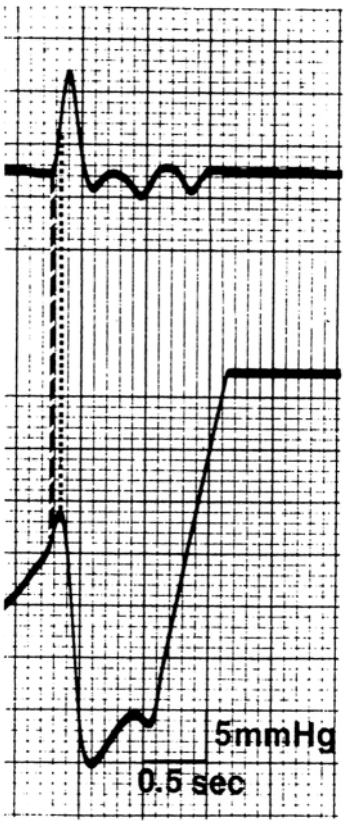

FIGURE 26-1 Delayed cricopharyngeal relaxation in a patient taking nitrazepam. The upper tracing represents hypopharyngeal contraction (onset at *broken line*); the lower tracing represents cricopharyngeal relaxation (onset at *dotted line*). Note that the hypopharyngeal contraction preceded the cricopharyngeal relaxation by 0.3 seconds. Reproduced with permission from Wyllie E et al.[6]

Even though myotonic muscular dystrophy usually has its onset in the adult, it begins in infancy or childhood with considerable frequency. It is characterized by myotonia, "myotonic facies," muscle wasting, frontal baldness, testicular atrophy, and cataracts. Pharyngeal muscle weakness is found in up to 92% of these patients,[26–29] although symptomatic involvement occurs in less than half.[26,27] Usually, however, myotonia or other evidence of disease is present for 2 to 15 years before the onset of dysphagia, and in addition to dysphagia, these patients also have involvement of the striated and the smooth esophageal muscle.[1,26] Pharyngeal weakness of contraction is the predominant finding[4] and reveals itself as barium stasis and hypomotility in the radiograph. The UES may be incompetent and is responsible for the appearance of a continuous column of barium in the pharynx and the upper esophagus.[1,26] Manometric studies reveal a reduction in the basal UES pressure, the duration of contractions in the amplitude, and amplitude progression, as well as in coordination[1,4,26,28,29] of pharyngeal and cricopharyngeal contractions.

Inflammatory Myopathies. Systemic diseases in which there is inflammation of the skeletal muscle usually also affect the striated muscle of the pharynx and esophagus. Dysphagia is a frequent symptom in these patients.[4] In a series of 152 patients, dysphagia was present in 54%,[30] and in about 2% it may be the initial symptom.[4] The dysphagia is related to pharyngeal muscle weakness. In one study of six patients with polymyositis and dysphagia, barium appeared to pass the oropharynx with difficulty, and all patients showed retained barium in the vallecula and piriform sinuses. Transport seemed to be affected only by gravity.[31] Manometric studies have revealed decreased cricopharyngeal pressure, with normal relaxation of the sphincter and poor amplitude of contractions in the pharynx and upper third of the esophagus.[31] It has been suggested that these abnormalities respond to the administration of steroids.[30] Recently, it has been reported that these patients may also have abnormalities in the distal esophagus.[32]

Neurologic Diseases

Arnold-Chiari Malformation. The Chiari malformation has been associated with dysphagia and radiographic evidence of UES dysfunction. Type II is usually associated with myelodysplasia, whereas type I is not. It has been suggested that up to 5% of patients with a Chiari malformation will develop symptoms as a result of brainstem dysfunction.[1,9]

Putnam evaluated the dysphagia in five children with Chiari malformation using esophageal manometry and barium esophagography.[1] The studies were done before and after craniocervical decompression. Preoperatively, three patients had failure of compete UES relaxation, one had pharyngo-UES incoordination, and the other had both. All patients had clinical and manometric resolution of the symptoms after surgery, suggesting that surgical decompression of Chiari malformations may lead to complete clinical and manometric resolution of the dysphagia owing to UES dysfunction. It is interesting to note that the dysphagia was the first symptom in two of the patients and was the only evidence for cranial nerve dysfunction in one, indicating that patients with unexplained cricopharyngeal dysfunction should be evaluated for Chiari malformations.

In another series of 46 patients who underwent craniectomy and laminectomy for Chiari malformations, 15 patients presented with symptoms of neurogenic dysphagia.[9] All patients had normal swallowing before the development of the dysphagia, and the symptom was progressive in all and in eight preceded other signs of brainstem involvement. Patients had widespread dysfunction of the swallowing mechanism, with a combination of pharyngoesophageal dysmotility, cricopharyngeal achalasia, nasal regurgitation, tracheal aspiration, and GER. Outcome after surgery varied according to the severity of the preoperative symptoms, with those patients with other signs of brainstem involvement having a poor result, whereas those with mild symptoms showed an excellent response. This finding suggests that early recognition of neurogenic dysphagia and expeditious intervention are crucial to ensure a favorable neurologic outcome.

In children with Arnold-Chiari malformation and neurogenic dysphagia, the treatment needs to be directed to improve the brainstem function, usually with craniectomy and cervical laminectomy.[1,9]

Motoneuron Disease. This is characterized by degeneration of the upper and/or lower motoneurons. Involvement of the bulbar neurons leads to paralysis of the tongue and the pharynx, which produces abnormalities in the buccal and pharyngeal phases of swallowing.[4] A recent survey in adults found that 73% of patients with motoneuron disease had difficulty in chewing and swallowing, with most problems being related to solid food rather than to liquids.[33] Cricopharyngeal abnormalities have also been found in adults with motoneuron disease.[33]

Bulbar palsy can occur in children. In the infant, this is usually supranuclear, with difficulty in sucking or swallowing and prominent drooling being the frequent symptoms.[22] The jaw jerk tends to be exaggerated, which is a diagnostic clue, and usually a picture of diffuse cerebral palsy and spasticity develops. In the lower motoneuron form of bulbar palsy, there are usually poor suck and nasal regurgitation of the formula. If facial bulbar paralysis is associated with facial diplegia, it constitutes Möbius syndrome.

Selective paralysis of the laryngeal nerve has been reported. Dysphagia and altered esophageal motility have been reported, and recovery usually occurs at the end of the first year.[22] Other processes that may induce motoneuron disease, with secondary problems in the pharyngeal and esophageal phases of swallowing, include neurosurgical procedures and tumors that involve the brainstem.[8]

Other Neurologic and Neuromuscular or Muscular Diseases

Myasthenia Gravis. This disease affects the motor end plate of the striated muscle, including the one situated in

the esophagus. In children, it can present in three clinical forms: transient neonatal myasthenia gravis, persistent neonatal myasthenia gravis, and juvenile myasthenia gravis, which is the most common form.[4,22] Dysphagia, choking, and aspiration of food are frequent clinical manifestations of the disease.[4] The description of the swallowing difficulty is characteristic: the patient is able to swallow normally at the beginning of the meal, but progressive difficulty appears with each swallow. Manometrically, these patients have been shown to have a decrease in the amplitude of peristaltic contractions, mainly in the upper esophagus, with the amount of decrease dependent on how severely each particular patient is affected.[34] At times, the proximal esophageal weakness may be apparent only with repetitive swallows, and cricopharyngeal dysfunction almost never occurs in these patients.[4] The distinguishing feature of this disease is the recovery of the manometric and clinical abnormalities with rest or the administration of anticholinesterase drugs. The intravenous administration of edrophonium chloride (Tensilon) in a dose of 0.2 mg/kg, up to 10 mg, produces prompt but transient relief in symptoms and radiographic and manometric abnormalities,[4] and it has been suggested that this diagnostic test should be employed in patients who show pharyngeal weakness without any obvious cause, particularly when ptosis is present.[4]

Other Neurologic Diseases. Swallowing difficulties are common in patients with multiple sclerosis.[4] In one study, they were reported to occur in 55% of the patients, and cricopharyngeal dysfunction can also occur. Even though cerebrovascular accidents are rare conditions in childhood, transient or persistent dysphagia is a frequent manifestation.[4] These problems occur when the lesions involve the swallowing center or the motor nuclei that control the hypopharynx, and dysphagia may be one of the predominant symptoms.

Poliomyelitis. Bulbar poliomyelitis may cause dysphagia, which is thought to be due to pharyngeal paralysis.[35–38] There are manometric and radiographic studies in which cricopharyngeal function has been reported to be normal,[36] although other studies, relying mainly on radiographic observations, have reported cricopharyngeal abnormalities and cricopharyngeal achalasia in some of these patients.[4,35,36,38] The swallowing problems of these patients have been treated with prolonged nasogastric intubation, cricopharyngeal dilatations, cricopharyngeal myotomy, and even cricopharyngeal denervation, all with good results.[4,35,36,38]

Poliomyelitis is a disease that has been eradicated from many parts of the developed world, but late postpolio sequelae have been reported recently in patients who suffered from the disease many years ago.[38] These late sequelae may occur more than 30 years after the original illness, and new symptoms include unaccustomed fatigue, new joint or muscle pain, new weakness in muscles affected and unaffected by polio, and new respiratory difficulties. Recently, dysphagia has been noted in nearly 18% of polio survivors.[35–38] This new dysphagia is worse for solids, and, radiographically, it seems to be related to decreased pharyngeal peristalsis. The dysphagia may be progressive, and it may be produced by a slow deterioration of the bulbar neurons.[38] A thorough evaluation is necessary to determine optimal feeding management and to search for treatable contributing factors.[37,38]

Botulism. The syndrome of infant botulism is characterized by peripheral muscle weakness, hypotonia, respiratory depression, and diminished suck and swallow.[39,40] Although descending flaccid paralysis of striated muscles is the most striking clinical feature of the syndrome, difficulty in swallowing and delayed evacuation of stool are often initial findings and are often overlooked.[39] The symptoms in this disease result from the irreversible binding of the botulinum toxin to peripheral cholinergic terminals, with the subsequent prevention of the release of acetylcholine at the neuromuscular junctions, as well as preganglionic and postganglionic synapses.[39] In a study of four infants with this disease, Cannon reported the results of esophageal motility in these infants.[40] The major effect of the toxin of esophageal motility was the disruption of the UES function and peristalsis in the proximal esophagus. There was a reduction in UES pressure and a marked reduction in the percentage of UES relaxation after swallowing (1–20% in patients compared with 80–100% in controls). The hypopharyngeal and proximal esophageal contractions were of low amplitude and poorly coordinated. Gradual return of esophageal motor function accompanied improvement in peripheral muscle strength and return of the gag and suck reflexes. Interestingly, the botulinum toxin had no significant effect on the LES and the distal esophagus.[40]

Drug Administration

Since the original report by Wyllie and colleagues, which described two children with delayed cricopharyngeal relaxation after nitrazepam administration,[6] Lim and colleagues performed a prospective study in 38 patients and found that 3 had delayed cricopharyngeal relaxation and high-peaked esophageal peristaltic waves.[41] They were also able to show in one patient that the manometric abnormalities disappeared after the drug was stopped. They postulated a CNS effect of nitrazepam promoting parasympathetic overactivity. These findings are important because they indicate that cricopharyngeal dysfunction may be responsible for the respiratory compromise seen in some of those patients and suggest that esophageal manometry should be considered in the evaluation of patients taking nitrazepam who have eating difficulties or aspiration pneumonia. If abnormalities of cricopharyngeal function are detected, the patient may be at greater risk for major complications, including sudden death.

Dysphagia has been described as being frequently associated with the administration of neuroleptics or other psychotropic medications.[42–44] The problem can be life threatening and is usually reversible once the medications are stopped.[42,43]

DISORDERS THAT AFFECT THE SMOOTH MUSCLE OF THE ESOPHAGUS

Primary Esophageal Motor Disorders

Achalasia. Achalasia is a motor disorder of the esophagus that presents as a functional obstruction at the esophagogastric junction (see Table 26-3).[45,46] It is characterized by the following abnormalities: a lack of esophageal peristalsis, increased LES pressure, and partial or incomplete LES relaxation.[45,47–49]

The illness is uncommon, with an estimated incidence of 1 case per 10,000 people.[47,50] Data suggest a worldwide incidence at between 0.03 and 1.1 of 10^5/yr.[47] It has been estimated that from all patients with achalasia, fewer than 5% manifest symptoms before the age of 15 years.[45,51,52] Moersch reported that of 690 cases seen in the Mayo Clinic, only 12 (1.7%) presented in the pediatric age group.[52] Olsen and colleagues reported that only 17 children (2.8%) of 601 patients were treated by them.[53] In a recent epidemiologic study of achalasia in children, Mayberry and Mayell, based on a study of 129 children, determined that in Ireland, the incidence was 0.31 cases per 105 children per year, and in England, it was 0.11 per 105 children per year.[51] No such epidemiologic information is currently available about children in the United States.

Etiopathogenesis. In idiopathic cases of achalasia, the incomplete relaxation of the LES is believed to be secondary to the fact that the postganglionic inhibitory nerves are absent, reduced in number, functionally impaired, or lacking in central connections.[54–56] The disease seems to involve the Auerbach plexus,[57] and absence of ganglion cells from the myenteric plexus in the involved portion of the esophagus with normal ganglion cells in the noninvolved segment has been described.[58–60] Ganglion cell degeneration appears to be prominent in the early years of the disorder, with progressive loss of neurons detected after a decade or more.[61] This progressive lesion of the plexus is accompanied by progression of the disease. Even though the most consistent neuropathologic lesion has been the ganglion cell degeneration or loss in the esophageal myenteric plexus, these findings are not a constant feature, and multiple instances of normal ganglion cells have been described.[62,63]

Many reports in children have found absent ganglion cells in the distal esophagus.[58–60,64] However, some other authors have described normal histology in some children with achalasia[62,65,66] or adequate numbers of ganglion cells but extensive perineural fibrosis. The presence or absence of ganglion cells may depend on the duration of the disease.[65]

Other neuropathologic findings that are frequently although inconstantly described include chronic inflammatory infiltrates in the myenteric plexus and degenerative changes in the smooth muscle or nerve fibers.[61] These findings could be secondary to neuronal degeneration and loss or to confounding variables.[61]

Most studies have noted no changes at the light microscopic level in the vagus nerves,[61] although some authors show evidence of nerve fiber degeneration at the electron microscopic level in the vagus nerve and its dorsal motor nuclei.[57,65] Electron microscopic studies on the muscle wall of achalasic LES have confirmed the specific nerve tissue damage, which light and electron microscopic studies on the intrinsic and vagal innervation of the LES had previously demonstrated.[67,68] The evidence of histopathologic studies points then to a primary neurogenic abnormality in patients with achalasia.[56,57,68]

The end result of all of those abnormal histologic findings is a decrease in the postganglionic cells, which mediate LES relaxation via the release of vasoactive intestinal polypeptide (VIP) and nitric oxide (NO).[69–71]

NO is an important mediator for the nonadrenergic, noncholinergic nerve effects in the human esophagus and LES.[70,72] Recent studies have demonstrated an absence of NO synthase in the myenteric plexus at the gastroesophageal junction of patients with achalasia, suggesting that NO deficiency may also be responsible for the lack of LES relaxation,[70,73] as it has been shown in other GI diseases that also involve a lack of inhibitory neurons, particularly in Hirschsprung disease.[74]

The evidence that suggests that VIP may also be important comes from the observation that it has been found to be reduced or completely lacking in patients with achalasia.[69,71,75] It has also been shown that the nerve endings in patients with achalasia are rare, contain few synaptic vesicles, and are particularly lacking in the large granular ones, which are those considered to contain VIP.[68] Furthermore, a study of six patients with achalasia, in which VIP was administered intravenously, showed that whereas normal controls did not show any changes in LES pressure after the VIP infusion, in patients with achalasia, there was a significant decrease in a dose-dependent manner. There was also a significant increase in the percentage of LES relaxation after swallowing. These findings suggest that there is a supersensitive response to VIP in the smooth muscle of patients with achalasia.[69]

Indirect evidence for the lack of inhibitory innervation in patients with achalasia has also been provided by hormonal studies. It was initially shown that in the cat, the effect of cholecystokinin-octapeptide (CCK-OP) on the LES involves two different opposing mechanisms: on the one hand, it elicits an indirect inhibitory effect by stimulating inhibitory nerves that mediate physiologic LES relaxation; on the other hand, it also has a direct excitatory effect by stimulating excitatory receptors located on the LES smooth muscle.[76] In cats, the net effect of CCK-OP administration is LES relaxation, but after pharmacologic denervation with tetrodotoxin, CCK-OP produced LES contraction. In normal human volunteers, the administration of CCK-OP results in LES relaxation.[76–78] Holloway and colleagues and others showed that when CCK-OP is administered to patients with achalasia, there is a paradoxic LES contraction in 90%, in contrast to the normal LES relaxation obtained in normal people, suggesting that this LES contraction in the patient with achalasia may be the result of the postganglionic inhibitory denervation of the LES that has been described in the cat.[54,55,68]

Even though the main problem seems to be neurogenic in origin, minor changes in the smooth muscle of the esophagus have also been noted.[57,79] It has recently been suggested that the interstitial cells of Cajal could also be involved in the pathogenesis of achalasia.[67] These authors found that the interstitial cells of Cajal present in patients with achalasia are fewer in number and more highly modified than those in normal patients or patients with other esophageal disorders. Other studies have found that there is a disruption in the contact between the nerves and the interstitial cells of Cajal, which may result in diminished conduction and resultant atrophy.[80,81] Because their function is not known, it is difficult to know which role they play, if any, in the pathogenesis of achalasia.

Although the denervation is mainly confined to the esophagus, abnormalities in other organs have been described. It has been shown that whereas 91% of achalasic patients studied had absent ganglia in the distal esophagus, 20% had no ganglia in the middle third of the stomach.[63] Also, in some patients, degenerative changes have also been found in extraesophageal vagal fibers and in the brainstem vagal motor nuclei, so it is expected that achalasic patients may have functional derangement of other alimentary tract organs under vagal control.[57,82,83] The exact nature of gastric involvement has been controversial. Different results have been found in gastric emptying studies, with some authors finding alterations with delayed solid emptying and increased liquid emptying, suggesting vagal dysfunction,[84] whereas others found normal patterns.[63] Other authors have found a decreased postprandial fundus relaxation, suggesting autonomic neural damage,[85] whereas others have described abnormalities in acid secretion although gastric emptying is normal, raising the possibility that the alterations observed are probably secondary only to intrinsic abnormalities in the myenteric plexus. Finally, some authors have reported abnormal gastric secretory responses to insulin stimulation and also an abnormal response in pancreatic polypeptide release after sham feeding, suggesting some denervation, but it was not clear if the problem was intrinsic to the stomach or secondary to vagal dysfunction.[57]

Other GI organs can also be affected. Abnormal gallbladder emptying has been described.[82] In a study of ambulatory jejunal motility in 13 patients, it was shown that they all exhibited abnormal findings that ranged from loss of cyclic activity, abnormal migration of phase III, abnormal fed patterns, and giant migrating contractions or retrograde contractions.[83]

Numerous theories exist regarding the pathogenesis of achalasia. Theories suggesting that the defect is genetic, neurogenic, myogenic, hormonal, or infectious have been postulated.[56,62] In the United States, the great majority of cases have no known cause,[57] although in certain parts of the world, Chagas disease of the esophagus can produce achalasia,[86] and achalasia has also been diagnosed in certain cases of carcinoma.[87,88] In one study, it was found by using deoxyribonucleic acid (DNA) hybridization that there was a significantly higher presence of varicella-zoster virus in tissue obtained at cardiomyotomy in three patients with achalasia, raising the possibility that this virus could be of etiologic importance.[89] This finding has not been replicated by others. Recently, myenteric neuronal antibodies have been isolated in some patients with achalasia, suggesting that there may be an autoimmune mechansim,[90] but the etiology is still unknown.

Genetics. The influence of genetic factors remains to be assessed. The clustering of esophageal motor disorders in families and the occurrence of achalasia in certain syndromes have led to the suggestion that genetic factors may play an important role in the etiology of the disease.[66] Many cases of achalasia in siblings have been reported, including in monozygotic twins,[91] and inheritance as an autosomal recessive trait has been suggested because of the lack of consistent vertical transmission, the clustering of cases in families, and the occurrence of the disease in father and son.[91–93] On the other hand, there is one report in which there was a lack of concordance of achalasia in monozygotic twins,[94] and the role of genetic predisposition seemed minimal when two large community-based studies failed to identify any family clusters.[47]

It has also been suggested that familial achalasia may be different from the nonfamilial variety, being either congenital in origin or more virulent.[66] Other arguments in favor of a possible congenital origin in subpopulations of patients are the occurrence of achalasia in the first 2 months of life[66] and a marked difference in sex ratio, with male preponderance among the familial cases. Also, a third of published familial cases are the product of consanguineous parents, a figure that again suggests a congenital disease caused by a rare recessive gene. It should therefore be recommended that all siblings of children with confirmed achalasia be studied, particularly if they are the product of consanguineous parents.

Others have proposed that there is an association of different human leukocyte antigen (HLA) types with idiopathic achalasia. Wong and colleagues found an association between HLA-DQW1 and patients with idiopathic achalasia,[95] whereas Verne and colleagues did not find an association with HLA-DQ1, although they did find an association between whites with achalasia and those with the DQB1*0602 allele.[96] Because the numbers are small in these studies, subgroup analysis is difficult, but it does provide hypotheses for future studies.

Associated Conditions. Achalasia has been associated with adrenocorticotropic hormone insensitivity and alacrima (triple-A or Allgrove syndrome).[97–99] The disorder seems to be inherited in an autosomal recessive manner and has been linked in some patients to markers on chromosome 12 q13.[100–102] In an article by Handschug and colleagues, all 47 families studied had a mutation in a 6 cM region on chromosome 12q13.[101] Usually, the alacrima has been present since birth, but hypoglycemia, usually associated with addisonian skin pigmentation, is the presenting symptom, starting before the age of 5 years. The achalasia is usually diagnosed either at the same time or after the cortisol deficiency, but its diagnosis may precede that of cortisol deficiency by 1 to 4 years. Investigation for glucocorticoid deficiency should therefore be undertaken in

cases of achalasia when parents are consanguineous, if the age at onset of symptoms is very young, and if the patient is male. In a study of 20 patients, neurologic abnormalities were also found, including hyperreflexia, muscle weakness, dysarthria, and ataxia, together with impaired intelligence and abnormal autonomic function, particularly postural hypotension.[97] These abnormalities indicate alterations in both central and peripheral neurons. Because there are many known similarities between the enteric and central nervous systems, it seems very likely that the alacrima, autonomic dysfunction, and abnormalities of the CNS have an origin in common with achalasia, and it is possible that there is a primary abnormality in parasympathetic function.[97–99] Achalasia has also been associated with an autosomal recessive syndrome consisting of deafness, vitiligo, short stature, and muscle weakness, as well as with familial dysautonomia with hypophosphatemic rickets (Rozychi syndrome).[97]

It has also been suggested that achalasia may be more frequent in patients with Down syndrome.[103,104] This has been studied prospectively by Zarate and colleagues, who found a high incidence of esophageal dysmotility in children and adults with Down syndrome. They found that in their 58 patients, 2 had achalasia by manometry, whereas a high proportion of other patients had other types of dysmotility diagnosed by manometry, nuclear medicine esophagography, or barium swallow.[105] A wide variety of esophageal abnormalities have been found in children with Pierre Robin syndrome; in a case series of 35 patients by Baujat and colleagues, 94% had abnormal manometries, of which 43% had LES hypertonia and 46% had an LES that did not relax. These children also had significant esophageal dyskinesia and UES dysfunction.[106] It may also be associated with pyloric stenosis, Hodgkin disease, and Hirschsprung disease. However, in a survey of 126 patients with achalasia and their first-degree relatives, it was found that there was no increased incidence of these conditions in either the patients or their relatives.[107]

Clinical Presentation. The usual age of presentation in adults is during the third and fourth decades of life. In a literature review of 167 patients, 57% were older than 6 years, with only 22% between 1 and 5 years, 15% between 30 days and 1 year, and 5.3% less than 30 days.[45] Even though it is a rare occurrence, the condition can present in the neonatal period,[60,62,108,109] with the youngest patient reported being a 900 g, 14-day-old premature infant.[93,110] The mean age at the time of diagnosis in the pediatric patients, taken from a recompilation of all of the pediatric series available (Tables 26-4 and 26-5),[45,52,53,58,59,62,64,65,92,98,110–148] was 8.8 years (range neonate to 17 years). The mean duration of symptoms prior to diagnosis was 23 months (range 1 month to 8 years). It has been suggested that in adults, there is a female preponderance, although in children, there is conflicting information, with authors describing a preponderance of males,[51,112] no difference,[111] and a female preponderance.[45] From the review, the female-to-male ratio was 1.1 to 1.

TABLE 26-4 CLINICAL SYMPTOMS IN 528 PATIENTS WITH ACHALASIA

SYMPTOMS	% OF CHILDREN
Vomiting	80
Dysphagia	75
Weight loss	64
Respiratory symptoms	44
Chest pain/odynophagia	45
Failure to thrive	31
Nocturnal regurgitation	21

This table is a recompilation of 23 pediatric series in which symptoms were reported.[45,58,59,62,64,65,92,111–124,146,148]

Table 26-4 presents the symptoms of presentation of achalasia in children, taking the pediatric series in which symptoms were reported.[45,58,59,62,64,65,92,111–24,146,148] The table summarizes the presentation of 528 children. Younger children tend to have symptoms of refusal to eat, although some may present as if they have GER. Respiratory symptoms predominate, with choking, recurrent pneumonias, and nocturnal cough. Older children have symptoms that are similar to adults, with dysphagia, regurgitation, and retrosternal pain being the most prominent.

As can be seen in Table 26-4, the most prominent symptoms on presentation are vomiting (80%) and dysphagia (75%). The dysphagia occurs initially with solids, but as the disease progresses, it occurs also with liquids.[149] Patients describe the sensation of food getting caught in the middle to lower chest,[45,112] and the children are usually noted to be very slow eaters and to swallow repeatedly to get food passed into the stomach.[59,112] Vomiting may manifest initially as food remnants on the child's pillow and progresses to severe vomiting and an inability to eat, with consequent weight loss.[112,117–119] The regurgitated food usually looks much as it did when it was swallowed and is not mixed with gastric juice.[117]

In contrast to adults, retrosternal pain does not seem to be a common complaint in children, having been reported in 1 to 50% of cases. From the 528 patients reported in Table 26-4, chest pain was present in 45%. When present, it is described as sharp and retrosternal and can be aggravated by the passage of food. Weight loss can be severe,[45] and, particularly in younger children, failure to thrive, aspiration, and recurrent pneumonia dominate the clinical history.[59,92,117,121,149] Sudden death from aspiration of esophageal contents has been reported,[109,149] and, overall, the respiratory complications in children are more prominent than those in adults.[45,149] The diagnosis in young infants can be difficult. The primary manifestations tend to be regurgitation and respiratory problems, so there is an overlap with infants who have other more common conditions such as GER.[150]

Methods of Diagnosis. R*adiography*. A plain chest radiograph may show a widened mediastinum and an air-fluid level and should be a clue to the diagnosis. Another feature that may be present is a lack of air in the stomach.[45] Radiographic features in a barium swallow include variable degrees of esophageal dilatation with tapering at the esophageal junction, which is sometimes referred to as beaking (Figure 26-2).[45,92,119] The esophageal dilata-

TABLE 26-5 TREATMENT OF ACHALASIA IN CHILDREN*

LEAD AUTHOR	PROCEDURE	NO. OF PATIENTS	COMPLICATIONS (%)	LATE RESULTS EXCELLENT (%)	GOOD (%)	FAIR (%)	POOR (%)	LATE COMPLICATIONS (%)	COMMENTS
Moersch[52]	Hydrostatic dilatation	12	0	12	0	0	0	NR	
Olsen[53]	Hydrostatic dilatation	11	0	7	0	0	4	NR	All poor results were in children < 9 yr old
Payne[120]	Hydrostatic dilatation	17	0	1	1	5	10	Dysphagia 58.8%	Multiple repeat dilatations needed (2–12); 4 of the 5 failed dilatations
	Modified Heller	5	1 lung empyema	4	1	0	0	0	
Paul[125]	Dilatation with Mosher bag	1		0	0	0	1	NR	
	Modified Heller	4	1 perforation	2	2	0	0	NR	
Swenson[59]	PD	6	0	4	0	0	2	NR	All required redilatation after 6 mo (mean no. of dilatations 2)
	Heller	2	0	2	0	0	0	NR	
Redo[126]	PD	2	0	2	0	0	0	NR	
	Heller	2	0	2	0	0	0	NR	
Polk[110]	Modified Heller	5	0	5	0	0	0	0	
Cloud[65]	Heller	7	0	6	1	0	0	Dysphagia (1)	1 required dilatation 4 yr after surgery
Tachovsky[122]	Modified Heller	14	1 empyema	12	1	1	0	Dysphagia (1); reflux (1)	All patients had dilatations before surgery; 1 required dilatations after surgery
Desai[58]	Modified Heller	6	1 perforation; 1 atelectasis	6	0	0	0	NR	
Ballantine[113]	Modified Heller	9	1 intussusception; 1 adhesion	7	0	2	0	Reflux (2)	3/9 also had a fundoplication; 2 without fundoplication developed GER and required the operation
Azizkhan[111]	PD (Mosher bag)	20	1 aspiration	2	3	2	13	Dysphagia (15); reflux(1)	The 5 who improved required an average of 2 dilatations each
	Modified Heller	12	1 bleeding	11	0	0	1	Dysphagia (1); reflux (1)	1 required a postoperative dilatation and eventually repeat myotomy
Accumulated case reports[111]	Modified Heller	20	NR	18	1	1	0	NR	
Boyle[112]	Hydrostatic dilatation	10	1 severe pain; 2 fever	6	2	0	2	NR	Some of these patients are probably also included in the study by Nakayama et al[119]
Berquist [45]	PD	10	1 oropharyngeal hematoma	6	0	0	4	NR	2 of the patients required more than one dilatation; 11 required postoperative dilatations; 4 had myotomy and fundoplications
	Modified Heller	12	0	1	0	0	11	Dysphagia (8)	
Koch[128]	Modified Heller	7	1 paraesophageal hernia	5	2	0	0	Dysphagia (2)	All also had fundoplications

continued

TABLE 26-5 Continued

LEAD AUTHOR	PROCEDURE	NO. OF PATIENTS	COMPLICATIONS (%)	LATE RESULTS EXCELLENT (%)	GOOD (%)	FAIR (%)	POOR (%)	LATE COMPLICATIONS (%)	COMMENTS
Buick[62]	Modified Heller	15	0	9	3	2	1	Reflux (3)	6 had myotomy and fundoplications; 3 without fundoplication developed GER and required the operation
Lemmer[92]	Modified Heller	6	0	6	0	0	0	0	3 had myotomy and fundoplication
Nakayama[119]	PD	15	1 severe pain; 2 fever	11	0	0	4	Dysphagia (4)	4 required myotomy; 7 required 2 dilatations;
	Modified Heller	8	1 epiphrenic diverticulum	6	2	0	0	Reflux (2)	1 required 4 dilatations; 1 developed a peptic stricture
Seo[127]	PD	10		4	0	0	6	Reflux (1)	Only study where reflux was
	Modified Heller	6		4	0	0	2	Reflux (2)	with pH probe; 1 developed a peptic stricture
Vane[124]	Modified Heller	21	42% minor	18			3	GER (3)	4 had unsuccessful dilatation;
	Nissen	3	2 perforations	3					2 required fundoplication; 3 had a second myotomy
Nihoul-Fekete[98]	Modified Heller	4		2			2	GER (2)	Largest pediatric series
	Heller and His	10		7			3	GER (3) (1 severe)	from a single institution;
	Heller and Nissen	21	3 perforations	20			1	Dysphagia (1)	follow-up 1–25 yr
Dohrman[129]	PD	5	NR	5					Follow-up 6 yr
Samarasinghe[121]	PD	4	NR	2			2	Dysphagia (2)	2 required repeated dilatation
Allen[114]	Heller and Dor-Gavriliu	6	1 pneumothorax	4	1		1	1 GER-Nissen; 1 dilatation	Bad results if neurologically abnormal
Emblem[116]	Modified Heller and Nissen	12	4 minor	9			3	Dysphagia (1)	1 had gastric interposition
	PD	2	Subphrenic abscess (1)	1			1	Dysphagia (1)	
Illi[64]	Modified Heller	1	4 needed dilatations because of obstruction				1	1 needed esophageal resection	Review of experience in Switzerland
	Heller and fundoplication	15		9			6		
Myers[117]	Transthoracic Heller with antireflux	13	NR	6			6	NR	No results reported in 10 patients
	Transthoracic Heller without antireflux	63		29			26		5 required repeat myotomy, 7 an antireflux procedure, 2 esophagocoloplasty
	Transabdominal Heller with antireflux	66		59			6		2 deaths
	Transabdominal Heller without antireflux	22		16			6		
Holcomb[130]	Laparoscopic Heller	2	0	2				0	Laparoscopic Heller; 18 mo follow-up
Perisic[132]	PD	12		10			2	NR	
	Myotomy	2		2					

continued

TABLE 26-5 Continued

LEAD AUTHOR	PROCEDURE	NO. OF PATIENTS	COMPLICATIONS (%)	LATE RESULTS EXCELLENT (%)	GOOD (%)	FAIR (%)	POOR (%)	LATE COMPLICATIONS (%)	COMMENTS
Hammond[131]	PD	4	0	3			1		Tandem PD; multiple dilatation required
Khoshoo[133]	Botulinum toxin	3	0	3				0	Short-term effect
Lelli[115]	Transthoracic Heller without antireflux	14	2 early dysphagia; 1 required esophageal dilatation	12	2			0	Experience of 21 yr
	Transthoracic Heller with antireflux	5	4/5 early dysphagia; all required esophageal dilatation postoperatively for 1 yr	3	2				Diagnosis made by UGI
Mattioli[134]	Laparoscopic Heller with Dor fundoplication	2	0	2				0	Laparascopic Heller
Morris-Stiff[118]	Modified Heller with Nissen	10	2 chest infections; 1 wound infection	8	1	1		0	5 had failed PD previously
Porras[135]	Heller	2	NR	2					Last 2 patients had failed multiple previous Heller myotomies at another facility
	Wendel	1	NR	1					
	Heyrowsky	1	NR	1					
Robertson[136]	Thoracoscopic esophagomyotomy	1	0	1				0	Had failed 5 PD; flu 12 mo
Walton[138]	Botulinum toxin	1		1					8-mo follow-up
Wilkinson[139]	PD	3		2			1	1 required a myotomy	All had multiple dilatations
Thomas[123]	Myotomy no fundoplication	2	1 death from intestinal obstruction			1	1	Respiratory problems	
	Heller with fundoplication	4		3		1			
Tovar[137]	PD	7	NR	1			6	Required Hellller	Follow-up 7.6–7.5 yr
	Heller myotomy	17		17					
Hurwitz[140]	Botulinum toxin	25			3		22	All required further treatment after the first injection; 17 needed either PD or surgery	Follow-up 1–36 mo
Ip[141]	Botulinum toxin	7			5		2	2 required Heller myotomy; all had more than 1 injection	
Babu[142]	PD	5	1 required multiple dilatations	4			1	0	Follow-up 1–3.5 yr
Mehra[143]	Laparoscopic or thoracoscopic myotomy without fundoplication	5	2 intraoperative perforations requiring conversion to open procedure	20		2		2 patients who had fundoplications required balloon dilatations postoperatively	
	With fundoplication	17							

continued

TABLE 26-5 Continued

LEAD AUTHOR	PROCEDURE	NO. OF PATIENTS	COMPLICATIONS (%)	LATE RESULTS EXCELLENT (%)	GOOD (%)	FAIR (%)	POOR (%)	LATE COMPLICATIONS (%)	COMMENTS
Rothenberg[144]	Laparoscopic or thoracoscopic myotomy without fundoplication	4	1 esophageal perforation	7		1	1	1 patient who received a thoracoscopic myotomy underwent a subsequent laparoscopic myotomy and fundoplication; 1 patient with persistent dysphagia	
	With fundoplication	5							
Patti[145]	Laparoscopic Heller myotomy with fundoplication	13	0	13					
Karnak[146]	Modified Heller myotomy without fundoplication	14	2 patients had stomach perforations; 1 patient had a transection of the vagus nerve	14	1		4	5 with abnormal peristalsis on UGI; 2 with esophageal stenosis; 1 lost to follow-up; 1 death from unknown causes	Authors did not discuss in which group (with or without fundoplication) the complications occurred
	Modified Heller with fundoplication	6							
Upadhyaya[147]	Hydrostatic dilatation	12	0	12				2 required a second dilatation but were then symptom free	Follow-up 3 mo–8 yr; patients' diagnoses by barium swallow only
Hussain[148]	Dilatation	7			0	0	9	All required myotomy	
	Rubber dilators	2		0				17/29 had Heller myotomy as their initial treatment; 6/7 required multiple injections	
	Pneumatic								2 had residual achalasia
	Heller myotomy	29		15	4	7			
	Botulinum toxin	7	1			6			
SUMMARY									
	PD	107	8 (7.5)	57 (53.2)	3 (2.8)	2 (1.9)	45 (42)	Dysphagia 31 (29); GER 3 (2.8); myotomy 40 (37.4); deaths 0	Many patients require multiple dilatations and may require surgery
	Modified Heller only	329	31 (9.4)	226 (68.7)	20 (6.1)	14 (4.3)	56 (17)	Myotomy only: dysphagia 16 (4.9%); GER 15 (4.6); fundoplication 12 (3.6); stricture 2 (0.6); second myotomy 13 (0.9); esophageal resection 3 (0.9); dilatation 15 (4.6); deaths 1 (0.3)	Total (all combined): dysphagia 26 (5.2); GER 19 (3.8); reoperation 17 (3.4); esophageal resection 4 (0.8); deaths 3 (0.6)

continued

TABLE 26-5 Continued

LEAD AUTHOR	PROCEDURE	NO. OF PATIENTS	COMPLICATIONS (%)	LATE RESULTS				LATE COMPLICATIONS (%)	COMMENTS
				EXCELLENT (%)	GOOD (%)	FAIR (%)	POOR (%)		
	Modified Heller with fundoplication	175	22 (12.6)	138 (78.8)	7 (4.0)	2 (1.1)	23 (13.1)	Myotomy and fundoplication: dysphagia 10 (5.7); GER 4 (2.3); reoperation 4 (2.3); esophageal resection 1 (0.6); deaths 2 (1.1)	
	Laparoscopic/ thoracoscopic myotomy ± fundoplication	47	3 (6.4)	43 (91.4)	0	3 (6.3)	1 (2.1)	2 patients required balloon dilatations after fundoplication, 1 required a second myotomy and fundoplication	
	Botulinum toxin	43	0	5 (11.6)	8 (19.0)	6 (14.0)	24 (56.0)	Repeated injections necessary; many needed PD or surgery	

*This represents a recompilation of all of the pediatric series. See the summary at the bottom.
GER = gastroesophageal reflux; NR = not reported; PD = pneumatic dilatation; UGI = upper gastrointestinal series.

tion may be severe, with the esophagus occupying the whole mediastinum, and it may assume an S shape, a form that some authors have called the sigmoid esophagus.[149] There may also be absence of peristalsis, tertiary contractions, and failure of LES relaxation.[119,151] Occasionally, an epiphrenic diverticulum may be observed. The barium swallow has been shown to be useful for the evaluation of patients after treatment.[151] Parkman and colleagues prospectively studied 96 adult patients referred for dysphagia. All of the patients received manometry, a barium swallow, and esophageal transit scintigraphy.[152] They found that, using manometry as the gold standard for the diagnosis of achalasia, the positive predictive value of a barium swallow to make the diagnosis of achalasia was 96%. The sensitivity was 100%, and the specificity was 98%.[152] Interestingly, although the barium swallow is good for diagnosis, the correlation of severity of disease assessed by barium swallow and the patients' symptoms is very poor.[153]

Endoscopy. The main clinical use of upper endoscopy in these patients is to exclude a malignancy or another cause of secondary achalasia, so the esophagogastric junction needs to be carefully examined. Berquist and colleagues and others reported that all of the children with achalasia who underwent endoscopy had esophageal dilatation and that the gastroesophageal junction did not distend with air insufflation.[45,148] Despite the achalasia, the endoscopists in Berquist and colleagues' study were able to get into the stomach in all patients.[45] Endoscopy also provides information about the esophageal mucosa before treatment is undertaken, particularly to assess the presence of inflammation or infection. It is also helpful to remove retained food particles.[148] Endoscopic ultrasonography can be used to evaluate the muscle thickness of the LES, but there is overlap between the thickness in patients with achalasia and control patients, so the technique is primarily used to rule out infiltrative disorders that may be causing the achalasia.[154]

Esophageal Motility. Esophageal motility remains the study of choice to make the diagnosis and provides quantitative information about the severity of the condition and the response to treatment (Figure 26-3).[117,155] Four manometric findings are characteristic of achalasia[48,49,117,137,155]:

Absence of Esophageal Peristalsis. This lack of esophageal peristalsis is the hallmark of the disease (see Figure 26-3).[48,49] Usually, the aperistalsis involves the entire length of the esophagus, and tertiary waves of low amplitude have been described.[48,49] If the amplitude of these tertiary contractions is greater than 50 or 60 mm Hg or if three or more pressure waves appear in response to a single swallow, the condition is usually known as vigorous achalasia.[48,49]

Increased LES Pressure. LES pressure has been described as being elevated, usually twice normal, in the majority of patients.[49,155,156] It is important to point out that even though, as a group, patients with achalasia have higher LES pressure, there is enough overlap with normal people that normal LES pressure does not exclude the diagnosis. Van Herwaarden and colleagues performed 24-hour pH/manometry recordings on adult patients and

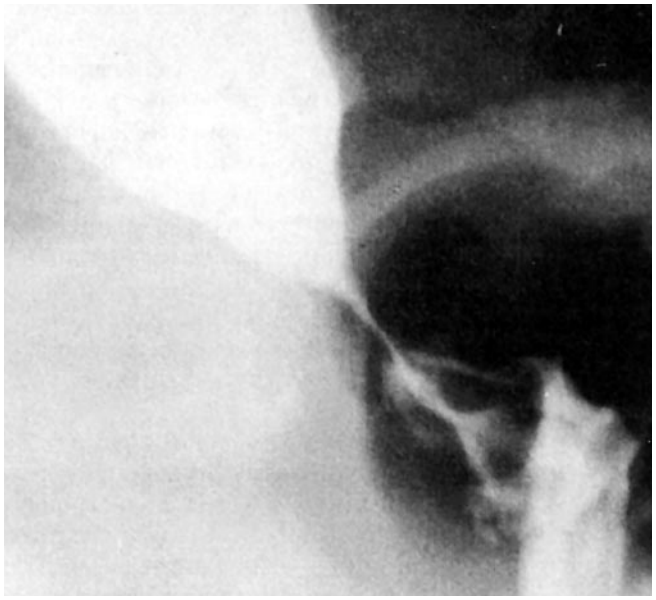

FIGURE 26-2 Barium swallow in a child with achalasia. Note esophageal dilatation and beaking.

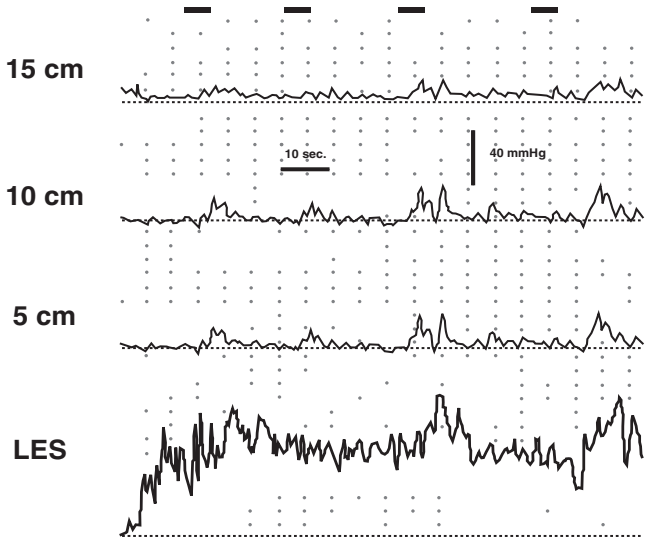

FIGURE 26-3 Esophageal manometry in a patient with achalasia. Note the high lower esophageal sphincter (LES) pressure, the lack of LES relaxation, and the lack of peristalsis after wet swallows. The distance above the LES is indicated in cm.

found that LES pressures were lower after meals and during sleep compared with preprandial recordings.[157] Hussain and colleagues reported in their series of 33 children with achalasia that 95.5% had increased LES pressures.[148]

Incomplete or Abnormal LES Relaxation. In normal individuals, LES relaxation is usually 100%, but in patients with achalasia, it usually represents less than 30% (see Figure 26-3).[48,49] However, the LES may also show complete relaxation. In a study of 23 adult patients with clinical and radiologic manifestations of achalasia, 30% had aperistalsis but complete LES relaxation.[158] Van Herwaarden and colleagues, using 24-hour manometry recording, showed that in 63.6% of their patients, there were at least occasional complete LES relaxations.[157] Katz and colleagues found that the relaxations were of shorter duration than the duration in normal controls, and esophageal emptying in these patients was delayed.[158] This study and others have shown that the relaxation was of shorter duration than the duration in normal controls, and esophageal emptying in these patients was delayed.[159]

Morera and colleagues examined the manometric tracings of 29 children who had achalasia diagnosed by barium swallow and absense of peristalsis by manometry. Fifty-seven percent of these patients had normal LES pressure. Additionally, 13.8% of children had no LES relaxation, whereas 87% of patients had some LES relaxation.[160]

It was suggested that incomplete LES relaxation may represent early stages of the disease, and one patient had progression from complete LES relaxation to incomplete relaxation over a period of 2 years. In another study of 135 adult patients without peristalsis, Vantrappen and colleagues noted that 81% had incomplete LES relaxation and 19% had intermittent normal LES relaxation.[48]

Elevated Intraesophageal Pressure Compared with Intragastric Pressure. This is the result of the functional obstruction at the level of the LES, and it is usually a useful clue to the diagnosis. In a study of 50 patients with achalasia, the esophageal pressure was 6.1 ± 0.7 mm Hg higher than the fundic pressure in 45 patients.[156] There was also no correlation between LES pressure and intraesophageal pressure.[156]

It has become apparent that to make the diagnosis of achalasia, absence of esophageal body peristalsis is necessary; other criteria are often fulfilled but are not required. Manometric abnormalities have been found in even the youngest patients. Asch and colleagues reported an infant who became symptomatic at 2 weeks of age in whom there was no esophageal peristalsis, and the LES pressure ranged between 25 and 40 mm Hg.[108] Some patients may initially present with nonspecific manometric findings but may progress manometrically to achalasia.[161]

Little information is available on UES function in patients with achalasia. Recent reports of cases have drawn attention to an association between acute airway obstruction and achalasia and prompted speculation that abnormalities of the pharynx and the UES may be present.[162,163] A study of 19 patients with achalasia found abnormalities in UES function. The major manometric finding was increased residual pressure. The researchers also found a reduction in the duration of UES relaxation with swallowing and a more rapid onset of pharyngeal contraction after relaxation.[163] They found that mean UES residual pressure was increased in patients and that the duration of UES relaxation was shorter. There was no correlation between the degree and duration of symptoms with the manometric abnormalities. Whether all of these abnormalities lead to impaired pharyngeal function is not known, and the clinical significance of these findings remains uncertain. Recently, it has been shown that the UES abnormal residual pressure observed in patients with achalasia decreases significantly after successful pneumatic dilatation (PD), suggesting that it is a secondary phenomenon.[162]

Repeat manometry is usually not necessary after therapy provided that the symptoms disappear. If manometry is done after treatment, the following changes can be expected: the LES pressure is lower, and the LES relaxation remains incomplete, although it is possible to see complete relaxation following a swallow.[137,164,165] It has been suggested that manometric findings after PD correlate with clinical response. In a study of 43 patients, it was found that a decrease of the LES pressure below 17 mm Hg or more than 40% of the pretreatment manometry was associated with a successful outcome.[166] It is still controversial whether peristalsis returns after successful treatment. Because treatment fails to correct the underlying motility disorder, it has always been assumed that the therapy is palliative at best and that the peristaltic defect is a permanent one.[167] There are case reports in which the return of peristalsis has been documented after PD.[48,167–169] In one study, it was found that aboral peristalsis returned in 22 of 69 patients with achalasia after PD.[48] In another study of 34 patients treated successfully with PD, it was found that in 20%, there was a return of distally progressive contraction waves following therapy.[167] There are also reports of return of peristalsis after successful myotomy,[168] and there is one report of a 14-month-old child with achalasia in whom esophageal peristalsis returned 6 months after she underwent a Heller myotomy.[169] These studies have found that there was no correlation between the return of peristalsis and clinical status, the decrease in LES pressure, or the radiographically measured diameter of the esophagus. This "return of peristalsis" has been explained as secondary to a reduction in the diameter of the esophagus (allowing the perfusion catheter to detect the pressure waves) as a result of dilatation or surgery rather than to a reversal of the degenerative process of the esophageal neurogenic structures.[48,137,168] As expected, not all studies have shown a return in peristalsis. In a study of 14 children who underwent stationary and ambulatory esophageal manometry before and after treatment, it was reported that there was no change in the abnormal peristalsis seen, even after long periods of follow-up.[137]

Radionuclide Tests. Recently, radionuclide tests have been used as a screening tool to assess esophageal emptying, particularly before and after therapy. The most commonly used method involves the ingestion of a solid meal labeled with technetium 99m sulfur colloid,[170–172] although it can also be done with the ingestion of liquid. They have also been useful in the differential diagnosis of achalasia and other conditions, such as scleroderma, because the pattern of retention is different, with the patients with achalasia retaining the tracer even in the upright position.[170,171] It is becoming clear that esophageal emptying studies using radionuclide techniques are a simple and noninvasive way to evaluate these patients and the result of the therapy.[170] Parkman and colleagues found that the positive predictive value of esophageal transit scintigraphy for the diagnosis of achalasia was 95%, the sensitivity was 91%, and the specificity was 98% using manometry as the gold standard for diagnosis.[152] The value of radionuclide esophageal emptying studies in the clinical follow-up of these patients remains to be determined. They do not, however, provide a way to predict accurately eventual success in individual patients, and they do not seem to be better than the symptom scores.[170,173] They do remain a useful, objective way to study esophageal function and may have an important future role in the objective outcome when comparing different treatments.

Provocative Tests. Two provocative tests have been used in the past to diagnose achalasia: the Mecholyl test and the administration of CCK-OP. The administration of acetyl-p-methacholine (Mecholyl) in patients with achalasia produces a rise in esophageal baseline pressure and the occurrence of high-amplitude, repetitive contractions in the esophageal body. The test is considered abnormal if there is a rise in esophageal pressure of greater than 25 mm Hg lasting for at least 30 seconds within 8 minutes after methacholine chloride (5 to 10 mg subcutaneously).[54] It is contraindicated in patients with asthma or heart disease, and the unpleasant cholinergic side effects and pain do not justify its routine use.

The administration of CCK-OP has been shown to produce paradoxical contraction in 90% of patients with achalasia.[54] In a study of 24 patients, CCK-OP produced paradoxical contraction in 21 patients and LES relaxation in 6 of 7 controls. These results were compared with those obtained after the Mecholyl test, and both gave a similar percentage of positive responses (90% of patients with achalasia had an abnormal contraction). If both tests are combined, in 94% of patients, either one or the other was abnormal.[54] The CCK-OP paradoxical response is not specific for achalasia because it might be expected to induce LES contraction in any condition in which the innervation to the LES is impaired, as in Chagas disease, diabetic neuropathy, and the neuronal form of pseudo-obstruction. This test has not been used in children, and the exact clinical role of both provocative tests in children remains to be determined, so their routine use should be avoided.

Differential Diagnosis. Achalasia has to be differentiated from other organic causes of esophageal obstruction, particularly infections[174] or benign or malignant neoplasms and benign strictures.[49] Payne and colleagues mention two children with a clinical diagnosis of achalasia who underwent surgical exploration and were found to have leiomyomas of the distal esophagus.[120] Usually, endoscopy and biopsy are the methods of choice to exclude this condition. In adults, a condition mimicking manometrically proven achalasia secondary to carcinoma has been described,[48,175] but this has not been found in children. Even though adenocarcinoma of the stomach is the most common tumor presenting as achalasia, even extraintestinal tumors such as oat cell carcinoma of the lung and pancreatic carcinoma have been reported.[175]

Chagas disease has to be part of the differential diagnosis in areas where it is endemic, particularly in South America.[86] The disease occurs from neuronal damage by the parasite *Trypanosoma cruzi*. The pathologic description of the megaesophagus seen in Chagas disease consists of neuronal destruction of Auerbach plexus, and this can also be seen in

other parts of the GI tract and can also manifest as megacolon. The LES of patients with Chagas disease has been studied by EM. The denervation has been attributed to direct infection by the trypanosoma, and the changes observed in the smooth muscle nerve endings are very similar to those reported in patients with idiopathic achalasia.[67,86] Usually, the damage to the neural plexus of the esophagus occurs late in the disease, and there seems to be a correlation between the severity of the ganglion cell destruction and the symptoms. Dantas and colleagues compared the manometries of patients with Chagas disease with those of patients with idiopathic achalasia. They found that patients with Chagas disease have lower LES pressures, fewer simultaneous contractions, shorter contraction duration, and higher numbers of ineffective contractions compared with patients with idiopathic achalasia.[176]

Patients originally diagnosed as having anorexia nervosa have also been shown manometrically to have achalasia.[64,112,177] In one study, esophageal motor activity was investigated in 30 consecutive patients meeting the standard criteria for the definition of anorexia nervosa, and it was found that 7 patients had achalasia instead of primary anorexia nervosa.[177] Analysis of their data shows that dysphagia for solids and liquids, as well as spontaneous vomiting and regurgitation, was more common among patients with abnormal esophageal motor function. Of interest is the fact that four of the seven patients with achalasia underwent mechanical dilatation, with subsequent disappearance of the symptoms. Two other patients with achalasia were successfully treated with nifedipine. It is important to emphasize that the evaluation of the patients suspected of having primary anorexia nervosa should always include careful assessment of upper GI function and, when clinically indicated, of esophageal motor activity.

Treatment. Because the etiology of achalasia is not well defined, treatment is generally directed at symptomatic relief of the functional obstruction at the level of the LES.[46,155] Multiple treatments aimed at reducing the resistance of the LES pressure have been tried,[178,179] but only invasive techniques have provided long-term benefit.[155,180] PD and surgical EM are still considered the best available options,[155,178–181] but the optimal treatment is still debated.[46,155,180] Because the complications after dilatation or EM may be significant, alternative therapies, such as botulinum toxin, are being sought.[16,164,178–183]

Table 26-5 shows the published reports of treatment of achalasia in children. Diet has no role in the primary treatment of the disease,[59] although a soft diet encourages more rapid esophageal emptying, and elevation of the head of the bed helps prevent nocturnal regurgitation.[155] Nutritional rehabilitation, on the other hand, has to be an important part of the overall management of the patient .

Pharmacologic Treatment. Anticholinergic drugs have been found to be of no value.[49,155] The manipulation of esophageal motility disorders using pharmacologic therapy was unsuccessful until reports on the use of isosorbide dinitrate (long-acting nitrate) and nifedipine (calcium channel blocker) appeared.[56,155,184–186]

Nitrates have the effect of relaxing the smooth muscle.[155,187] Isosorbide dinitrate (5–10 mg) has been shown to cause significant LES relaxation in patients with achalasia and has allowed most patients to eat normal meals. It usually decreases LES pressure by 30 to 65%, resulting in symptom improvement in 53 to 87%.[155] This improvement has been confirmed manometrically and with radionuclide esophageal transit measurements.[187] Long-term use of the drug is associated with a high incidence of either side effects, particularly headache in up to one-third, or early or late failure to respond, raising to 50% the number of patients who have some problems with this drug.[187] In a report of 15 adult patients, 5 patients received isosorbide dinitrate therapy successfully for 8 to 15 months.[187] There is one report of the successful short use of an isosorbide dinitrate patch in an 8 year old.[188]

Calcium channel blockers have been used. Because calcium is directly responsible for the activity of the myofibrils and, consequently, the tension generated, the idea that their use may produce a reduction in LES pressure seems logical. Their usefulness, however, remains limited.[186] Overall, calcium channel blockers decrease LES pressure by 13 to 49% and improve smptoms by varying amounts, which range from 0 to 75%.[56,155] Nifedipine has been shown to decrease LES pressure and the amplitude of the esophageal contractions in normal volunteers.[184] Studies in patients with achalasia have shown that nifedipine (10–20 mg) significantly decreases LES pressure and improves esophageal emptying.[184–186] Nifedipine also decreases the amplitude of esophageal contractions. A report of long-term use of nifedipine from 6 to 18 months showed an excellent response in two-thirds of the patients and rare side effects that included venous dilatation, ankle swelling, heat, and systemic hypotension in one case, all appearing soon after ingestion of the drug. In all cases, with continued use, the side effects either disappeared or decreased in severity. In a double-blind study, nifedipine significantly reduced LES pressure (28% reduction) and had no effect on esophageal emptying. Side effects were common.[184] A comparison between isosorbide dinitrate and nifedipine[187] in adults showed that isosorbide had a more pronounced effect on symptomatic relief, early LES pressure fall (63.5% versus 46.7%), and improvement of esophageal emptying compared with nifedipine. These aspects, however, become blunted because of the higher incidence of side effects and of failure to respond with isosorbide. In one study, it was suggested that patients with slight esophageal dilatation (< 5 cm) on radiography and a good manometric response to nifedipine may be good candidates for its long-term use. The drug was given as 10 to 20 mg sublingually 30 to 45 minutes before each meal. Of 56 patients, 17 did not achieve a response or developed side effects; of the 39 who continued, 13 were still on therapy and 26 stopped after an average of 2.8 years: 17 had PD or EM, 4 for unknown reasons, and 5 recovered.[186]

The experience with nifedipine in children is limited, consisting mainly of case reports.[62,185,189] In the largest report, four adolescents were administered 10 mg of nifedipine 15 minutes before each meal and before

esophageal manometry.[185] In all cases, there was a good clinical response and a manometrically proven fall in LES pressure. Two children developed mild transient headaches, and it was noted that they all experienced a recurrence of their symptoms if the medication was not taken.

Recently, sildenafil has been used to decrease LES pressure, initially in healthy volunteers and then in patients with achalasia.[190,191] Sildenafil blocks phosphodiesterase type 5, an enzyme that is responsible for the destruction of cyclic guanosine monophosphate. When this enzyme is not destroyed because of sildenafil, there is resulting inhibition of smooth muscle. Bortolotti and colleagues, in a double-blind study, gave 14 patients, ages 21 to 64 years old, with idiopathic achalasia either sildenafil or placebo. They found a decrease in LES tone, pressure wave amplitude, and residual pressure compared with baseline in the treatment group. More studies must be done to determine the clinical utility of this class of drugs.[190]

The exact role of long-term pharmacologic therapy remains to be determined. The clinical response appears to be short acting, with prominent side effects and partial remission of symptoms.[155] Long-term pharmacologic therapy may therefore have a role for patients who are very early in their disease with a nondilated esophagus, who are not candidates for PD or EM,[46,155] and who refuse invasive treatment, or who need to achieve some weight gain before more aggressive therapy,[187] as well as for various reasons when definitive therapy has to be postponed, such as to wait for school vacation.[185]

Botulinum Toxin. It has recently been suggested that the intrasphincteric injection of botulinum toxin may be a simple and effective treatment.[46,133,155,179,180,182,192] Botulinum toxin is a neurotoxin that binds to presynaptic cholinergic terminals, thereby inhibiting the release of acetylcholine at the neuromuscular junction and creating a chemical denervation.[193,194] It has been used therapeutically in humans, including children, for a variety of other medical conditions since 1980[193,194] and has been found to be of therapeutic value in the treatment of a variety of neurologic and ophthalmologic disorders. The clinical effect is due primarily to its action at the neuromuscular effective junction, but it can be transported to the spinal cord or brainstem by retrograde delivery.[193,195]

Although the innervation of GI smooth muscle is different from that of striated muscle,[196] the local injection of this neurotoxin in the abnormal LES of patients with achalasia has some theoretical basis.[46,179,180,182] The esophageal abnormalities seen in achalasia result from a loss of inhibitory neurons in the myenteric plexus, resulting in unopposed excitation of the smooth muscle of the LES.[196] This excitatory effect is probably mediated by acetylcholine because the pressure in the LES can be reduced by anticholinergic drugs and increased by drugs that inhibit cholinesterase activity.[54] Botulinum toxin has been shown to reduce LES pressure in patients with achalasia,[164,182] and it does so by decreasing the excitatory cholinergic innervation to the sphincter.[16,197]

Experience with botulinum toxin for the treatment of achalasia is growing.[46,133,155,179,180,182,192] Initially, an open-label study and, later, a double-blind placebo-controlled trial showed that it is an effective, safe, and simple method for the treatment of achalasia in adults.[182,192] The long-term follow-up of the first 31 patients treated has been reported.[196] A good initial response was observed in 28 patients, but during the first 2 to 3 months after injection, 11 patients reported a relapse of their symptoms and required a second injection. Only 20 patients had sustained responses beyond 3 months, 17 after a single injection (54.8%). Nineteen of those 20 patients relapsed and 15 received a second injection, with satisfactory results in only 60%. This experience indicates that frequent injections are necessary and that the response to subsequent treatments may diminish. This need for repeated treatments and loss of effectiveness with each treatment in some patients are also well documented when botulinum toxin is used in patients with skeletal muscle disorders.[193,195]

Since those initial reports, many other uncontrolled[198] and controlled studies have duplicated the effectiveness of botulinum toxin.[155,164] Annese and colleagues completed a randomized controlled trial of botulinum toxin in 118 adult patients.[199] The patients were randomized to receive one of three doses of botulinum toxin (50, 100, and 200 U). The patients who received 100 U were given a second injection 30 days after the first one. The authors found that a much higher percentage of patients who received two doses of 100 U remained in remission compared with those who received a single dose of either 50 or 200 U (68% versus 29% of patients receiving 50 U and 27% of patients receiving 200 U). The severity of the disease prior to injection did not predict who would respond to therapy. These researchers also found that there was no difference in the percentage of patients who were asymptomatic in the group receiving 50 U compared with those receiving 200 U. They found that the only predictor of response to botulinum toxin is the presence of vigorous achalasia.

The information on children appears only in case series.[133,138,140,141] Khoshoo and colleagues reported the first experience with botulinum toxin for the treatment of achalasia in three children.[133] Since then, there have been two larger case series of children with achalasia who received botulinum toxin. The first series by Hurwitz and colleagues examined the efficacy of botulinum toxin in 23 children using data pooled from multiple institutions.[140] They found that 83% of patients had an improvement or resolution of symptoms. Of the 19 patients, approximately 16% required subsequent balloon dilatation, 58% required or were scheduled for surgery at the time of publication, and 16% had balloon dilatation and then surgery. The mean duration of effect for the botulinum toxin was 4.2 ± 4.0 months. Sixty-three percent of patients required more than one injection.

Ip and colleagues published a series of seven children.[141] All patients had an improvement in their symptoms and all patients required repeat injections. In this series, the median length of response was 4 months. The authors reported that the patients who had higher LES pressures at the time of the first injection had a shorter duration of response to botulinum toxin compared with those children with lower initial LES pressures.

With such a limited number of patients, however, it is difficult to know if the response to treatment in children will be similar to the adult experience. It has been noted that younger adults and adults with classic achalasia do not respond as well,[196] raising the possibility that the response rate in children may be even lower. Even though in the adult studies there was no relationship between the response to botulinum toxin and a history of previous dilatations,[182] the only child with a sustained response in the present series had initially undergone one pneumatic dilatation and relapsed. This observation needs to be replicated in controlled studies because it raises the possibility that botulinum toxin in children may have a role in those who failed previous dilatations.[46]

Botulinum toxin has been used in thousands of patients with ocular and spastic muscle disorders, at various doses and without serious reactions,[193,200] and no major morbidity or mortality has been reported with its use for achalasia or other disorders.[193] Occasional chest pain or transient skin rashes have been reported.[193,200] Retrograde axonal transport of trace amounts of botulinum toxin to spinal cord segments has been detected in animals, but the implication that this has for patients, in particular children, is not known but is probably insignificant.[46,193,200] It has also been noticed that in some patients, botulinum toxin stimulates the formation of antibodies, which may neutralize the physiologic effect.[46,193,200]

One concern is that botulinum toxin injections as an initial therapy may affect any future surgical outcomes should these injections fail to provide symptom relief.[183,201] Patti and colleagues began to address this concern.[201] They looked at the outcomes of Heller EM and Dor fundoplication in adults who received no therapy, PD, and botulinum toxin injection prior to surgery. They found that in those patients who received botulinum toxin injections and had symptom improvement for at least 4 months, the surgery was significantly more difficult owing to periesophageal inflammation and fibrosis of the LES. The surgical complication rate in this population was much higher than that of the other study groups and the rates of dysphagia postoperatively were higher in the botulinum toxin responders. These results are very interesting, but, because the numbers in this study are small, a future study would be crucial to help clarify the role of preoperative botulinum toxin. Most authors agree that even though it is useful, it is limited by its short-term efficacy and need for repeated treatments.[180,200,202] Botulinum toxin may have a role in the management of those patients who, for medical reasons, are poor candidates for surgery.[183] It has been suggested that because of its short-term duration of action, it may be useful in guiding therapy before considering more invasive treatments.[202] Candidates for this approach may be patients with symptoms compatible with achalasia but insufficient manometric criteria to establish the diagnosis, complex situations in which there are factors in addition to achalasia contributing to the symptoms that may require a different treatment, atypical manifestations of achalasia, and advanced achalasia in which it is unclear that sphincter-directed therapy (versus esophagectomy) would be of benefit, as well as after Heller myotomy.[202] In a description of this approach, 11 patients with the above characteristics received botulinum toxin, and 10 showed a good response.

In children, botulinum toxin may have the advantage that, depending on the age, it may be administered without general anesthesia and is relatively noninvasive.[46] The main disadvantages include the fact the duration of treatment is short-lived, with the need for multiple treatments over time[46,180,183] and the possibility that surgical therapy may be more difficult.[183]

Before this therapy can be recommended as a first-line treatment for children, more information needs to be gathered. For now it may be a good alternative in those places in which performing PD is not possible, for those children who refuse more invasive therapies, in those in whom the diagnosis is not clear, for those children who may be at high risk for the other procedures or their complications, or for those who have failed other therapies.

Nonpharmacologic Treatment. There have been two main successful modalities to decrease the pressure of the LES and to relieve the functional obstruction. One is surgical, with an EM, and the other involves forceful PD.[155,203] In a simple way, they can be thought of as an approach that tries to relieve the obstruction from the exterior of the esophagus (surgery) and as an approach to do it from within the lumen (PD).

Pneumatic Dilatation. The use of bougienage has been uniformly disappointing,[45,111,119,155,203] and in one series, an incidence of 6% of esophageal perforation was described.[204] Payne and colleagues reported the use of bougienage in eight children, in whom it rarely provided more than very temporary relief.[120] Because of the short-term benefits and the complication rate, this type of therapy is no longer advocated.

PD tries to produce a controlled tear of the LES, resulting in relief of the distal esophageal obstruction and clinical improvement.[155,205] It consists of positioning a dilating device at the level of the LES, with the purpose of inflating it to apply direct pressure on the esophageal muscle to the muscle fibers of the LES.[45,62,121,155] Even though the purpose is to tear the LES fibers, it is interesting that in experimental animals, there is no evidence that the muscle fibers actually tear and do not just stretch. Vantrappen and Janssen have subjected dogs and monkeys to balloon dilatation of the LES, and histologic examination failed to distinguish the sphincter segments of treated animals from those of controls.[206]

The initial experience was reported with the Mosher bag, the Browne-McHardy dilator, and others.[155,206] In general, these are no longer used, and at this point, PD is performed most commonly with Rigiflex dilators (Microvasive, Boston Scientific Corp, Boston, MA).[155] They have been successful, showing high success rates from 70 to 93% and low complications.[164,181,207,208] This is also the most common method of PD currently employed in children. These balloons have a predetermined size and are made of a modified polyethylene polymer mounted on a flexible polyethylene catheter. In children, the most common range used goes from 20 to 30 mm Hg. If necessary, 35 and 40 mm Hg are also available. To

introduce the dilator, a guidewire is placed through the biopsy channel of the endoscope into the stomach. The endoscope is then removed, leaving the guidewire across the esophageal junction. The dilating balloon is introduced over the guidewire and placed so that it is across the LES. The position is then checked fluoroscopically. The balloon is then inflated rapidly in an attempt to obliterate the waste.[155,205] In most centers, it is then maintained inflated for 1 minute and then deflated. The procedure is repeated up to three times with each diameter, and the dilator is removed. The dilation is followed by the administration of contrast to exclude esophageal perforation.

Despite its widespread use, the practice of PD has not been standardized. Recent studies compared the use of different balloon sizes (30–35 mm), number of balloon inflations (one or two), and duration of the inflations (15, 20, 40 or 60 seconds) and found that the outcome did not depend on the technique used.[209–211] In another prospective study, balloon inflations of either 60 or 6 seconds were compared. Significant and sustained improvement was observed in both groups, and there were no perforations.[210] The higher-size balloons have been associated with perforations more frequently.[155]

In adults,[155,212] and in many pediatric centers, PD[112,119] is performed under intravenous sedation, whereas general anesthesia is routinely used in others. In younger children, it is difficult to ensure that movement will be restricted, so general anesthesia may be safer.

The results of PD in adults have been satisfactory, with an overall response rate that varies from 60 to 95% depending on the technique used.[132,155,213] The cumulative experience with the old dilatation methods (Mosher bag, Browne-McHardy dilator, and others) was recently reported as showing symptom improvement in 72% of 2,418 treated patients. The perforation rate was 2.5%.[155] With the use of the Rigiflex, symptomatic relief was reported to be 67 to 93% of patients after a mean follow-up of 0.3 to 4 years.[155]

In a report of their experience with 455 dilatations, Vantrappen and Hellemans found that 77% of patients had excellent results, 8.7% had moderate results, and 14.4% had poor results.[165] There was an overall improvement in 93% of the patients. In 41 of the patients, symptoms recurred and another series of dilatations had to be performed, and approximately 50% of those had a good or an excellent result.

To assess long-term success with PD, Katz and colleagues reviewed their cases of PD between 1971 and 1996.[214] All patients who had a PD were sent a questionnaire to evaluate for persistent symptoms. The mean time from dilatation to follow-up was 6.5 years. Eighty-five percent of the patients had a successful dilatation, which was defined as no further therapy required after two dilatations. Fifteen percent of patients required further therapy such as surgery or botulinum toxin injection. In long-term follow-up, there was no difference in the dysphagia scores between those who had successful PDs compared with those who had dilations followed by surgery or botulinum toxin injections. The authors found no difference in the long-term success across ages, gender, duration of symptoms prior to dilatation, or duration of follow-up.

In a prospective study of 54 adult patients, it was reported that outcome was worse in younger patients and that postdilatation LES pressure was the most valuable factor for predicting the long-term clinical response.[215] In some series, successful long-term results after PD[216] with symptomatic improvement have been lower, around 40%.[216] It has also been shown in another series that 40% of patients treated by a single dilatation required a second dilatation after 5 years.[213] Patients who have undergone a second and a third dilatation required subsequent dilatations in 40 to 75% of cases, respectively.[213] It has also been reported that if the first dilatation is unsuccessful, 38% and 19% improve after a second or third dilatation,[155] suggesting that patients who have poor results originally or a rapid recurrence of their symptoms respond less to subsequent dilatations.

The results of dilatation in children have been variable and are difficult to compare because of the different techniques used. PD has been used successfully in children for a variety of conditions other than achalasia, particularly for peptic strictures, anastomotic strictures, and restrictive Nissen repairs.[217] The use of PD for achalasia in children was first reported by Moersch in 1929.[52] As can be seen in Table 26-5, many authors have used PD with varying results. Overall, there are approximately 107 children reported, in whom there was an overall improvement rate of 56% (60 patients). Perforations were the most frequent complication. Long-term dysphagia persisted in 29% and GER in 2.8%, and 37.4% required a myotomy. Of course, it is difficult to compare different studies and techniques, and the rate of improvement after PD among the different series varies from only 35%[111] to 100%.[52] Moreover, like adults, many children require more than one dilatation to respond. Nakayama and colleagues reported that of their 15 patients, 7 responded to one dilatation, 7 required two, and 1 required four, and in the patients who failed, the duration of symptom relief was much shorter than in the patients with a good response (6 weeks versus 18 months).[119] It has also been suggested that if symptoms recur after PD in the first 6 months, the patient is likely to require a second dilatation[45] or eventually surgery.[127] Some authors have suggested that children who respond to PD are older than 9 years,[111] although many reports of successful PD in children younger than 5 years have appeared.[45,132,218]

Originally, it was also suggested that after a failed myotomy, PD had a prohibitive risk. This, however, has not been the recent experience even in children,[45] five of whom underwent successful PD without complications after EM. Although both PD and EM are safe procedures with very low complication rates, both are potentially dangerous.[46,119]

Complications after PD. Esophageal perforation after pneumatic dilatation has been described in 1 to 12% of cases.[155,164,165,213,219] It has been suggested that older age; the presence of a hiatal hernia, epiphrenic diverticulum, esophagitis, malnutrition, or high-amplitude esophageal contractions; and one or more previous dilatations may increase the risk.[219] Balloon instability or inflating the dilation balloon to 11 psi or higher may also increase the risk.[219,220] Perforation rates also seem to be greater when the larger balloon sizes are used and to be lower if the smaller

balloons are used; only the large sizes are used when the patient does not respond to initial dilatation.[207] In a compilation of adult series, there were 7 perforations in 345 patients treated with Rigiflex balloons.[155] Sixty percent occurred after a 4.0 cm balloon was used, whereas a 3.5 cm balloon was responsible for the other 40%.[155] Perforation, however, also occurred after the 3.0 cm balloon.[155,221]

Okike and colleagues, in their study of 899 patients with achalasia, found that the rate of esophageal perforation following PD was 4%, and following EM, it was 1%.[222] Reviewing their own experience, Vantrappen and Hellemans reported an incidence of 2.6% of esophageal perforation and 4.3% of other complications (fever, pain, or pleural effusion) in a total of 537 patients with three or four dilatations each.[165] A survey of 1,224 instances of PD for achalasia completed by the American Society of Gastrointestinal Endoscopy noted a 1.8% incidence of esophageal perforation.[223] The perforation usually occurs at the anterolateral esophageal wall.

The exact incidence of this complication in children is not known, although a recent study reported perforation in 3 of 50 procedures (6%).[221] Two occurred after the 3.5 cm dilator was used and one occurred after the 3.0 cm dilator was used. As can be seen in Table 26-5, there was a reported incidence of perforation of 5.3%. The symptoms after perforation include severe and persistent chest pain (longer than 4 hours), usually fever, and unexplained tachycardia. Dysphagia and subcutaneous emphysema may also be seen.[155] A plain chest radiograph may reveal subcutaneous or mediastinal emphysema or a left-sided pleural effusion. A definitive diagnosis is made with the use of a water-soluble contrast esophagogram.[155] Some centers recommend the routine use of a postdilatation esophagogram[224] because there are case reports of an esophageal perforation that was missed because of the paucity of symptoms, resulting in the patient's death.[222] In a prospective series of 41 patients undergoing PD and an early postprocedure esophagogram, it was found that there were two immediate and two delayed esophageal perforations, for a perforation rate of 9.5%.[224] This illustrates the fact that delayed perforations of the esophagus can occur and may not be present after the first study, emphasizing the need to closely observe all patients and to obtain or repeat contrast studies if symptoms develop. By performing early contrast studies, these researchers also found six intramural hematomas, one of which progressed to a free perforation, whereas the other five resolved spontaneously. They also showed that there is no correlation between the postdilatation appearance of the esophagus and the clinical outcome, suggesting that the esophagogram should be performed only to exclude complications and not to assess the adequacy of the forceful dilatation or to predict clinical outcome. Prior PD and the use of more than 11 psi with a Browne-McHardy dilator were found as risk factors for perforation.[220] Also, patients with a tortuous esophagus, esophageal diverticula, or previous surgery at the gastroesophageal junction may have a higher risk for perforation.[155,225]

The mortality after a perforation has been reported to range from 0 to 50%.[165,219,222] It is now recognized that if the perforation is diagnosed and treated promptly, the outcome is similar to that of patients who undergo elective myotomy.[221,226] Some authors prefer a nonoperative management of esophageal perforations,[165,221] but others continue to advocate aggressive surgical management.[227] Miller and Tiszenkel described their surgical management of six patients with esophageal perforation after PD. They advocated suturing the perforation followed by a modified Heller procedure, reporting excellent results.[227] Vantrappen and Hellemans reported that 10 of 13 patients with esophageal perforation healed with total parenteral nutrition and broad-spectrum antibiotics for 2 weeks.[165] In 4 patients, a pleural effusion was evacuated by puncture or drainage under local anesthesia. Two other patients needed surgical drainage, and the last patient died. In a summary of the literature, Wong and Johnson found 53 perforations reported, with only 17 patients having undergone surgery.[228] A mortality of 4% was noted, and, overall, 68% of the patients were treated conservatively. In the only series in children, perforation was successfully managed with the use of total parenteral nutrition and intravenous antibiotics.[221] These children were again eating by mouth 7 to 10 days after the perforation. It is now suggested by some that medical therapy may be appropriate for small perforations without significant mediastinal contamination, when the cavity drains back into the esophagus, and when there is an absence of communication with the pleural space and no evidence of sepsis.[221,229] Medical therapy needs to include measures to avoid mediastinal contamination (by giving nothing by mouth), neutralization of gastric acid, and administration of intravenous antibiotics.[221,226,227]

GER can also be a late complication of PD. This complication has been described in adults to range from 1 to 9%.[155,165] Disabling reflux in children has also been described,[111] and some other reports show an incidence of 12% compared with 36% after myotomy.[127] In a prospective study comparing pH measurements before and after PD, the percent pH < 4 increased from 2.9 ± 4.9 to 10.2 ± 15.9% after PD.[230] As can be seen in Table 26-5, GER in children was reported to occur in close to 2% of the patients.

Another complication that has been rarely reported after PD is bleeding. In a review of 1,261 cases, there was an incidence of 1.1% of moderate hemorrhages.[165]

Surgery. The surgical treatment varies, but it involves performing a myotomy, with or without an antireflux procedure.[155] The most common EM employed is the Heller myotomy and its variants, which consist of either a thoracic or an abdominal approach and a vertical incision of the esophagus extending along the serosal surface of the distal esophagus and transecting the circular muscle fibers that make up the LES.[56,231] The recent advent of minimally invasive surgery is changing the surgical approach, resulting in shorter patient hospital stay, reduced morbidity, and quick return to activities.[56,155]

Good to excellent results have been obtained after EM in 64 to 94% of adult patients.[17,56,222,231–234] In their report of 468 patients with achalasia who underwent myotomy, Okike and colleagues reported excellent to good long-term results in 85% of the surgically treated patients compared with 65%

of a group of 431 patients treated by PD.[222] In a recent review of the literature, Vaezi and Richter reported an overall good to excellent symptom improvement in 83% of 2,660 patients undergoing EM through the abdominal approach and in 83% of 1,210 patients who had a transthoracic EM.[155] LES pressure was reduced by a similar degree (74% versus 79%), and the operative mortality from both procedures was low (0.2% versus 1%).[56,155] When surgery has been performed after failed PD, the results have also been satisfactory, and previous dilatation does not seem to alter the response to surgery. Furthermore, the literature shows that after a failed dilatation, surgery is usually successful.[119,222]

As can be seen in Table 26-5, from 526 children evaluated after surgery, 77.5% had excellent to good results. The improvement rate varies among series, going from as low as 10%[45] to as high as l00%.[64,98,110,125,126] The youngest successful patient reported was 6 weeks of age,[60] and there are numerous reports of successful EM in infants less than 6 months.[98,117] The results after surgery are not uniformly good. In the series of Berquist and colleagues, of five patients who underwent an EM as primary therapy, four had recurrence of symptoms 1 month to 5 years following surgery, and either repeat dilatation or surgical intervention was necessary to relieve the symptoms.[45] Most series, however, report excellent results, and it is interesting to note that most of the recently reported series of achalasia in children have used an EM as a primary treatment.[98,114,116,123,124,145] In the largest published series from one institution with 35 patients, the primary treatment was myotomy.[98] In their first four patients, it was performed without an antireflux procedure and later with a fundoplication. The long-term results were excellent, with 34 of 35 remaining without dysphagia. In six patients, GER developed and was particularly severe in those patients without fundoplication.

The most common Heller myotomy is done using a transabdominal approach.[56] In a worldwide survey of pediatric surgeons that compiled 164 patients treated surgically with different methods, a fair clinical response to open transthoracic EM and a good to excellent result from open transabdominal EM were reported.[117] Even though most of the experience has been with using the transabdominal approach, some authors have also reported good long-term experience in children after the transthoracic Heller myotomy. In a series of 19 children, 17 had excellent and 2 good long-term results after a mean follow-up of 9 years.[115]

The surgical approach has changed recently with the advent of minimally invasive surgery.[46,56,130,233] The results obtained with the use of minimally invasive surgery have been very encouraging, with a reduction in the duration of hospitalization and a more rapid recovery of the patients.[56,130,235–237] The EM can be performed thoracoscopically or laparoscopically. Simultaneous endoscopy provides insufflation and light, which allow the surgeon a better visualization of the esophageal wall during EM. The endoscopist can also determine if a complete EM has been accomplished and can alert the surgeon if there has been a violation of the esophageal or gastric lumen. The experience with thoracoscopic myotomy is limited,[56,155,236,237] with good to excellent symptomatic improvement reported cumulatively in 84% of 82 treated patients.[155] There has been, however, a high incidence of dysphagia (up to 18%) and GER (50%), which has suggested that the laparoscopic approach may be more effective.[56,155] The results after laparoscopic myotomy have shown a cumulative good to excellent clinical response rate in 94% of 254 treated patients (range 83–100%).[56,155] Reviews have shown that it reduces LES pressure by 59% (range 42–72%); GER was reported in 11%, and there was no mortality.[155] However, the long-term outcome is still not known.

The first case reports in children have started to appear; within the last 2 years, there have been three case series published about pediatric laparoscopic and thoracoscopic myotomies.[143–145] These series report good to excellent results in 90.9% of their pediatric patients.[143–145] Because the results after EM in children have been very good, some authors have recently suggested that the primary treatment for children with achalasia should be surgical.[98,114,117]

Complications after Surgery. Esophageal perforation can also be a complication of myotomy. Most commonly, it is secondary to inadvertent perforation of the mucosa at the time of the myotomy, which occurred in 14% of 502 collected cases.[165] Such a perforation can be closed with one or two sutures. Nevertheless, in 2% of those 502 patients and 1.7% of other series, it resulted in a fistula formation.[155,165,206,222]

Other rare complications include phrenic nerve paralysis, massive hemorrhage, and necrosis of the stomach and esophagus owing to herniation.[165] The overall rate of severe complications after EM is said to be 3 to 4%.[56,165]

As can be seen in Table 26-5, there was an incidence of 10.5% of complications after EM. There were seven perforations (1.5%) and other children with lung empyema, atelectasis, intussusception, adhesions, bleeding, paraesophageal hernia, wound infections, epiphrenic diverticulum, pneumothorax, and subphrenic abscess. Three patients died, 4 children required esophageal resection (0.8%), and 21 required reoperation (4.6%).

There are two main long-term complications after EM[56,165]: failure to relieve the obstructive symptoms and GER. Failure to relieve the obstruction is usually secondary to an inadequate myotomy, usually owing to failure to carry the muscle division distally enough to divide all of the obstructing circular muscle fibers.[56,238]

This has made some authors recommend that the EM should include the entire length of the gastroesophageal junction, thus requiring extension onto the stomach cardia for about 1 to 1.5 cm,[56] although different authors recommend different distances because excessive distal extension of the EM onto the stomach contributes to the major long-term complication of myotomy, which is GER.[165]

Significant dysphagia after the operation has been reported in 1 to 9% of patients, although mild dysphagia may be found in up to 20%.[56] This dysphagia represents the most frequent indication for reoperation in up to 58% of patients.[56,165,238] In the evaluation of those patients, manometry and endoscopy are important to permit the differentiation between an inadequate myotomy (high LES pressure still present) and a complication from the operation (eg, severe esophagitis and a stricture).[56,165,238] In children, the

incidence of postoperative dysphagia varies. As Table 26-5 shows, it was reported as a long-term complication in 4.6%, being equally distributed between those children who did and those who did not have a concomitant fundoplication.

The true incidence of GER is unknown. Most authors' estimates vary from 3 to 21% (mean 10%) of the cases.[56,155,165,222] In a long-term study, it was estimated that 52% of patients suffered from it.[239] This latter report is important because of the long follow-up, with an average of 7 years. They also reported that the incidence of reflux increases with the passage of time, 24% after 1 year and 48% after 10 years, and becomes stable only after 13 years. In another study of 70 patients after EM, asymptomatic GER was detected in 14.3%, symptomatic GER in 8.6%, peptic strictures in 5.7%, and Barrett esophagus in 4.5%.[240] In a recent prospective study in which a pH probe was used, it was estimated that 35 to 40% of patients having undergone PD or thoracic myotomy without a fundoplication may develop significant reflux.[230] In the myotomy group, the total percentage of time of pH < 4 rose from 3.7 ± 4.4 to 8.6 ± 9.2%.[230]

It is thought that the incidence of GER is related to the surgical technique.[232] The authors with the largest experience have suggested that the anterior myotomy should not extend more than a few millimeters beyond the gastroesophageal junction because further dissection into the anterior wall of the stomach results in an incompetent gastroesophageal junction.[222,232] In general, less GER is noted in studies that specified that the anterior myotomy extended up to or less than 1 cm from the gastroesophageal junction.[222,232]

The scope of this problem in the pediatric patient is unknown. In a pediatric series of 10 patients studied by pH monitoring, it was reported that GER was present in 36% of children postmyotomy compared with 12% after PD,[127] and other series have reported severe and disabling reflux after EM (see Table 26-5).[62,119,120] As can be seen in the table, GER was reported in 5% of patients after a myotomy without fundoplication, and 80% of those required a fundoplication.

Peptic strictures can develop as a complication of GER in 3 to 6% of patients.[165,240] Ellis, in a 22-year follow-up, stated that GER was a problem in nine patients, six of whom developed a stricture, with three eventually requiring a colonic interposition.[232] Barrett esophagus has also been described as a complication of the GER that can occur after myotomy.[240] In two series totaling 70 patients treated with EM, three cases of Barrett esophagus (4.3%) were found 5, 8, and 15 years after the procedure.[240,241] In one case, an adenocarcinoma was reported to have developed in a Barrett esophagus many years after a Heller myotomy,[241] and in another two cases, dysplastic changes were found.[240] Peptic strictures secondary to disabling GER after EM have also been described in the pediatric literature.[116,119,127]

Because of the development of postoperative GER and the severe sequelae that can accompany it, the need to perform an antireflux procedure at the time of the EM has been argued by some. This is also controversial and depends on the experience and results of the different surgeons.[56,165] The point merits careful consideration, particularly if one considers that, overall, the results from surgical therapy tend to be better, but the long-term outcome can be dramatically affected by GER complications.

One side of the discussion claims that a precise EM carried just onto the stomach relieves dysphagia effectively and does not cause reflux.[222] These researchers also mentioned that the additional dissection necessary to do an antireflux repair is meddlesome, and the fundoplication runs the additional risk of causing a distal obstruction, thereby defeating the purpose of the myotomy. They suggested that the only indication for an antireflux procedure is the presence of a hiatal hernia. In their long-term follow-up from the Mayo Clinic, Okike and colleagues reported a 3% incidence of GER-related complications and suggested that an antireflux procedure is not routinely necessary.[222] It has been suggested that an antireflux procedure should be performed only in certain circumstances: presence of hiatal hernia, presence of pretreatment esophagitis and GER, or when the integrity of the esophageal mucosa is compromised (excision of diverticulum or perforation).[56,165,203]

Skinner recommended the routine use of a fundoplication, arguing that to perform an adequate myotomy, a greater area of exposure is necessary and that the use of fundoplication does not change the long-term effectiveness of the surgery but avoids the GER complications.[242] Nissen fundoplications, Belsey and Collins repairs, and anterior gastropexy or a Dor procedure have been reported with varying degrees of success.[114,165]

It has been suggested that if pathologic GER is documented preoperatively, an antireflux procedure should be done at the time of the myotomy. In a prospective study of five patients, it was found that in two patients, reflux occurred 16.8% and 53.3% of the total time, with prolonged episodes in the supine position. Smart and colleagues cautioned that the use of preoperative 24-hour pH monitoring may be altered by the presence of food residue. In a prospective study of 17 patients with achalasia before PD, they found that 1 patient had evidence of typical episodes of GER by 24-hour pH monitoring and 9 patients had increased times of acid exposure.[243] They showed that this increased exposure was secondary to lactic acid from food residue and not from refluxed acid, so they concluded that preoperative esophageal pH studies do not offer a valid means for the selection of patients in whom an antireflux procedure should be combined with the cardiomyotomy.

It is difficult to draw conclusions because it is usually difficult to compare one surgical technique with another. However, in one adult series that compared patients after EM with or without a Nissen fundoplication, symptoms of GER were present in 10 of 12 patients after EM alone and in none of the Nissen patients, suggesting that an antireflux procedure should have been done.[244] Recently, however, 24-hour pH determinations were done in a prospective study of five patients who underwent a successful myotomy for achalasia, and even though in two of five patients there was more GER while they were supine, there was no difference between the patients and the controls in the total amount of reflux or total number of reflux episodes, supporting again the notion that a fundoplication is not routinely necessary.[245] Things may be different

after laparoscopic myotomy, where the LES is freed from its surrounding ligamentous attachments.[56] Therefore, many authors consider the performance of an antireflux procedure as necessary after laparoscopic myotomy.[236,237] It has been suggested that a Toupet fundoplication may control reflux more effectively in those patients.[56,155,246]

Some authors have not considered fundoplication necessary when children undergo an EM.[65,111,120,122] In some series, however, GER has developed when an EM was performed without a fundoplication,[62,98] and some have suggested that there is an increased risk for the appearance of reflux with the passage of time.[113]

Nihoul-Fekete and colleagues reported that they initially performed only EMs, but after their first patients developed severe GER, a His reconstruction was added in the next 10 patients. GER continued to be problematic and 3 patients needed a Nissen fundoplication, so it was added in the subsequent 21 patients, with only 1 patient developing dysphagia, but none with GER. Similar good results after adding a Nissen fundoplication were reported by Emblem and colleagues.[116] Myers and colleagues reported in their worldwide survey that the best surgical results were observed in those children who had a transabdominal myotomy with a fundoplication.[117]

It is clear, then, that the argument for the need for antireflux surgery following EM centers on the small percentage of patients who develop complications from GER. Because this percentage is small and because it is not possible to predict reliably who those patients are going to be, it is probably not necessary to subject all patients to a longer and more difficult procedure to prevent the few cases of troublesome reflux; this is also the latest recommendation from most of the adult series.[232] By reviewing the available literature in children, it can be noted in Table 26-5 that 329 children have had an EM alone and 175 had an EM with fundoplication. In the group without an antireflux procedure, there were 4.6% with GER and 4.9% with dysphagia, and 4.6% required esophageal dilatation, 3.6% a fundoplication, 0.9% a repeat myotomy, and 0.9% an esophageal resection. These compare with an incidence of 5.7% with dysphagia, 2.3% with GER, a 2.3% reoperation rate, and 0.6% requiring esophageal resection who had a myotomy and a fundoplication.

Dysphagia also occurs from 10 to 28% after a fundoplication is made during laparoscopic myotomy.[56,155,236,237]

Zaninotto and colleagues looked at adult patients who had Heller EMs and anterior partial fundoplications who developed symptoms of dysphagia or chest pain after the surgery.[247] In their series of 113 adults, 8.7% of patients had these syptoms. When they performed manometries on those who had the surgery and were asymptomatic compared with those patients who were symptomatic, they found that the syptomatic patients had a longer LES postoperatively compared with preoperatively, had a longer LES overall compared with that of the asymptomatic patients, and had higher pressures in the esophagus proximal to the surgery.

In a second series of 102 adults who underwent Heller myotomies and Dor fundoplications by Patti and colleagues, 11 patients had persistent or recurrent dysphagia. The etiologies for this dysphagia were myotomies that were too short (36%), fundoplications that were too tight (36%), and stricturing of the esophagus owing to prior dilatations or botulinum toxin injections (27%) that was not recognized prior to surgery.[234]

Obstructive symptoms are also common in children after fundoplication.[45,98,111,128] This potential complication has led to the recommendation of a partial fundoplication.[62,114] Interestingly, in children, dysphagia has been reported in around 5% (see Table 26-5) and, as can be seen in the table, in an equal percentage when comparing those with or without a fundoplication. One problem with surgery is the fact that if it fails and multiple operations are needed, eventually, it may be necessary to perform an esophageal resection (0.9%; see Table 26-5).

Medical versus Surgical Therapy. The choice of initial therapy is still controversial.[46,56,155]

PD versus EM. The results obtained with any of the main treatments vary from center to center, and the most extensive experience comes from the adult literature. In one of the largest adult series, the Mayo Clinic reported the results on 899 patients and compared the late results of treatment with dilatation and EM.[222] They concluded that EM was more successful and safer than dilatation, with poor results being obtained twice as often with dilatation as with EM. As mentioned before, the adult experience reports a success rate of 65 to 75% after PD and of 85 to 90% after EM.[222]

In the only large prospective randomized study, Csendes and others randomized patients to EM or Mosher bag dilatation.[212] A total of 81 patients were included, and after a follow-up of 5 years, 95% of the operated patients were asymptomatic and two had mild heartburn, whereas only 65% of the dilated patients were asymptomatic. Positive acid reflux tests were noted in 28% of the postoperative patients versus 8% after dilatation. They also found a lower LES (less than 10 mm Hg) in the surgical group. They concluded that surgery is more efficacious. This study has been criticized on the grounds that the technique of dilatation may have been suboptimal and that the study may have been biased against the dilated patients because of their more pronounced dysphagia.[248] In the only study that compared EM with the Rigiflex dilators, the authors found equal effectiveness (88% versus 89%) in symptom improvement in 45 patients.[249]

Most authors still recommend PD as the initial therapy in adults.[155,203,248] Also, in a retrospective analysis based on the treatment and follow-up of 123 patients, Parkman and colleagues estimated that the cost of EM was five times greater than the first PD and still two to four times greater, adding to the PD the cost incurred by all of the patients who required multiple dilatations.[213] In another recent cost-effective analysis comparing PD, laparoscopic surgery, and botulinum toxin, it was found that PD was the most cost-effective approach.[155,225]

As can be seen in Table 26-5, the experience in children also varies from center to center. Grouping all of the studies, with an understanding of the problems inherent in

doing so, both treatments look similar, although the long-term results may be slightly better after EM. The percentage of improvement was higher after EM than after PD (up to 82.8% with fundoplication versus 56%), and the complication rate was similar (7.5% after dilatation and up to 12.6% after myotomy). It has also been noted that in long-term follow-up, 29% of patients had dysphagia and 2.8% had GER after PD, whereas in the surgical group, 5.7% had dysphagia and 4.6% had GER. Also, 37.4% of the children who had PD required a myotomy, whereas of those who had a myotomy, 3.4% required a second operation, 3.6% required fundoplication, and 0.8% required an esophageal resection, and there was a mortality of 0.6%.

Both medical and surgical therapies are effective.[203] On the one hand, PD is safe, effective, and cheaper unless repeated dilatations are necessary and is usually an outpatient procedure. The risk of perforation is always present, and improvement of the dysphagia varies among studies but is probably not as great as that occurring after EM. On the other hand, EM generally results in a more complete and longer duration relief of the dysphagia, and GER is a major complication. The cost and morbidity associated with the surgery are significant.

PD versus Botulinum Toxin. Recent randomized studies comparing botulinum toxin with PD have appeared.[164,208,250,251] When comparing botulinum toxin with PD done with Witzel dilators, there was a 6-month clinical remission of 89% following PD compared with 50% after botulinum toxin.[250] In another trial, there was a symptomatic response rate of 80% at 12 months after two injections of botulinum toxin, although the effectiveness was only 13% after one injection. The response to PD was reported as 100% initially.[208] Muehldorfer and colleagues found that dysphagia improved in 75% of patients who received botulinum toxin and in 83% of patients who had PD. Long term, however, the patients who received botulinum toxin had significantly higher rates of regurgitation and dysphagia than did the PD group.[251] In the largest prospective study, 42 patients were randomized to either PD or botulinum toxin.[164] Both therapies were equally effective at 1 month. PD resulted in a significant higher cumulative remission rate. At 12 months, 14 of 20 patients (70%) after PD and 7 of 22 patients (32%) after botulinum toxin were in symptomatic remission. PD also resulted in significant reductions in symptom scores, as well as objective parameters (LES pressue, esophageal diameter, barium swallow), whereas botulinum toxin decreased symptom scores but did not change objective parameters. Also, the failure rates were similar initially, but failure over time was higher after botulinum toxin.[164] In another comparative study of 42 patients receiving botulinum toxin compared with 26 receiving PD, it was shown that retreatment was necessary in 50% of botulinum toxin patients after 265 days compared with < 40% after 2 and 5 years of follow-up after PD. However, if repeated injections are compared, the benefits approximated those obtained by a single PD.[252]

The information published so far indicates that despite the initial enthusiasm for botulinum toxin, it seems to be inferior to PD or myotomy, and recent data indicate that it is less cost-effective.[225,253] Therefore, botulinum toxin therapy needs to be reserved only for those patients who represent a poor risk for the other invasive therapies, for those who refuse other therapies, or for those in whom it may help clarify an indication for definitive therapy.[46,155,202]

Therapy in Children. The therapy of choice for esophageal achalasia in children has not been established and will still depend in large part on the expertise present at each institution. Some centers advocate PD,[45,46,111,119,139,142] whereas others advocate surgical treatment.[62,92,98,113,122] As can be seen in Table 26-5, the results obtained with different modalities vary from center to center, and there are no comparative studies. Although surgery may provide better results and a longer duration of remission in children, it is costly and may be associated with serious short- and long-term complications. Because PD is less invasive, is effective in more than 50% of patients (range 35–100%),[46] and has a low rate of short- and long-term complications, with nearly absent mortality, many feel that a trial of PD should be a first-line therapy. If repeated dilatations become increasingly necessary, with short periods without symptoms between them, a surgical procedure should be done.[46,119,164] If surgery is necessary, the modified transabdominal Heller EM without a fundoplication has been the procedure of choice.[56,98,235] Recently, however, the successful advent of laparoscopic EM has provided a new, effective, and less invasive alternative.[56,130,134,136,143–145,203] In some centers, laparoscopic myotomy is now being offered as the initial treatment,[56,134,155] but long-term studies are needed, and experience in children is still very limited.[46,130,134,136]

Pharmacologic therapy with nifedipine is not a good long-term option and can be used to buy time to prepare for the definitive treatment and to adjust to the child's school schedule. Botulinum toxin should be reserved for those children who are high-risk candidates for invasive procedures or for those in whom the nature of the problem is not clear, and before undertaking a definitive procedure, the response to an intervention that is self-limited needs to be evaluated.[46,202]

Long-Term Follow-up. After successful therapy, a recurrence of symptomatic achalasia may develop, even after a symptom-free period of many years.[165] This may occur after either PD or EM without any evidence of peptic or stenosing esophagitis. The reason for this is unknown. It may be due to slow progression of the degenerative process of the myenteric plexus and other nervous structures. Some patients seem to be resistant to therapy, and it has been noted that some patients who fail PD respond poorly to EM and that forceful dilatations are less successful in patients with previous PD or EM.[165]

Esophageal cancer, particularly squamous cell carcinoma, has been considered by some authors to be a late complication of achalasia.[254] Numerous case reports and retrospective reviews have appeared to substantiate the association between achalasia and esophageal carcinoma,[255] with most series estimating an incidence of 5% of esophageal carcinoma in these patients.[255] Esophageal cancer arises at a younger age in these patients, with a mean of 48 years, and the mean time from diagnosis of the achalasia to the occurrence of the malignancy is 17 years.[255] In a

population-based study, it was estimated that the prevalence of this complication was 1%,[256] and an autopsy study reported a prevalence of 1.5%.[257] A follow-up study of 147 patients treated with EM (mean 23.2 years, range 6–41 years) found that 10 of 23 patients who died of cancer had a malignant tumor in the esophagus, with an overall mortality rate of 66.1% and with 11.9% of the deaths caused by esophageal cancer, concluding that achalasia is a risk factor.[255] It has been suggested that this complication is more likely in patients who have had a long course without treatment or have failed therapy, raising the possibility that mucosal irritation from stasis of different substances may be an important factor. A recent report found a prevalence of 9.21%, using endoscopy with Lugol staining. They found a higher incidence in patients with more than 20 years of evolution, enlarged esophagus with knees, and marked retention.[254] Because of this possibility, some authors have advocated frequent endoscopy with cytology and biopsy or with Lugol staining every 3 to 5 years,[254] particularly because any warning symptoms are more likely to occur late as a result of the dilated esophagus. It is also not known what role other risk factors (eg, alcohol, tobacco) play in the development of this complication.[254]

Brucher and colleagues published one of the most recent series of patients with achalasia and their experience with the development of esophageal cancer.[258] They found that esophageal cancer was diagnosed 17.8 to 42.5 years after the diagnosis of primary achalasia in 3.2% of patients. Conversely, when they examined all of the patients who presented at their institution for the treatment of esophageal squamous cell carcinoma, 1.5% had a preceding history of primary achalasia; the average length of time between the diagnosis of achalasia and carcinoma in these patients was 32 years. When they examined all of the patients at their institution who had adenocarcinoma of the esophagus, 0.2% (one patient) had preceding primary achalasia diagnosed 22 years previously.[258]

There is currently no information about the incidence of this complication in patients who developed achalasia as children, although, as far as we can tell, no cases of esophageal carcinoma have arisen in adults who had achalasia as children. At this time, with our current knowledge, it is believed that a surveillance program in adults would not be cost-effective, not only for the amount of psychological trauma that would be inflicted but also because there are no data showing that such an approach can alter the outcome.[259] It is recommended that diagnostic tests in treated achalasia patients rely on clinical criteria, with particular attention paid to recurrence of symptoms or newly developed dysphagia or pain. Because there is no information about the long-term prognosis of children with achalasia, these children should be followed closely, and the threshold for initiating an evaluation should be low.

OTHER PRIMARY MOTILITY DISORDERS

Advances in esophageal manometry and more widespread use of this technique in the evaluation of patients with noncardiac chest pain or dysphagia have shown that patients with primary esophageal motor disorders do not fit into the simple classification of achalasia and diffuse esophageal spasm.[165] A variety of motor alterations have been described in the adult literature, and even though some distinct clinical entities, such as diffuse esophageal spasm (DES) and nutcracker esophagus, have been identified, a larger proportion of patients remain in the category of nonspecific esophageal motility disorders.[260,261]

Diffuse Esophageal Spasm and Nutcracker Esophagus

DES is a primary disorder of the motor activity in the smooth muscle portion of the esophagus.[260–262] It represents a group of disorders in which there are high-amplitude, repetitive, nonperistaltic esophageal contractions. DES is a distinct clinical entity in adults, but its incidence in children is unknown, and there are only case reports.[263] The pathophysiology is still unknown, although it has been suggested that it is the result of damage to the inhibitory esophageal nerves and the consequent increase in excitatory input. In adults, this syndrome is characterized by chest pain and/or dysphagia that is usually not progressive or associated with weight loss.[260,262] Because of the predominance of the chest pain, these patients are usually evaluated first for coronary artery disease.[260] The pain may be initiated by the ingestion of very cold or very hot meals, dysphagia is usually present in 30 to 60% of the patients, and in contrast to the pain in achalasia, it is not constant. The barium swallow may show frequent nonpropulsive contractions indenting the barium column, usually only in the lower third. There may also be delayed transit documented either by radiography or emptying scans. The diagnosis, however, is established manometrically using the following criteria: (1) repetitive, simultaneous (nonperistaltic) contractions, at least 10% of wet swallows; (2) periods of normal peristaltic sequences; (3) alterations in the contraction waves (increased duration and amplitude), although there are patients who can have normal amplitude; and (4) a normal LES in most patients, although incomplete LES relaxation or a hypertensive sphincter has been described.[260–262] This alteration in the LES and some long-term follow-up of patients with DES confirming the rare evolution of some cases of DES into achalasia suggest that the two disorders may represent points on a continuum of esophageal motility dysfunction.[48,260] It has been suggested that compared with stationary motility, 24-hour ambulatory motility is more sensitive and specific for diagnosing DES.[261] When high-frequency intraluminal ultrasonography is done during the manometry, patients with DES (and nutcracker esophagus) have thicker muscles in the area of the LES and throughout the esophagus compared with normal subjects. Both the circular and longitudinal muscles are thicker than those of controls.[264]

In children, this is an extremely rare entity.[263,265] In infants, the presentation is usually with apnea and bradycardia; in young children, it is aspiration pneumonia; and in older children, the presentation is similar to that in adults. There is a particularly well-documented case

reported by Fontan and colleagues in which a newborn was found to have bradycardia, obstructive apnea, and central apnea, all of which were consistently associated with manometric findings typical of DES.[263] They suggested that the symptoms in this infant were secondary to vagally induced responses because the infant showed an immediate decrease in heart rate after esophageal spasms, with a lack of a temporal relationship between the apnea and bradycardia and subsequent good clinical response to the use of anticholinergic medication. Glassman and colleagues reported a retrospective study in 83 children with chest pain who underwent esophageal manometry and endoscopy.[265] They found that 47 had normal studies, 15 had esophagitis with normal motility, 13 had dysmotility and normal histology, and 8 had abnormalities in both esophageal motility and esophagitis. In the group with motility disorders, they identified 7 patients with achalasia and 4 with DES, indicating that children with unexplained chest pain should have an evaluation to exclude primary motility disorders.

Using the above-mentioned criteria, it is estimated that only 10% of adult patients referred for esophageal motility because of chest pain have DES.[260,266] The most frequent finding is what is now called nutcracker esophagus. This is a clinical entity presenting as noncardiac chest pain and occasionally dysphagia. The barium swallow and radionuclide emptying scans are usually normal, and the diagnosis is made manometrically, with high-amplitude peristaltic waves being the hallmark of the disease.[260,266] This is in contrast to DES, in which the high-amplitude contractions do not result in peristalsis. The mean peristaltic amplitude with nutcracker esophagus is usually greater than 120 mm Hg, and there is usually an increased duration of esophageal waves.

A large number of patients with noncardiac chest pain have been shown to have manometric abnormalities that cannot be classified as one of the above disorders. These patients are now classified as having nonspecific esophageal motility disorders, and the manometric findings can vary from a "hypertensive LES" with normal relaxation and peristalsis to minor abnormalities in the peristaltic sequences with repetitive contractions or isolated prolonged contractions.[260,266] Patients with these nonspecific abnormalities also show a delay in esophageal emptying for solids, indicating that the manometric findings are not only a laboratory curiosity.[267]

Esophageal dysmotility in infants has also been linked to the "near-miss" sudden infant death syndrome in a report of four infants with radiographically proven esophageal dysmotility.[268] The alterations consisted of distal esophageal spasm and esophageal strictures. No manometric studies were performed, so it is difficult to know the type of motor alterations. They were all born prematurely, the symptoms occurred after feeding, and they all had esophageal strictures, suggesting that they all had long-term GER. Of interest is the fact that two patients developed apnea during the barium study in relation to esophageal dysmotility and that they all improved after aggressive antireflux treatment was undertaken.

The therapeutic armamentarium for the primary motility disorders, with the exception of achalasia, is greatly limited.[269] Treatment is directed toward symptom reduction. Calcium channel blockers and nitrates have been shown in uncontrolled trials to improve the symptoms of patients with spastic disorders.[270,271] Peppermint oil may also be helpful in reducing the simultaneous contraction seen with esophageal spasm.[272] Of particular interest is the recent observation that different antidepressants produce improvement and reduce distress from esophageal symptoms.[262,273] In some studies, this improvement was not related to manometric changes, emphasizing that manometric abnormalities may not be solely responsible for the symptoms.[273] The fact that antidepressants have such a marked influence over these disorders raises the issue of the importance of psychiatric alterations in these patients and their relationship to esophageal motor disorders. Previous studies have revealed that a psychiatric disorder at some point in the patient's lifetime is present in approximately 80% of patients with esophageal contraction abnormalities.[262]

If conservative treatment fails, the use of PD, surgery, or, recently, botulinum toxin has been advocated to treat patients with spastic esophageal disorders.[17,269,274,275] PD has been reported to improve the dysphagia of 40% of the patients with severe manometric abnormalities, particularly if there is incomplete LES relaxation.[269,276] In a series of 20 patients treated with PD, good results were obtained in 14 and poor results, including an esophageal perforation, in 6.[276] Botulinum toxin injection of the LES was used in 15 patients, and there was a significant improvement in chest pain, dysphagia, and regurgitation. One month after treatment, 73% had a good to excellent response. At a mean follow-up of 10 months, 33% continued to have good to excellent results, whereas 67% required additional treatment with botulinum toxin or PD.[274]

Surgery should be the last resort; a long EM is generally advocated for intractable cases, and good results have been reported,[17] although, in general, the results are poorer than those of surgery for achalasia.[17]

SECONDARY ESOPHAGEAL MOTOR DISORDERS

Motor Disorders in Esophageal Atresia and Tracheoesophageal Fistula

Primary repair of esophageal atresia permits the restoration of GI continuity but does not ensure normal esophageal function. Even though a complete consideration of esophageal atresia is beyond the scope of this chapter, we briefly discuss some of the important aspects that relate to esophageal motor function.

Early surgical intervention allows the neonate to survive, but it has become clear that many children surviving the repair of esophageal atresia frequently have symptoms related to esophageal motor dysfunction, including regurgitation, vomiting, heartburn, dysphagia, and chronic respiratory symptoms, such as nocturnal wheezing and recurrent pneumonias.[277–280] Up to a third of patients followed long term report impaired quality of life.[280] Some patients also develop strictures near the anastigmatic site, which have been attributed by some authors to reflux esophagi-

tis.[277] Long-term follow-up of these children with esophageal atresia repair has revealed disordered esophageal motility in almost all of the children studied.[277,279–283] Manometric tests and long-term pH monitoring have proved to be the most sensitive indicators of esophageal abnormalities in these patients,[277,279,282] although barium esophagrams[284] and radionuclide scans have been used as well.[283] Most series have shown almost universal alterations in the peristalsis of the esophagus, with rare reports of normal esophageal function.[282] The alterations described have been a low LES pressure and a lack of peristalsis, with esophageal contractions that tend to be simultaneous and weak, although at times of normal amplitude, especially in the lower esophagus.[279,282,283] It has been suggested that a low LES pressure in these patients correlates with the development of worse reflux[283] or aspiration pneumonia,[283] although two studies found no correlation between abnormalities in pulmonary function tests in these patients and the presence of GER, esophagitis, or esophageal dysfunction.[285,286]

Long-term esophageal function has been described by Biller and colleagues, who studied 12 adult patients who had their original repair in the first week of life.[286] The mean age was 26 at the time of the evaluation. During manometry, they found a mean LES pressure of 21.3 ± 2.8 mm Hg, with only two patients having LES pressure less than 15 mm Hg (both had esophagitis). Ten had complete LES relaxation after swallowing, and the amplitude of the contraction was also low throughout the esophagus, although the duration was normal. All patients had at least one portion of aperistalsis, with most patients showing a diffuse abnormality. The UES pressure and function were normal. The peristaltic function of 22 adolescents and adults with tracheoesophageal fistula repairs was described with the use of ambulatory 24-hour pH manometry.[281] All had diminished contractile activity, disorganized propulsive activity, and abnormal and ineffective peristalsis. This indicates a poor capacity for acid clearance and may explain the frequent dysphagia and GER-related problems experienced by these patients.

The etiology of abnormal esophageal motor dysfunction is unclear.[287] Some authors have suggested that there is damage to the esophageal branches of the vagus during the surgical repair.[282,288,289] Shono and colleagues described two patients with a long gap esophageal atresia without tracheoesophageal fistula in which manometry was performed before the operation.[288,289] Both patients showed peristaltic contractions in the proximal esophagus, which was always followed by coordinated contractions of the distal esophagus and a normal LES relaxation. After the operation, the repaired esophagus demonstrated abnormal motility and LES relaxation, suggesting that the alterations were secondary to intraoperative mobilization and probable denervation.[288,289] Experimental evidence in dogs in which the esophagus was divided and the vagus nerve was damaged in half gives support to this observation. Motility studies showed that normal peristalsis was preserved only in those in which the nerves were not affected.[288,289] Also, it has been shown in dogs that even though cervical vagotomy produces low LES pressure, thoracic procedures, including vagotomy, resection of the esophageal branches of the vagus, phrenic nerve resection, and midesophageal resection, have no significant effects on the LES.[290]

The fact, however, that there is dysmotility not only in patients who have had surgery but also in those with a fistula without atresia who have not had surgery, or in the different segments before the operation, has led others to believe there may be a congenital abnormality.[279,287] To further support the theory that the esophageal motility alterations are present since birth, before the operation, Romeo and colleagues reported esophageal motility studies of both the proximal and the distal segments in 20 newborns with esophageal atresia before surgical repair.[291] They found that the LES length varied from 8 to 14 mm, and the pressure was between 22 and 35 mm Hg in 84%. The LES pressure was low in 16.7% of the patients, and there was incomplete relaxation in 8.4%. There was also incomplete relaxation of the UES in 12.5% of the patients. The esophageal body in the proximal and distal segments showed a positive basal tone with total motor incoordination. Nakazato and colleagues demonstrated preoperatively abnormalities of the myenteric plexus, suggesting that there is abnormal development of neural tissue in these children.[292] Experimental evidence in a rat model of esophageal atresia has also shown abnormalities not only in the course and branching of the vagus nerve but also of the intrinsic esophageal innervation (both excitatory and inhibitory).[287] These studies suggest that there may be intrinsic abnormalities and that the esophageal motor incoordination is an intrinsic part of the congenital malformation, although it may be exacerbated by damage to the extrinsic inervation during surgery.[287]

Chronic Idiopathic Intestinal Pseudo-obstruction

This syndrome is characterized by intermittent symptoms and signs of intestinal obstruction without evidence of actual mechanical blockage and is discussed in Chapter 46.4, "Chronic Intestinal Pseudo-obstruction Syndrome." Various motility abnormalities throughout the body have been described; besides the small bowel, the esophagus is frequently involved.[293–295] At least 85% of patients have abnormal esophageal motility. Most patients exhibit aperistalsis and often decreased or absent LES. In the recent national survey reported by Vargas and colleagues, esophageal motility studies were performed in 14 children.[294] All patients had low LES, with failure to relax with swallowing. Low-amplitude waves occurred in the esophageal body, with lack of propagation and the presence of tertiary contractions in all 14 patients. Because none of the patients had histologic studies done to correlate whether they had a myopathic or a neuropathic form, no further conclusions can be reached. In another report of 20 children, 18 had abnormal esophageal peristalsis, consisting mainly of simultaneous contractions, short-lasting, or low-amplitude waves.[293] It can therefore be concluded that because of the high incidence of esophageal motor abnormalities in these patients, esophageal motility can be used as an initial

screening test, particularly if small bowel motility studies are not available where the patient is located.[293,295]

Caustic Ingestion

Ingestion of harmful household compounds is a frequent accident in toddlers and can lead to severe esophagitis and life-threatening acute complications.[296,297] In the subacute and chronic stages, strictures of the esophagus and dysphagia are common, and it is known that the severity of the symptoms does not correlate with the degree of esophageal stenosis. Long-term follow-up has shown motility abnormalities.[297,298] Cadranel and colleagues have shown that the severity of symptoms correlates with impairment of esophageal motor function and delayed esophageal transit. The basis of the motor abnormalities seems to be damage to the deep muscle esophageal layers with fibrosis, with subsequent loss of normal motility. Manometrically, they found signs of segmental hypoperistalsis, usually with normal UES and LES function. Esophageal transit was altered, with abnormalities observed in the middle and lower esophagus.[297]

Endoscopic Injection Sclerotherapy and Band Ligation

Endoscopic variceal sclerotherapy (EVS) of esophageal varices and, recently, endoscopic variceal ligation (EVL) are effective treatment modalities for patients who have had variceal hemorrhage.[299] They have also been successfully used in children. Following EVS, the histologic changes in the esophageal wall include thrombosis of the submucosal vessels, esophagitis, ulceration, and, subsequently, fibrosis. Persistent dysphagia after EVS has been uncommon despite extensive damage to the esophageal mucosa, although there are reports of esophageal strictures following the procedure.[300] The results of esophageal motility studies in patients undergoing EVS have varied. A recent review of the literaure concluded that esophageal varices reduce the mean amplitude of contractions, particularly in the lower third, and increase the mean duration of peristaltic waves but have little effect on LES function.[299] Nonpropagating simultaneous contractions may appear and may result in chest pain and/or dysphagia in the absence of stricture. Pathogenesis of the abnormal motility remains poorly understood, and no correlations were demonstrated between esophageal motor parameters and doses of sclerosant.[301] In some studies, the abnormalities have been reversible, suggesting that sclerotherapy injections acutely impair the motility of the esophagus and indicate that the motor function is partially restored 4 weeks after their completion.[301]

The information after EVL is limited, but it appears to have little impact on esophageal motility.[299,302] In one study comparing EVS and EVL, the LES pressure did not change, but the amplitude of contractions changed significantly after either two sessions of EVS or EVL variceal therapy. There was also an increase in nonperistaltic waves, but there was no correlation between the presence of ulcers and dysmotility and there was no difference in the changes between the EVS and EVL groups, suggesting that both affect esophageal motility.[302]

Collagen Vascular Disorders

Of all of the collagen vascular disorders, scleroderma shows the most marked esophageal abnormalities.[303,304] Scleroderma is a systemic disease of unknown etiology characterized by excessive deposition of collagen and other connective tissue components in skin and other target organs, most notably the GI tract.[303,304] Esophageal abnormalities are present in half to three-fourths of patients with scleroderma, a much higher frequency than in patients with other collagen vascular disorders.[303,304] Prominent digestive symptoms in these patients are dysphagia, weight loss, and diarrhea alternating with constipation. In advanced cases, the esophageal involvement can be easily diagnosed by cinefluoroscopy, whereas at an early stage, it can be detected by esophageal manometry.[303,304] The manometric findings are quite suggestive of the diagnosis, although not pathognomonic, because severe reflux esophagitis can have the same manometric appearance.[303–305] The characteristic esophageal manometric findings are (1) incompetent LES, (2) low-amplitude esophageal contractions in the smooth portion of the esophagus, and (3) later alterations in the striated muscle section.[303,304,306,307] The incompetent LES fails to provide an effective barrier against the gastric acid, and the abnormal peristalsis provides an inadequate acid clearance, predisposing the patients to severe complications from GER.[305] It is not surprising then that patients with scleroderma may have heartburn as a prominent symptom.[303,304,307]

Ling and Johnston looked at the yield of various GI tests in the evaluation of the esophagus in connective tissue disease in adults.[308] Their patient population included 47 patients with scleroderma; calcinosis cutis, Raynaud phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia (CREST); mixed connective tissue; and other connective tissue diseases (lupus, rheumatoid arthritis, dermatomyositis, myasthenia gravis, Still disease). These patients all had manometry and some combination of an upper GI series, endoscopy, and/or a pH probe. Of all of the tests, endoscopy had the lowest diagnostic yield, with only 33% of patients with positive findings on the test. Sixty-six percent of patients had abnormal manometry, 56% of patients had an abnormal upper GI series, and 100% had an abnormal pH probe, although only 3 of the 47 patients underwent a pH probe in the study.[308]

The severity of the esophageal motor dysfunction in scleroderma varies.[304] It has been suggested that alterations are more severe in patients with progressive systemic sclerosis (PSS) than in those with localized scleroderma (LS). By studying the relationship between the severity and extent of esophageal acid exposure and manometric abnormalities in patients with systemic sclerosis, it was concluded that the severity and extent of GER are closely related to the integrity of the distal peristalsis. It has also been reported that Raynaud phenomenon is closely associated with the loss of esophageal peristalsis and that there is a significant association of esophageal dysmotility with

reduced lung volumes.[304] It is possible that the pulmonary damage is due to GER or to simultaneous involvement of the esophagus and the lungs in the disease process.[304]

In a study of children with scleroderma, Flick and colleagues studied seven children with PSS and two with LS.[307] The most frequent symptoms in patients with PSS were regurgitations, heartburn, and dysphagia. They showed that in 72% of the patients with PSS (mean age 15 years; range 10 to 18 years), there was a decreased LES pressure, tertiary waves, or feeble contractions. They found a strong correlation between the presence of Raynaud phenomenon and esophageal symptoms but no correlation with disease duration. There was a correlation between dysphagia and the presence of esophageal motor abnormalities. In contrast to adults, they also did not find any correlation between the presence of esophageal motor abnormalities and Raynaud phenomenon. Both patients with LS had no esophageal symptoms and minimal nonspecific alterations in the esophageal motility (tertiary waves and variability in amplitude). Of interest is the fact that after 4 years of follow-up, none of the patients with LS had either progression of their motor abnormalities or the appearance of symptoms, challenging the suggestion that nonspecific esophageal abnormalities may indicate which patients will develop a more progressive illness.

Because of the link between motor disturbances and reflux disease, Weber and colleagues examined 14 children with scleroderma for evidence of reflux disease using pH probes.[309] They found that 64.3% of the children had pathologic reflux, defined as reflux for greater than 4.5% of the day. They also found that 85.7% of the patients had more frequent episodes of reflux and 50% had longer episodes of reflux than normal standards.

The pathogenesis of the esophageal motor disorder in these patients remains unknown. In some pharmacologic studies, Cohen and colleagues showed that the LES pressure in patients with intact peristalsis is higher and demonstrates an increase in sphincter pressure after the administration of methacholine (a cholinergic agonist) or edrophonium (a cholinesterase inhibitor).[305] In patients with reduced or absent peristalsis, the sphincter responds to methacholine but shows a diminished response after the administration of edrophonium. Some patients with absence of peristalsis did not show any response to methacholine, suggesting extensive muscle atrophy. All patients with scleroderma showed a diminished response of the LES to gastrin injection. They suggested that patients with scleroderma have an early abnormality in cholinergic neuronal function, which later is compounded by smooth muscle atrophy. It has also been shown that scleroderma patients with autonomic dysfunction also have esophageal dysmotility, also supporting the hypothesis that there is a neurogenic defect.[310] It is possible that a neural lesion occurs early in scleroderma and disrupts esophageal motor function before the development of significant smooth muscle disease.[305] Studies of motility abnormalities in the small bowel, colon, and anorectal area seem to support this hypothesis.[305,311] Other authors suggest that the LES hypotension is secondary only to a primary muscle defect, although the finding of abnormal motor function in areas of normal muscle in several studies suggests that atrophy is a later stage of the disorder.[307]

At present, there is no specific treatment for the esophageal alterations secondary to scleroderma. The treatment is symptomatic, trying to prevent the complications from GER.[304,305,307] Cimetidine, metoclopramide, cisapride, and, recently, omeprazole have been shown to cause symptomatic improvement.[304,305,307,312]

Even though the esophageal motor abnormalities of scleroderma have been well characterized, other connective tissue diseases also have motor alterations,[306,313] particularly in patients with systemic lupus erythematosus (SLE) and those with mixed connective tissue diseases (MCTDs). In a study of 150 patients with different rheumatic disorders who underwent esophageal motility studies, it was common to find functional involvement of the esophagus.[306] The frequency differed by disease: in scleroderma patients, abnormalities were found more frequently in the LES (81.8%) and in the esophageal body (84.8%), whereas patients with dermatomyositis and MCTD overlapped. In SLE, there did not seem to be abnormalities of the LES, and the most specific problem was an isolated abnormal peristalsis. The authors concluded that the simultaneous involvement of the esophageal body and the LES is discriminant between nonlupus connective tissue disease and SLE.[306] Other studies have suggested that esophageal symptoms and aperistalsis are not prominent manifestations of SLE. Patients with SLE can also have esophageal motor abnormalities (approximately 50%). In a series of 14 patients, 3 had low LES, 1 had aperistalsis, 2 had changes similar to PSS, and 4 had minor esophageal abnormalities.[237] Even though heartburn was a very common symptom, no correlation between motility abnormalities and symptoms could be established.

In a study of 17 adults with MCTD, Gutierrez and colleagues showed that 82% of patients had abnormal esophageal motility.[314] The mean LES and UES pressures were lower than in controls, and the amplitude of esophageal contractions was significantly decreased. In 53% of patients, there was total aperistalsis of the esophagus, including the upper third, reflecting involvement of the striated muscle. They suggest that the diagnosis of MCTD should be entertained when total aperistalsis in the esophageal body is found, although this pattern can be seen occasionally in patients with PSS and SLE.[314] In a recent study of four children with MCTD, three had low LES pressure, two had tertiary waves, and two had feeble contractions. They found no evidence of aperistalsis, in contrast to the findings in the above-mentioned study.[307] The question of whether steroids will improve the esophageal motility problems remains unanswered. It has been suggested that the abnormal peristalsis in MCTD returns to normal after steroid treatment, although in the study by Gutierrez and colleagues, 14 patients had been receiving steroids for a mean of 6.9 years, and all had abnormal peristalsis.[314] This question needs to be addressed with a prospective study.

Sjögren syndrome, which is characterized by xerostomia and keratoconjunctivitis, can have involvement of

other organs, including the GI tract.[313] Esophageal function has been studied in these patients, and it was shown that they have minor motor differences compared with controls (shorter peristaltic contraction time and faster peristaltic velocity in the distal part of the esophagus). Webs were found in 10%, and it was concluded that the dysphagia that was reported by 73% of the patients is probably related to the lack of saliva, making the solid bolus passage difficult. In a study of esophageal manometry in 21 patients with Sjögren syndrome, abnormalities were present in 33%, and these did not correlate with the dysphagia or the extraglandular involvement.[313] The esophageal motor abnormalities included a high LES pressure in seven, aperistalsis in two, and nonspecific motor problems in one.

There has been controversy around the possibility that women with silicone breast implants may have an increased incidence of rheumatologic problems.[315] Even though this is a problem that is not encountered in the pediatric population, it has been suggested that breastfed children of mothers with silicone breast implants may be at risk of having esophageal motor problems with absent distal peristalsis and decreased LES pressure.[316,317] The initial reports described 11 children with chronic GI symptoms, including abdominal pain, vomiting, and poor weight. Eight children had been breastfed (mean age 6.1 years; range 1.5 to 9 years) and three bottle-fed, and they compared their esophageal manometry with a control group of 20 children. Six of eight breastfed infants studied demonstrated significant esophageal motor abnormalities, which are typical of those seen in both children and adults with scleroderma. The changes included abnormal peristalsis in the lower third and decreased LES pressure, findings that persisted over time in at least three children who had repeated manometric studies done a mean of 10 months later. They found that only 21% of swallows propagated to the lower third of the esophagus. The mean LES was 13.1 ± 5.9 mm Hg in breastfed children compared with 24.8 ± 11.9 mm Hg in controls. UES pressure and function were normal. Endoscopic biopsies failed to reveal either granulomas or silicone crystals,[316] and a search for a variety of autoantibodies yielded no differences.[318] These children were followed prospectively, and 7 of 11 had subjective clinical improvement on prokinetics. In 8 of 10 children, the esophageal biopsies were normal or showed mild esophagitis. LES and UES pressures and percent propagation were not significantly different at follow-up, whereas the wave amplitude significantly increased.[317]

The physiopathology of this problem is not clear. The fact that bottle-fed infants were unaffected suggested that the exposure was postnatal. It is unclear if the passage of silicone itself, other by-products released by the implants, or immunologic factors may have contributed to the esophageal dysmotility. In a case-control study in which urinary NO metabolites and neopterin were compared between 38 infants breastfed by mothers with silicone implants and controls, it was found that the breastfed infants had increased urinary NO metabolites and neopterin, as well as in vitro macrophage activation after silicone exposure.[319] Also, there was a significant inverse relationship between urinary neopterin excretion and the severity of the esophageal dysmotility. These findings suggest that in those breastfed children, there is evidence of macrophage activation, and this may be associated with esophageal dysmotility.[319] After long-term follow-up, the urinary levels of neopterin decreased significantly, whereas the urinary nitrates were unchanged.[319] The implications of the above-mentioned findings are not clear. More data are needed to confirm these findings, particularly if one takes into account that the population studied was a very self-selected sample and that the benefits of breastfeeding are well established, so that it may be premature to state that women with silicone breast implants should not breastfeed.[320]

Hirschsprung Disease

Patients with short segments of Hirschsprung disease usually do not have any problems related to esophageal function. However, it has recently been stated that they have minor abnormalities in esophageal motility, including abnormal tertiary and double-peaked contractions that persisted after surgery.[321] Isolated case reports of an association between Hirschsprung disease and achalasia have also been published.[322]

Patients with total colonic aganglionosis usually have feeding difficulties, and a recent study described abnormalities in both duodenal and esophageal motility in 11 children.[323] They found normal UES and LES function and abnormalities in the lower third of the esophagus, with abnormal and double-peaked waves and absence of propagation in more than 20% of swallows, as well as abnormalities in small bowel function, suggesting that patients with Hirschsprung disease have diffuse digestive dysmotility.[323] It is not clear if this dysmotility is related to antenatal chronic obstruction, the surgical repair itself, or a primary motor disorder. Clearly, more studies will be necessary to further define the nature of the esophageal dysmotility in these patients.

Graft-versus-Host Disease

Dysphagia, painful swallowing, and severe retrosternal pain can develop in patients with chronic graft-versus-host disease (GVHD). In one series, 8 of 63 patients developed this problem, including a 12-year-old and a 19-year-old patient.[324] No infectious pathogens were identified, and in all patients, an endoscopy showed desquamative esophagitis in the upper and middle esophageal third and, frequently, webs. The distal esophagus was described as normal in all but two patients. Seven underwent esophageal manometry and five had abnormalities, including two with aperistalsis, one with simultaneous contractions and aperistalsis after swallowing, and two with high-amplitude, long-duration peristaltic contractions. They suggested that reflux esophagitis was present in at least three patients, including two with distal strictures; that the esophageal motor disorder is secondary to the immunologic response to GVHD; and that, as a result of delayed acid clearance, these patients are prone to develop complications from acid reflux. It is interesting that the

pathologic description of the esophagus of those symptomatic patients who died did not indicate any abnormalities of the myenteric plexus or the smooth muscle, with only severe mucosal abnormalities, further reinforcing the impression that the motility disorder is secondary to the mucosal lesion. Whether the abnormal motility is secondary to the mucosal lesion that occurs with GVHD, to reflux esophagitis, or to both is not known, but these esophageal motor alterations are of interest because they seem to be secondary to immunologic phenomena.

SUMMARY

This discussion is the clinical extension of that in Chapter 25, "Esophagitis." In this chapter, the pathophysiology and clinical manifestations of specific motor disorders of the esophagus, including achalasia, were discussed. In addition, a detailed approach to the treatment of these conditions affecting the esophagus was provided.

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CHAPTER 27

INJURIES OF THE ESOPHAGUS

Jean-Pierre Olives, MD

Injuries of the esophagus in children are most often due to ingestions and to traumatic lesions secondary to thoracic contusion, crush syndrome, blunt trauma, or iatrogenic perforations occurring during investigational procedures or surgery.[1,2] Ingestions of foreign bodies, coins, disk batteries, corrosive substances, and drugs are accidental in the majority of cases,[3–6] but child abuse, poisoning, or Munchausen syndrome by proxy should be considered.[2] Retrosternal pain, dysphagia, hypersalivation, and emesis are typical symptoms of esophageal lesions,[3–6] but in the young and nonverbal or retarded child and sometimes in older children, esophageal symptoms are not always obvious.[2] For example, large esophageal blunt objects might predominantly cause respiratory symptoms[7–9] and be misdiagnosed as tracheobronchitis, bronchopneumonia, or asthma.[8] Moreover, small impacted foreign bodies and even intramural perforations or infections can be, in infants and toddlers, completely asymptomatic.[3,10–13]

The management of esophageal injuries has changed significantly over the last two decades with the improvement of diagnostic procedures; 30 years ago, only radiologic studies (chest radiographs, barium swallow, or conventional tomography) were performed. Nowadays, sophisticated procedures are available in the majority of pediatric hospitals: ultrasonography, computed tomography (CT) (if necessary with three-dimensional reconstruction), nuclear magnetic resonance imaging, and, of course, fiberoptic endoscopy, which allows a thorough examination of the esophagus lumen but also provides the opportunity to perform interventional procedures.[1,2,14]

INGESTED FOREIGN BODIES

Foreign object ingestions in children occur commonly. The management of these patients remains controversial; each team probably has its own protocol; therefore, the type of intervention and the risk of complication vary with the site of initial health care contact.[5] The majority of foreign bodies will progress through the gastrointestinal tract without any problem and will be excreted by the feces. Although deaths caused by foreign body ingestion have rarely been reported, mortality rates have been extremely low, with recent large series reporting no deaths among 852 adults and 1 death among 2,206 children.[10,11,14,15] Most studies showed no gender predilection[2] or a slight prevalence of male ingesters[3]; surprisingly, the prospective study from Paul and colleagues reported that 60% of the patients were female.[5]

Infants and young children explore their environment by placing objects in their mouth; around 10% of the children seem to be recidivists.[5] Childhood curiosity and carelessness appear to be the major risk factors for accidental ingestion. Because it appears that foreign bodies are often easy for children to reach, parents need to place more emphasis on environmental safety than on their own vigilance. In only 51% of the cases reported by Paul and colleagues, the ingestion was witnessed.[5] This might suggest that the true incidence of accidental swallowing is, in general, underestimated because the majority of ingested foreign bodies do not cause symptoms. As a consequence, it can be supposed that the caretakers of the child are not aware of accidental ingestion in an important number of cases.[11]

Esophageal foreign body impactions should be removed as soon as possible because of the increased risks of perforation and aspiration, especially when they are lodged in the upper third of the esophagus.[2,6] Foreign objects that arrive in the stomach are likely to be eliminated between 2 and 30 days. Nevertheless, a high incidence of complications has been reported with large objects (> 5 cm in length and > 2.5 cm in diameter), sharpended foreign bodies, and batteries located in the stomach or duodenum.[2,3] In considering the outcome of foreign bodies located in the gastrointestinal tract, the composition, size, shape, and number of ingested objects should be taken into account but also the existence of a number of specific anatomic barriers. The transit is most likely to be retarded or blocked at the cricopharyngeal ring, aortic arch, lower esophageal sphincter, pylorus, duodenal curve, ligament of Treitz, Meckel diverticulum, ileocecal valve, appendix, and rectosigmoid junction.[2,3,14] There exist as well possible associated conditions, such as esophageal stenosis, achalasia, or previous abdominal surgery, that may alter spontaneous passage.

The evaluation of children with an ingested foreign body should include a careful clinical history, including age, previous digestive symptoms, the type of ingested object, and the interval from ingestion to consultation.

Pharyngeal and Cricopharyngeal Foreign Bodies

Foreign bodies that lodge in the pharynx or at the level of the cricopharyngeal ring are often coins, tokens, toy

parts, or fish bones. Standard fluoroscopy is useful to detect metallic objects; plain films of the neck should be taken in both posteroanterior and lateral views. Flat objects, such as coins or tokens, that lodge in the hypopharynx are seen on edge on the lateral film of the neck, whereas those lodged in the upper airway are seen on edge on the frontal film. The accuracy of radiologic examination to locate fish bones has been validated for nine species when taped to the neck of a control patient[2] and for 14 fish bones embedded in a tissue phantom.[16] Coins and flat objects are easily removed using a Magill forceps, a Foley catheter,[17,18] or a magnet[3,11] or by suction retrieval.[19] Fish bones can usually be extracted with a curved forceps or with long tweezers.

Esophageal Foreign Bodies

Children can put inedible things in their mouth. Sometimes the object is swallowed and consequently becomes a foreign body when it enters into the gastrointestinal tract. Impaction, obstruction, and perforation most often occur at areas of physiologic narrowing.[14] The esophagus is a natural "filter": the majority of foreign bodies pass spontaneously if they are less than 2 cm in diameter.[2,3,6,20,21] Blunt, long, or sharp-pointed objects are most likely to be retained in the cervical esophagus (Figure 27-1), at the level of the aortic arch or just above the lower esophageal sphincter. The type of ingested foreign body varies with age: in young children, they are largely coins, toy parts, crayons, jewelry, and ballpoint pen caps, whereas older children and adults commonly tend to have problems with meat and bones (Table 27-1).[2–6,10,11,14,15,20–28]

Small coins (15–20 mm) are less likely to get stuck in the esophagus than are larger coins (20–35 mm), which lodge at the cricopharyngeal ring in 60 to 65% of cases (Figure 27-2), at the level of the aortic arch in 10 to 15% of cases, or above the lower esophageal sphincter in 20 to 25% of cases.[2] Radiologic investigations reported by Gryboski in a review showed that the coins passed from the esophagus to the stomach between 1 and 20.5 hours in two studies, whereas another prospective study showed that 62% of the coins were in the stomach 6 hours after ingestion.[2]

TABLE 27-1 DIFFERENT KINDS OF FOREIGN BODIES LODGED IN THE ESOPHAGUS

Coins
Buttons, press studs, zips
Bones*
Button battery disks
Crayons
Meat*
Toy parts
Tooth picks*
Fruits
Jewelry
Ballpoint pen caps
Salad vegetable leaves*
Needles
Can pop tops
Kernels
Nails
Bottle tops
Dental retainers, crowns*
Screws
Marble
Plastic leaves
Safety pins
Stones
Spoons*
Straight pins
Drug vials and bags*
Toothbrushes*
Tacks
Small electric bulbs
Razor blades

Adapted from references 2 to 6, 10, 11, 14, 15, 20 to 28.
*More frequently ingested by teenagers and adults.

Symptoms

Older children are able to identity the object swallowed and point to the location of discomfort. In young or noncommunicative (mentally impaired or psychiatrically deranged) children, the sudden onset of dysphagia, wheezing, or respiratory distress should suggest ingestion of a foreign body. The most common symptoms of an impacted foreign body are choking, hoarseness, refusal to eat, vomiting, drooling, bloodstained saliva, or respiratory distress. Less common complaints are pain on swallowing, chest pain, and local-

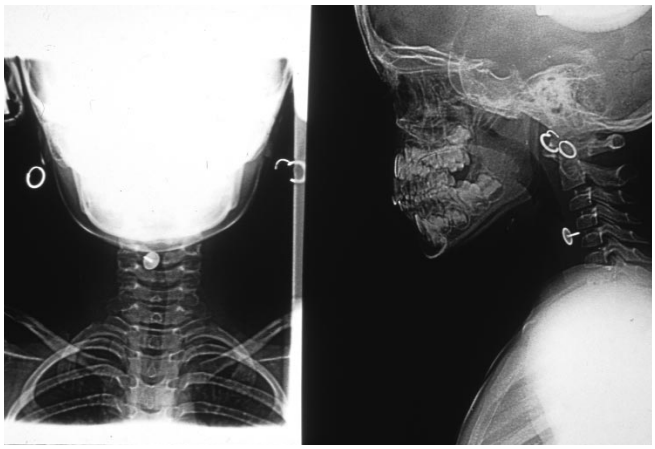

FIGURE 27-1 Ingested thumbtack at the level of the cricopharyngeus.

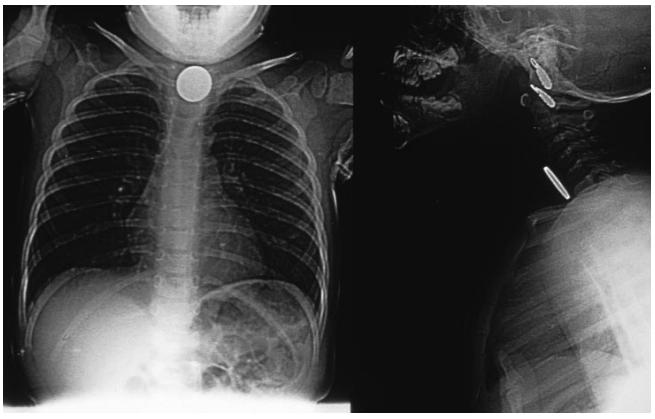

FIGURE 27-2 Anteroposterior film showing the flat surface of an ingested coin within the cervical esophagus. Lateral film showing the edge of the coin.

ization of the level of impaction within the chest, which is usually not reliable. Long-standing esophageal foreign bodies can present as a neck mass, chronic cough or stridor, and dysphagia. Swelling, erythema, tenderness, or crepitus in the neck region may be present with oropharyngeal or proximal esophageal perforation.[2]

Diagnosis

Radiographs usually identify most true foreign objects, bones, and the majority of complications secondary to perforation such as pneumomediastinum, pneumothorax, mediastinitis, and pneumoperitoneum.[2,14] Plain films of the neck, chest, and abdomen are generally recommended and should be taken in both posteroanterior and lateral views.[2,3,20,21] The lateral projection confirms location in the esophagus and may reveal the presence of more than one disk-shaped object (coins, button batteries, tokens).[10]

Handheld metal detectors are useful to spot the majority of swallowed metallic objects and may be of use as a screening tool in pediatric patients.[23–28] Nevertheless, it depends on the location of the foreign body: in an emergency room study, in 14 children, the presence or absence of coins was correctly identified by a metal detector in 13; when compared with radiologic studies, the remaining child had a coin lodged in the rectum. The same results were published by Saccheti and colleagues; of 20 coins, 1 was missed in the right iliac fossa.[24] More recently, in a large prospective study, radiopaque metallic foreign bodies were detected by use of a metal detector in 79% of the cases (183 of 231) when only 181 were identified by radiologic studies. In the remaining 45 children, in whom ingestion was suspected but not definitive, both a metal detector and radiology confirmed the presence of a foreign body in only 4.[26]

If symptoms are not clear or specific, a cautious contrast study may be appropriate to clarify the presence of a foreign body or its location (Figure 27-3).[27,28] CT may be necessary in some cases but may be negative with radiolucent objects; the yield may be improved with the use of three-dimensional reconstruction.[14,29] Drug packings have been studied by radiography, CT, and ultrasonography. Cannabis and cocaine packages were easily detected by plain radiographs and CT scans showing a high-density shadow surrounded by a gas halo. Heroin packages are difficult to localize, but on sonograms, they appear as round, echogenic structures.[29]

Treatment

The management of an esophageal foreign body is influenced by the child's age, body weight, some clinical criteria (ie, the size, shape, number, and classification of ingested objects), the anatomic location in which the object is lodged, and, finally, the armamentarium, skilfulness, and technical capacities of the endoscopist.

For coin ingestions, in many circumstances, noninternational protocols are applied. For instance, in a home-based study, 52 of 61 children having swallowed a coin were managed at home without calling a physician.[2] Two studies proposed a management based on location.[30,31] Radiologic study documented the site of the coin. If it is in the upper third of the esophagus (including the cricopharyngeus region), it should be removed urgently. If it lies in the middle of the lower esophagus, a repeat film should be taken in 12 to 24 hours if the child has remained asymptomatic because most coins pass into the stomach within 24 hours.[30] For Maksimak and colleagues, children without symptoms were treated conservatively with observation and sips of water or clear liquid to promote passage of the coin. This protocol was successful in 83% of the cases (18 of 21 children).[31] If the coin is still in the esophagus after 24 hours, it should be retrieved. If the coin is located below the diaphragm or not visible, the family may be reassured; the child is discharged, and the stools are examined and strained to identify the coin's passage. If, after a week's time, the child has not passed the coin, an abdominal film should be obtained. If the coin remains in the stomach after a delay of 4 to 6 weeks, it should be removed endoscopically.[2,3,30] In removing a foreign body from the esophagus, the most important point of treatment is the maintenance of an airway at all times.[2,21] For that, endotracheal anesthesia, coupled with endoscopy, is the safest method. Rat-tooth and alligator-jaws forceps are very efficient to ensure coin retrieval if the child is correctly sedated. If the patient does not have an endotracheal tube, the Trendelenburg position should be used to keep the coin out of the trachea.[21]

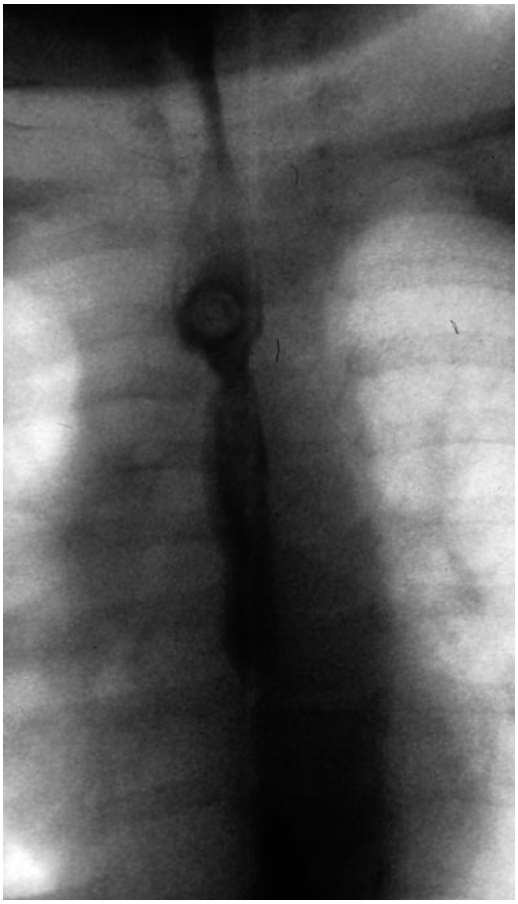

FIGURE 27-3 A 9-month-old boy who had an esophageal atresia repair has a plastic pearl impacted at the level of the anastomosis.

The "through-the-scope" balloon can also be used to extract a coin, but this is rarely necessary. However, blunt foreign bodies, such as marbles, that cannot be grasped with instruments can be removed easily under direct vision by using through-the-scope esophageal dilating balloons.[21] The Roth retrieval net, which is a polypectomy snare with a net, can be used to capture round or oval foreign bodies with ease.[2,20,21] It should be stressed that if one is having difficulty retrieving a foreign body from the esophagus and it is less than 2.0 cm in diameter and 5.0 cm in length, it can be gently pushed into the stomach and should pass through the gastrointestinal tract without difficulty.[2,3,14]

An alternative method for removing coins and blunt foreign bodies from the esophagus is the use of the Foley catheter.[32–34] Campbell and colleagues successfully removed blunt foreign bodies from 98 of 100 infants and children by this method.[32] In a survey of pediatric radiologists, 2,500 successful removals were recorded, with only one reversible complication.[33] This procedure is performed in the radiology department under fluoroscopy, with the catheter being placed orally, rather than nasally, to keep the foreign body out of the nasopharynx. The radiograph table is placed in a deep Trendelenburg position to minimize the chance that the foreign body will enter the larynx.[21] The Foley catheter technique provides no control of the foreign body as it is removed.[2,21] Another disadvantage is that the pathology, if present, cannot be assessed. If the foreign body has been present for longer than 24 hours, or if edema is present, this technique should not be used. The Foley catheter technique is recommended only if endoscopy is not available. The magnet can be used for removal of metallic objects, but the disadvantages are identical to those observed with the Foley catheter. Paulson and Jaffe, however, have reported successfully removing metallic foreign bodies in 34 of 36 cases using this technique.[35]

Sharp and pointed foreign bodies, as well as elongated foreign bodies, can be difficult to manage; fortunately, they are not common. When considering sharp and pointed foreign bodies as a separate group, morbidity and mortality figures are higher.[2,4,6,10,15,21] The most common foreign bodies in this group are toothpicks, nails, needles, bones, razor blades, and safety pins. The open safety pin represents a major problem (Figure 27-4). If a safety pin is in the esophagus with the open end proximal, it is best managed with the flexible endoscope by pushing the pin into the stomach, turning it, and then grasping the hinged end and pulling it out first. An alternative is to close the pin using a polypectomy snare. The closed safety pin, once in the stomach, will pass without difficulty. An overtube or a rigid esophagoscope may be necessary with large, open safety pins.

The ingested razor blade is a challenging experience for both the patient and the endoscopist. This foreign body can be managed with the rigid esophagoscope in both the child and adult by pulling the blade into the instrument. One also can use a rubber hood or a piece of rubber glove on the end of the endoscope to protect the esophagus from sharp or pointed foreign bodies.[2,20,21]

The straight pin ingested by infants and children is an exception. Those longer than 5 cm may fail to pass through the duodenal loops and may perforate with the risk of hepatic hemorrhage or infection.[2,6] The use of an overtube or an endoscopic end protector hood will prevent laceration during removal. Crack tubes or body bags of heroin or cannabis must be retrieved very cautiously using a basket or a net because forceps grasping carries the danger of tearing the bag, with intraluminal release of the narcotic substance.[2]

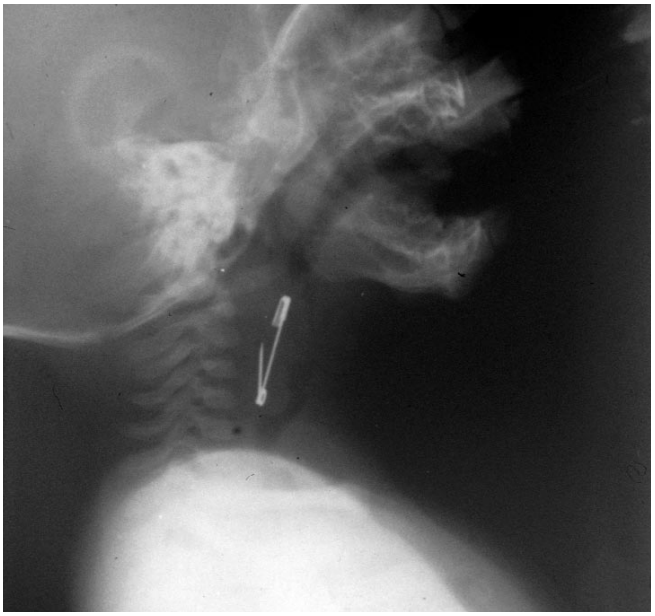

FIGURE 27-4 Open safety pin at the level of the cricopharyngeal ring.

Radiolucent objects such as pieces of glass, bone fragments, aluminum (eg, canned drink pop tabs), plastic, and pieces of wood can often be difficult to see in the hypopharynx and cervical esophagus on routine radiographs.[21] It the patient has complained of swallowing a foreign body and it is not seen on routine radiographs, thin barium is used to try to outline the object.[2] If the foreign body is identified radiographically, endoscopy is performed. If no foreign body is seen radiographically but the patient remains symptomatic, endoscopy is also performed (Figure 27-5). If no foreign body is seen radiographically and the patient has become asymptomatic, the endoscopy is not mandatory.[21]

Complications

Esophageal examination in children with coin ingestions of less than 24 hours duration may show normal mucosa in the majority or minimal erythema or abrasion in some cases.[36] Complications increase with the length of time the foreign body remains in the esophagus, with perforations, particularly from sharp bodies, occurring after 24 hours.[2,31] Fewer than 1% of esophageal foreign bodies cannot be retrieved by endoscopy, and esophagotomy must be employed. Perforation of the esophagus after foreign body and coin ingestions may be either acutely symptomatic or asymptomatic.[37] Aspiration pneumonia, lobar atelectasia, hemoptysis, perforation with neck abscess, mediastinitis with lung abscess, acquired esophageal pouch, pseudodiverticula, tracheoesophageal fistula, esophageal-aortic fistula, perforation of the heart by an

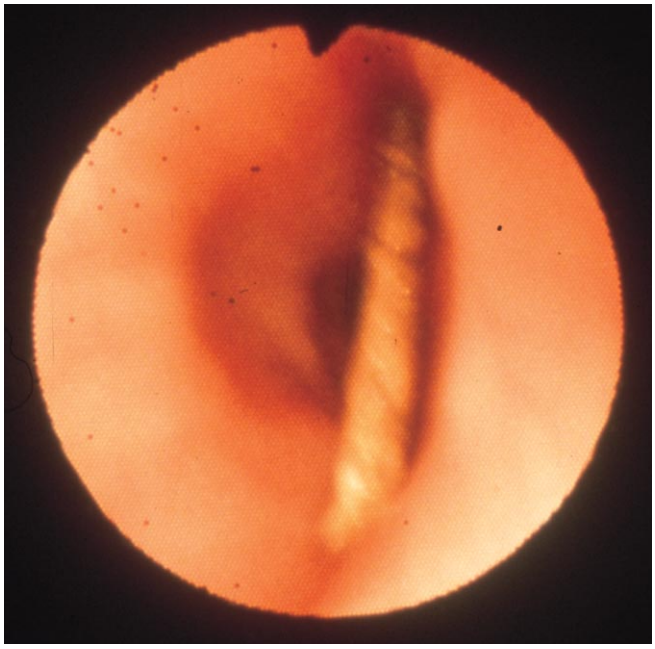

FIGURE 27-5 Endoscopic view of a rabbit bone stuck in the esophagus.

open safety pin, and aortic pseudoaneurysm are less common complications, occurring months to years after impaction of the foreign body.[2]

The CT scan is useful in confirming the location of impacted fish bones and radiolucent foreign bodies, as well as identifying inflammatory changes in adjacent structures.[29,38] Indications for thoracotomy for removal of a long-retained foreign body are poor endoscopic visualization of the foreign body because of inflammatory tissue and herald bleeding during endoscopy.[2]

Nickel dermatitis and associated gastritis and copper and zinc toxicity have been reported after coin ingestion, usually by retarded or schizophrenic adults.[2,39]

Food Impaction

Food bolus obstruction might occur in children with an esophageal stricture or with digestive motility disorders. Children who are in severe distress or unable to swallow oral secretions require immediate intervention. If the patient is not uncomfortable, not at risk for aspiration, and able to handle his or her secretions, then intervention need not be emergent and can be postponed to a reasonably convenient time because food impactions will often pass spontaneously.[21] However, endoscopic intervention should not be delayed beyond 24 hours from presentation because the risk of complication may increase.[6,15,20]

The initial endoscopic examination should verify and locate the site of the impaction. The food bolus can usually be removed en bloc or in a piecemeal fashion.[14]

An overtube may facilitate multiple passes of the endoscope, protect the esophageal mucosa, and minimize the risk of aspiration. Nevertheless, in small children, this technique is difficult to use because of the risk of esophageal injury during the overtube insertion. Once reduced in size, the bolus can often be passed under endoscopic visualization and direction. The high incidence of underlying esophageal pathology in this setting increases the risk associated with the practice of blindly pushing an impacted food bolus with the endoscope or a dilator.[14] The application of a cautery current applied to a bipolar snare to cut into, grasp, and retrieve an impacted food bolus in the esophagus has been reported in two articles.[40,41]

Enzymatic digestion with papain should not be used because it has been associated with hypernatremia, erosion, and esophageal perforation.[20,21] The administration of glucagon 0.5 to 1.0 mg intravenously, in an attempt to relax the esophagus, is generally safe and may promote spontaneous passage of an impacted food bolus while definitive endoscopic therapy is being coordinated. However, its use should not delay definitive endoscopic removal.[14] The use of Coca-Cola has even been suggested to "digest" a food bolus trapped in an esophageal stricture to avoid endoscopy.[42]

Food-Related Esophageal Trauma

Food-related trauma of the esophagus in children is rare. In adults, esophageal hematoma or laceration has followed swallowing or impaction of tortilla chips, taco shells, bagels, or bay leaves. Ingestion of hot pepper sauce can cause esophageal burns; inflammation of the mucosa is associated with a significant increase of esophageal peristalsis.[2]

Disk Battery Ingestion

The disk button battery is a single cell usually used to power digital watches, photographic equipment, toys, hearing aids, car electronic keys, handheld calculators, and even musical greeting cards.[2,43] Although these cells are sealed, they contain corrosive and toxic chemicals. Lodgment in the esophagus can lead to mucosal damage, and exposure to gastric acid is associated with a remote risk of leakage of the cell contents.[44,45]

There are four main types of button cell: mercury, silver, alkaline manganese, and lithium. Lithium cells are mostly used in watches in which replacement is normally by a specialist, thereby limiting access to children. Lithium cells exhibit a potential of 3 V against the 1.5 V of the other systems but are more resistant to corrosion than other button cells. Used batteries are potentially far less toxic than new ones. Discharged cells are less liable to leak or cause tissue injury, and in discharged mercury cells, the mercuric oxide will be largely converted to elemental mercury, which is not absorbed.[43,45] Finally, the mechanisms that corrode the cell container also discharge the cell, so the contents of a discharged cell are the most relevant.[43]

Because of a then estimated 510 to 850 ingestions yearly, a National Button Battery Ingestion Study was established in 1982 in the United States to provide guidelines to therapy. A 1995 review of 119 ingestions during an 11-month period determined that the majority of buttons contained silver or mercuric oxide.[46] Less common were those of magnesium dioxide, zinc or air, or lithium content, with all containing a 20 to 45% solution of potassium or sodium hydroxide. Those most frequently ingested were the 7.9 and the 11.6 mm batteries. Ingesters were primarily young, with

71.2% less than 5 years, and with 1 to 2 year olds at greatest risk. Sixteen percent were between 6 and 12 years and 4.5% were between 13 and 17 years, all with a male preponderance. Nearly half of the children found the battery loose or discarded, and a surprising source (39%) was from hearing aids belonging to the children or to others. More than one battery was ingested by 8.5% of patients. Older children or adults, while testing the viability of the battery by touching it to the tongue, have inadvertently swallowed it. A 1992 update from the Registry described 2,382 cases with similar findings.[47] The authors estimate a minimum of 2,100 battery ingestions per year based on their figures of 2.1 to 8.5 ingestions per million population in different states. The battery removed from a child's own hearing aid was the most common (44%) of ingested batteries. Lithium batteries with larger diameters and greater voltage cause the more severe injuries. Mercuric oxide cells were more likely to fragment.[2,47]

Although 89.9% of batteries passed spontaneously between 12 hours and 14 days, with 31% requiring more than 48 hours, those lodged in the esophagus (4.2%) required early removal. Most of the batteries that lodged in the esophagus were 20 to 23 mm. Esophageal injury is attributable to electrolyte leakage from the battery, alkali produced from external flow of current causing liquefaction necrosis (it is estimated that when lodged in the esophagus, 26 to 45% will leak sodium or potassium hydroxide), mercury toxicity, pressure necrosis, and direct flow of 1.5 or 3.0 V current to cause low-voltage burns.[2] Although most batteries show corrosion, they do not disassemble, although 2% in the series of Litovitz and Schmitz did fragment, and 11% had severe crimp dissolution or extensive perforations.[47]

Esophageal damage occurs rapidly, and burns are noted as early as 1 hour after ingestion. Within 4 hours, there may be involvement of all layers of the esophagus. In experimental studies in dogs in whom batteries were placed within the esophagus, by 8 hours, there was mucosal abrasion or necrosis under the muscular layer without evidence of battery leakage.[2] In rabbits, esophageal injury was created by placing a 3 V battery in the esophagus for 9 hours. Injury was more severe on the alkaline side when the battery was placed with the cathode directed toward the trachea. More severe damage is produced by a lithium battery than a button alkali one, with damage occurring within 15 minutes.[2]

Symptoms

Usually, children are asymptomatic after button battery ingestion.[44,46] Nevertheless, the immediate symptoms might be coughing, gagging, nausea, vomiting, and chest or abdominal pain.[2]

Diagnosis

The battery must be identified and distinguished from a coin radiologically. In the anterior projection, it shows a double-density shadow owing to its bilamellar structure (Figure 27-6). On lateral films, the edges are round and show a step-off at the junction of anode and cathode.

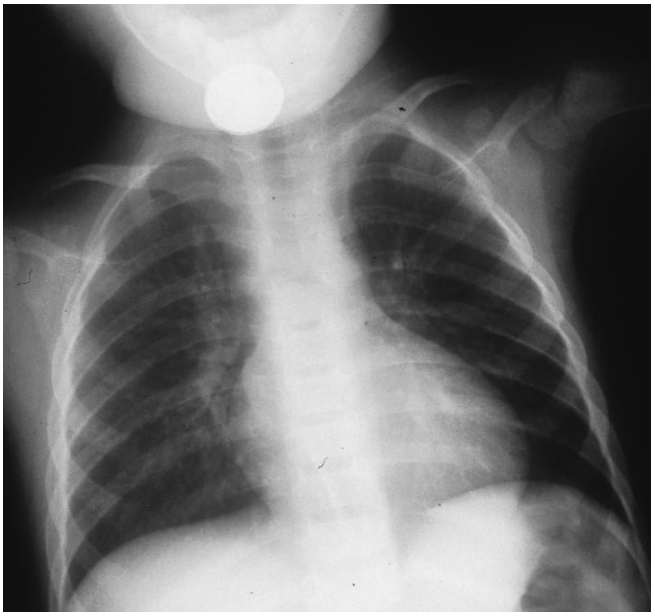

FIGURE 27-6 Anteroposterior film of a large disk battery (20 mm) with the halo sign (double-density shadow).

Treatment

Ipecac is not recommended to induce vomiting because it may lead to aspiration and impaction of the disk in the respiratory tree or to retrograde advancement from the stomach to the esophagus. Its use further carries the risk of perforation of the stomach or esophagus if the battery has caused a significant mucosal burn.[2] Administration of neutralizing solutions or charcoal has not been helpful. The treatment protocol recommended by the Button Battery Study advocates nothing by mouth and an initial radiologic study to determine the location of the battery.[47] If the battery is lodged within the esophagus, it should be removed immediately.[2,44,47] Endoscopic retrieval rates are 33 to 100%, and in children, endoscopic removal is performed using general anesthesia (to avoid aspiration) and may include the use of a polyp snare, a Roth retrieval net, a basket, or a through-the-endoscope balloon.[2] Endoscopy may also assess the degree of esophageal trauma. If there is evidence of tissue damage, a follow-up barium study should be performed 10 to 14 days later to rule out stricture or fistula formation.[47]

Success to remove disk batteries lodged in the esophagus has also been reported with the use of a Foley catheter, a balloon, or a magnetized catheter (Figure 27-7). In this author's experience, button batteries located in the esophageal and gastric areas can easily be extracted with a magnet attached to an orogastric tube in 88% of cases. My colleagues and I reported the management of 64 cases of accidental ingestion of button batteries in which magnetic removal proved to be successful in 49 of 56 attempts (14 of 14 cells lodged in the esophagus).[44] Failure of extraction occurred for 6 batteries located in the duodenum and solely for 1 in the stomach. This type of device is very cheap (50 dollars) and can be used by all pediatricians under fragmentary fluoroscopy. Because magnetic removal of disk batteries is very easy, and leakage, although extremely rare,

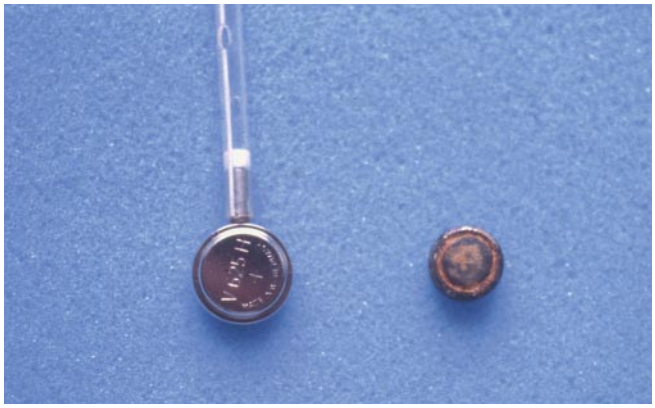

FIGURE 27-7 The new battery on the left is attracted with the magnet tube. The battery on the right shows severe corrosion after 6 hours in the upper gastrointestinal tract.

would result in life-threatening complications, especially when lodged in the esophagus, we recommend extracting emergently batteries if they are in the esophagus or still in the stomach at the first health care contact.[44] Management of a battery lying in the stomach remains controversial; for several authors, the child can be discharged home with normal eating and activity, with the parents instructed to strain the stool for retrieval of the battery and to report any pain, fever, or vomiting.[43,45,48] A prokinetic agent such as metoclopromide or a laxative may hasten battery transit through the stomach and small intestine. If the battery remains within the stomach after 1 week, it should be retrieved by endoscopy. If the battery fails to move through the intestine or if the child develops pain or peritoneal symptoms, it should be removed surgically.[2]

Complications

Reported complications of esophageal lesions are tracheoesophageal fistula, perforation, stricture, and even death.[2,49,50,51] A battery may lodge in a Meckel diverticulum and cause perforation.[2] Mercury toxicity is possible but rare, with only one mild case having been reported. Elemental mercury is produced by the action of gastric acid and iron from the casing, and this, in contrast to mercuric oxide, is readily absorbed. Elevated mercury levels have been reported in some patients after battery ingestion, but there were no signs of mercury toxicity.[48] The highest levels were found in children in whom the batteries split before or after passage and who had evidence of radiopaque droplets within the gastrointestinal tract. Rashes owing to presumed nickel hypersensitivity occur in approximately 2% of children.[2]

CAUSTIC INGESTIONS

Esophageal injuries caused by ingestion of caustic agents occur frequently in young children. The peak incidence is below the age of 5 years. The estimated frequency of admissions for caustic ingestion ranges from 1,000 to 20,000 per year in industrialized countries; the majority of cases are children.[52] Severe caustic injuries have been reported from developing countries, especially in northern Africa, with a very high incidence.[53] The male-to-female ratio is 1.2 to 1.4:1, although a female preponderance has also been reported, mainly owing to occupational habits. After a rise in frequency in the 1960s, there has been a decreasing incidence owing to legislation and because of an increasing awareness of the hazards of storing caustic products in an inappropriate way, such as within the reach of young children or in food and drink containers.[52] Inappropriate storage occurred in 10 to 15% of the accidental ingestions.[54]

Etiology

In the majority of cases, ingestion of caustic agents in children is accidental.[2,52–54] Only a minority of cases are intentional as an attempt at suicide or, rarely, as an attempt at homicide.[52]

Caustic products ingested by children most often are strong alkaline or acidic agents. Liquid forms cause more significant injuries than solid products, which are more irritating and difficult to swallow.[2,55] Strong alkali are used in the household as cleaning agents for the dishwasher (sodium metasilicate, sodium tripolyphosphate), oven, drain, or toilet bowl or as a declogging agent (sodium hydroxide, potassium hydroxide). Sodium hydroxide tablets are used for medical (Clinitest tablets) and sometimes pseudomedical purposes (a homemade mixture to predict gender during pregnancy) and cause caustic as well as thermic injury, leading to devastatingly deep burns.[55] Ammonia causes not only caustic esophageal injury but also chemical pneumonitis and pulmonary edema. Less frequently, caustic esophagitis is caused by ingestion of strong acids (eg, hydrochloric, sulfuric, formic, or phosphoric acid used in the household as cleaner for coffee makers, irons, and toilet bowls but also used as soldering fixes, antirust compounds, battery liquids, cleaners for swimming pools, milking machines, and slate). Detergents and bleach are reported to be ingested most commonly by children. This is not confirmed by many reports. Household bleach (Na hypochlorite, pH 6.0) is considered an esophageal irritant but does not cause tissue necrosis because of its low concentration (5%) and seldom causes clinically significant esophageal injury.[2,52,54]

Pathophysiology

The physical form and pH of the corrosive agent play a significant role in the location and type of resultant injuries. Crystalline drain cleaners (lye) tend to adhere to the oropharynx or become lodged in the upper esophagus, where they cause most of their damage.[55,56] High-density liquid drain cleaners usually pass rapidly through the oropharynx and upper esophagus and cause more significant problems in the lower esophagus and stomach. Strong acids usually pass rapidly through the esophagus and cause their most significant damage in the stomach and duodenum.[56]

Because corrosive substance ingestions are rarely fatal and the injured part is seldom removed surgically during the immediate postinjury phase, little histopathologic information is derived from human specimens. Much of what is known is derived from studies in experimental ani-

mals. Acid burns cause a coagulation necrosis that usually limits acid penetration and results in damage limited to the mucosa.[2,56] Both cats and dogs have been used to study the effects of sodium hydroxide on the esophagus. Initially, there is liquefaction necrosis with destruction of the epithelium and the submucosal layer. Hemorrhage, thrombosis, and a marked inflammatory response with significant edema are seen within the first 24 hours of injury (early acute phase). Depending on the extent of the burn, inflammation may extend through the muscle layer, and perforation may occur. After several days, the necrotic tissue is sloughed, edema decreases, and neovascularization begins. This early reparative or subacute phase extends from the end of the first week through the second week after injury, and if the insult has been relatively minor, function begins to return. The cicatrization phase begins in the third week when fibroblast proliferation replaces the submucosa and muscularis mucosa; there stricture formation begins. During this time, adhesions form, and narrowing or obliteration of the esophageal lumen may occur. If there has been a significant penetrating injury, adhesions to surrounding mediastinal tissues may also occur. Re-epithelialization begins during the third week and is usually complete by the sixth week after injury.[56]

Household bleach, which is moderately alkaline, has been, for a long time, a matter of debate regarding its potentiality to induce caustic lesions.[2,52,53,56] To clarify the conflicting observations that sodium hypochlorite bleaches cause significant burns identifiable at endoscopy and that serious long-term morbidity and stricture formation are exceedingly unusual, Yarrington studied the effect of sodium hypochlorite bleach on the canine esophagus.[57] Large volumes of bleach and prolonged contact time (longer than 2 minutes) were necessary to induce injuries detectable by endoscopy. Animals were killed 1 week after injury, and significant histologic changes were identifiable only in animals subjected to a large volume (30 mL) or significant exposure time (5–10 minutes). None of the dogs had penetrating injury or involvement of the muscularis. Yarrington concluded that although ingestion of household bleach may induce mucosal burns and edema, extensive necrosis and stricture formation do not occur.[57]

Symptoms

The clinical presentation of caustic esophageal injury shows a wide spectrum: in many cases, the child will have no complaints, and the physical examination may be normal. At the other end of the spectrum, the child can present with shock or severe respiratory distress.[58–60]

Respiratory distress and stridor following laryngeal injury occur more frequently in children below the age of 2 years and are not related to the nature of the caustic. Vomiting, dysphagia, drooling, epigastric pain, abdominal pain, and refusal to drink may inaccurately predict the presence or the severity of esophageal injury.[52] A close inspection of the patient will show irritation or frank burns periorally, on the lips, but also on other parts of the body, for example, the thorax and/or the extremities if the caustic agent has been vomited. Inspection of the mouth and pharynx can show edema, ulceration, or white, fragile, easily bleeding membranes over the buccal mucosa, tongue, uvula, and tonsils. Laryngoscopy can reveal laryngeal edema or more severe lesions. Fever may occur, and in 30%, slight leukocytosis is present.[52]

Serious esophageal burn, even perforation, can occur in the absence of oropharyngeal burns or abdominal complaints. On the other hand, burns to the mouth do not provide evidence of an esophageal burn.[2,52,54,59,60]

Diagnosis

Ingestion of caustic agents by children is often unwitnessed. The first element in diagnosing esophageal burn is a good history taking. Every effort should be made to document the possible agent, its physical and chemical characteristics, and its volume to estimate not only the caustic properties but also the noncaustic toxicity.[2,52] However, adequate information often is not available. The simplest way to obtain the information is to have the product itself.

If there is any suspicion of ingestion of a caustic agent, the child needs immediate evaluation by a physician. Burns of the mouth or pharynx are inaccurate as indicators of esophageal injury or its extent and mandate endoscopy. Conversely, the absence of oropharyngeal burn does not eliminate the need for endoscopy.[2,52,54,59,60]

Esophagoscopy with a flexible fiberoptic pediatric endoscope, allowing complete examination of both the esophagus and the stomach, is accepted as the single most accurate method of assessing esophageal injury. It should be performed within 12 to 36 hours after ingestion or suspicion of ingestion.[2,52] Because there is no correlation between esophageal and peribuccal or oropharyngeal burns, esophagoscopy should be done under general anesthesia. Not all authors agree with the need for systematic endoscopy.[2,52,54,55] Two recent studies concluded that endoscopy is not mandatory for children living in developed countries who are asymptomatic after nonintentional caustic ingestion.[61,62]

Caustic esophageal lesions are graded endoscopically as grade 0, normal; grade I, erythema and edema; grade II-A, noncircumferential superficial mucosal ulcerations with necrotic tissue and white plaques extending over less than one-third of the esophageal length; grade II-B, the same as grade II, with deep or circumferential ulcerations extending over more than one-third of the esophagus; grade III-A, mucosal ulcerations and area of necrosis in a circumferential pattern extending over less than one-third of the esophagus length; and grade III-B, extensive necrosis over more than one-third of the esophagus.[63] Some authors include a grade IV, that is, with signs of transmural necrosis: shock, coagulopathy, and metabolic acidosis. Some others do not take into account the circumferential appearance of the lesions. Distinguishing grade II from grade III then can be difficult because there is no clear definition of how the depth of an injury is determined (Figure 27-8).[52]

The opinion that endoscopy is the most accurate way to diagnose caustic esophageal lesions is not shared by all, and esophagography, in an early stage as well as during follow-up, is believed to provide better information.

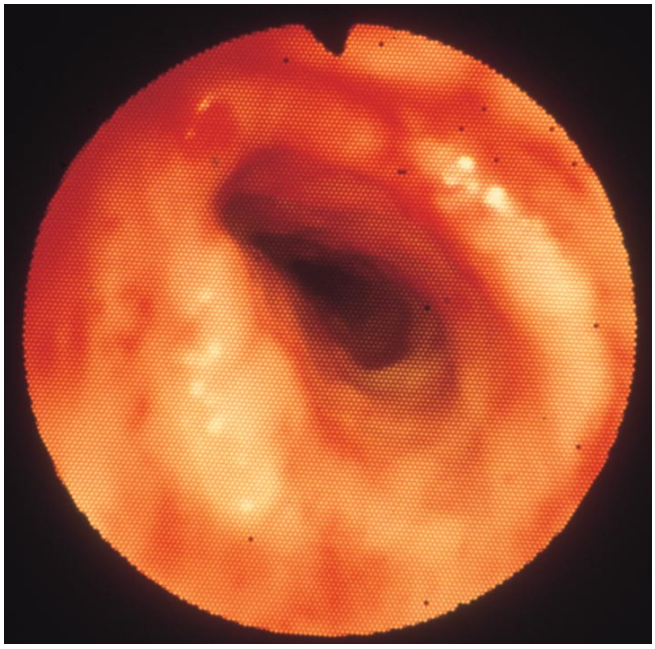

FIGURE 27-8 Endoscopic view of a grade III caustic esophagitis.

Indeed, radiology is adequate in the management of caustic esophageal injury.[52,64] Barium swallow, when performed early, is useful in assessing perforation in patients with suggestive symptoms; however, it is rarely sensitive enough to detect early mucosal injury or to allow therapeutic or prognostic decisions. When performed 2 to 3 weeks after ingestion, assessment of stricture formation can be made.[65] Cine-esophagography has been recommended as the examination of choice in assessing esophageal damage sufficient to provoke stricture formation. Mucosal and submucosal injuries are then seen as irregularities along the contact margin. Alterations in esophageal motility, resulting from injury to the myenteric nerve plexuxes, are indications of severe burn and accurately show evolution to stricture formation.

Outcome

Sequelae of caustic injuries of the esophagus are stricture formation, development of achalasia, brachyesophagus, gastroesophageal reflux, and, as a late complication, development of malignancy.[2,52] Esophageal motor function is disordered for days to years after lye ingestion and shows weak to absent peristaltic contractions, nonpropulsive contractions, gastroesophageal reflux, and dysphagia. Studies with pH monitoring, esophageal manometry, radiology, and esophageal transit scintigraphy with technetium 99m or krypton Kr 81m showed that the severity of the dysphagia was not correlated to the importance of the residual stenosis but rather to specific patterns of esophageal motility.[66,67] Pyloric stenosis can occur after gastric lesions, mainly owing to acidic agents.[52,54] The incidence of esophageal strictures in children is reported to be up to 15%, but figures range from 9 to 18%.[52,59,60,65] Most strictures result from grade III lesions and to a lesser degree from grade II-B and, rarely, from grade II-A. However, management is not consensual; many authors agree that grade I lesions heal without stricture, regardless of treatment, and that grade III lesions progress to stricture, regardless of treatment.[52,54] Acute inflammatory strictures appear at about 21 days (or earlier); complete stricture formation can take 30 to 45 days (Figure 27-9).[54,56]

Treatment

Some suggest that patients who are asymptomatic after unintentional ingestions are not at risk for complications and do not necessarily have to undergo endoscopy.[61,62] Many, however, agree that all children suspected of caustic ingestion should be admitted to the hospital for observation.[2,52,54,56,60] Intravenous fluids are administered, and the child is not permitted to drink until the decision for endoscopy.[2,52,54,55,65]

If there is a reasonable suspicion of caustic ingestion, regardless of the symptomatology, the child should be brought immediately to the emergency department, even if there is doubt that ingestion has occurred.[52] If medical advice is asked via the telephone, the first treatment that should be recommended is not to make the child vomit or give any acid or alkali to neutralize the agent ingested, as is sometimes recommended on the package of caustic agents. The latter can cause marked exothermal reactions and additional injury. Water can be used to wash away residual caustic from the buccal mucosa and face.[52,56] There is no role for diluents, emetics, lavage, smectite, aluminum phosphate, or charcoal. The use of a nasogastric

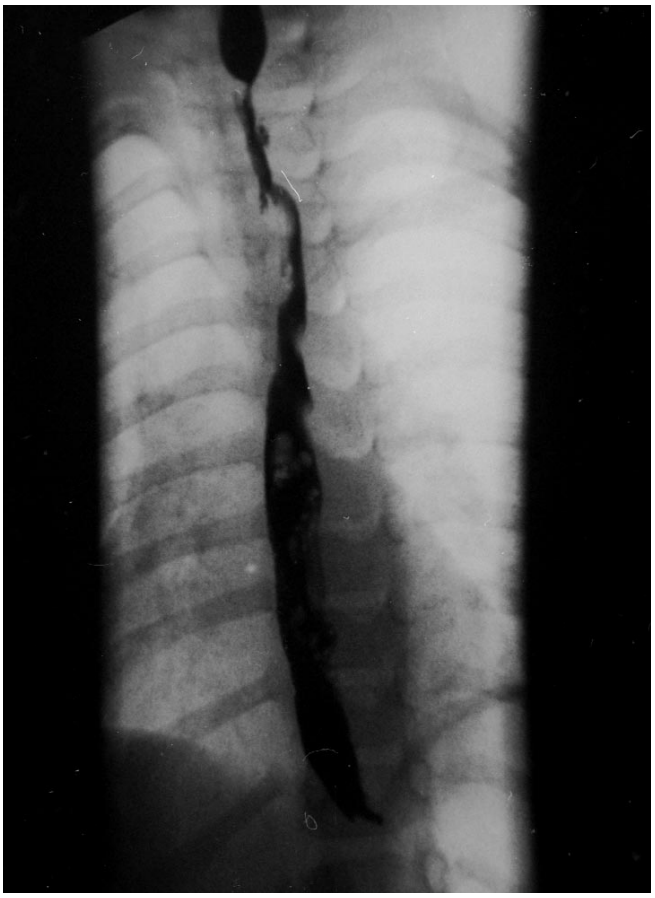

FIGURE 27-9 Severe irregular stricture of the upper third of the esophagus.

tube for gastric lavage is contraindicated because of the risk of aggravation or esophageal perforation. For suspicion of ingestion of bleach or mild household detergents, endoscopy is not mandatory, except when signs or symptoms suggest mucosal injury.[2,52,57,61,62]

After admission to the hospital, when severe general symptoms are present in cases of perforation, laryngeal obstruction, or pulmonary edema, immediate treatment consists of resuscitation, airway control, administration of fluid, plasma expanders, or blood. Physicians agree on the necessity of keeping children with severe lesions under strict surveillance in an intensive care unit during the first week after the accident. Early surgical treatment in cases of esophageal or gastric perforation or laryngeal edema, or for feeding gastrostomy in cases of very severe injury, is rarely indicated.[65] Emergency esophagectomy is indicated only if massive quantities of a strong caustic agent are ingested with esophageal necrosis, occurring almost exclusively in intentional ingestion. Gastric resection, if indicated, must be sparse and limited to the antrum if possible.[52]

When no burns or grade I lesions are present on endoscopy, no treatment is indicated, and the patient is discharged. Children with grade II-A lesions can be observed for 1 to 3 days in the hospital. In some centers, they receive no treatment, but in others, they are given an oral broad-spectrum antibiotic and, in some cases, antacids or histamine$_2$ (H_2) blockers.[2]

Once definitive evidence of grade II-B or grade III lesions is established, treatment of esophageal lesions is directed toward the prevention of stricture formation. Optimal nutrition is imperative during the acute healing phase.[2,52,54,56] Liquids are given by mouth as soon as the child is able to swallow. Oral intake, if possible, is started with antacids and dairy products. The diet is progressively increased as tolerated. If the patient is unable to eat, feeding gastrostomy or parenteral nutrition is indicated. Total parenteral nutrition is sometimes given systematically in grade II and III lesions for at least 3 weeks and continued if there is still no healing of the lesions.[68]

The treatment regimen of significant caustic esophagitis seems to have less influence on the development of stricture formation than the degree of injury immediately after ingestion. Several treatment regimens have been recommended. Since the early 1950s, evidence from animal experiments has shown that systemic or locally injected steroids prevent stricture formation, although a high mortality resulted from infections.[52,56,66] Steroid treatment, prednisone 2 mg/kg or its equivalent, has since been widely used but also rejected as a therapy because of lack of proof of efficiency or because of the risk of bacterial infection, serious varicella infection, or suppression of the hypothalamic-pituitary function.[2,52,54–56] In a review of 14 clinical studies,[69] in which over 2,000 patients were involved, and in a more recent controlled study,[64] no significant difference could be shown in the incidence of stricture formation between the patients treated with steroids and those not treated. Following the beneficial results of early treatment with high-dose steroids in spinal cord injury, a study was conducted in which patients with severe caustic esophageal lesions were treated with dexamethasone 1 mg/kg/d.[70] Compared with those treated with prednisone 2 mg/kg, the patients receiving high doses of dexamethasone developed less esophageal stricture. However, patients in this group had less severe lesions than the patients in the other groups. On the other hand, there was a marked reduction in the number of dilatations needed to treat stricture formation when the high-dose dexamethasone was given immediately after the dilatation. In continuation of this experimental protocol, Cadranel coordinated a study reported by Beddabi in three pediatric gastroenterology centers (two in Tunisia and one in Belgium) in which only grade 3 caustic esophagitis was considered[71]; the children were treated either following classic management without steroids (n = 15) or with daily intravenous shots of 1,000 mg/1.73 m^2 of methylprednisolone (n = 21) for 6 to 12 days, depending on the results of the endoscopic follow-up after 1 week. Of those children managed classically, 14 of 15 developed a secondary stricture; on the other hand, a prevention of secondary stricture was observed in 15 of 21 children managed with early high doses of steroids. This protocol has been enlarged to a working group of the French Speaking Society of Pediatric Hepatology, Gastroenterology, and Nutrition; at the present time, more than 40 children with severe corrosive esophagitis were treated, with a 75% success rate in prevention of stricture formation (J. P. Olives, unpublished data, 2003).

The use of antibiotics is also controversial.[65] In all cases in which steroids are included in the therapy, antibiotics are associated with the prevention of infection.[2,52] In some reports, antibiotics without steroid treatment are used, whether systematically or if indicated.[52,58,68] However, bacteremia does not occur even in severe reflux esophagitis, and it is very rare in caustic lesions, except in cases of perforation. It can occur, although rarely, after esophageal dilatation. If antibiotics are used, mostly ampicillin 50 to 100 mg/kg/d is given.[2,52] Because caustic injury causes esophageal dysmotility and gastroesophageal reflux, antacids and H_2-blocking agents are indicated.[52,54]

The use of an esophageal stent to prevent stricture formation was tried in cats and later in a clinical setting in the 1970s.[52,72] It has since then been widely used with variable degrees of success in preventing stricture formation.[2,52,54] The rationale is that stenting inhibits synechial formation in ulcerated zones, inhibits excessive granuloma formation and retraction of fibrous tissue, and facilitates epithelialization. It is believed to give a continuous, atraumatic, and early dilatation; it permits gastric feeding, facilitates later dilatation if necessary, and can avoid gastrostomy. Stenting was performed in the past with a silicone rubber nasogastric tube. Poor tolerance of the stent has been reported.[52] More recently, expending stent devices inserted through the endoscope have been tried with success. The stent must be kept in situ for 3 to 4 weeks, although durations of up to 3 months have been reported.[52] Stenting is also used in combination with steroids. Possible disadvantages of stenting are its enhancing gastroesophageal reflux and inflammatory reaction, thus provoking stenosis or delaying healing. Furthermore, there is a risk of perforation when placed blind.[52]

Gastrostomy, whether or not in combination with a stent, is performed in severe burns to allow feeding and to facilitate dilatation. Additionally, it allows superficial exploration of the stomach.

Endoscopic or radiographic evaluation after 2 to 3 weeks establishes healing of the lesions or development to stricture formation. If no healing of the lesions occurred, the treatment has to be continued. If stricture formation is developing, dilatation is initiated. This should not be started before 1 to 2 months after ingestion. If performed at high enough a frequency, it can avoid major surgery. Dilatations have been performed in an antegrade way with Eder-Puestow dilators, Savary bougies, and mercury-filled Hurst-Maloney bougies or in a retrograde way with Tucker dilators using an endless guidewire via a gastrostomy.[2,52,54,55] Recent studies have shown no difference between bougienage or balloon dilatation regarding the risk of esophageal perforation, although the balloon technique seems less hazardous and more efficient.[2,52] Septicemia, brain abscess, and meningitis have been reported in children after dilatation of the esophagus for stenosis after caustic ingestion.[72] Dilatation is performed under general anesthesia; it is repeated every 2 or 3 weeks, if possible, starting at every new session with the size that was used when the last session was stopped.[52,72] When the dilatation progresses, the frequency is reduced and, according to the result, is eventually stopped. The goal of the treatment is to dilate so that the child is able to take a normal diet by mouth. However, dysphagia, a common symptom in esophageal stricture, does not correlate with the esophageal caliber but with the esophageal transit time, as measured by scintigraphy, and with the esophageal function, as measured by manometry.[66,67] Complications of esophageal dilatations are traumatic perforation and tracheoesophageal fistula. When serious complications occur, the dilatations should be interrupted.[52,72] Dilatation is not indicated in patients showing clear evidence of developing severe extended stenosis, even early after ingestion, or in patients not able to swallow saliva. Some patients need ongoing dilatations. Failure of dilatation is defined as the need for continuous dilatation after completion of a 12- to 18-month dilatation program or as the psychological burden on the child has become too important.[2,52] Recently, very promising results have been reported by Afsal's group in London using topical mitomycin C for the prevention of restenosis after dilatation in the management of intransigent esophageal strictures.[73] Alternative ways of stricture treatment are resection of the stenosis, which proved to be inefficient, and parietal fibrosis resection. Failure to obtain a sufficient dilatation is considered an indication for esophageal replacement.[52]

Esophageal replacement should not be performed within 6 months after conservative treatment.[2,52] Colonic interposition is the most frequent therapeutic procedure, but other techniques (eg, gastric interposition or gastric tube formation) are also used. Gastric tube formation can be performed only if there is no gastric lesion. The colon transversum and the left or right hemicolon are anastomosed in an isoperistaltic or an antiperistaltic way.[2,52] The antiperistaltic anastomosis is preferred by some because it inhibits gastroesophageal reflux, although it appears to make very little functional difference. Additionally, pyloroplasty, Nissen fundoplication, or an anterior cologastric anastomosis can be performed to prevent reflux.[52] Perioperative or early postoperative complications are perforation or torsion of the colonic transplant, ischemia of the colon, tracheal tear, pneumothorax, cervical hematoma, and redundancy of the colon. The most frequent late complications are anastomotic leak or fistula, cervical anastomotic stenosis, anastomotic bleeding, and gastroesophageal reflux but also pyloric stenosis, transient dumping syndrome, eventration, and mediastinitis. There is some discussion as to whether esophagectomy should be performed simultaneously with the esophageal replacement.[2,52] Because patients with severe esophageal stricture are at risk for malnutrition, adequate nutrition during the pre- and postoperative period is crucial.[52]

Risks of Cancer

Carcinoma of the esophagus occurs in a thousandfold increased incidence in patients with a history of caustic lesion.[54] According to different series, the percentage of caustic esophageal lesions in which carcinoma eventually develops is 1 to 2%, up to 5%, but figures as high as 20 to 30% have been cited.[2,52] In a large series of 846 patients with esophageal squamous cell carcinoma registered between 1941 and 1981, 12 (1.4%) had previously ingested a caustic agent.[74] In another series of 2,414 patients with carcinoma of the esophagus, aged 28 to 79 years and registered between 1945 and 1970, 63 had ingested lye at the age of 0 to 56 years.[75] The time interval between the ingestion and the diagnosis of carcinoma was 13 to 71 years. The latent time between the corrosive accident and the diagnosis inversely correlated with the age at the time of ingestion. Carcinoma can also develop in the isolated strictured esophagus, left in situ after replacement therapy.[52] The risk of malignancy has led some authors to strongly advocate early surgery in severe caustic esophageal injury, even in children.[2,52] A major contributory factor in the evolution to malignancy after caustic esophageal injury possibly is repeated stricture dilatation. It is therefore recommended to limit the duration of dilatation therapy.

THERMIC AND ELECTRIC BURNS

Burns may follow the drinking of hot beverages. In infants, intensive warming up of bottles may induce acute burns of the throat or esophagus. Because microwave ovens rapidly cook through to the center of heated foods, children may suffer esophageal burns, not appreciating that the interior of a heated dish is intensely hot, although the outer covering is only moderately warm.[76]

Electrical burns of the esophagus in adults have been reported in situations of esophageal temperature monitoring during general anesthesia. Experimentally, they have been produced during pill electrode transesophageal pacing when current levels above 75 mA are applied over a period of less than 30 minutes. A deliberate electrical burn

followed by stricture was reported in a suicide attempt by ingestion of an active electrical wire.[2] Electrical burns of the esophagus in children are extremely rare. Charged disk-shaped or cylindrical batteries may produce a direct flow of 1.5 to 3.0 V current. Low-voltage burns have been described with batteries lodged in the esophagus; endoscopy showed lesions ranging from mucosal edema to necrotic or hemorrhagic deep ulcerations.[2,45] In experimental studies in animals, esophageal damage occurs rapidly, and burns are noted 1 hour after contact with the mucosa; lesions of the submucosa and the muscle layers were noticed after 8 hours.[2]

RADIATION-INDUCED INJURY

Because some children need radiation therapy for thoracic tumors, the risk of esophageal damage is important, although the esophagus has been considered relatively radioresistant for a long time. Deleterious effect is enhanced by the combined effect of chemotherapeutic agents.

Pathologic examination of samples of irradiated esophagus shows early degenerative changes: inhibition of mitosis in the germinal cells of the squamous epithelium and dilation of the capillaries with edema and leukocytic infiltration. Epithelial cells may slough, and some glandular cells are distended with secretions, whereas others are atrophic. Endothelial cells proliferate. Regeneration begins at the end of therapy and continues for 3 or more months. Late effects are dependent on the dose, length of irradiation, and length of the esophagus exposed to it. Injury results from damage to capillaries and disturbances of microcirculation in the tissues, causing tissue hypoxia, "oxidative stress," and loss of parenchymal cells.[2] The most frequent complications are altered motility, ulcerations, pseudodiverticula, and stricture owing to fibrosis of the lamina propria and submucosa.[77–79] Secondary changes such as altered motility and stricture may occur 10 to 15 years later.[79,80] These chronic lesions are secondary to subepithelial and arteriolar-capillary fibrosis, causing epithelial atrophy and ulcerations. Chronic ulceration, fistula, and stenosis may be late complications. Esophageal squamous cell carcinoma is a late complication and, in one instance, developed 30 years after mediastinal irradiation.[81]

SYMPTOMS

Acute manifestations of radiation-induced damage are mucositis, dysphagia, and odynophagia, which can appear 10 to 12 days after the beginning of therapy. Some patients experience occasional sharp chest pains that radiate to the back and may be lessened by interruption of treatment for 2 or up to 7 to 10 days. Radiologic examination of the esophagus at this time may show fine mucosal serrations and absence of primary peristalsis. Radiologic double-contrast studies, which are more sensitive than single-contrast ones, performed 13 to 87 days after the initiation of therapy showed multiple, small, discrete ulcers and a granular appearance of the mucosa; in 13 asymptomatic patients investigated at the end of this period, 3 developed significant strictures.[82]

TREATMENT

Treatment during irradiation with sucralfate alone has been of limited efficacy in the treatment of esophagitis, particularly if used after the onset of symptoms. The combination of sucralfate given all along the therapy with fluconazole initiated during the fourth week of treatment seems to diminish oral discomfort and pain.[2] Treatment for acute esophagitis consists of viscous xylocaine, diphenhydramine for topical anesthesia, and the use of an antacid containing metoclopromide, bethanechol, or nifedipine. Experimentally, indomethacin and aspirin have proven protective in preventing esophagitis in pretreated experimental animals, presumably through the release of prostaglandin leukotriene and cytokines. Strictures are treated by stenting, bougienage, or balloon dilatation.

PILL-INDUCED ESOPHAGEAL INJURY

Esophageal injury caused directly by prolonged mucosal contact with tablets or capsules ingested in therapeutic dosage was first reported in adults in 1970.[83] Since that time, a variety of drugs have been identified as causing esophagitis or obstruction (Table 27-2).[84] Many cases of pill-induced esophageal injury probably remain unrecognized and unreported because most patients fully recover. Medication-induced esophageal injury is obviously rare in children, first, because the presence of an underlying systemic disease or esophageal transit abnormalities is not frequent and, second, because dosage forms and formulation forms are different from those for adults. Pills and gelatin or cellulose capsules have a great tendency to adhere to the esophageal mucosa; on the contrary, aqueous suspensions, syrups, or powders are less likely to stick to the mucosa and to induce esophageal injury.[85] Nevertheless, some cases of drug-induced esophageal damage have been reported in children.[86–88]

In adults, the most common site of esophageal injury has been near the level of the aortic arch, an area characterized by external compression from the arch itself, a tran-

TABLE 27-2 ORALLY ADMINISTERED DRUGS CAUSING ESOPHAGEAL DAMAGE

Doxycycline
Other antibiotics: tetracycline, clindamycin, oxytetracycline minocycline, erythromycin, phenoxymethylpenicillin, lincomycin, tinidazole, rifampin, metronidazole
Ephedrine
Emeprodium bromide
Potassium chloride
Ferrous sulfate or succinate
Alendronate
Alprenolol chloride
Quinidine and chloroquine phosphate
Indomethacin
Aspirin, phenacitin, acetaminophen
Phenylbutazone
Prednisone
Birth control pills
Ascorbic acid

Adapted from Gryboski JD,[2] Kikendall JW,[83] and Jaspersen D.[84]

sition from skeletal to smooth muscle, and by physiologic reduction in amplitude of the esophageal peristaltic wave, all of which might contribute to pill retention.

The sharp demarcation of esophageal injury seen in most cases suggests that this injury results from mucosal contact with a potentially caustic agent. This premise is supported first by the fact that 25% of reported patients sensed that the swallowed pill had stuck in the chest and, second, by the occasional observation of pill fragments within a region of injury.[83,84]

About 40% of patients take their pills with little or no fluid, and the primary cause of injury is adherence of the pill to the esophageal mucosa. Further predisposition to injury is decreased salivation and decreased swallowing if the pill is taken at night and if the patient lies down shortly after its ingestion.[2]

Symptoms

Continuous retrosternal pain and dysphagia occur shortly after pill ingestion. Less common are abdominal pain, weight loss, hematemesis, and dehydration. Lesions of the lower esophagus are less frequent, and their symptoms may be erroneously attributed to gastroesophageal reflux. Doxycycline, ferrous sulfate, and emepronium bromide produce an acid pH (less than 3) even when dissolved in water and may injure the buccal mucosa if held in the mouth for a protacted time. Doxycycline further accumulates in the buccal layer of squamous epithelium. Stricture may be an eventual complication of this type of esophageal burn.[2,83]

Endoscopy shows circumferential lesions, well-delineated ulcers, or longitudinal exudate with necrotic epithelial shreds covering linear ulcerations. Histologically, changes vary from intense inflammatory reaction to erosion or necrosis.

Conventional barium studies are positive only in cases with deep ulcerations or strictures. Double-contrast studies may show the discrete, clustered, ovoid ulcerations and subtle mucosal abnormalities of edema and irregularity.

Treatment

To avoid esophageal injury, pills should be taken when upright rather than supine, with adequate water, and not at bedtime when saliva production and swallowing are decreased.[2,83,84] The offending drug should be either discontinued or given in liquid or parenteral form. In patients with severe symptoms, and in a few truly severe cases, intravenous fluids and nutrition may be required. Strictures that develop require dilation.[2]

TRAUMATIC RUPTURE AND PERFORATION OF THE ESOPHAGUS

Esophageal perforation is a rare occurrence in children that can cause considerable diagnostic difficulty. Two etiologic types of rupture can be defined: traumatic and spontaneous.

Traumatic

Children. In children, perforation may occur as a complication of operative procedures, for example, during the process of immobilizing the esophagus to effect hiatal hernia repair or vagotomy, impaction of a sharp foreign body when perforation may occur at the time of its attempted removal, external penetrating injuries, gunshot and stab wounds, indirect trauma to chest and abdomen (automobile injuries), corrosive damage, and compressed air injuries (Figure 27-10).[89] Cardiac massage, neonatal resuscitation, the Heimlich maneuver, improperly positioned seat belts (even in minor accidents), boxing blows to the stomach, and even vomiting sometimes associated with anorexia have led to rupture of the esophagus.[2] Traumatic perforation of the esophagus in children is most commonly a complication of instrumentation: endotracheal intubation, nasogastric tubes, biopsy, dilating procedures, variceal sclerosis, and esophagoscopy.[90] Complications arise during a procedure by blind advancement of the fiberoptic endoscope with failure to maintain the tip of the instrument in the midline, particularly when passing it through the cricopharyngeal lumen.[2] The perforation is usually located on the posterior wall of the esophagus. Balloons used for variceal tamponade or for dilating procedures for stricture or achalasia are less frequent causes of perforation.[91] Sclerosing of esophageal varices has been associated with both hematoma and perforation, with the latter often related to frequent procedures. Perforation occurs in 2 to 6% of patients with pneumatic dilation of the esophagus.[89,91] In a review of esophageal damage after pneumatic esophageal dilation for achalasia, Molina and colleagues reported an incidence of transmural perforation in 4% and of linear mucosal tears in 8% of patients.[91] In a review of perforations encountered after dilations for caustic strictures in 195 patients, Gershman and colleagues found that 75% of perforations occurred during antegrade

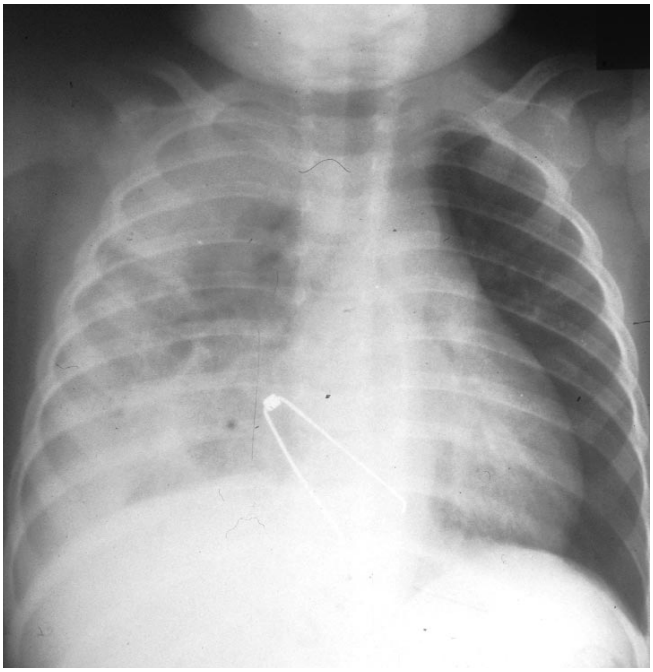

FIGURE 27-10 Perforation of the lower esophagus by a large open safety pin ingested 6 weeks ago. Note the enlargement of the mediastinum and the empyema of the right pleura.

dilations with a stiff woven dilator, and most occurred in the first, second, or third dilations.[92]

Newborns. Although very uncommon, instances of iatrogenic esophageal perforation in the newborn, particularly preterm, have been increasingly reported since the introduction of more intensive resuscitative procedures for low birth weight babies.[93] The injury is usually located in the area of the pharyngoesophageal junction and may be either transmural or intramural (submucosal).

Transmural Perforation. This complication should be suspected in any newborn who develops rapidly increasing respiratory distress. Subcutaneous crepitus of the neck and clinical signs of a pneumothorax may be demonstrable, although they will mostly be difficult to elicit because the patient is likely to be an ill preterm infant under intensive care for respiratory distress syndrome. The esophagus may be torn or perforated by too vigorous suctioning of the neonate in which nonregulated wall suction has been implicated.[2,94] Other causes of neonatal perforation have been stiff suction catheters, nasogastric tubes, traumatic laryngoscopy, or endotracheal intubation.[94] In a review of 12 cases of neonatal pharyngoesophageal perforation, 10 of whom were in premature infants, Bonnard and colleagues noted that repeated attempts at postpartum suctioning, airway intubation, or gastric aspiration preceded perforation.[93] Esophageal atresia was the initial erroneous diagnosis in five cases. Six were treated nonoperatively, five underwent thoracotomy, and one had a gastrostomy. One infant developed an esophageal stricture, and two infants died. A case of esophageal rupture in a 5-month-old child followed inadvertent placement of a Foley gastrostomy tube into the esophagus at the time of tube change. One infant had cervical esophageal rupture as a result of abusive blunt trauma. The esophageal obturator used in cardiopulmonary resuscitation has been the source of perforation owing to distention of the occlusive balloon at the level of the tracheal bifurcation. Similarly, compression of the esophagus by a tracheostomy tube cuff may result in perforation and creation of a tracheoesophageal fistula.

Intramural (Submucosal) Perforation. In some newborns, the esophageal rupture may be incomplete and result in a pseudodiverticulum. Following a mucosal breach, there occurs extensive dissection along the submucosal layer, separating the mucosa from the muscle wall.[2] This separation may extend for a considerable distance along the esophagus. On radiologic examination with contrast medium, both the esophageal lumen and false track may fill, giving the appearance of a double-barreled esophagus or a pseudodiverticulum. There is no pneumothorax.

Clinical presentation can simulate that of esophageal atresia. Affected infants present with excessive outpouring of oral mucus and saliva with immediate choking and cyanosis whenever feeding is attempted; aspiration pneumonia is a common complication. It is usually impossible to pass a nasogastric catheter into the stomach. The differential diagnosis from esophageal atresia is important because treatment is nonsurgical.

Spontaneous

Spontaneous rupture of the esophagus (Boerhaave syndrome) is a well-established entity in adults. In children, the condition is uncommon, but instances of this catastrophe have been reported.[95] All have occurred in the newborn.

The usual story is of a full-term infant who, after appearing well at birth, develops increasing respiratory distress and cyanosis within the first 48 hours owing to the development of a tension pneumothorax.[2] There is typically no preceding history of intubation or other resuscitatory procedures. An initial chest radiograph taken with the infant held upright will reveal a hydropneumothorax or tension pneumothorax; a pneumomediastinum is an unusual finding. Contrast radiography of the esophagus will show extraluminal extravasation of contrast material and will enable the site of rupture to be localized. This is almost always located in the lower esophagus just above the hiatus.

Rupture almost always occurs into the right pleural cavity.[2,95] On chest aspiration, serosanguineous fluid is obtained that may be contaminated with either amniotic fluid or orally administered feed, for example, milk or glucose water.

Early diagnosis and immediate relief of the tension pneumothorax by intercostal drainage and underwater seal are essential to a successful outcome. These measures need to be followed by prompt surgical closure of the esophageal deficit supplemented by broad-spectrum antibiotic, appropriate intravenous fluid therapy, and parenteral nutrition.

In adults, spontaneous rupture usually follows a bout of forceful retching and vomiting. This has led to the theory that in these patients, the cricopharyngeus fails to relax during the act of vomiting, resulting in a sudden, steep rise in intraluminal pressure sufficient to split the relaxed esophageal wall at its lowest and weakest point, that is, the left posterolateral aspect just above the diaphragm.[2] Infants differ in that spontaneous rupture usually occurs into the right pleural cavity, and there is characteristically no preceding history of vomiting.[95] Greater pressures are needed to rupture an infant's esophagus than that of an adult. It is likely, therefore, that in newborn infants, factors other than esophageal overdistention are operative, and the area of esophagus adjacent to a perforation is avascular and friable and shows changes of necrotizing esophagitis. Some form of local devitalizing lesion may thus be an important predisposing factor. It has also been suggested that the cause may be a localized congenital defect in the wall of the esophagus by analogy with cases of spontaneous perforation of the stomach.[95]

Symptoms

The symptoms related to trauma are immediate, whereas those attributable to iatrogenic causes may not be obvious for an hour or more. With endoscopic perforation of the piriform sinus or the cervical esophagus, there is direct extension into the mediastinum. The symptoms are pain and tenderness in the neck, tenderness under the neck, swallowing, tachycardia, and fever. Crepitus usually does not appear for several hours, after fever is evident. Cold water polydipsia is frequent in patients with cervical perforation as an effort to relieve throat discomfort. Perfora-

tions associated with procedures may occur anywhere in the esophagus and are accompanied by pain, fever, and tachycardia. If the thoracic esophagus is perforated, there is chest pain worsened by inspiration or swallowing or on motion, back pain, fever, dyspnea, and tachycardia.[2]

Diagnosis

The diagnosis is made by plain cervical and chest films, which show mediastinal widening[13] or air in the paracervical region or near the esophagus. An esophagogram using a water-soluble contrast will identify the site of perforation in most patients but in only 62% of those with cervical perforation.[2] A barium study may reveal perforation when studies with water-soluble contrast are unremarkable. CT is helpful in demonstrating extraluminal air, periesophageal fluid, esophageal thickening, extraluminal contrast, and mediastinal fluid and air. Endoscopy is the diagnostic procedure of choice to localize precisely a linear mucosal tear or a bluish submucosal mass bulging in the lumen in case of intramural hematoma.

Treatment

The type of treatment varies with the type and location of the perforation and with the condition of the patient and the time elapsed after injury.[2,93] Pharyngoesophageal perforations can be treated successfully with broad-spectrum intravenous antibiotic therapy, peripheral or parenteral nutrition, and no oral feeds. Patients with a penetrating injury of the hypopharynx below the arytenoids or of the cervical esophagus should have neck exploration and drainage.[89] Those with a small esophageal tear and minimal contamination can be treated conservatively. If there is mediastinal air, neck drainage may be necessary, and some feel that mediastinal drainage can be avoided. For large perforations and extensive contamination of the mediastinum and pleura, as from gunshot wounds where there is extensive tissue damage, esophageal exclusion through ligation of the distal esophagus gastrostomy and cervical esophagostomy with parenteral nutrition are the treatment of choice. High gastric fundoplication has also been used effectively to cover a lower esophageal perforation. Perforations of the intrathoracic esophagus that are confined to the mediastinum are treated conservatively, and those of the intra-abdominal esophagus should be treated by closure or diversion, even if this requires esophageal resection.[2,89]

Complications

Fulminant mediastinitis is the major threat in esophageal perforation.[12] Although most often attributable to rupture of the thoracic esophagus, it may also occur in cervical rupture if drainage is delayed and the infection spreads along the periesophageal planes into the mediastinum. Delayed complications are tracheoesophageal, esophagocutaneous, and carotid-esophageal fistula, which may cause important hemorrhage.

Mortality rates for penetrating perforation of the cervical esophagus range from 9 to 15% for those treated immediately to 25% for those in whom treatment was delayed. Patients with perforation after sclerotherapy for esophageal varices are at particularly high risk owing to their underlying liver disease, and in those, mortality may reach 83%. In children, most cases can be closed primarily and the esophagus salvaged, despite late presentation, with a mortality rate of 4%, significantly less than in adults (25–50%). There is little difference in the mortality between iatrogenic perforation and Boerhaave syndrome as long as the diagnosis is made early and the treatment is prompt.[2,95]

MALLORY-WEISS SYNDROME

Although this syndrome of laceration of the esophagus has been reported in few children, its frequency is probably underestimated[2,96] because the diagnosis is confirmed only by endoscopy.

The fissuration of the mucosa results from forceful or prolonged vomiting. The laceration is located at the esophagogastric junction and cardia of the stomach. The tear is sometimes double, extending only through the mucosa along the longitudinal axis of the organ. There is little inflammatory reaction or fibrosis, but some granulation tissue is apparent with healing. Bleeding is most intense when the gastric and the esophageal mucosa are involved.[2]

Symptoms

There is a history of vomiting, either of several episodes or of a duration of several days. Suddenly, the vomitus contains small or large amounts of blood. Dark blood may alternate with bright red blood. In some patients, there is a history of achalasia or hiatus hernia.

Diagnosis

This tear is not detected by radiologic examination. At endoscopy, fresh lesions appear as longitudinal cracks in the mucosa with little inflammatory reaction. They may be so thin as to be missed by the endoscopist so that several passages of the endoscope are often needed to identify them. After 24 hours, the tear will appear as a white, raised streak with some erythema and granulation tissue. If through and through perforation does occur, it involves the distal esophagus.[2]

Treatment

In the majority of children, bleeding stops spontaneously and only rarely does the patient require transfusion, unless there is an underlying coagulopathy. The stomach should be lavaged to prevent gastric distention, and many patients are treated with H_2 antagonists. If hemostasis does not occur, aggressive treatment must be undertaken using vasopressin infusion, balloon tamponade, sclerotherapy ligation, clipping, injection of drugs such as epinephrine or thrombin, bipolar electrocoagulation, neodymium-yttrium-aluminum-garnet laser, or angiographic embolization of the left gastric artery. Endoscopic sclerotherapy using 1:10,000 adrenaline + 1% polydocanol is often successful in achieving hemostasis.[97]

In children, management of bleeding is effective with medical treatment; in contrast, 25% of adults require surgical control of hemorrhage.[2]

ESOPHAGEAL LESIONS IN EPIDERMOLYSIS BULLOSA

Epidermolysis bullosa describes a group of genetically determined mechanobullous disorders that vary in course and severity, ranging from relatively minor disability to death in early infancy. They are characterized by the excessive susceptibility of the skin and mucosa to separate from the underlying tissues following minimal mechanical trauma to form bullae. The affected areas can be considerable in size because the bullae enlarge by expanding and tracking along the natural tissue planes. Like all blisters, they can be extremely painful. A painful cycle of repeated trauma, secondary infection, and subsequent healing, which results in scarring and deformity in those with the dystrophic type of epidermolysis bullosa, leads to a host of complications.[98]

Children with severe forms of epidermolysis bullosa lead very disrupted lives. Those worst affected are disabled and excluded from normal physical activities; they may be unable to attend school regularly, are frequently in pain, and are often admitted to hospital.

Health care professionals who come into contact with children with epidermolysis bullosa should be aware of the general management of the condition and of the necessary techniques to minimize bullae formation and further complications.[2,98]

There are over 20 types of epidermolysis bullosa described, with three major subtypes, dystrophic, simplex, and junctional, with each broad category of epidermolysis bullosa containing several subtypes. Each type of epidermolysis bullosa is distinguished on the basis of the skin level in which the characteristic blistering occurs.[98]

Oral, pharyngeal, and esophageal blistering is common in epidermolysis bullosa. The recurrent blistering leads to progressive contraction of the mouth (causing limited opening) and fixation of the tongue. The associated pain and resulting dysphagia lead to a reduction of nutritional intake because eating is a painful, slow, and exhausting experience. Gastroesophageal reflux is common in patients with epidermolysis bullosa, and esophageal scarring leads to dysmotility and the formation of strictures or webs,[99,100] which contributes to the dysphagia by aggravating oral, pharyngeal, and esophageal ulceration and also by increasing dental decay.[98]

Epidermolysis bullosa sufferers are prone to dental caries owing to oral infection and compounded by poor dental hygiene owing to the pain of teeth brushing. Chronic intraoral infection and gum disease, plus the absence of the normal physical cleansing effect of food owing to a more or less liquid diet often high in sucrose, also contribute. Fixation and shrinkage of the tongue also lead to a loss of normal teeth cleansing.[97]

Diagnosis

Radiologic study is the safest diagnostic procedure, but because strictures may be multiple and often associated with a web, care must be taken in interpreting the data because visualization below a web or stricture is poor. During the phase of active mucosal erosion, there are ulcer niches and detectable edema. Strictures vary from less than 3 mm to several centimeters in length and appear either smooth or irregular.[2]

Treatment

To overcome dysphagia and nutritional problems, bottle-fed infants need the softest available teats. Vitamin and mineral supplements are often required. A nasogastric tube may be used as a short-term measure in severe dysphagia. Gastrostomy (open rather than percutaneous because the endoscope causes significant shearing damage to the oropharynx and esophagus) may be necessary if nutrition cannot be maintained. Good dental hygiene must be encouraged. A conservative approach to dental treatment is taken rather than widespread extraction, but postoperative dysphagia is common, especially after conservation treatment owing to the prolonged duration of the procedure and damage to the throat on removal of the throat pack.[98]

Treatment in the acute bullous stage is aimed at decreasing bullous formation, and nutrition is of primary concern. Corticosteroid therapy is given as large doses of prednisone, 2 mg/kg/d, or the intravenous equivalent of hydrocortisone and usually results in a decrease in dysphagia within 3 to 14 days. Nevertheless, systematic use of corticosteroids should not be recommended. Peripheral parenteral hyperalimentation should be used while treatment is ongoing. After cessation of symptoms, steroids are tapered over 6 to 8 weeks, and oral hyperalimentation is initiated. Antacids and oral antibiotic suspensions also help to minimize symptoms and prevent superinfection.[2]

Dilation of strictures must be performed carefully because mouth injury and esophageal trauma are ever-present hazards. Balloon dilation is now the recommended procedure,[99,100] but perforation is likely to occur. The best results of dilation are in patients whose blistering is minimal because of either quiescence of the disease or of treatment. In those with long strictures, esophagectomy or bypass using right colon interposition or gastric tube replacement has been therapeutic for esophageal symptoms.[2]

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CHAPTER 28

CONGENITAL ANOMALIES

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Congenital anomalies of the stomach are very rare as opposed to those of the duodenum or, indeed, the intestine as a whole. Most gastric or duodenal defects are sporadic, isolated, and of unknown etiology, but some are inherited or form part of recognized syndromes. Notable associations include duodenal atresia and trisomy 21. A careful examination looking for associated defects must therefore be carried out and appropriate management instituted. Clinical presentation is variable and depends on the anomaly. In general, given the proximal position in the gastrointestinal tract, feed-associated symptoms predominate and invariably include vomiting and reflux with or without compromise of the respiratory tract. Presentation may be more subtle and diagnosis delayed even to adult life if anomalies are less conspicuous (eg, gastric diaphragm or diverticulum).

Broadly, congenital anomalies can be divided into the following:

- *Atresia and stenosis*. Atresia refers to complete obstruction of the gut either by a membrane or fibrous band or complete separation of the adjacent sections. Stenosis refers to incomplete obstruction caused by intrinsic narrowing of the internal lumen of the gut.
- *Duplications and cysts*. Duplications are cystic or tubular malformations of the gut. They may be multiple and often communicate with and share the blood supply of the adjacent gut. Usually, they are composed of intestinal or ectopic gastric mucosa, submucosa, and smooth muscle coats. Presentation may be delayed until enlargement causes compression of adjacent structures or may be revealed following volvulus, intussusception, or investigation of gastrointestinal hemorrhage or perforation. All duplications should be surgically excised to prevent complications.
- *Abnormal rotation and fixation*. The entire gut assumes its normal postnatal position within the abdominal cavity following a carefully sequenced process of physiologic herniation out of the abdominal cavity in the sixth week of embryonic life. This is followed by elongation, counterclockwise rotation, return into the abdomen in week 10, and fixation of the duodenum and ascending colon to the posterior abdominal wall. Obstruction and volvulus tend to occur when such rotation and fixation are incomplete or abnormal (malrotation) and can lead to ischemia and infarction of the bowel. Total nonrotation results in the duodenojejunal loop on the right and cecocolic loop on the left side of the abdomen. Symptoms and signs at presentation are variable from those of acute obstruction or compromise (eg, volvulus to vague chronic gastrointestinal symptoms).

GENERAL MANAGEMENT

As for most intestinal malformations, surgery is the definitive management. This may be done electively or as an emergency within hours of presentation. Early recognition and diagnosis not only of the defect but of the clinical state of the child, resuscitation, and stabilization of the child are vital. Valuable time is often lost in stabilizing a child for surgery in whom initial resuscitation has been overlooked or inadequate in the eagerness to diagnose and transfer the child. Intravenous fluid, keeping patients nil by mouth, drainage of gastric or intestinal contents by passage of a nasogastric tube, and careful monitoring are the initial management of almost all children presenting with intestinal malformations. Sepsis must always be considered in an unwell baby or child, appropriate microbiologic cultures taken, and antibiotics commenced. Intestinal perforation or intraoperative spillage of contents may occur, and broad-

spectrum antibiotics to cover sepsis from gut organisms are usual during the perioperative period. Postoperative care involves close monitoring and the maintenance of various drains and vascular lines. Recommencement of feeding will depend on the operative procedure undertaken, healing, and clinical signs of recovery of bowel function.

NORMAL EMBRYOLOGIC DEVELOPMENT OF THE STOMACH AND DUODENUM (PATTERN FORMATION AT THE PYLORIC SPHINCTER)

The gastrointestinal tract (or gut) is formed very similarly in all vertebrate species early in embryogenesis. The gut starts out as a simple tube of mesodermal tissue surrounding an endodermal core. Later differentiation results in smooth muscle and epithelial development. Neural tissue in the gut comes from colonization of specialized neural crest cells, which form the enteric nervous system. While these tissues are differentiating, the gut tube develops regional specification both in anatomy (gross and microscopic) and physiology (function). All of these events must occur in proper spatial and temporal order to complete a normal (nonanomalous) gut. Although many of these events are understood at the molecular level, much work needs to be done to understand the coordination of these processes to ensure normal development. In this section, we review what is known about the molecular controls of gut development, focusing on the development of the stomach and duodenum. Our assumption is that the misregulation of these molecular controls results in anomalous development. Although there is no known specific genetic cause of the congenital anomalies we discuss, inferences into their etiology can be derived by understanding the normal genetic controls of stomach and duodenal development.

Early gut tube development is choreographed in synchrony with the turning and folding movements of the embryo during and immediately following gastrulation. Critical in early gut formation is the invagination of the definitive endoderm and the subsequent growth and differentiation of the subjacent splanchnic mesenchyme. A sequence of two invaginations, one at the anterior end (anterior intestinal portal [AIP]) followed temporally closely by a posterior invagination (caudal intestinal portal [CIP]), forms the two ends and begins the internalization of the gut. The endoderm of early gut tube stages is remarkably uniform in its morphology along the length of the primitive gut tube. There are no morphologic differences between the portions of tube formed by elongation of the AIP or by the CIP. The primitive gut tube is lined by a single layer of a cuboidal/columnar endoderm/epithelium and encircled by a thin layer of splanchnic mesoderm. As the mesoderm grows and differentiates into smooth muscle, the gut tube alters its gross morphology, resulting in clear demarcations that have been categorized as the foregut, midgut, and hindgut. These regions can be defined by embryologic, anatomic, vascular, functional, and molecular criteria. Each of the three major gut regions is composed of subregions: esophagus and stomach from foregut, small intestines (duodenum, jejunum, and ileum) from midgut, and colon from hindgut. Boundaries between these regions typically include valves or sphincters (the pyloric sphincter [PS] at the foregut-midgut boundary and the ileocecal valve at the midgut-hindgut boundary). The gross phenotype and the overall "gut plan" along the anteroposterior axis is quite well conserved among all animal species and remarkably so among vertebrates.

Embryologically, the stomach is one of the first structures that differentiates grossly, in nearly all vertebrates, by a left-right axis asymmetry and a hypertrophy or dilatation of the otherwise straight gut tube. The region just caudal to the stomach appears to be clearly demarcated anatomically and molecularly. The small intestines begin distal or posterior to this boundary. The rostral and caudal boundaries of the small intestines are defined in this chapter based on functional and anatomic boundaries that correlate well with molecular expression boundaries[1] and therefore are used as the boundaries for this chapter.

Because much of the work deciphering the molecular controls of gut development has used the chick embryo as a model system, we briefly review the chick gut anatomy. The avian species has a specific adaptation in the stomach region. Avian stomachs are composed of two structures, the proventriculus and the gizzard (Figure 28-1). The anterior chamber, the proventriculus, is the glandular stomach that expresses stomach-specific enzymes such as pepsinogen[2–4] and is therefore most homologous to the human stomach. Posterior to the proventriculus is the gizzard, a specialized avian adaptation to replace mastication that may act as a sphincter as well owing to its muscular anatomy. The gizzard is characterized by thick muscle and a specialized stratified keratinizing squamous epithelium covered by a thick keratin layer.[5] The gizzard at its posteriormost boundary has a specialized region homologous to the human PS. The chick PS can be discerned histologically, anatomically, and molecularly.

The luminal epithelial morphology lags significantly behind the gross gut pattern in its regionally specific differentiation. In some vertebrates, the gut epithelium continues to be plastic, often undergoing functional differentiation after birth before forming the adult phenotype.[6] The gut has the remarkable ability to continue epithelial growth and differentiation throughout the life of the organism along its radial axis. It is this axis in which the regionalization of the gut is often distinguished because morphologic differences are easily discernable.

The PS lies at the caudal end of the foregut at the foregut-midgut boundary (see above). This structure acts as a valve to control the flow of food from the stomach to the small intestines, thereby ensuring proper gastric digestion. The anatomic phenotype and physiologic functional importance of this structure vary considerably among species, but the development of this boundary appears to be remarkably conserved.[7] The molecular boundary (expression limits) of developmentally important factors shows a "hot spot," of sorts, at the PS (Figure 28-2). Many candidate control genes are expressed limited caudally, cranially, or at the PS. Examples include factors with expres-

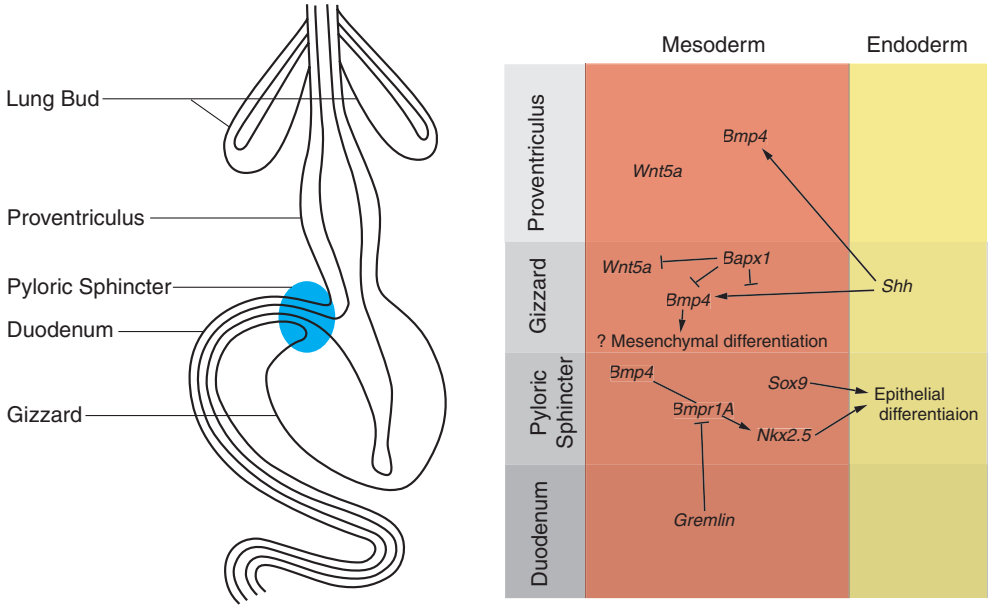

FIGURE 28-1 Cartoon of E7 chick foregut-midgut showing the major structures, with the pyloric sphincter highlighted in blue. Mesodermal (red) and endodermal (yellow) gene expression and proposed model are on the right (see CD-ROM for color image).

sion restricted to the mesoderm anterior to the PS in the stomach (or gizzard), *Bapx1/Nkx3.2*; those restricted to the mesoderm posterior in the small intestine, *Wnt5a* and *Bmp4*; and that crossing the PS, *Nkx2.5*. Additionally, some factors are expressed in the endoderm at this region (those restricted to the PS and posterior, *CdxA* and *Pdx1*; those

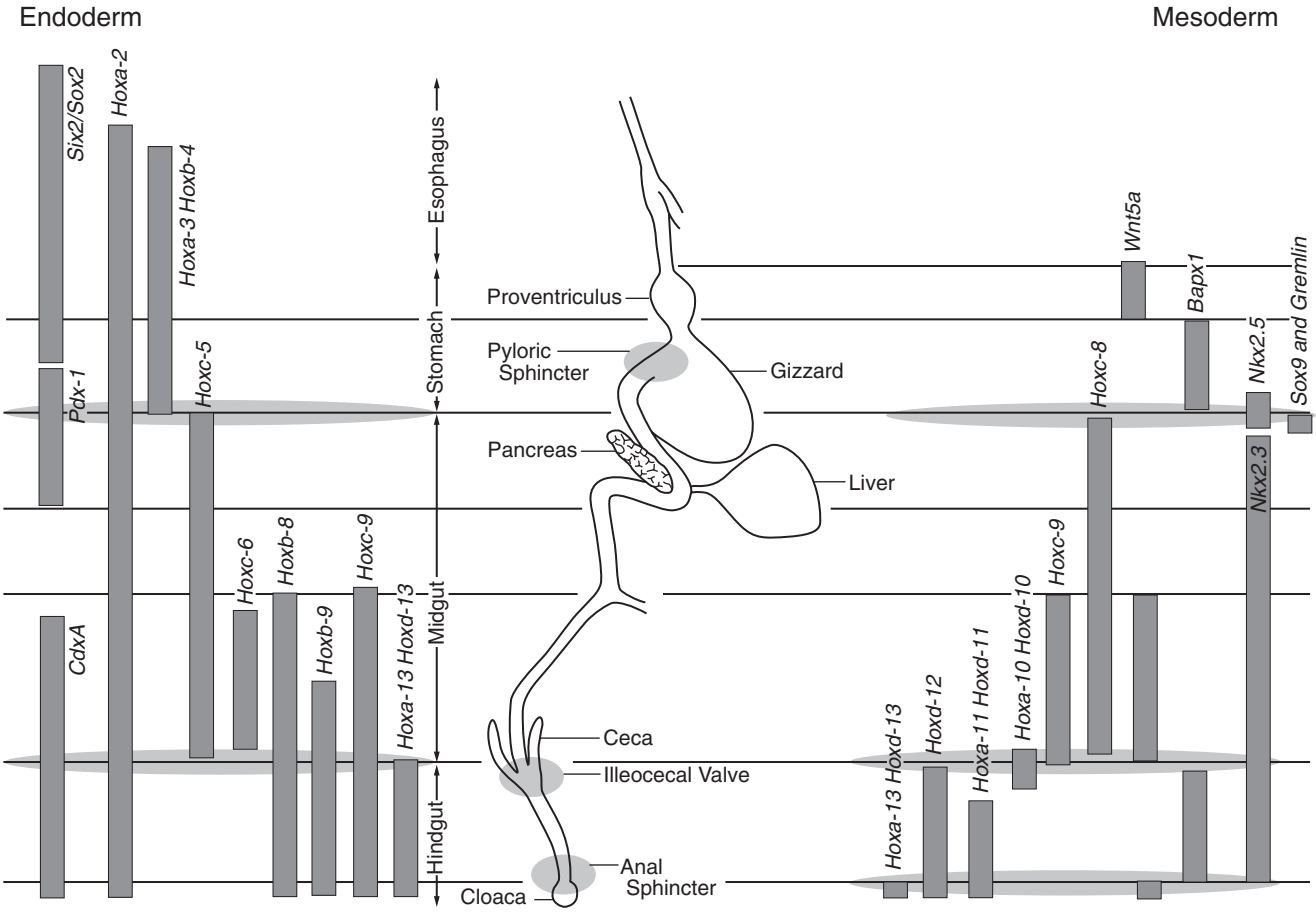

FIGURE 28-2 Cartoon outline of an E10 chick gastrointestinal tract showing major structures. Gene expression boundaries are demarcated by black bars on the right (mesodermal expression) and left (endodermal expression). Adapted from Roberts DJ.[1]

restricted to the PS and anterior, *Sox2* and *Six2*).[1,5,8–11] Other factors are apparently expressed just at the PS: gremlin and Sox9 (data not published).[12]

These expression boundaries appear to be important in ensuring proper development and placement of the PS.[5,8,9] When these expression boundaries are disturbed, the gut develops with malformations in the PS and adjoining structures. These are discussed below, but first a general overview of gut development is necessary.

It has been known for decades that the gut cannot develop normally without an interaction between the endoderm and the mesoderm.[13–15] The direction of these endoderm-mesoderm interactions has been the focus of much investigation. Cultures of primitive foregut endoderm cannot differentiate without coculture with mesodermal tissues.[3] There is a developmental window after which the primitive gut endoderm, although still morphologically undifferentiated, is committed and develops into its regionally specified epithelium when cultured with a variety of tissues, including the vitelline membrane.[16] However, at an earlier developmental time, the ultimate differentiation of primitive endoderm will depend on the anteroposterior region of its adjacent mesoderm. For example, early gizzard endoderm can differentiate as proventricular epithelium if cocultured with proventricular mesoderm.[3] Many studies have confirmed that the mesoderm directs the ultimate epithelial pattern in the gut,[14,17–19] but the endoderm also has inductive capacities. Definitive endoderm cocultured with somitic mesoderm stimulates smooth muscle (splanchnic or visceral) rather than skeletal muscle development as assayed by histology and by induction of visceral mesodermal proteins (eg, tenascin[20] and smooth muscle actin[21,22]). Other factors clearly modulate this interaction, including hormonal and basement membrane proteins.[23,24]

The mesodermal influence on endoderm patterning involves primarily specification of morphology that may not include all of the epithelial cytodifferentiation. Most of the endodermal gut regions studied appear plastic to influence from mesoderm in both morphologic and cytologic differentiation, except for the midgut region. Some midgut-specific epithelial cytodifferentiation appears to have cell-autonomous or cell-specific features. Specific midgut epithelial expression of digestive enzymes is maintained even when influenced by heterologous mesoderm.[2,11,25–27] This difference between the ability of the midgut and foregut endoderm to undergo complete heterologous differentiation may be an endogenous characteristic of the endoderm.

Some of the molecular controls of early endodermal-mesodermal events have been described. *Sonic hedgehog* (*Shh*), a vertebrate homolog of *Drosophila hedgehog* (*hh*), encodes a signaling molecule implicated in mediating patterns in several regions of the embryo.[28–32] *Shh* is expressed in the endoderm of the gut and its derivatives[11,33–38] and is a candidate for an early endodermally derived inductive signal in gut morphogenesis because its earliest endodermal expression is restricted to the endoderm of the AIP and CIP before invagination occurs.[39] *Shh* is not the signal that initiates the invagination of the AIP or CIP because murine null mutants for *Shh* develop a gut, although severe foregut abnormalities are present.[40] These mutants have malformed esophagi with enlarged lumens and disorganized or absent subjacent mesoderm.[34,41] This finding suggests that the endodermally derived signal from *Shh* is involved with mesodermal development, recruitment, or other aspects of mesodermal foregut patterning. Indeed, *Shh* must act as a signal from the endoderm to the mesoderm because its receptor is present only in the gut mesoderm,[11,35,37] and overexpression of *Shh* in the early primitive gut leads to a mesodermal (not endodermal) phenotype.[11]

In each organ in which the endoderm-derived tissue expresses *Shh*, there is closely associated mesenchymal mesoderm that expresses a homolog of *Drosophila's dpp*.[38,39,42] Of the vertebrate homologs of *dpp* expressed in the gut, only *Bmp4* is expressed at the earliest stages of gut development. In the primitive hindgut, at the earliest time *Shh* expression can be detected in the CIP region (even before invagination is apparent), *Bmp4* is expressed in the subjacent mesenchymal mesoderm.[39] In misexpression studies, *Shh* induces *Bmp4* in the splanchnic mesoderm of the developing gut.[11,35,39] An endodermal role of *Shh* is to induce *Bmp4* expression in the splanchnic mesoderm, which then controls aspects of smooth muscle development in the gut.[8,11,35,39] These aspects of patterning also play a key role in the development of the PS and the foregut-midgut boundary.

At early patterning stages (< E7 in the chick), *Bmp4* is expressed in the mesoderm of all regions of the developing gut mesoderm but is excluded from expression in the primitive gizzard.[5,9,11,42] Several bone morphogenetic protein (BMP) receptors are expressed in the intestinal mesoderm in a position to mediate Bmp signaling in this portion of the gut. The type I receptor, *BMPR1B*, is specifically expressed in the gizzard mesoderm from E2.5,[5] despite the fact that no *Bmp* is expressed in the early gizzard mesoderm. With ectopic expression of *Bmp4* early in primitive gizzard development, a thinning of the smooth muscle layer results.[9,11] *Bmp4* may affect the mesoderm by negatively regulating growth and hypertrophy or facilitating differentiation to smooth muscle.[8,9] The factor inhibiting *Bmp4* expression in the gizzard is *Bapx1*, an NK-2 class transcription factor also known as *Nkx3.2*.[9] *Bapx1* has the inverse expression pattern of *Bmp4* in early gut development, expressed only in the gizzard mesoderm from the earliest stages examined.[9] Misexpression of *Bapx1* either anteriorly into the proventriculus or posteriorly into the duodenum results in a gizzard homeosis of these structures (Figure 28-3).[9] The resulting pattern includes transformation of the normally thin-walled muscular proventriculus or duodenum into a thick-walled mesoderm (such as the gizzard), as well as an epithelial transformation from glandular (as in the proventriculus) or villous (as in the duodenum) into gizzard-like stratified squamous epithelium (see Figure 28-3). At the posteriormost boundary of *Bmp4* expressing versus nonexpressing mesoderm, it has been shown that this role of the Bmp signaling system is to pattern the foregut midgut boundary, the PS.[5,8] Signaling by

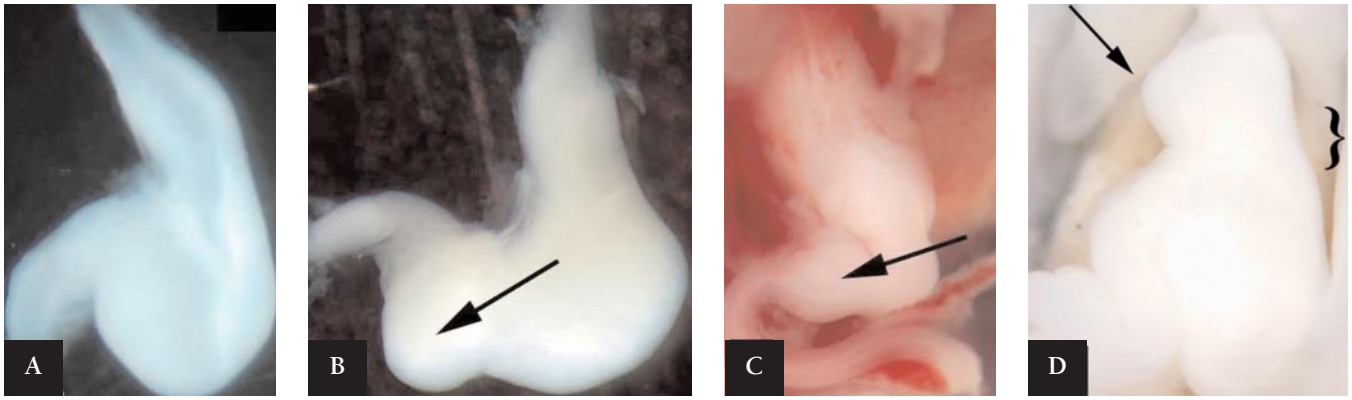

FIGURE 28-3 Panel of E7 chick stomach complexes. *A* is wild type. *B* to *D* are *Bapx1* ectopically expressing regions; *B* and *C* are ectopically expressing *Bapx1* in the duodenum (*arrows* point to enlarged gizzard-like duodena). *D* shows *Bapx1* ectopically expressed in the proventriculus with gizzard-like hypertrophy (*arrow*). The bracket indicates the proventriculus gizzard boundary. Adapted from Nielsen C et al[9] and de Santa Barbara P et al.[90]

Bmp from the avian midgut induces the cells of the adjacent gizzard primordium to form a sphincter,[5] and Bmp signaling is important in the phenotype at this boundary.[9] One of the roles of the Bmp signaling is mediated by induction of a transcription factor necessary for PS formation.

Nkx2.5 is a specific marker for the mesoderm of the pyloric sphincter in the chick embryo (see Figure 28-2). In the early stages, it is expressed adjacent to the *Bmp4*-expressing area and overlaps with the posterior expression domain of *BMPR1B*. By misexpressing *Bmp4* into the primitive chick gizzard using a retrovirus containing the *mBMP-4* complementary deoxyribonucleic acid (DNA), expression of Nkx2.5 was induced, and the PS border was anteriorly shifted.[5] If PS mesodermal *Bmp4* expression was inhibited using a *Noggin* (encoding a specific Bmp antagonist) retrovirus, Nkx2.5 was down-regulated at the border of the gizzard and the small intestine.[5] Because the Noggin-infected embryos did not survive long enough to allow morphologic analysis, the presence or absence of the PS could not be determined.[5]

Using the same retroviral misexpression system to extend the expression of *Nkx2.5*, the same alteration of the PS placement resulted if *Nkx2.5* was expanded anteriorly. With posterior infection, no alteration in gut patterning was observed.[8] This suggests that other factors are important in regulating PS patterning or responsiveness to *Nkx2.5*. Because there are other known factors expressed differentially at this boundary (see Figure 28-2), one or more of these factors must play a role in PS patterning. Although none of these factors has been *directly* implicated in development of the PS in the avian misexpression studies or in murine null transgenics, midgut malformations have been described in mice in which the anterior limit of expression of *Hoxc-8* is shifted cranially: a portion of foregut epithelium misdifferentiates as midgut.[43] This can be interpreted as shifting the foregut-midgut boundary anteriorly, although in this study, the PS as an anatomic structure was not described.

Other factors expressed in a spatially temporally mediated manner at or near the PS likely also play a role in the development of this structure. Mutations in any of them may play a role in the development of PS malformations, as discussed above.

CONGENITAL ANOMALIES OF THE STOMACH

Gastric Atresia or Stenosis

This most commonly affects the pylorus or antrum of the stomach and occurs either as a true atresia of the stomach or secondary to complete or partial occlusion of the lumen by a circumferential mucosal membrane or diaphragm. Overall, these conditions are extremely rare, accounting for approximately 1% of intestinal atresias, with an incidence of 1 in 100,000 newborns.[44,45] Embryologic events leading to these defects are not clear. Most cases are sporadic, but some may be inherited as autosomal recessive. Although a specific genetic cause of this anomaly has yet to be described, one can hypothesize that misregulation of the BMP signaling pathway may play a role because experimental inhibition of signaling results in a hypertrophied muscle at this region.[5,8,9] Defects can be isolated or occur in association with other genetic defects (eg, junctional epidermolysis bullosa, multiple intestinal atresias, Down syndrome, or aplasia cutis congenita).[46,47] Obstruction of the gastric outlet may be caused by extrinsic pressure from annular pancreatic tissue or congenital peritoneal bands.

Clinical Presentation. Onset of symptoms will depend on the degree of gastric outlet obstruction. Complete obstruction results in persistent nonbilious vomiting within a few hours of birth, whereas in cases of partial obstruction (eg, secondary to membranes, stenosis, or duplication cysts), symptoms may not appear until childhood or even adulthood. Upper abdominal distention may be present, and recurrent vomiting may result in metabolic derangement similar to hypertrophic pyloric stenosis. In incomplete obstruction, failure to thrive and upper abdominal discomfort may also occur. In association with multiple atresias of the small and large bowel or with epidermolysis bullosa letalis, the outcome is usually fatal.

Diagnosis. In cases of complete obstruction, plain abdominal radiography reveals a "single bubble" appearance with a large distended stomach and absence of distal intestinal gas.

Together with the symptoms, this is very suggestive of gastric atresia, and contrast studies are often unnecessary. In partial obstruction, additional studies using contrast and/or ultrasonography are valuable. An incomplete prepyloric membrane is seen as a thin, linear filling defect on contrast studies. On a sonogram, this appears as an echogenic band extending centrally from the lesser and greater curvatures in the prepyloric region.[48] Endoscopy may be used to directly visualize the defect, and gastric emptying studies may aid diagnosis.

Management. If the defect is a thin membrane, excision along with pyloroplasty is the treatment of choice. More complex atresias may require resection and formation of a gastroduodenostomy, pyloroplasty, or, less commonly, gastrojejunostomy. The stomach is kept decompressed postsurgery, and enteral nutrition is commenced in the first week in the absence of complications. If there is little delay of gastric emptying, conservative management consisting of low-residue feeds and gastric emptying drugs may be successful, although careful long-term follow-up is essential. Surgery involves complete excision, or endoscopic transection, of the web. Balloon dilatation and laser ablation are newer therapies. Complications are minimal. Other bowel atresias should be excluded

Gastric Duplication

Foregut duplications (esophagus, stomach, and duodenum) account for approximately one-third of all congenital duplications of the gastrointestinal tract,[49] with gastric duplications accounting for between approximately 4 and 8%.[50,51] They are more common in females. Gastric duplications occur most commonly along the greater curvature but can arise from the posterior or anterior wall or pylorus. They usually share a common blood supply and outer smooth muscle coat with the stomach, although most do not communicate with the gastric lumen. Duplication cysts are most commonly lined with gastric or other alimentary-type epithelium, but respiratory-type epithelial lining has been described.[52] Associated anomalies are common and reported in approximately 50% of patients.[53] These include other intestinal tract duplications, most commonly esophageal and vertebral anomalies, and aberrant pancreatic development.[54,55] Although the exact embryologic mechanism is not known, various theories have been proposed, including the "split notochord theory."[53]

Clinical Presentation. Presentation classically occurs in infancy, although presentation at any age is possible. Symptoms depend on the size and location of the cyst and any communication. Common symptoms are vomiting (classically nonbilious), weight loss, failure to thrive, and abdominal pain and distention. An abdominal mass may be palpable on examination. Pyloric duplication may be mistakenly diagnosed as hypertrophic pyloric stenosis. Enlargement of the cysts can present with obstruction of gastric emptying or compression of adjacent structures. Ulceration, bleeding, or inflammation of the mucosa within the cyst or of the adjacent gut results in either local complications, overt gastrointestinal hemorrhage, or perforation with peritonitis or fistula formation. Ectopic pancreatic tissue is common in gastric duplications and may be associated with raised amylase levels and pancreatitis.

Diagnosis. Duplications are often difficult to diagnose preoperatively. Plain abdominal radiography may reveal a soft tissue mass, and ultrasonography is useful to reveal the cystic nature of the duplication. Contrast studies may reveal the presence of a mass with displacement of adjacent bowel and is useful if a communication exists with the lumen of the gastrointestinal tract. Direct visualization of the mass by endoscopy has also been used.[56] Computed tomography is often used to define the nature and location of duplication cysts, but endoscopic ultrasonography and magnetic resonance imaging are becoming increasingly popular.[57] The presence of an echogenic inner rim and hypoechoic outer muscle layers is very suggestive of a duplication.[58] Prenatal diagnosis of gastric duplications by ultrasonography or magnetic resonance imaging has been reported.[59,60]

Management. Excision of the duplication cyst is the treatment of choice and can be done with minimal loss of adjacent normal stomach, although the common vasculature and wall often complicate this. A communication can be created between the cyst and gastric lumen. In complete or tubular duplications, the normal stomach can be preserved by stripping the mucosal lining, along with variable excision of the cyst. Resection of aberrant or ectopic pancreatic tissue may also be required. Laparoscopic resection of gastric duplication cysts has been reported.[61] The outcome following surgery is usually excellent.

Gastric Volvulus

Gastric volvulus was first described by Berti in 1866 and is thought to be relatively rare in the newborn period and in infancy,[62,63] although in its chronic form, it is likely to be underdiagnosed.[64] Recognition is essential, however, because it constitutes a surgical emergency. In normality, the stomach is resistant to abnormal rotation, being fixed at the gastroesophageal junction and pylorus, in addition to four gastric ligaments. As a result, congenital gastric volvulus is associated with disruption of one or more of these, although in a proportion, no cause is identified.[65] Gastric volvulus results in abnormal rotation of one part of the stomach around another, with resulting obstruction at the pylorus or cardia and possible ischemia. This rotation is either organoaxial (around the longitudinal esophagogastric-pyloric axis), mesentroaxial (around a transverse axis through the greater and lesser curvatures), or combined.[63,66] The majority of congenital gastric volvuli are secondary to gastric malfixation, especially at the gastroesophageal junction, diaphragmatic complications (eg, congenital diaphragmatic hernia), and absence or laxity of gastric ligaments.[63,67,68] Splenic anomalies are common.

Clinical Presentation. Gastric volvulus in childhood tends to present within the first few months of life, with symptoms depending on the degree of rotation and obstruc-

tion.[64,69] Classic symptoms of Borchardt triad (unproductive retching, localized epigastric distention, inability to pass a nasogastric tube) may be difficult to elicit in younger children, and Borchardt triad should be considered in the presence of other chronic symptoms (eg, gastroesophageal reflux, recurrent vomiting, failure to thrive).

Diagnosis. Radiographic features are most reliable for diagnosis, showing abnormalities in the position and contour of the stomach in the abdomen or chest and position of the pylorus in relation to the gastroesophageal junction.[63,70,71] Contrast studies may be more informative.

Management. Acute gastric volvulus, especially intrathoracic, is a surgical emergency to prevent gastric ischemia, necrosis, and perforation and to prevent cardiorespiratory compromise. At surgery, the volvulus is reduced, and the viability of the stomach is assessed. If the stomach is viable, it is fixed by gastropexy to the abdominal wall, or a gastrostomy is fashioned. Repair of any associated defects (eg, diaphragmatic defects) is undertaken. Successful laparoscopic surgery has been reported in acute gastric volvulus.[72] The treatment of chronic cases remains controversial, although surgery is indicated in persistently symptomatic individuals. Gradual improvement over time has been reported in conjunction with conservative treatment (eg, positioning infants in the prone or upright position after meals) in less affected children.[71]

Microgastria

Microgastria results from a failure of gastric enlargement during embryogenesis, resulting in a tubular stomach of reduced capacity. It is extremely rare, with approximately 45 cases described in the literature since its first description in 1842.[73] It appears to occur sporadically, with a slight female preponderance,[73] and is almost always associated with other congenital anomalies. In a review of the literature, Kroes and Festen could identify only 2 of 39 cases in which microgastria appeared to occur as an isolated defect.[74] The associated malformations include intestinal (84%), cardiovascular (43%), pulmonary (33%), skeletal (31%), urogenital (28%), and neuronal (12%) pathologies,[73] many of which share a mesodermal origin. The predominant anomalies include intestinal malrotation, asplenia, transverse liver, tracheoesophageal anomalies, atrioventricular septal defects, upper limb and spinal deformities, micrognathia (including Pierre Robin sequence), renal dysplasia or aplasia, corpus callosum agenesis, and anophthalmia. Microgastria should be excluded in patients presenting with VACTERL (vertebral, anal, cardiac, tracheal, esophageal, renal, and limb) association and midline defects. The genetic cause of microgastria is unknown, but the BMP signaling pathway may play a role. Overexpression of *Bmp4* results in a microgastria phenotype in the chick.[5,8,9]

Clinical Presentation. Symptoms are mainly related to the markedly reduced capacity of the stomach to retain contents. Thus, postprandial vomiting and gastroesophageal reflux are common, with aspiration leading to recurrent chest infections. Rapid gastric emptying can lead to diarrhea. Nutrition is compromised, and malnutrition, failure to thrive, and growth retardation are very common. Developmental delay is often evident.

Diagnosis. The diagnosis is usually made on the basis of an upper gastrointestinal contrast study, which shows a small, tubular stomach in an abnormal position, usually midline. A dilated, poorly peristaltic esophagus and gastroesophageal reflux are frequently evident. Attention should be given to excluding associated anomalies with appropriate investigations.

Management. Management is designed to ensure adequate nutrition and prevention of aspiration and, if possible, to create an adequate gastric reservoir. Failure to thrive and gastroesophageal reflux are the greatest problems. Surgery is usually attempted only if a feeding strategy of frequent small-volume, high-calorie feeds fails to achieve adequate growth or symptoms of reflux are prominent. Nasojejunal or jejunostomy feeding has also been used, with variable success.[75] The aim of surgery is to increase the capacity and drainage of the stomach and prevent or resolve dilatation of the esophagus as a compensatory reservoir. This can be achieved by attaching a jejunal pouch to the stomach and forming a distal Roux-en-Y jejunojejunostomy (Hunt-Lawrence pouch).[76] Outcome is variable.[74] Associated gastrointestinal anomalies may need correction concurrently.

Gastric Diverticulum

Congenital gastric diverticulae are very uncommon. They occur most commonly in the posterior wall, antrum, and pylorus, usually comprising all layers of the stomach wall. They may be associated with hiatus herniae and aberrant pancreatic tissue.[77] Presentation is usually in adult life, although children may present with recurrent abdominal pain and vomiting.[78] Upper gastrointestinal endoscopy or contrast studies revealing the outpouching are usually sufficient to make the diagnosis. It should be differentiated from ulcers and malignancy. Treatment is by surgical excision.

Complete or Partial Absence of Gastric Muscle

This is a rare condition characterized by complete or partial absence of gastric muscle coats. The body of the stomach is most commonly affected. Muscular agenesis likely has many genetic causes, but it is known that in the gut, the hedgehog-bone morphogenetic protein signaling pathway plays a critical role and may be one of the causes of this rare disorder. Gastric perforation is the main complication, often occurring soon after birth. The clinical presentation is of intestinal perforation, often with cardiovascular collapse. Abdominal distention may be marked and may lead to respiratory compromise. Radiography reveals free intraperitoneal air. Fluid resuscitation with or without emergency decompression of intraperitoneal air may be required prior to surgery.

CONGENITAL ANOMALIES OF THE DUODENUM

Congenital duodenal obstruction most commonly results from duodenal atresia. Other causes include extrinsic compression from annular pancreas, Ladd bands or preduodenal portal vein, midgut volvulus, and duodenal webs.

Duodenal Atresia and Stenosis

The duodenum represents one of the most common sites for atresia in the gastrointestinal tract. The reported incidence for duodenal atresia is approximately 1 in 10,000 to 30,000 live births. Most atresias occur at the level of the ampulla of Vater and the obstruction owing to either a complete mucosal membrane or diaphragm without discontinuity of the muscle coats (type 1) or blind-ending proximal and distal segments of duodenum. These segments are either connected by a fibrous band (type 2) or separated by a gap (type 3). Duodenal stenosis is again the most common of gastrointestinal stenoses and occurs when a hole is present through the mucosal diaphragm.

Embryologically, the cause is thought to be a failure of canalization of the duodenum, which normally occurs after the seventh week of gestation. Although gut atresias are thought to be related to ischemic events early in gut development,[79] genetic causes may also play a role. Associated anomalies are common and occur in about 50% of cases of atresia. The most common is Down syndrome, which is present in one-third of affected infants. Other anomalies include esophageal atresia, midgut malrotation, annular pancreas, and biliary tract, anorectal, cardiac, genitourinary, and vertebral anomalies. Prematurity, intrauterine growth retardation, and polyhydramnios are more common.[80]

Clinical Presentation. Presentation of atresia is within the first few days of life and usually follows the first feed. The major symptom is vomiting, most commonly bile stained, given that most atresias occur distal to the ampulla of Vater. Gastric distention with visible peristalsis may be present, with the former easily reducible by nasogastric aspiration. Abdominal distention is not usual. There is an increased incidence of jaundice. In duodenal stenosis, presentation may be delayed, and recurrent vomiting and failure to thrive are more common symptoms.

Diagnosis. In atresia, the classic radiographic sign is the "double bubble" sign on abdominal radiography, denoting the higher, larger, left-sided stomach bubble together with the lower, smaller, right-sided bubble of the dilated proximal duodenum. No gas is visible throughout the distal intestine. With such an appearance, there is no need for an upper gastrointestinal series, especially because these contrast studies carry the additional risk of gastrointestinal perforation and aspiration of contrast.[48,81,82] Frequent vomiting may result in an absence of air in the stomach and duodenum, making the diagnosis difficult. To confirm the diagnosis in such cases, a small amount of air can be injected into the stomach via a nasogastric tube prior to radiography.[81] Prenatal diagnosis is possible with the use of ultrasonography to demonstrate the presence of a fluid-filled double bubble in the fetal abdomen in association with polyhydramnios.[83] In such cases, the fetal karyotype and a careful search for other anomalies should be instigated. Direct visualization of defects by endoscopy may be useful in the diagnosis of duodenal stenosis, although contrast studies are most useful. A "windsock" sign on contrast studies may be produced when peristalsis and movement of gut content protrude the membrane distally into the lumen of the third or fourth part of the duodenum or even proximal jejunum. The presence of gastric emphysema or duodenal pneumatosis may suggest a diagnosis of duodenal obstruction.[84,85]

Management. Following resuscitation, surgery is performed by laparotomy, although, more recently, laparoscopic surgery, including resection of a duodenal membrane, has been described.[86,87] At surgery, the usual approach is via a supraumbilical transverse abdominal incision. The entire duodenum is visualized by mobilizing the right colon. This allows identification of the obstruction and exclusion of associated malrotation. In malrotation, peritoneal (Ladd) bands extending from the cecum to the right upper quadrant may obstruct the duodenum. A side to side or end to side duodenoduodenostomy or duodenojejunostomy is carried out. Resection of any obstructing membranes is likely to damage the bile duct or pancreatic duct and is therefore usually avoided. The presence of other small bowel atresias is excluded at operation. The stomach and bowel are kept decompressed postoperatively using nasogastric or nasojejunal tubes, which can also be used for feeding. Survival rates are above 90% in the absence of chromosomal or cardiac defects. Long-term follow-up is needed to monitor development of complications such as ulceration and duodenal stasis.

Duodenal Duplication

Duplications of the duodenum are rare. They tend to occur on the mesenteric border of the first two parts of the duodenum. Presenting symptoms usually relate to duodenal obstruction. Ulceration, hemorrhage within the cyst, pancreatitis, and biliary obstruction may also occur. On contrast studies, the duodenum may appear to be compressed by a mass in the concavity of the duodenal C loop.[48] Ultrasonography, computed tomography, and magnetic resonance imaging are useful to further characterize the mass and determine its location. Surgery to excise the duplication may be complicated by its close proximity to the pancreatic and biliary tree.

Gut duplications are fascinating malformations because they are nearly always mesenteric and often have gastric epithelial differentiation.[88] This suggests that the gastric phenotype may be the "default" gut phenotype and occurs when gut development occurs out of the normal spatiotemporal and anatomic controls of development. If this is true, then the molecular controls of stomach development (see Figure 28-2) may be "ectopically" expressed in duplicated regions of gut. Gastric epithelial differentia-

tion does express embryologic factors when present in adults, as in Barrett esophagus and Meckel diverticulum.[89] Investigation of the expression of these factors in duplications of the gut may help in deciphering their etiology.

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CHAPTER 29

GASTRITIS

1. Helicobacter pylori *and* Peptic Ulcer Disease

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GASTRITIS AND PEPTIC ULCER DISEASE

The surface of the gastric mucosa is lined by a simple mucus-secreting columnar epithelium punctuated by gastric pits within which the gastric glands are situated. From a functional and histologic point of view, the stomach can be divided into three areas: cardiac, fundus or oxyntic, and antral, according to the predominant gland type within each zone (Table 29.1-1). In the normal gastric mucosa, the glands are separated by little or no extracellular matrix, and few or no mononuclear cells are present.[1] Gastritis is defined as microscopic evidence of inflammation affecting the gastric mucosa. The intensity of the inflammatory response is variable. Mild mucosal inflammation may be difficult to distinguish from normal mucosa and often requires review by an experienced pathologist.[2,3]

Duodenitis is characterized by the presence of neutrophils in the lamina propria, crypts, or surface epithelium, in addition to an increase in the number of mononuclear cells. There may be associated villous blunting. Duodenitis is graded from mild to severe, depending on the number of neutrophils present. Histologic assessment of the mucosa bordering primary duodenal ulcers usually reveals active duodenitis, and this histologic appearance may also be seen in symptomatic patients without overt ulceration. Therefore, duodenitis and duodenal ulceration likely represent different manifestations of a disease spectrum sharing a common underlying pathogenesis.

Peptic ulcers, by definition, are deep mucosal lesions that disrupt the muscularis mucosa coat of the gastric or duodenal wall.[4] Peptic erosions, on the other hand, are superficial mucosal lesions that do not penetrate the muscularis mucosae. Most gastric ulcers are located on the lesser curvature of the stomach. More than 90% of duodenal ulcers are found within the duodenal bulb.

Gastritis and peptic ulcer disease can be divided into two major categories, primary and secondary, on the basis of the underlying etiology.[5] This division is relevant because the natural history of primary gastroduodenal inflammation or ulceration is different from that of the secondary type.[5] Most cases of primary or unexplained gastritis are now known to be caused by gastric infection with the organism *Helicobacter pylori*.[6–11] Secondary gastritis and secondary ulceration are clinically and often histologically distinct from primary peptic disease.[6] Secondary ulcers may be gastric or duodenal in location and are discussed in Chapter 29.2, "Other Causes."

Gastritis and peptic ulcer disease were previously considered distinct entities. Over the past 20 years, it has come to be understood that these two conditions are closely related. In 1983, Warren and Marshall reported an association between the presence of spiral organisms on the gas-

TABLE 29.1-1 ANATOMY AND PHYSIOLOGY OF THE STOMACH

ANATOMIC/ GLAND AREA	CELL TYPE*	SECRETORY PRODUCTS†
Cardiac	Mucous	Mucus, pepsinogen
	Endocrine	—
Fundus/oxyntic	Parietal	Hydrochloric acid
	Chief	Pepsinogen
	Enterochromaffin	Histamine, serotonin
	G	Gastrin
Antral/pyloric	G	Gastrin
	D	Somatostatin
	Enterochromaffin	Histamine, serotonin

Adapted from Soll AH.[4]
*All three anatomic areas contain mucus-secreting cells in addition to endocrine cells.
†Endocrine cells in the stomach produce a variety of different products. For cells such as enterochromaffin cells and D cells, the secretory product has been identified, whereas the function of others is not as well defined.

tric mucosa and antral gastritis in adults.[12] Subsequent studies in adults and children have confirmed the etiologic role of *H. pylori* in primary antral gastritis and have demonstrated a strong association between *H. pylori*–associated gastritis and duodenal ulcer disease.[6–11]

Duodenal ulcer disease is reported as one of the more common chronic diseases in adults, occurring in 5 to 10% of the population. However, the prevalence of peptic ulcer disease in all age groups is changing. Duodenal ulcer disease, unusual prior to the turn of the twentieth century, increased steadily in the 1900s, reaching a peak in the 1950s.[13,14] Since then, the incidence of gastric and duodenal ulcers has decreased.[13–15] Nonmalignant primary gastric ulcers are now uncommon in adults.

There are no accurate figures on the incidence of peptic ulcer disease in children. Primary duodenal ulcer disease is very rare in children under 10 years of age, but prevalence increases in adolescence.[6] Large medical centers for children typically diagnose only four to six cases of peptic ulcer disease per year. As the prevalence of *H. pylori* infection decreases, the relative number of non–*H. pylori*–associated duodenal ulcers will increase, but, to date, very few non–*H. pylori*–associated duodenal ulcers have been reported in children.[7,16,17] Primary gastric ulcers rarely, if ever, occur in children.[5]

HISTOLOGIC DIAGNOSIS OF HELIOBACTER PYLORI GASTRITIS

In the past, there has been considerable confusion surrounding the histologic terminology used to classify gastritis. This is due to the use of such terms as "acute," "chronic," and "chronic active" to describe gastritis. The Sydney classification of gastritis aims to incorporate topographic, morphologic, and etiologic information into a clinically relevant scheme.[18,19] This classification and grading, which now incorporates the use of a visual analogue scale,[20] is accepted as the standard method by which all gastric biopsies from adult patients should be assessed. Classification is based on the location (antrum or corpus) and presence of a number of histologic parameters that are graded semiquantitatively as mild, moderate, or marked. These parameters are inflammation, activity, atrophy, intestinal metaplasia, and *H. pylori* infection.

When the Sydney system is used, it is recommended that two antral biopsies from within 2 to 3 cm of the pylorus, two corpus biopsies, and one biopsy from the incisura be obtained at endoscopy. The usefulness of the updated Sydney classification in a pediatric setting has not been formally assessed, but the underlying principles can be applied.

Inflammation and Activity

A precise definition for chronic gastric inflammation is difficult owing to a lack of agreement on the number of mononuclear cells present in the normal gastric mucosa of adults or children.[18] However, chronic inflammation is generally considered to be present if there are more than two to five lymphocytes, plasma cells, and/or macrophages per high-power field.[18] In children with *H. pylori* infection, substantial numbers of plasma cells and lymphocytes are present in mucosal biopsy sections. The inflammatory cell infiltrate is usually superficial in location, with panmucosal inflammation present in a small number of cases.[6,21]

The term "activity" is used to characterize the presence of neutrophils in the gastric biopsy. Neutrophil activity is almost always present in adults in association with *H. pylori* infection, and the density of the intraepithelial neutrophils has been correlated with the extent of mucosal damage.[18] Neutrophils have been identified as early as day 5 in an adult with acute infection,[22] but in children and in animal models, the active or neutrophil component of the histologic response is less than that reported in adults.[6–8,21,23]

Atrophy

Atrophy of the gastric mucosa, defined as loss of glandular tissue, is extremely rare in children.[21,24] When damage to the gastric glands is such that they lose their ability to regenerate, a repair process consisting of fibroblast recruitment and deposition of extracellular matrix occurs. The space previously occupied by the glands becomes replaced by fibrosis.[1] However, the presence of an inflammatory infiltrate and lymphoid follicles in the lamina propria may alter the architecture of the gastric mucosa, particularly in the antrum, where the glands are tortuous. Therefore, it may be difficult to distinguish loss of gastric glands from mere displacement secondary to increased numbers of inflammatory cells. For these reasons, inter- and intraobserver agreement among pathologists for the diagnosis of atrophy is poor.[25,26]

Intestinal Metaplasia and Lymphoid Follicles

Intestinal metaplasia is common in adults with chronic gastritis attributable to any cause and increases in prevalence with disease duration.[20] Intestinal metaplasia is an independent process, and although it is often present with atrophy, these conditions may occur independently. Intestinal metaplasia is rarely, if ever, found in children.

Lymphoid follicles with germinal centers are very suggestive of *H. pylori* infection in adults and children. If specifically sought, they are found in 100% of adult patients with *H. pylori*.[20] However, sampling error may occur unless sufficient biopsy specimens are taken. If lymphoid follicles and inflammation are present in the absence of *H. pylori*, it is likely that the organism has been missed.[20]

Helicobacter pylori

Colonization of the gastric antrum by *H. pylori* is graded as mild, moderate, or marked. In children, the number of bacteria present on the gastric mucosa is usually less than that in adults. Identification of *H. pylori* is facilitated by the use of special staining techniques (see below).

Successful treatment for *H. pylori* is accompanied by rapid and complete disappearance of bacteria and neutrophils. The presence of even a small number of neutrophils after treatment is very suggestive of treatment failure even if *H. pylori* is not identified.[27] Assessment of biopsy specimens for the presence of small numbers of *H. pylori* is

more difficult following treatment.[27] Chronic inflammatory changes may take a year or more to resolve.[27] Lymphoid follicles decrease very slowly and are still present 1 year after treatment. Lymphoid follicles, in the absence of active inflammation in the adjacent mucosa, are strongly suggestive of *H. pylori* eradication.[27]

DISEASES ASSOCIATED WITH HELICOBACTER PYLORI

Gastritis

H. pylori is a gram-negative spiral flagellated bacterium. It is found within and beneath the mucous layer on the gastric epithelium.[6–11] Infection of gastric epithelium has been reported not only in the stomach but also in areas of gastric metaplasia in the duodenum,[28] esophagus (Barrett esophagus),[29,30] and ectopic gastric mucosa at various sites in the gastrointestinal tract, including Meckel diverticulum[31] and the rectum.[32] However, *H. pylori* does not colonize tissue of nongastric origin.

There is strong evidence implicating *H. pylori* as a cause of chronic gastritis in children.[6–9]All children colonized with *H. pylori* have chronic gastritis. *H. pylori* is not an opportunistic colonizer of inflamed gastric tissue[33,34] because children with secondary gastritis owing to Crohn disease or eosinophilic gastritis are not consistently colonized with *H. pylori*. Eradication of *H. pylori* from the gastric mucosa results in healing of gastritis in children[6–8,35] and adults.[36] Further evidence implicating *H. pylori* as a gastric pathogen has come from volunteer studies. Two adult volunteers who ingested the organism developed gastritis, and gastric colonization was demonstrated.[37–39]

Despite the universal presence of chronic gastritis in infected individuals, the majority remain asymptomatic; however, up to 15% of infected individuals will develop peptic ulcer disease, and a further 1 to 5% may develop gastric cancer.[40–42]

Duodenal Ulcer Disease

H. pylori is found on the antral mucosa of almost 90% of children with duodenal ulcer disease.[34] Studies on adult patients have similarly indicated that 80% of individuals with duodenal ulcer disease are colonized with *H. pylori*.[10] Eradication of *H. pylori* from the gastric mucosa leads to long-term healing of duodenal ulcer disease in both adults[43,44] and children.[6–8,35] It is not known why a bacterial infection of the antral mucosa is critical in the pathogenesis of duodenal ulcers or why only a minority of those colonized with *H. pylori* develop ulcers. It has been hypothesized that *H. pylori* colonizes areas of ectopic gastric tissue (gastric metaplasia) in the duodenum, with the subsequent development of duodenal inflammation and possibly ulceration. In children, *H. pylori* infection of the antral mucosa and gastric metaplasia in the duodenum were each found to be significant risk factors for duodenal ulceration.[24,45] The presence of both *H. pylori* in the antrum and gastric metaplasia in the duodenum greatly increases the risk of duodenal disease.[24]

Gastric Ulcer Disease

There is also an association between *H. pylori* antral gastritis and gastric ulcer disease in adults.[10] The organism is found in approximately 60% of adults with gastric ulceration. This lower correlation may be due to the fact that a significant number of gastric ulcers are secondary, being related to drug and other ingestions. Gastric ulceration is extremely rare in children; when it occurs, it is usually secondary.[5,6] Therefore, there are no studies on any possible association between gastric ulceration and the presence of *H. pylori* on the gastric mucosa in children.

Gastric Cancer

Gastric cancer is the second most frequent cancer worldwide and the second leading cause of death from cancer.[46–48] Based on data from seroepidemiologic studies that suggested a two- to sixfold increase in risk for gastric cancer among infected individuals,[49–51] *H. pylori* was classified as a group 1 carcinogen in 1994 by the World Health Organization.[52] Early studies may have underestimated the risk of gastric cancer associated with *H. pylori* infection because later studies using immunoblotting or biopsy-based tests to diagnose infection suggest that the risk of gastric carcinoma may be greater.[53–55] A recent report from Japan, where the prevalence of gastric cancer is extremely high, suggests that up to 5% of *H. pylori*–infected individuals will develop gastric cancer in contrast to noninfected controls.[41] The risk of gastric cancer was highest in patients with corpus-predominant gastritis, gastric atrophy, and intestinal metaplasia. Uemura and colleagues also confirmed the previous findings of Hansson and colleagues[56] that patients with duodenal ulcer disease (who have antral-predominant gastritis rather than pangastritis) do not develop gastric cancer.

There is some evidence that genetic factors have a role in the development of gastric cancer, including a family history in 10 to 15% of cases, an elevated risk among first-degree relatives of gastric cancer patients, and reports of clustering of cases across several generations of the same family.[57–61] Furthermore, there is an increased prevalence of gastric atrophy in the relatives of gastric cancer patients.[62,63] Studies have also demonstrated an association between proinflammatory interleukin (IL)-1 genotypes and gastric cancer,[64–69] which suggests that host genetic factors are important in determining the outcome of infection with *H. pylori*.

Much attention is now focused on the possibility that treatment of *H. pylori* infection will prevent the development of gastric cancer. In a Japanese study, Uemura and colleagues suggest that patients who were not infected or who received treatment for *H. pylori* did not develop gastric cancer.[41] However, the duration of follow-up was much shorter in this group of patients compared with the patients who did develop gastric cancer.[41] Whereas a number of studies have suggested that eradication of infection can reverse atrophy and intestinal metalpasia,[70–72] others suggest that if eradication is to be successful, it must be prior to the development of atrophy because, by definition, atropy is irreversible.[73,74] Randomized trials in adults of

H. pylori eradication for the prevention of gastric cancer are currently in progress worldwide. Although a pilot study in Japan suggests that such studies are feasible, in practice, the Japanese group found that patients infected with *H. pylori* are unwilling to participate in a randomized placebo-controlled trial of cancer prevention.[75]

Mucosa-Associated Lymphoid Tissue Lymphoma

H. pylori has been implicated as an etiologic factor in mucosa-associated lymphoid tissue (MALT) lymphomas of the stomach.[76–78] Normal gastric mucosa is devoid of organized lymphoid tissue. For lymphoma to develop, the gastric wall must first acquire organized lymphoid tissue, which occurs as a reaction to infection with *H. pylori*.[79] Gastric MALT lymphoma is often multifocal, and most tumors are located in the antrum or distal body of the stomach. Eradication of *H. pylori* leads to complete resolution of 75% of gastric MALT lymphomas.[79,80] Staging of the tumor may help to determine the response to anti–*H. pylori* treatment because only those in the early stages respond to treatment.[79] Liu and colleagues demonstrated that MALT lymphomas, regardless of stage of disease, with the translocation t(11;18)(q21;q21) do not respond to *H. pylori* eradication and require conventional chemotherapy.[81] Less commonly, *Helicobacter heilmannii* has been implicated as a cause of MALT lymphoma.[79,82] There have been a number of case reports of MALT lymphoma in children.[83–86]

EPIDEMIOLOGY

It is accepted that *H. pylori*, in common with most enteric infections, is generally acquired in childhood and usually before the age of 5 years.[87–90] In the absence of treatment, infection is usually lifelong.[91–93] Adults rarely become infected with *H. pylori*, with seroconversion rates varying between 0.33 and 0.5% per person-year.[92–94]

Whereas most studies have reported no difference in infection rates between men and women, a large cross-sectional study from Bristol suggests that infection is more prevalent among men than women (29% vs 26%), and this remained statistically significant after controlling for other risk factors of infection.[95] Diseases associated with *H. pylori* infection are historically more common in men.

The major risk factor for infection is poor socioeconomic conditions in childhood.[96–98] In developing countries, the prevalence of infection is as high as 80% in children under 10 years of age.[99] In developed countries, although the overall prevalence of infection in young children is less than 10%, up to 50% of those children living in poor socioeconomic conditions may be infected.[96] Other markers of poverty, such as bed sharing and large sibships, are additional risk factors for infection.

The prevalence of infection increases with age, from 10% at age 10 years to 60% at age 60 years in developed countries.[99] The high prevalence seen in adults is consistent with a birth cohort effect, whereby adults acquired the infection as children because they lived in much poorer socioeconomic circumstances in the last century.[100] As socioeconomic conditions have improved in successive generations in the developed world, the prevalence of *H. pylori* has declined.

The exact age at which infection is acquired has not been determined. Cross-sectional studies in both developed and developing countries indicate that young children are the group that becomes infected. Ethiopian children appear to become infected between 2 and 4 years of age, with 60% of 4-year-old children being infected.[101] In a study from the United Kingdom, 23% of 5-year-old children were infected, and there was only a small increase in the prevalence of infection among 8-year-old children, suggesting that infection was acquired prior to school entry.[87] The rate of infection in children over 5 years of age in a large Chinese study was 1% per year, similar to the rate of infection in adults.[89] A cohort study from the United States again confirmed that most children became infected before the age of 4 years.[90] Malaty and colleagues also suggested that 9 of 58 (15%) children seroreverted during the study period.[90] This would suggest that infection with *H. pylori* may be transient in young children. However, the reproducibility of serologic assays limits their usefulness in longitudinal studies.[102] This would suggest that the seroreversion rates in children and adults are much lower than previously considered. Spontaneous clearance followed by subsequent reacquisition of infection in young children also has been reported using repeat ^{13}C urea breath tests (UBTs). However, it is now accepted that the UBT is not accurate in young children.[103]

There have been a number of individual case reports of *H. pylori* infection in very young children.[104,105] However, under 1 year of age, children rarely become infected with *H. pylori* even when they are exposed to infected mothers.[106] Many groups have reported a high prevalence of *H. pylori*–specific immunoglobulin (Ig)G antibodies in the first few months of life, with subsequent decline by 6 to 12 months.[107,108] Elevated *H. pylori*–specific IgG titers in these children reflect maternal transfer of *H. pylori*–specific antibodies to the fetus.

TRANSMISSION

The mode of transmission of *H. pylori* is poorly understood. A clear understanding of the most common route of *H. pylori* transmission would help elucidate the epidemiology of this infection and is essential for the prevention of infection.

The only known reservoir for *H. pylori* is the human stomach. Person-to-person spread currently appears to be the most likely mode of transmission. Evidence for person-to-person transmission includes clustering of *H. pylori* in families[33] and in institutions for the mentally handicapped.[109] Whether infection is spread from adult to child or from child to child is unknown. In some studies, strain identification using deoxyribonucleic acid (DNA) digest patterns has shown the same strain infecting different members of the same family, suggesting a common source of infection, whereas others have reported colonization by different strains.[110–112] Indirect evidence from a number of studies has suggested that transmission may be from mother to child,[113, 114] whereas other studies have sug-

gested that transmission is more likely from father to child.[115,116] Goodman and Correa suggested that transmission of infection is from older to younger siblings.[117] However, in this study from Columbia, 61% of firstborn children were also infected, which suggests that older siblings are not the only source of infection. In contrast, Tindberg and colleagues suggested that the prevalence of infection among mothers is the most important determinant of infection in children and that day care and school are unlikely sources of infection.[114] They also suggested that socioeconomic status and family size are risk factors for infection only when the mother was infected.

If transmission is from person to person, then the possible routes of transmission are fecal-oral, oral-oral, or gastric-oral. The fastidious growth requirements of *H. pylori* have hindered attempts to establish the relative importance of these potential routes of transmission. Polymerase chain reaction (PCR) is a very sensitive technique for detecting microbial DNA in clinical samples, but the use of PCR on feces, saliva, and dental plaque for the identification of *H. pylori* has limitations. Although a PCR diagnosis confirms the presence of DNA, it does not confirm the presence of viable organisms. Inhibitors present in fecal samples, such as acidic polysaccharides and metabolic products, can interfere with the PCR reaction.[118,119] False-positive reactions can occur because of the presence of unidentified *Helicobacter* species or other urease-producing organisms.

Using a novel approach for stool culture, Thomas and colleagues were the first to report the successful culture of *H. pylori* from feces in 9 of 23 Gambian children under 30 months of age and in 1 adult.[120] Hypochlorhydria occurs in association with acute infection.[22,38,121] It has been speculated that successful culture in these children may have been possible because of gastric hypochlorhydria. In animal studies, Fox and colleagues found that *Helicobacter mustelae* could be detected in the feces of infected ferrets when gastric pH was increased using omeprazole but not when acid secretion was normal.[121]

Using the same technique as Thomas, Kelly and colleagues cultured feces of 12 dyspeptic adults in the United Kingdom.[122] However, *H. pylori* was identified in only 3 of the 12 cultures using PCR. Others have not succeeded in culturing *H. pylori* from the feces of adults by this method.[123,124]

Parsonnet and colleagues failed to culture *H. pylori* from normal stool but cultured *H. pylori* from 7 of 14 infected volunteers following administration of a cathartic.[125] Stools passed late in the catharsis were more likely than early stools to grow *H. pylori*, but the number of organisms present was very low. This further supports the finding of Graham and Osato that *H. pylori* may not survive normal transit through the gastrointestinal tract owing to interference from bile acids.[126]

Oral-oral transmission has also been postulated. *H. pylori* has been cultured on one occasion from saliva,[127] and there are a number of reports of culture from dental plaque.[128–130] The complexity of oral flora is a major drawback in attempting to isolate *H. pylori* from the oral cavity. Evidence against oral-oral transmission is that there is no increased prevalence in teenagers and that *H. pylori* does not appear to be spread between couples.[131,132] Furthermore, although gastroenterologists have a higher prevalence of infection than expected,[133] dentists do not,[134,135] suggesting that exposure to oral secretions is not a risk factor for infection.

Gastric oral transmission has been postulated in young children in whom reflux and regurgitation are common occurrences. Recently, Leung and colleagues have reported isolation of *H. pylori* from vomitus on one occasion in a 6-year-old child.[136] Parsonnet and colleagues cultured *H. pylori* from all 16 infected adult volunteers with organism growths of greater that 1,000 colony-forming units (CFU)/mL of vomitus.[125] *H. pylori* was also cultured from saliva (9 of 16) and from air sampled close to the patient (6 of 16) in this study.[125]

Waterborne transmission has also been investigated. *H. pylori* has been cultured from waste water in Mexico,[137] and there have been a number of reports of PCR identification of *H. pylori* DNA in water from Peru, Sweden, Mexico, and Japan.[138–141] Water as a source of infection is a possibility where drinking water is untreated. It is unlikely that *H. pylori* can persist in treated drinking water systems under normal disinfectant concentrations.[142] However, in developing countries, the possibility of transmission through contaminated water must be considered.

Evidence for vector transmission is based on reports that laboratory houseflies can harbor viable *H. pylori* on their bodies and in their intestinal tracts.[143] *H. pylori* has also been detected in wild houseflies using a PCR assay to detect *H. pylori*–specific isocitrate dehydrogenase.[144] Vector transmission has some biologic plausibility because the midgut of the housefly (*Musca domestica*) has a pH of 3.1 and may be able to provide an ecologic niche for *H. pylori*.[143]

REINFECTION

Adults in developed countries rarely become reinfected with *H. pylori* following successful treatment, with reinfection rates being less than 1% per year.[43,44,145] This low rate of reinfection is not surprising because primary infection in adults is also uncommon. However, a recent study from Bangladesh suggests that reinfection in adults in developing countries may be as high as 13% per annum.[146] Reinfection in children in developed countries is also uncommon.[147–149] In children over 5 years of age, the rate of reinfection was only 2%.[147] This low rate of reinfection prevailed despite continued exposure of the children to *H. pylori*–infected family members (with 65% of siblings and 85% of parents infected).[147] Further studies are required to determine if this low rate also applies to children under 5 years of age.

MECHANISMS OF DISEASE

The mechanisms involved in the spectrum of diseases associated with *H. pylori* infection are not understood. The recent characterization and deposition in the public domain of the complete genomic sequences of two

H. pylori isolates undoubtedly will further the understanding of this organism.[150] The pathogenicity of *H. pylori* appears to result from its ability to (1) synthesize products that directly or indirectly damage the gastric mucosa, (2) cause a persistent inflammatory response, and (3) alter the regulation of acid secretion.[151] The specific bacterial virulence factors and/or host responses that determine the clinical outcome remain largely uncharacterized.

Bacterial Virulence Factors

There are many potential virulence factors that may contribute to the ability of *H. pylori* to induce gastric inflammation and ulcer disease. These include motility, adherence of the organism to the gastric mucosa, urease activity, toxin production, and subversion of host cell signal transduction.[9,152] The potent urease activity of *H. pylori* is an important virulence factor for this organism.[153] Urease-negative *H. pylori* mutants are incapable of colonizing the gastric mucosa in animal models.[153] Urease appears to play a vital role in protecting the bacteria against gastric acid.[154] The UreI protein, expressed from a gene of the urease gene cluster, has been identified as a pH-sensitive urea channel.[155] As the external pH falls, UreI increases urea transport to the bacterial cytoplasmic urease complex, providing the organism with a mechanism to maintain a neutral and viable intracellular pH despite its variable and often highly acidic milieu in the stomach.

Flagella are also important for the virulence of *H. pylori*. Flagella confer motility on the organism, allowing it to move through the gastric mucus.[152] Isogenic mutants that do not express flagella are incapable of colonizing gnotobiotic piglets.[156]

The cytotoxin-associated gene *CagA* has been identified as a possible marker for more virulent *H. pylori* strains.[157] The gene is part of a pathogenicity island (*cag* PAI) on the *H. pylori* genome encoding a so-called type IV secretion apparatus that serves to translocate bacterial products, notably CagA, into host cells.[151] Subversion of host cell processes is an emerging paradigm among bacterial enteric pathogens. In the case of *H. pylori*, it appears that within the host cytoplasm CagA is tyrosine phosphorylated and causes cytoskeletal reorganization in models of infection in vitro.[158]

The precise relevence of CagA translocation to the pathogenesis of *H. pylori*–associated diseases in vivo remains to be defined. The prevalence of CagA-positive strains varies in different parts of the world.[159] In many Western countries, CagA-positive strains are more commonly found in association with peptic ulcer disease than are CagA-negative strains.[160] However, ulcer disease can occur in association with CagA-negative strains. Furthermore, in some parts of the world, almost all strains are CagA positive, but the prevalence of peptic ulcer disease is not increased in these countries. In children, CagA is not a marker for specific disease development.[161–163]

H. pylori strains also differ in their ability to produce vacuolating cytotoxin (VacA). In some studies, toxin-producing strains also have been more commonly associated with ulcer disease.[164,165] However, as with CagA, ulcer disease may occur in association with VacA-negative strains.

H. pylori adheres only to gastric epithelium.[152] This strict tissue tropism suggests the importance of specific adhesion-receptor interaction for the maintenance of bacterial adhesion to the gastric epithelium. Several epithelial structures are potential receptors for *H. pylori*, including lipids, gangliosides, and sulfated carbohydrates.[152] A number of potential adhesins on the bacterial surface have been identified, but there is no convincing evidence to date that any of these potential adhesins or receptors are important in *H. pylori* colonization of the gastric epithelium in vivo.[152]

Host Inflammatory Response

Following infection with *H. pylori*, the human host mounts a strong local and systemic immune response. Despite this immune response, infection persists for life.[91,92] Although how *H. pylori* evades eradication is unknown, evidence is accumulating for a complex interaction between the organism and host defense mechanisms.[151] For example, some strains of *H. pylori* may be able to subvert the normal phagocytic mechanisms of the innate immune system.[166,167] *H. pylori* also has been associated with increased apoptosis and altered proliferation in epithelial cells[168] and may induce inappropriate effector T-cell responses.[151]

IL-8 recruits neutrophils and leukocytes by chemotaxis and then activates them.[169] The severity of the mucosal injury in *H. pylori* gastritis is directly correlated with the extent of neutrophil infiltration,[20] and *H. pylori* has been shown to stimulate IL-8 production in vitro and in gastric epithelial cells in vivo.[170] IL-8 production is enhanced by *cag* PAI *H. pylori* strains and by the presence of *cagE* in particular. In children, *cagE*-positive *H. pylori* strains have been associated with the development of ulcer disease.[171] However, non–*cag*-related bacterial factors also may influence IL-8 production.[172] The exact role of these bacteria-induced inflammatory changes in affecting disease outcome in *H. pylori* infection in vivo is unknown.

Gastric Acid Secretion

Acute infection with *H. pylori* is associated with a transient hypochlorhydria that may last for several months, as demonstrated by volunteer studies[37–39] and by reports of accidental infection.[22] Hypochlorhydria following acute infection has also been demonstrated in animal models.[170,173,174] The mechanism for this hypochlorhydria and its importance in determining colonization of the gastric mucosa are not understood. This acute hypochlorhydria is thought to facilitate transmission of infection.[175] More recently, it has been suggested that hypochlorhydria may become chronic in some people and may not respond to eradication of *H. pylori*.[176]

The gastric antrum plays an important role in the regulation of normal gastric acid secretion (see Table 29.1-1). G cells located within the gastric mucosa and duodenum produce gastrin, which, in turn, stimulates parietal cells to produce acid.[177,178] D cells are found within the antral mucosa in close proximity to G cells and also in the fundal mucosa close to parietal cells. D cells secrete somatostatin, a hormone that inhibits gastrin release and, therefore, acid secretion.[177,178] The factors that control acid secretion are

regulated through complex pathways. Gastrin release is stimulated by cholinergic innervation, gastrin-releasing peptide, and cytokines. If excessive amounts of acid are produced, then somatostatin is released in response to a low intraluminal pH.

H. pylori gastritis causes an increase in gastrin release, which returns to normal following treatment.[177] There is evidence that the increased release of gastrin caused by *H. pylori* is secondary to the infection depleting somatostatin-producing D cells in both adults[179] and children.[180] Eradication of *H. pylori* is associated with an increase in D-cell density and an increase in mucosal somatostatin baseline concentrations in children.[181] Changes in D-cell density and somatostatin levels are characteristic of all children with *H. pylori* gastritis and not just duodenal ulcer patients.[180]

The relationship between chronic *H. pylori* infection and acid secretion is not straightforward.[182,183] There is a large overlap in the levels of acid production between normal noninfected individuals, individuals with *H. pylori* gastritis alone, and individuals with *H. pylori*–associated peptic ulcer disease.[184] Most researchers now accept the fact that basal acid output does not differ markedly between infected and noninfected healthy controls.[177] However, patients with *H. pylori*–associated duodenal ulcer disease have an increased basal and maximal acid output when compared with infected healthy volunteers.[177] This increase in acid output may be due to the increased parietal cell mass seen in duodenal ulcer patients rather than to any direct effect of *H. pylori* on hydrochloric acid production.[183]

Pepsinogen

Oderda and colleagues were the first to report that serum pepsinogen 1 was elevated in children who were infected with *H. pylori* and that following treatment of infection, there was a significant fall in pepsinogen 1 levels.[185] This finding has since been confirmed in adult studies.[186] Earlier reports of a genetic predisposition to hyperpepsinogenemia are probably erroneous and reflect increased pepsinogen levels in several members of the same family owing to clustering of *H. pylori* infection in the family.

SYMPTOMS

Gastritis

There is no evidence that *H. pylori* gastritis in the absence of duodenal ulcer disease causes symptoms in children. *H. pylori* infection occurs frequently in asymptomatic children both in the developed and the developing world, and its eradication is consistently associated with improved symptoms only in children who have duodenal ulcer disease and not in those with gastritis alone.[187] In a large non–hospital-based epidemiologic study, Bode and colleagues have shown that abdominal pain is more common in children who are not infected with *H. pylori* than in those who are infected.[188]

There is no evidence for an association between *H. pylori* infection and recurrent abdominal pain (RAP) in children. RAP is a common condition of childhood, affecting 15% of children between 4 and 16 years of age.[189] In the search for a possible relationship between RAP and *H. pylori*, it is important that there is a clear definition of RAP, as stated by Apley and Naish,[190] and that both the patient and the researcher are unaware of the *H. pylori* status of the child when symptoms are assessed. In a meta-analysis, MacArthur and colleagues found that RAP is not associated with an increased prevalence of *H. pylori*–associated gastritis.[191]

Duodenal Ulcer Disease

Primary duodenal ulcer disease is associated with chronic or recurrent symptoms.[5,35,192,193] Most children present with episodic epigastric pain that is frequently associated with vomiting and nocturnal awakening.[5,35,192,193] When only patients with ulcer disease diagnosed at endoscopy are evaluated, up to 90% of children have abdominal pain, and in 55% of these children, abdominal pain is the sole presenting symptom.[5] However, this pain is often not typical of ulcer-associated pain, as described in adults. For example, a temporal association with mealtimes is present in only 50[194] to 75%[35] of children with duodenal ulcers.

Nocturnal awakening, which should be differentiated from difficulty in falling asleep, is an important feature in distinguishing abdominal pain associated with peptic ulcer disease from RAP.[187] Similarly, recurrent vomiting in association with upper abdominal pain should be considered suggestive of ulcer disease.

An acute episode of hematemesis may indicate primary or secondary ulceration. In such patients, a history of recent nonsteroidal anti-inflammatory drug ingestion should be sought. However, hematemesis occurring with a history of chronic abdominal pain is highly suggestive of primary duodenal ulcer disease.

In the past, up to 80% of children with primary peptic ulcer disease had symptoms that persisted into adult life.[192,193] The discovery of *H. pylori* has dramatically altered the prognosis of such patients. Successful treatment of *H. pylori*–infected children who have duodenal ulceration results in long-term healing of the ulcer and complete resolution of symptoms.[35,195]

Nongastrointestinal Manifestations

There have been a number of case reports describing children with refractory sideropenic anemia that responded to treatment only after the eradication of *H. pylori*.[196–202] A trial of *H. pylori* eradication therapy for iron deficiency anemia has been reported from Korea.[203] The numbers in the different groups in this study were very small, and although there was an increase in ferritin levels among those who were treated for *H. pylori* infection, serum ferritin levels at the end of the trial still suggest clinically depleted iron stores. A number of studies on adults also suggest that *H. pylori* may interfere with iron metabolism, with serum ferritin levels reduced in *H. pylori*–infected adults.[204–207] The mechanisms postulated include an increased demand for iron because *H. pylori* requires iron for growth, *H. pylori* may sequester iron, or *H. pylori* infection may result in hypochlorhydia, which would inhibit the reduction of iron to its ferrous state for absorption.[208] Because only 5 to 20% of ingested iron is absorbed, it is

unlikely that insufficient iron is available from dietary sources for *H. pylori*. Capurso and colleagues found that 51% of patients with *H. pylori*–associated iron deficiency anemia had a pangastritis compared with 20% of *H. pylori*–infected controls.[209] They suggest that this pangastritis may be responsible for changes in intragastric pH, which results in suboptimal iron absorption. Although pangastritis does occur in adults, there are no reports in the pediatric literature of pangastritis. Care must be used in interpreting studies of *H. pylori* and iron deficiency anemia because poor socioeconomic status is an important risk factor for both conditions. Furthermore, many of these studies use different ferritin levels to define anemia, and the duration of iron therapy is variable. Of the 21 pediatric cases reported with *H. pylori*–associated anemia,[196–202] 16 (75%) were male. Infant boys have a significantly higher risk of iron deficiency anemia than infant girls have.[210] Because children have lower iron stores than adults, *H. pylori* may contribute to the development of anemia rather than cause the anemia.

DIAGNOSIS OF H. PYLORI GASTRITIS AND ULCER DISEASE

Upper gastrointestinal endoscopy is the investigation of choice for the diagnosis of gastritis and peptic ulcer disease.[211] It has been shown to be both safe and effective, even in small infants.[212] The detection of ulcers using radiographic studies is often difficult in children. Single-contrast barium examinations correctly identified only 1 of 7 (14%) endoscopically proven duodenal ulcers in children.[5] Furthermore, the risk of false-positive diagnoses with barium studies is especially high in children. Miller and Doig failed to demonstrate any abnormality at endoscopy in 56 of 89 children with abdominal pain who had peptic ulcers diagnosed by barium meal.[213] Double-contrast barium techniques are more sensitive, but even in adults, this procedure failed to demonstrate 55% of gastric ulcers and 30% of duodenal ulcers.[214] In children, double-contrast barium studies are more difficult to perform, particularly in young children, and such studies involve increased radiation to the patient.

The endoscopic appearance of a peptic ulcer depends on the stage of the disease. Florid, active ulcers are usually round or oval, with a white base composed of debris and fibrin. The ulcer border may be hyperemic and elevated. Duodenal ulcers are often associated with spasm of the pylorus and a deformed pyloric outlet (Figure 29.1-1).

The endoscopic appearance of the stomach often correlates poorly with the presence or absence of gastritis.[2,3,6–8] Histologic evidence of mucosal inflammation is essential to establish a diagnosis of gastritis, and it frequently aids in the differential diagnosis of gastritis. Therefore, biopsies of the antrum and corpus, as well as the duodenum, should be obtained from children who undergo endoscopy.

A nodularity of the antral mucosa has been described in association with *H. pylori* gastritis in children.[6–8] These nodules give the antrum a cobblestone appearance (Figure 29.1-2). Hassall and Dimmick reported that nodularity of the antrum was present in their study in all 23 children with *H. pylori*–associated duodenal ulcers.[7] Nodularity of the antral mucosa is also seen in the majority of children with *H. pylori*–associated gastritis who do not have duodenal ulceration. The reason for this appearance of the gastric mucosa in association with *H. pylori* in children is unknown.

HISTOLOGY

The characteristic appearance and unique location of *H. pylori* allow a presumptive diagnosis of *H. pylori* colonization in children to be made by identifying spiral organ-

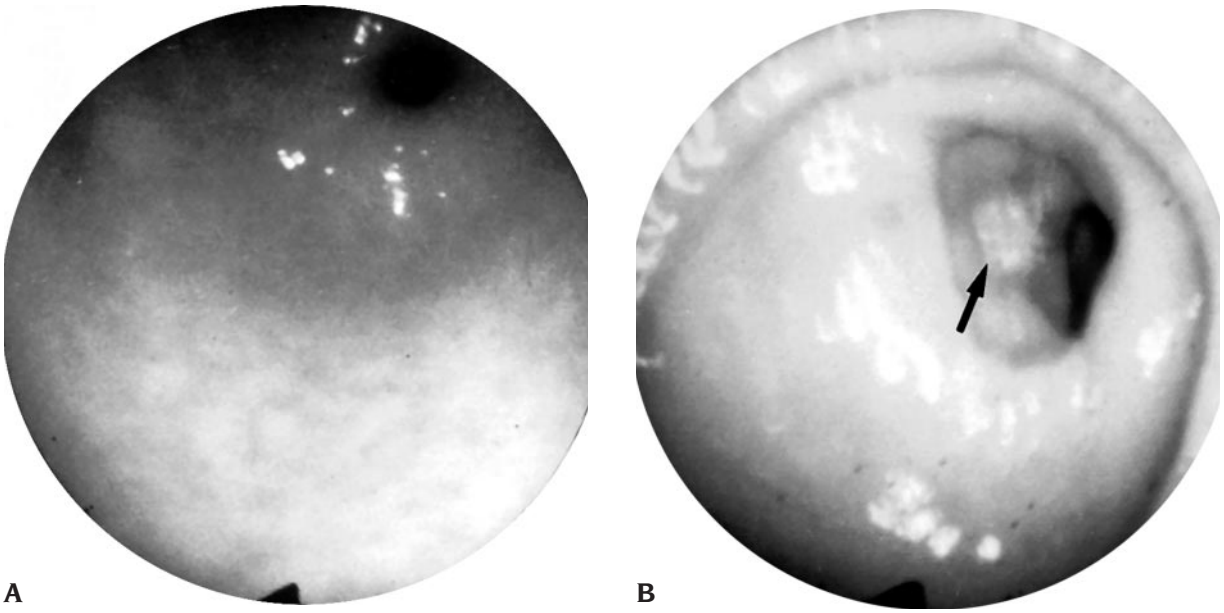

FIGURE 29.1-1 *A*, Endoscopic photograph showing normal-appearing antral mucosa: histologic examination of an antral biopsy, however, revealed evidence of acute gastritis and colonization with *Helicobacter pylori*. *B*, A duodenal ulcer (*arrow*) is visible through a patent but irregular pyloric channel.

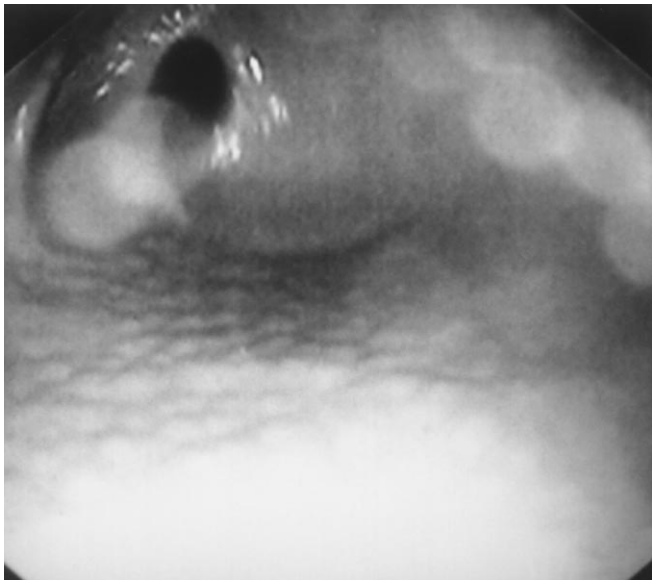

FIGURE 29.1-2 Endoscopic view of nodularity of the gastric antrum, which is seen in association with *Helicobacter pylori* infection.

isms on histologic sections of the gastric mucosa. Historically, the organism has been identified using a Warthin-Starry silver stain (Figure 29.1-3).[12,34] Silver stains are usually very sensitive and specific for identifying the presence of *H. pylori* in children, but they are expensive and difficult to perform. A modified Giemsa stain or a cresyl violet stain is both sensitive and specific for the organism and is much easier to perform than silver staining.[6] The Genta stain allows simultaneous visualization of the bacteria and the histologic features of gastritis.[215] Ultrastructural studies show the spiral morphology of organisms present on gastric epithelium (Figures 29.1-4 and 29.1-5).

Culture

Because *H. pylori* is extremely fastidious in its growth requirements, routine culture is difficult in a clinical practice. A more practical "gold standard" for the diagnosis of *H. pylori* is either a positive culture or the identification of *H. pylori* on both histology and urease testing.

Culturing of *H. pylori* is performed by inoculating minced biopsy specimens into blood agar plates that are held under microaerophilic conditions at 37°C. Media that contain nalidixic acid or vancomycin (ie, Skirrow medium) are frequently used to minimize overgrowth of organisms from the oropharyngeal flora. Visible colonies usually require 5 to 7 days of culture. The long incubation period is particularly important in children because the number of organisms present on their gastric mucosa is often very low. The organism is identified as *H. pylori* if it is positive for urease, catalase, and oxidase and produces a negative reaction for hippurate hydrolysis and nitrate reduction.

For optimal recovery rates of *H. pylori* from gastric biopsy specimens, the viability of the organism must be maintained during transportation to the laboratory. It was

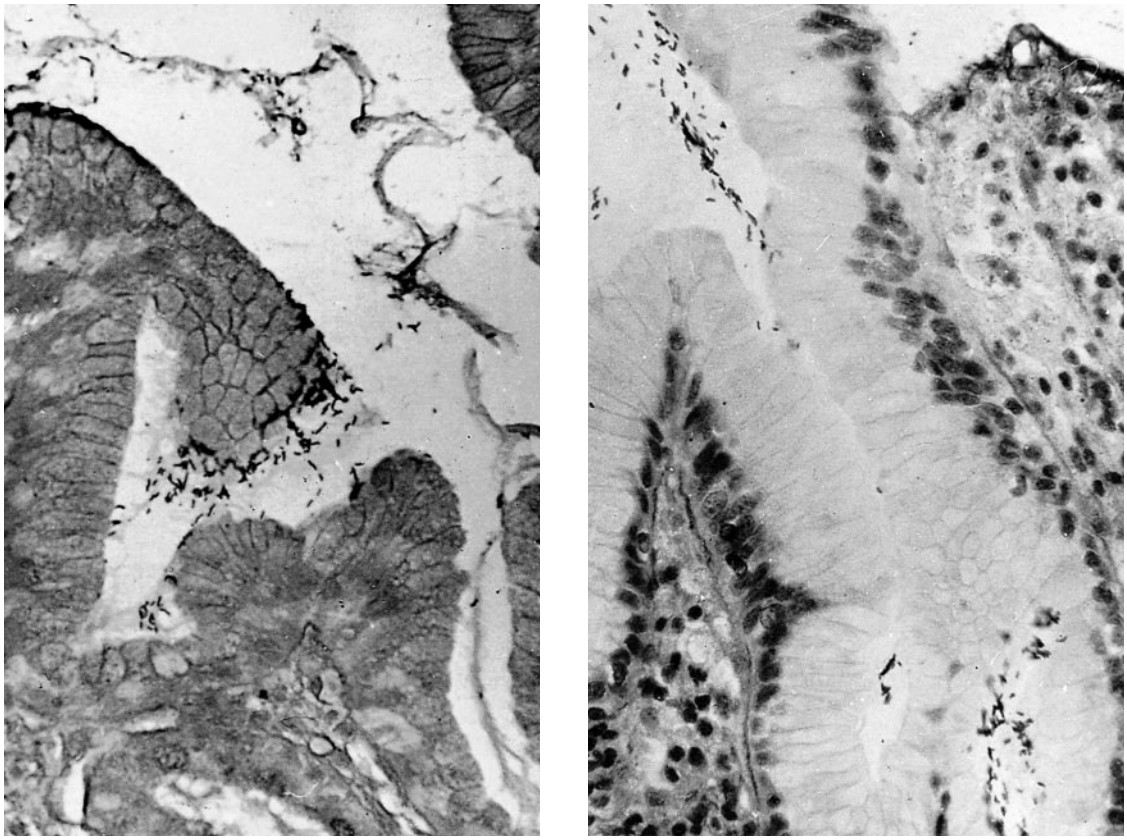

FIGURE 29.1-3 Modified silver impregnation method of antral mucosa (with histologic evidence of active gastritis) demonstrates gastric *Helicobacter*-like organisms. Reproduced with permission from Drumm B et al.[34]

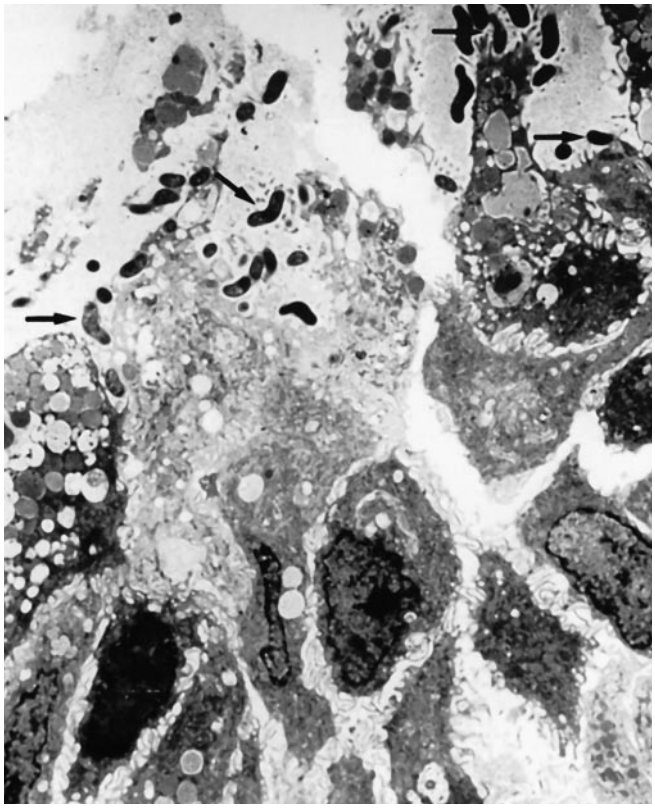

FIGURE 29.1-4 Transmission electron photomicrograph of antral mucosa shows spiral-shaped *Helicobacter*-like organisms (*arrows*) adherent to surface epithelial cells and contained in the overlying mucous layer. Courtesy of Dr. Ernest Cutz, Department of Pathology, The Hospital for Sick Children, Toronto, ON.

previously considered necessary for biopsy specimens to be delivered to the microbiology laboratory within 1 hour, but successful culture is possible even after 24 hours when a suitable transport medium is used.[216] It is essential that the biopsy specimens are placed directly into the transport medium and not exposed to room air. When carefully performed, culture of the organism is successful in almost 100% of specimens.[33,34]

Urease Test

Because *H. pylori* produces high levels of the enzyme urease, this property can be exploited to detect the presence of bacteria in antral biopsy specimens and also in UBTs. To rapidly identify *H. pylori* on the gastric mucosal specimens, a specimen is placed on urea medium; hydrolysis of urea leads to a color change of the medium, from tan to pink. The color change may occur as soon as 30 minutes after inoculation, but if the number of bacteria is small, the color reaction may take up to 24 hours to develop. Urease tests in children occasionally have not been as sensitive as in adults,[34,217] perhaps reflecting the lower number of bacteria present on biopsy specimens from children. When a full biopsy specimen (rather than a fragment of the specimen) is placed in the urea medium, the sensitivity of this test increases and is close to 100%.[218] Commercial kits based on similar principles are available for use in the endoscopy suite.

Urea Breath Tests

The ^{13}C UBT is a safe and noninvasive method for the diagnosis of *H. pylori* infection in adults and children.[219–225] Isotopic urea (^{13}C) is ingested by the patient; if *H. pylori* is present in the stomach, breakdown of the labeled urea by *H. pylori* urease results in the production of labeled carbon dioxide, which is measured in the expired air. In contrast to ^{14}C (a radioactive label), ^{13}C urea is labeled with a naturally occurring stable isotope of carbon. Tests using stable isotopes are ideal for use in children, but they are more expensive because they require the use of a mass spectrometer.

The UBT for use in adults involves an overnight fast and the administration of a test meal to slow gastric emptying.[219,221,222] The use of a prolonged fast and a test meal makes the test difficult to perform in children and limits its usefulness in both research and clinical practice.

We have shown that a simplified test protocol is very successful in children over 2 years of age.[223] After the patient fasts for 2 hours, the UBT is performed by collecting a baseline sample of expired air, followed by ingestion of ^{13}C urea (50 mg for children < 50 kg or 75 mg for children > 50 kg) with 50 mg of a glucose polymer in 5 to 10 mL of water. It is important that the solution of urea is swallowed quickly and not held in the mouth. This is not a problem in the older child but may present difficulties for younger children. A second breath sample is collected 30 minutes later. In older children, as in adults, samples may be collected by blowing directly with a straw into a glass

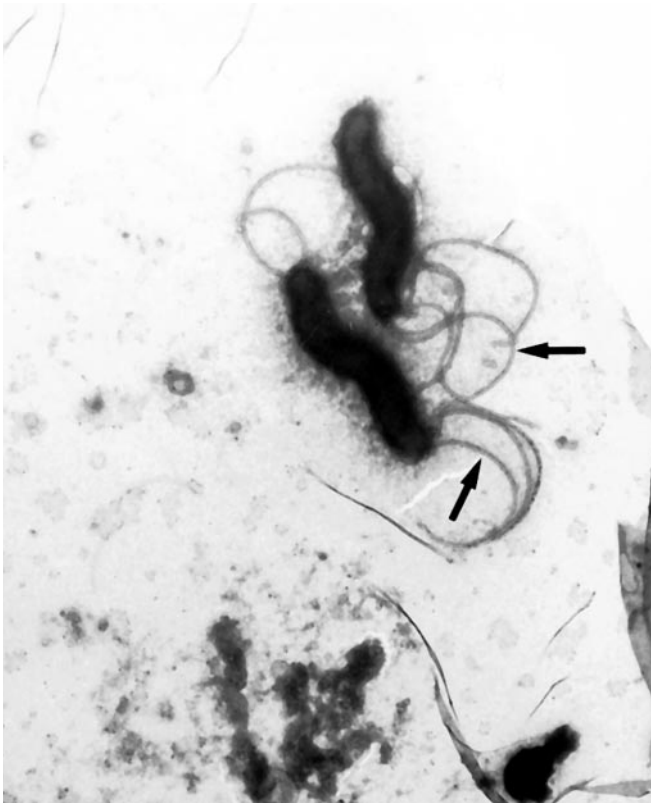

FIGURE 29.1-5 Transmission electron photomicrograph after negative staining of *Helicobacter pylori*. Note the spiral shape of the organism (comparable to intestinal *Campylobacter*) and the presence of flagellae (*arrows*).

tube that can be sealed. For younger children, a closed system such as a rebreathing bag with tap (Childerhouse Medical, London) is required, and the expired air is transferred into an evacuated glass tube. The ratio of ^{12}C to ^{13}C is measured in both the baseline and the 30-minute sample, and the difference between the samples is calculated by subtraction. This value is referred to as excess delta or delta over baseline. In children, an excess delta of 5.0 is indicative of *H. pylori* infection.[103]

The UBT was shown to be 100% sensitive and 92% specific for the diagnosis of *H. pylori* infection in older children.[223] The sensitivity and specificity were achieved after a 2-hour fast and without the use of a test meal or drink. This test has an excellent capacity to distinguish infected from noninfected children, with a clear separation of excess delta values between the two groups.[224] In children under 2 years of age, the UBT may have a reduced specificity.[103,226] Children in this age group have more borderline and false-positive results than older children. Urease-producing organisms in the mouth may interfere with the test in very young children.[103,227] When the test was carried out using a nasogastric tube (in situ for clinical indications), there were no false-positive results in children under 2 years of age.[103]

Samples collected at 15 or 20 minutes often give false-positive results, perhaps because of interference from oral urease-producing organisms.[223] Therefore, the second breath sample should be collected 30 minutes after ingestion of substrate. Because the UBT measures the ratio of ^{12}C to ^{13}C, the volume of expired breath collected is not critical. However, the effect of crying or dead space on the test in young children has not been determined. Breath samples from children are stable for up to at least 7 months; therefore, transport and storage of specimens for analysis at a later time are possible.[224] Following treatment, the UBT is 100% sensitive and specific in assessing *H. pylori* status.[223] It has replaced endoscopy as the investigation of choice for assessing treatment success in children. Breath tests should not be carried out for at least 1 month after the completion of treatment.

Isotope ratio mass spectrometry is an expensive technique for the analysis of ^{13}C. Nondispersive infrared mass spectrometry has been shown to be equally accurate and less expensive.[225] However, much larger volumes of breath are required, making this method more difficult for young children.

Serology

Infection with *H. pylori* provokes a specific serum IgG response. The initial antibody response in children is to low-molecular-weight antigens in the 15 to 30 kD range and may take up to 60 days to develop.[228,229] The mean antibody levels in young children are significantly lower than in older children and adults.[230–232] Antibody titers in children may not reach their maximum levels until the age of 7 years.[233]

Serologic tests in children must therefore be standardized using children's sera.[233] If the assay is based on adult antibody levels, less than 50% of children will be correctly diagnosed because the cutoff point is higher for adults than for children. Commercially available serologic tests do not have the sensitivity or specificity to accurately diagnose *H. pylori* infection in children under 12 years of age,[232] with second-generation serologic tests failing to diagnose up to 20% of children under the age of 10 years.[234]

Everhart and colleagues have shown that even using adult sera, the reliability of serology on repeat analysis of the same sample is poor and could easily explain the 1 to 2% seroreversion and seroconversion rates reported from adult seroepidemiologic studies.[102] Therefore, in all seroepidemiologic studies, there is a need for caution in interpreting results.

Measurement of *H. pylori*–specific serum IgA antibodies in children is not a sensitive indicator of gastric colonization. Czinn and colleagues found that only 45% of children with *H. pylori* colonization of the gastric mucosa had increased *H. pylori*–specific serum IgA antibodies.[235] Also, *H. pylori*–specific serum IgM antibodies are not consistently elevated in children with *H. pylori*–associated gastritis.[235]

Measurement of Salivary IgG Antibodies

The *H. pylori*–specific antibody response can also be detected in saliva. In adults, varying sensitivities and specificities have been reported, depending on the assay used.[236,237] In children, Luzza and colleagues have reported a sensitivity of 93% and a specificity of 82% with an in-house enzyme-linked immunosorbent assay.[238]

Stool Antigen Enzyme Immunoassay

The detection of *H. pylori* antigens in stool using polyclonal and, more recently, monoclonal antibodies provides a noninvasive method for identifying infected adults and children. The *H. pylori* stool antigen test (HpSA) (Premier Platinum HpSA test, Meridian Diagnostics Inc, Cincinnati, OH) is based on a polyclonal antibody and in adults is 91 to 98% sensitive and 83 to 100% specific for the diagnosis of infection.[239] Initial results also suggested the HpSA to be very sensitive and specific following treatment of *H. pylori* in adults,[240–242] but more recent studies would have called into question the overall accuracy of the test in this situation.[239,243,244]

Preliminary reports suggest that in children, the HpSA test is less accurate and that the cutoff values may have to be adjusted for each population studied, rendering the test of little use in a routine clinical setting.[245–249] The HpSA has a specificity of only 70% following treatment for *H. pylori* in children.[249]

The development of a monoclonal antibody has provided much greater accuracy in stool antigen testing.[250–252] This is particularly so in children in whom the sensitivity, specificity, positive predictive values, and negative predictive values were 98%, 99%, 98%, and 99%, respectively, in a large multicenter European study involving 302 previously untreated children using a monoclonal stool antigen test (*H. pylori* CnX, FentoLab, Martinsreid, Germany).[253] Although the accuracy of the monoclonal stool antigen test has not yet been evaluated in children following treatment for *H. pylori* or in the diagnosis of infection in children under 2 years of age, this approach appears to hold particular promise as a diagnostic tool in childhood *H. pylori* infection.

TREATMENT OF PEPTIC ULCER DISEASE

In adults, both the National Institutes of Health consensus statement[254] and the Maastricht consensus report[255] recommended that *H. pylori* should be eradicated in adults who have *H. pylori*–associated peptic ulcer. More recently, the Maastricht 2-2000 consensus report suggested treatment of *H. pylori* infection in first-degree relatives of gastric cancer patients.[256]

Children with peptic ulcer disease who are infected with *H. pylori* should receive treatment to eradicate the infection.[257] However, the majority of children infected with *H. pylori* do not have peptic ulcer disease and are not symptomatic.[6] The diagnosis of *H. pylori* infection is often an incidental finding at endoscopy, and the management of these children is therefore controversial. Recently, two pediatric consensus conferences on *H. pylori* have stated that there is no evidence demonstrating a link between *H. pylori* gastritis and abdominal pain except in those children in whom an ulcer is present.[257–259] If infection is incidentally diagnosed, it should be treated, but there is currently no indication to screen children for *H. pylori* infection except at endoscopy, when peptic ulcer disease is investigated.

Specific Treatment Regimens

Several regimens used to treat *H. pylori* in children are listed in Table 29.1-2.[187,260–266] Because peptic ulcer disease is much less prevalent in the pediatric age group, treatment studies in children are usually open trials with small numbers of participants. Treatment regimens have evolved from using a single antibiotic with a bismuth preparation for 4 to 6 weeks to 1-week treatment regimens using two antibiotics and either bismuth or a proton pump inhibitor for 1 week. The most frequently used antibiotics in children and adults are amoxicillin, metronidazole, tinidazole, and clarithromycin. Selection of optimal antibiotic combinations should be based on known antibiotic sensitivities for *H. pylori* in the local population. Following treatment, children should have a UBT carried out to determine if the treatment has been successful.

Currently, there is only one reported multicenter prospective randomized controlled trial of *H. pylori* treatment in children.[266] In this study, a combination of omeprazole, amoxicillin, and clarithromycin for 1 week was compared with dual therapy of amoxicillin and clarithromycin. On an intention-to-treat analysis, 74% of children were successfully treated with omeprazole, amoxicillin, and clarithromycin compared with 9% of those treated with antibiotics alone. Although this study clearly demonstrates that triple therapy is more effective than dual therapy, it also suggests that the combination of omeprazole, amoxicillin, and clarithromycin is less than optimal in children. Adult guidelines suggest that treatment regimens with less than an 80% success rate are unacceptable for use in clinical practice.[255] The reason for a much

TABLE 29.1-2 TREATMENT OF *HELICOBACTER PYLORI* INFECTION IN CHILDREN

STUDY	YEAR	ELIGIBLE CHILDREN	TREATMENT REGIMEN	DURATION (D)	ERADICATION RATE (%)	95% CI (IF STATED)
Gottrand et al[266]	2001	31	Omeprazole 10–20 mg bid Amoxicillin 25 mg/kg Clarithromycin 7.5 mg/kg	7	23/31 (74.2) ITT 20/25 (80) PP	59–90
		32	Amoxicillin 25 mg/kg Clarithromycin 7.5 mg/kg		3/32 (9.4) ITT 3/28 (10.7) PP	46–83
Tiren et al[261]	1999	38	Omeprazole 0.3 mg/kg Amoxicillin 50 mg/kg Clarithromycin1 5 mg/kg	14	24/32 (75)	60–90
Behrens et al[265]	1999	63	Omeprazole 1 or 2 mg/kg Amoxicillin 50 mg/kg	14	27/52 (52)	
		73	Omeprazole 1 or 2 mg/kg Amoxicillin 50 mg/kg Clarithromycin 20 mg/kg	14	44/53 (83)	
Casswall et al[262]	1998	32	Omeprazole 10 or 20 mg/d Clarithromycin 7.5 mg/kg Metronidazole 7.5 mg/kg/d	7	28/32 (87)	
Moshkowitz et al[263]	1998	35	Omeprazole 20 mg bid Clarithromycin 250 mg bid Metronidazole 500 mg bid	7	25/35 (71)*	
Walsh et al[260]	1997	28	Bismuth 480 mg/1.73 m^2 Clarithromycin 15 mg/kg Metronidazole 20 mg/kg	7	21/22 (95)	77–100%
Kato et al[264]	1997	22	Omeprazole 0.6 mg/kg Amoxicillin 30 mg/kg	14	15/22 (70)	
			Omeprazole 0.6 mg/kg Amoxicillin 30 mg/kg Clarithromycin 15 mg/kg	14	11/12 (92)	

ITT = intention-to-treat analysis; PP = per protocol analysis.
*Eight children had received previous eradiaction therapy.

lower success rate for the combination of omeprazole, amoxicillin, and clarithromycin compared with other open trials of treatment in children (see Table 29.1-2) or with similar studies in the adult literature is unclear.[267–269] In children, clarithromycin resistance is usually much higher than that reported in adults, yet in this study, resistance to clarithromycin was low at 7.7% and did not account for treatment failures. However, the antibiotic dosages reported in this study are lower than in many other pediatric studies (Table 29.1-2) and may account for the reported differences in efficacy.

Bismuth. Bismuth formulations have been used in the management of peptic ulcer disease for over 100 years. Their precise mechanism of action in eradicating *H. pylori* from the gastric mucosa is not known. Bismuth compounds have some antibacterial properties, and bismuth monotherapy clears *H. pylori* from the gastric mucosa in about 50% of cases. The rate of ulcer relapse after bismuth therapy alone is significantly lower than that after treatment with histamine$_2$ receptor antagonists.

In Europe, colloidal bismuth subcitrate is the bismuth preparation most commonly used to treat *H. pylori* infection, whereas bismuth subsalicylate is used in North America. There has been concern about the use of bismuth salts in children because of potential toxic effects. However, bismuth does not appear to have any toxic effects in children other than those already well described in adults. Encephalopathy and acute renal impairment after chronic use of high-dose bismuth are reported, but these side effects, which are reversible, have not been reported in children treated for *H. pylori*–associated gastritis or ulcer disease.[6] Serum bismuth levels remain within the normal range for children when colloidal bismuth subcitrate is prescribed as either 480 mg/1.73 m^2 of body surface area per day or 120 mg twice daily (240 mg twice daily for children over the age of 10 years). There is a concern about the presence of salicylate and therefore the risk of Reye syndrome with bismuth subsalicylate. A 30 mL dose of bismuth subsalicylate contains 230 mg of salicylate.

Proton Pump Inhibitors. Proton pump inhibitors inhibit the gastric acid pump (hydrogen-potassium–exchanging adenosine triphosphatase) in a dose-dependent manner.[270–272] Proton pump inhibitors are rapidly absorbed, with peak concentrations occurring 2 to 4 hours after oral administration.[273] The precise mechanism of action of a proton pump inhibitor in inhibiting *H. pylori* is unknown.[273] Proton pump inhibitors have some antibacterial activity in vivo. More important, by inhibiting gastric acid secretion, these drugs may promote the increased effectiveness of acid-sensitive antibiotics, such as clarithromycin, in triple-therapy regimens.

The side effects of omeprazole, which include headache, diarrhea, abdominal pain, and nausea, are self-limiting. Bacterial overgrowth in the stomach and small intestine by oral and colonic flora has been reported.[274,275] Proton pump inhibitors are metabolized completely by the polymorphic cytochrome P-450 system. Although drug interactions with warfarin, diazepam, and phenytoin theoretically could occur, none have been reported to date.[273]

Treatment Failure

Numerous factors are responsible for treatment failure, but the most important are poor patient compliance, inadequate drug delivery, and antimicrobial resistance. Ingestion of less than 75% of the prescribed medication results in decreased eradication rates.[276] Walsh and colleagues achieved excellent compliance in children, using special boxes in which the drugs for each dose were compartmentalized.[260] This level of compliance, although desirable, is unlikely in clinical practice.

In treating children, the availability of suitable drug preparations is very important. Although bismuth has been widely used in children and comes as a liquid preparation, the strong taste of ammonia from liquid bismuth may reduce compliance in children. In clinical practice, therefore, the treatment regimen with the simplest dosing requirement and fewest side effects is to be preferred, assuming similar eradication rates.

Antibiotic Resistance

The development of antibiotic resistance by *H. pylori* is an important variable in the success of treatment regimens. Resistance to antibiotics may be primary, or it may develop during the course of treatment. The development of secondary resistance is usually associated with suboptimal treatment regimens. Agar dilution is considered the method of choice for resistance testing.[277] Disk diffusion techniques are simple and less expensive but, although less suitable for slow-growing bacteria such as *H. pylori*, are a more realistic option for everyday laboratory use.[277] The E-test is a semiquantitative variant of disk diffusion, and although there is excellent correlation between the different methods when testing for clarithromycin resistance, discrepancies have been reported for metronidazole resistance between E-test and disk diffusion, with rates of metronidazole resistance being higher for E-tests.[256,278,279] In determining antibiotic sensitivities, it is recommended that isolates of *H. pylori* should be recovered during the active phase of growth within 3 days and that the inoculum size of 10^8 CFU/mL (equivelant to McFarland 4) is used.[277] The conditions (anaerobic vs microaerophilic) under which resistance is determined may also influence the outcome of resistance testing.

Metronidazole resistance greatly reduces the efficacy of metronidazole-based regimens in both adults and children.[268,280,281] Primary resistance to metronidazole may be a nonstable phenomenon[282] and may explain why treatment is more successful than anticipated in a number of studies in which metronidazole was used.[283] Goodwin and colleagues were the first to demonstrate that metronidazole resistance in *H. pylori* arose from mutations within the *rdxA* gene.[284] It has also been suggested that other reductase-encoding genes, including *frxA* and *fdxB*, are associated with metronidazole resistance.[285–287]

Rates of metronidazole resistance vary from 33% in Europe[278,288,289] to 20 to 50% in the United States[290] and Aus-

tralia[291] to up to 70% in developing countries.[283] Women are more likely to harbor resistant strains, as are migrants from developing countries.[288,289] The higher resistance among women and in developing countries may be explained by the use of metronidazole for gynecologic and diarrheal diseases in these groups. The rate of metronidazole resistance in adults appears to be relatively constant over time.[291,292]

The presence of metronidazole-resistant strains has been reported as 26 to 37% in French children,[266,281] a finding similar to that seen in French adults.[202] Metronidazole resistance rates are 18% in Belgian children[293] and 24% in Japanese children.[294]

The mechanism of action of clarithromycin is to bind ribosomes and disrupt protein synthesis. The development of resistance is attributed to various point mutations in the two 23S ribosomal ribonucleic acid (rRNA) genes of *H. pylori*. It is thought that clarithromycin needs effective acid control to achieve high eradication rates. Primary clarithromycin resistance is less common in adults than metronidazole resistance and ranges from 10 to 15% of strains in adults. There is concern that clarithromycin resistance rates may be increasing, particularly among children. In 1998, Raymond and colleagues reported a 4% rate of clarithromycin resistance in children,[295] and a more recent report from Kalach and colleagues suggests that the prevalence of clarithromycin resistance has increased to 18%.[296] Similarly, in Belgian children, the prevalence of clarithromycin resistance increased from 6.0% prior to 1995 to 16% in the following 5 years.[293] In this same period, metronidazole resistance remained stable at around 18% in children.[293] In Japan, clarithromycin resistance has been reported as 30% in children, with 92% of strains having an A2144G mutation in the 23S rRNA gene. Clarithromycin resistance may be becoming more common in children because of the widespread use of clarithromycin in pediatric practice. With this increasing prevalence of resistance to clarithromycin, careful consideration should be given to its inclusion as a first-line treatment in children.

Until recently, it was thought that *H. pylori* did not develop resistance to amoxicillin. Stable amoxicillin resistance has been reported from the Netherlands in an 82-year-old man. Previous reports of amoxicillin-tolerant strains have come from Italy and the United States. The major concern in the study by van Zwet and colleagues is that amoxicillin resistance could be transferred to susceptible strains, suggesting the possibility of the spreading of amoxicillin resistance. To date, amoxicillin resistance has not been reported in children.

Resistance to tetracycline has also been reported, which may greatly hinder its use as a low-cost first- or second-line treatment.[297] Mutations of the 16S rRNA genes are thought to be responsible for resistance,[297–300] but a number of mutations may be necessary to confer clinically significant resistance.[299]

Second-Line Treatment

Current treatment regimens fail to eradicate *H. pylori* infection in 5 to 30% of children. Therefore, an important question that needs to be considered is the management of children in whom *H. pylori* treatment has been unsuccessful. Currently, there are no published data or guidelines for children. The Maastricht 2 guidelines suggest that treatment for adult patients infected with *H. pylori* should adopt a planned approach, with a therapeutically effective second-line treatment regimen available if the first-line treatment fails. They suggest that first-line therapy should include a proton pump inhibitor or ranitidine bismuth citrate with amoxicillin, metronidazole, or clarithromycin, whereas second-line therapy should include a proton pump inhibitor, bismuth, metronidazole, and tetracycline (quadruple therapy). A number of recent adult studies report excellent eradication rates with quadruple therapy when prescribed twice daily for 7 days as a first- or second-line treatment.[301–306] Well-conducted randomized controlled trials remain to be conducted for second-line treatment regimens in adults.

The use of tetracycline is contraindicated in children before the age of 12 years at least. This and the increasing problem of clarithromycin resistance limit the choices available for the treatment of *H. pylori* in children. Optimal treatment protocols for *H. pylori* infection in children should be developed for individual pediatric units based on local antibiotic resistance rates and the age of children who require treatment.

H. heilmannii Gastritis

H. heilmannii (formerly *Gastrospirillum hominis*) is known to cause gastritis in humans. It can be distinguished morphologically from *H. pylori* on histologic sections by its larger size and its characteristic shape with five to seven regular spirals.[307,308] The frequency of gastric infection with *H. heilmannii* varies from 0.08 to 1% of adults undergoing routine endoscopy[307]and has been reported as 0.3% in one pediatric series.[309] There also have been a number of individual reports of *H. heilmannii* in children.[309–311] *H. heilmannii* gastritis is similar to *H. pylori* gastritis in that it mainly involves the antrum, but the mononuclear inflammatory response is described as mild, and there is no neutrophil activity. The bacteria do not adhere to the gastric epithelium but are usually seen within the mucus layer.[307] The importance of *H. heilmannii* as a gastric pathogen in children is not yet clear.

CONCLUSION

The discovery of *H. pylori* over 30 years ago has revolutionized our knowledge of peptic ulcer disease and gastric cancer. However, there are still many areas of great uncertainty in relation to the pathogenesis and epidemiology of this infection. *H. pylori* displays marked trophism for gastric tissue, yet we do not understand the mechanism of adherence or the role of the host in adherence of the organism to the gastric mucosa and the development of disease. There is now a substantial body of indirect evidence that suggests that infection is mainly acquired in preschool children. However, the mode of transmission of infection in children is unknown. This is a very important question, especially for children living in poor socioeconomic condi-

tions, if we want to develop strategies that will prevent infection. Further work on the optimal treatment regimens is required for infected children because resistance to clarithromycin is increasing and because some of the adult treatment regimens are not licensed for use in children.

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2. *Other Causes*

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The term *gastritis* is often used somewhat loosely. Not infrequently, clinicians will refer to a patient with epigastric pain or dyspepsia as having "gastritis," whereas radiologists may diagnose "gastritis" on the basis of nonspecific radiologic changes, such as mucosal irregularity or swelling.

However, gastritis is neither a clinical nor a radiologic diagnosis. Most often it is a purely histologic diagnosis, made by the use of random or targeted endoscopic biopsies.

Whereas some conditions that injure the gastric mucosa may result in inflammation, others do not. Thus, gastritis, as the suffix *-itis* implies, is characterized by the presence of inflammatory cells. In contrast, the term *gastropathy* is used to refer to conditions in which inflammation is not a prominent feature, although there may be epithelial damage and regeneration. In gastropathies, there are almost always visible abnormalities of the mucosa at endoscopy, with or without histologic changes; sometimes the mucosal appearance at endoscopy is typical or diagnostic of a particular condition. Therefore, in the case of gastritis, the diagnosis is always based on biopsy, whereas with few exceptions, gastropathy is usually an endoscopic diagnosis. Strictly speaking, the term *gastropathy* refers to any and all disorders of the stomach, but in this chapter, we use its more recent application.

The categorization of entities into gastritides and gastropathies is an important concept and helps narrow the diagnostic possibilities in a given case. However, for the sake of simplicity, in this chapter, the term *gastritis* is often used generically to refer to both gastritides and gastropathies.

Although gastritis and gastropathy are often clinically important in and of themselves, they are sometimes part of a continuum that includes ulcer disease.[1]

In this chapter, we propose a classification of pediatric gastritis and an approach that is pertinent to practicing pediatric endoscopists. Attention to key elements will facilitate consistency of communication between endoscopists and between endoscopists and pathologists, hopefully resulting in increased diagnostic accuracy in the area, that is, "high-yield gastroscopy." The key elements are endoscopic landmarks, terminology, and acquisition of biopsies and are discussed in detail below.

HIGH-YIELD GASTROSCOPY

Once a child is scheduled for diagnostic upper gastrointestinal (GI) endoscopy, the endoscopist has an obligation to maximize the diagnostic potential of the procedure. Central to this must come the recognition that the ability to make a diagnosis based on macroscopic appearances alone is limited.[1–7] For example, gastric mucosa that appears normal at endoscopy may harbor marked inflammation on microscopy. The converse is also true, that is, a markedly red mucosa at endoscopy may be normal or may result from contact with the endoscope; redness may reflect underlying microvascular congestion without the presence of inflammatory cells. In other words, there is often poor correlation between endoscopic and histologic findings. It follows, therefore, that targeted gastric biopsies must be an integral part of a proper examination of the gastric mucosa. In children, gastritis remains underrecognized and poorly characterized largely because of the flawed tendency to rely on macroscopic appearances at endoscopy and an evident reluctance by pediatric endoscopists to pay attention to the quantity and quality of tissue obtained at endoscopy.

A high yield of accurate diagnoses, including the definitive ruling out of mucosal disease, largely depends on the endoscopist's care and attention to detail in the following areas.

Clinical Status and Drug Therapy

Endoscopy with biopsy is no substitute for a thorough history and physical examination. Too often endoscopy is used as a means to "rule out acid peptic disease" when a history of lower abdominal pain is present and constipation is the cause or when upper GI symptoms are relatively mild, and a trial of a low-level therapy might be appropriate. Although a definitive diagnosis can often be made with endoscopy, many endoscopic and histologic findings are nonspecific and require interpretation in light of the patient's presenting complaint and overall clinical condition.

Close communication with a pathologist well versed in GI pathology is essential to do the patient and procedure justice. Drug therapy may cause disease in the stomach, change the nature and pattern of the disease, or mask the disease. Therefore, for interpretation of endoscopic and biopsy findings, the clinical context is key, and this includes what drugs the patient is taking or has recently taken. It is the responsibility of the endoscopist to communicate this information to the pathologist; this is best done in writing, on the pathology form that accompanies tissue to the laboratory. Drugs such as bismuth (PeptoBismol, DeNol), antibiotics, acid-suppressing drugs, and nonsteroidal anti-inflammatory drugs (NSAIDs) all may significantly change endoscopic and histologic findings. In patients with suspected acid-peptic disease, it is advisable to have the patient off acid-suppressing drugs for at least 2 weeks prior to endoscopy.

Endoscopic and Histologic Zones

In reporting endoscopic appearances, the endoscopist is describing the gross pathology of disease, and different disorders have predilections to involve different zones of the stomach. Familiarity with the gross and histologic zones is important (Figure 29.2-1) to avoid poor targeting of biopsies and therefore erroneous or missed diagnoses.

The largest region of the stomach is the gastric body or corpus, characterized at endoscopy by thick mucosal folds or rugae; the body or corpus extends distally to the incisura or angulus on the lesser curvature. The fundus is the dome-shaped area immediately above the gastric body and abutting the diaphragm; both the corpus and the fundus are composed of oxyntic mucosa. This consists of tightly packed glands, with little lamina propria between. The foveolae, or gastric pits, occupy the upper 20 to 25% of the mucosa, with the glands comprising the rest. The glands contain parietal cells that secrete acid and intrinsic factor and chief cells that secrete pepsinogen. The parietal cell zone occupies approximately half of the upper gland mass, with chief cells occupying the basal half. Most of the endocrine cells present are enterochromaffin-like cells that secrete histamine; some D cells that secrete somatostatin are present, as are some enterochromaffin cells that secrete serotonin. The endocrine cells are small and are present in the lower one-third or so of the glands, squashed between the basement membrane and the bases of the parietal and chief cells. The mucosal thickness is around 0.5 to 1.5 mm, thicker than other zones of the stomach. The greater curve at midbody is the thickest zone and thus the best zone to assess for atrophy.

The gastric antrum occupies the lower quarter or third of the stomach; when the stomach is distended with air to allow visualization, the antrum is seen to begin on the greater curvature where folds of the gastric body end. The antrum ends at the pylorus and consists of clear-staining mucus glands and endocrine cells. The antral mucosa is 200 to 1,000 μm thick. The foveolae or pits are deeper than those in the body, occupying about half of the mucosal thickness. The glands are clear staining, coiled, and mucus producing. The antral mucosa is also referred to as "pyloric-type" mucosa. The predominant endocrine cell is the G (gastrin producing) cell, with fewer numbers of D cells (somatostatin) and enterochromaffin cells (serotonin). These cells are primarily present at the junction of foveolae and glands.

The gastric cardia comprises a short zone of mucosa, immediately distal to and abutting the normally located Z line. Although the cardia is variously defined in different adult studies, we regard the cardia as the anatomic 0.5 to 1 cm below the Z line.[7] The anatomic cardia may be composed of purely clear-staining mucous glands (the same as those of the antrum) or clear-staining glands with occasional parietal cells; the latter is a transitional mucosa, betweeen purely fundic and purely cardiac-type mucosa. Much less commonly, pure fundic mucosa may directly abut the Z line. The cardia does not contain endocrine cells.

Although the different histologic zones of the stomach correspond to the different gross anatomic zones thereof, there is always some overlap and interdigitation of histologic zones at areas of transition—hence the term *transitional mucosa* for these. Examples of transitional zones are the antrum-body interface, especially the region of the incisura, and the cardia.

More detailed descriptions of normal gastric anatomy and histology are available elsewhere.[8,9] One of the difficulties in pediatric gastric histology is establishing what is "normal" because there are no pediatric data on "normal volunteers." Pediatric patients come to endoscopy because they have upper GI symptoms or a suspected systemic disorder in which upper endoscopy is performed to look for gastroduodenal pathology. Where "normal values" for cellularity of the lamina propria are described,[10] they are "retrospectively normal."

Terminology: Reporting of Endoscopic Findings

The endoscopy report is, in fact, a description of gross anatomic pathology or normality. Precision in description

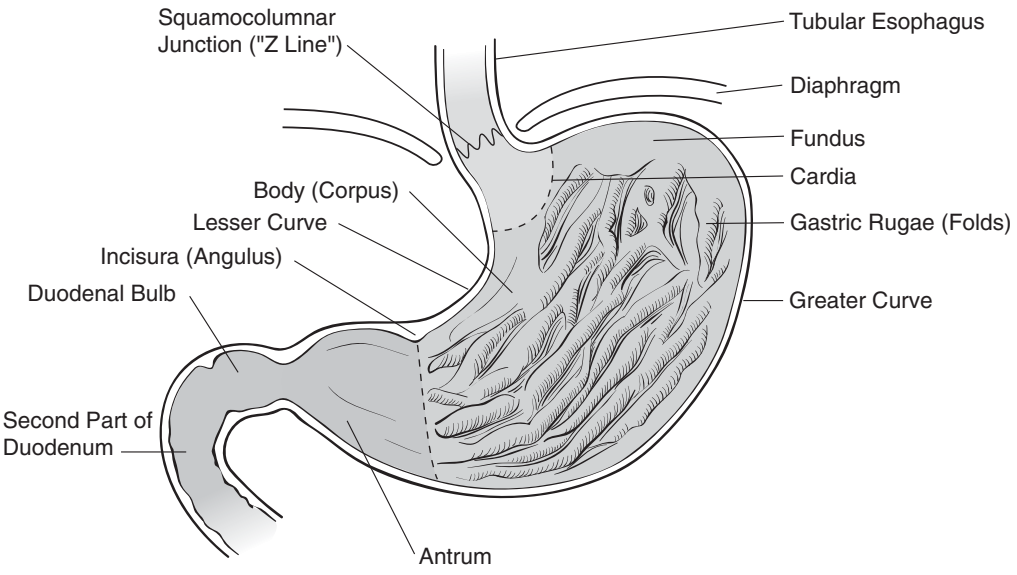

FIGURE 29.2-1 Anatomic regions of the stomach. Adapted with permission from Anatomy and histology: stomach. Rudolph CD, Rudolph AM, Hostetter M, editors. Rudolph's pediatrics. 21st ed. New York: McGraw Hill; 2003. p. 1306.

is therefore important; even when endoscopic photographs are available, words complement the images. The endoscopist should report only what he or she sees using terminology that is standard, factual, and unambiguous; the report should be descriptive rather than interpretive. Jargon, or *-itis* terms, should be avoided in the objective part of the endoscopy report. For example, use of the term *gastritis* could mean anything from erythema to distinct erosions; if the mucosa is red, it should be documented as "red" or "erythematous" (mild/moderate/intense or hemorrhagic) and not as antritis or gastritis because inflammation may not be present.[2,3] Another example is that of antral nodularity. This may indicate *Helicobacter pylori* gastritis, past or present; the nodules may persist for months or years after eradication of *H. pylori* and resolution of gastritis, and inflammation may not be present on biopsy. Therefore, the term *nodular gastritis* is to be avoided. After all, the endoscope is not a microscope; we cannot "see" inflammation at endoscopy.

An erosion is a mucosal break that does not penetrate the muscularis mucosae, whereas an ulcer extends through the muscularis into the submucosa. Endoscopists cannot accurately determine depth of lesions, but there are some clues; erosions are often multiple and usually have white bases, and each erosion is usually surrounded by a ring of erythema. When erosions have recently bled, their bases may be black. *Hemorrhage* refers to the bright, shiny red appearance of the mucosa in patches, streaks, or discrete petechiae, not associated with a visible mucosal break. Although the term *submucosal hemorrhage* is sometimes used, endoscopists cannot see through the muscularis mucosae; therefore, the term *subepithelial hemorrhage* is preferable to allow for varying depths of hemorrhage. Other confusing terms used for subepithelial hemorrhage and best avoided are *acute gastritis*, *hemorrhagic gastritis* (inflammation is usually absent from hemorrhagic lesions), or *hemorrhagic erosion* (usually no erosion present).

If gastric rugae are large, accurate terms of description are thick folds or swollen folds, not edematous folds or hypertrophic folds, because edema and hypertrophy are histologic not endoscopic findings; the swelling might be due to infiltrative disease, edema, or hypertrophy.[3,9,10] The folds remain thick in appearance despite adequate insufflation of air into the stomach at endoscopy. Causes of swollen folds are shown in Table 29.2-1.

It is also important to carefully and accurately describe nodules in the stomach because the different types and distributions have different implications. For example, a nodule or patch of nodules may be seen occasionally, especially at the antral-body junction; these may represent a prominent areae gastricae[11] and are usually unimpressive. In contrast, when a continuous diffuse carpet of nodules is present throughout the antrum, this has a high positive predictive value for *H. pylori* infection, past or present.[12,13] Absence of nodules, however, does not have a high negative predictive value for *H. pylori* infection or ulcer disease. When *H. pylori* gastritis is associated with duodenal ulcer in children, a striking diffuse nodularity of the antrum is always present; however, when *H. pylori* causes gastritis alone (primary gastritis), this nodularity is seen only in some 50 to 60% of cases.[12] We have not seen this nodularity in cases of true non–*H. pylori* duodenal ulcer disease[14] or in any of the some 8,000 upper GI endoscopies at our institution at which neither ulcer disease nor *H. pylori* was present over the last 18 years. Nodularity is sometimes not visible at first examination of the antrum, but once biopsies have been taken, oozing blood acts as a vital stain, making visible a confluent carpet of nodules. The term *hematochromoendoscopy* has been applied to use of blood as a vital stain in this circumstance.[1]

The nodules of chronic varioliform gastritis (CVG) are again different; they are larger in diameter and more raised than *H. pylori*–related nodules in the antrum and discrete, not in a continuous carpet. They often have an umbilicated central erosion or shallow ulcer, which predominates along the folds of the gastric fundus and body (Figure 29.2-2).[15–17] Large discrete nodules, more like blebs, may occur in the proximal stomach with cytomegalovirus (CMV) gastritis[3]; similar nodules in the antrum (and to a lesser degree in the body) may occur in eosinophilic gastritis and in Henoch-Schönlein disease.[18,19] In these three conditions, the mucosa is often hemorrhagic appearing, with erosions or ulcers, whereas in *H. pylori* disease, the

TABLE 29.2-1 CAUSES OF SWOLLEN OR THICK FOLDS

Ménétrier disease
Chronic varioliform gastritis
Helicobacter pylori
Chronic granulomatous disease
Eosinophilic gastritis
Adenocarcinoma
Mucosa-associated lymphoid tissue lymphoma
Plasmacytoma

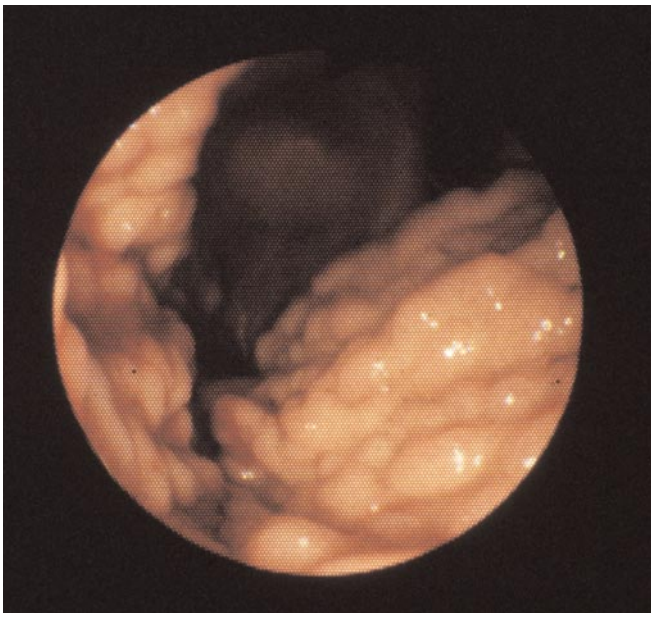

FIGURE 29.2-2 Endoscopic view of the proximal corpus to show the striking, large, "juicy" nodules typical of chronic varioliform gastritis. Typically, these are present in the corpus and fundus and not in the antrum.

mucosa is often just nodular, with or without erosions, but otherwise usually looks quiescent.

Some children with the rare systemic disorder cystinosis may have a unique *H. pylori*–negative diffuse fine "reptile-skin" nodularity throughout the stomach on gastroscopy (Figure 29.2-3).[20]

Nodules are different from polyps or pseudopolyps. Polyps may occur in a variety of conditions and are mentioned below. Pseudopolyps in the stomach may occur in inflammatory conditions such as allergic gastroenteroenteritis and Crohn disease. In these conditions, the lesions, although still sessile, are generally quite a bit larger than the nodules of the above conditions; they are blebs—areas of swollen mucosa. They are usually localized to the antrum and are discrete, not continuous.

Multizone Biopsy Sampling

Different disorders often have a predilection for one topographic zone or another of the stomach. However, the same agent may cause different patterns of injury in different populations (eg, *H. pylori*). Sometimes there may be disease in more than one zone of the stomach (eg, *H. pylori*, Crohn disease, eosinophilic gastritis, atrophic gastritis, CMV), as indicated below. In addition, distribution of disease may be influenced by treatment. Thus, the topology of endoscopic or histologic findings may give important clues to the etiology. Although the antrum is the major repository of histologic abnormalities for many pathologies that occur in children, biopsies should be taken from different topographic zones of the stomach.[1,7,21–37]

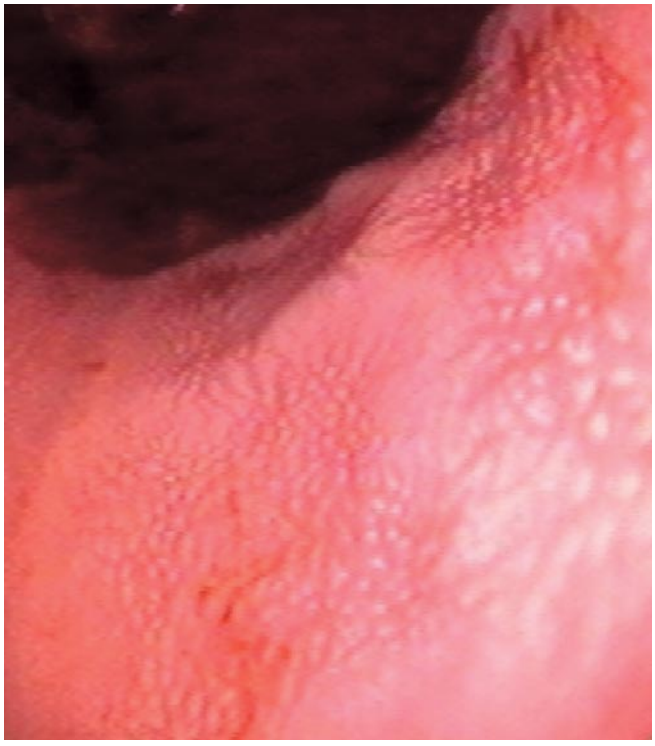

FIGURE 29.2-3 Cystinosis. Pangastric confluent finely nodular appearance in the stomach of a 3-year-old girl with cystinosis. She presented with severe abdominal pain, nausea, and vomiting and was unable to tolerate oral cysteamine.

The absence of histologic abnormalities is also helpful. For example, it is now well recognized that a significant percentage of duodenal ulcer disease in adults and children that is not due to NSAIDs, Crohn disease, or hypersecretory syndromes is truly non–*H. pylori* related.[14,38,39] In these cases, a major factor in children distinguishing the etiology of the ulcer disease from *H. pylori* is that there is an absence of gastritis.[14]

Even with careful handling of biopsies by trained personnel, endoscopic biopsies may sustain crush or other artifacts; thus, when biopsy is indicated, at least two biopsies should be taken from a particular lesion or zone of the stomach. Although the optimum number of biopsies has yet to be determined, when mucosa appears normal, our practice is to take at least two biopsies from the prepyloric or midantrum and two from the greater curve of the midbody. Others advocate taking two from the antrum-body transition zone of the lesser curve (a zone in which inflammation and metaplasia occur in adults).[9,21–27] In addition, biopsies should be taken from the gastric cardia when *H. pylori* infection, gastroesophageal reflux disease (GERD), or mucosa-associated lymphoid tissue (MALT) lymphoma[34] may be present. When the Z line is "prominent"or particularly asymmetric, that is, has one or more tongues that extend beyond most of the circumference into the tubular esophagus, this should be biopsied to determine whether this mucosa is stomach or Barrett specialized metaplasia. Whether an "extension" or "prominence" of the Z line is a variant of normal stomach with gastric cardiac or transitional mucosa or short-segment Barrett esophagus can be determined only by biopsy.[33,40–42]

Inflammation of the gastric cardia is an area of considerable interest because of the increasing incidence of cancer of the cardia and the esophagus and the potential for detecting preneoplastic changes (ie, intestinal metaplasia). Whereas some studies in adults have indicated that carditis and intestinal metaplasia are due to *H. pylori* infection as part of a pangastritis,[28,29,43,44] others indicate that the cause is GERD.[30,31] Yet others suggest that both may be etiologies[32,33]; the difference may lie in the definition of cardia and where the biopsies are taken. A study in children indicates that the most important cause of carditis is *H. pylori* infection (although GERD may also be a cause) and that the cardia may be the most sensitive area to detect *H. pylori*.[7]

When endoscopic findings are puzzling or a lesion is present, more biopsies should be taken randomly and from the lesion or its edge. The size of biopsies is also important. Biopsies taken with "pediatric" forceps are often of little value; they are tiny and difficult to mount, and the amount of useful interpretable tissue is very limited. In most children over 2 or 3 months of age, an endoscope with a 2.8 mm biopsy channel can usually be used, and biopsies with these forceps are often quite adequate if several specimens are taken. In contrast, each biopsy taken with "jumbo" or large-cup forceps offers at least two or three times the amount of mucosa for diagnosis, and these can often be obtained in older children. Issues regarding mucosal biopsy in children are dealt with in more detail elsewhere.[36]

On occasion, usually with rare disorders, even large endoscopic biopsies may be insufficient to make a diagnosis, and endoscopic mucosal resection or full-thickness surgical biopsies are required. This may be the case in infiltrative disorders, which can present with thick folds, a mass, or an ulceration, such as cancer, lymphoma, plasmacytoma,[45] or leiomyoma or leiomyosarcoma, or with certain gastric polyposes.[46] These disorders are not discussed in this chapter.

CLASSIFICATION

Previously, a scoring system was described to quantify the severity of gastritis in children,[12] but only recently has a classification of gastritis in children been proposed.[47] By placing specific pediatric conditions into a conceptual framework and describing the entities and their differential diagnosis, a system was created to facilitate understanding and diagnosis of gastric mucosal disorders.

No classification of gastritis can satisfy everyone because the published classifications have different objectives. Some are a glossary of appearances[21]; others list clinical disorders.[3] Another approach is that of the Sydney system, which essentially is a checklist of histologic findings to aid the pathologist to review biopsies and uniformly report findings by use of a visual analogue scale[22–25]; thus, it is aptly named a system rather than a classification. Although the general principles of this system can be applied to gastritis in children, its major focus is histologic grading of the severity of chronic gastritis, atrophy, and intestinal metaplasia. Although atrophy and intestinal metaplasia do occur in children occasionally, their occurrence is uncommon; therefore, the Sydney system is of limited usefulness in the pediatric age group. Although an early version of the Sydney system had an endoscopic component, this is no longer used, and it remains a purely histologic system. In addition, it does not integrate histopathology with endoscopic appearance and does not address noninflammatory conditions. Atrophy and metaplasia are addressed in Chapter 29.1, "*Helicobacter pylori* and Peptic Ulcer Disease."

We have classified mucosal disorders of the stomach in children primarily by their endoscopic appearances. In this system, gastritis is classified into two groups: erosive and/or hemorrhagic gastritis or gastropathy and nonerosive gastritis or gastropathy (Table 29.2-2). Although some disorders can present as either erosive or nonerosive, each is classified by its most common presentation. The disorders in each group are placed in approximate sequence of their prevalence in the practice of the authors. Each disorder is then described by etiology and any distinctive clinical, endoscopic, and histologic features. We believe that the advantages of this approach are simplicity and ease of use for the practicing pediatric endoscopist. Based on this approach, a diagnosis or differential diagnosis may be made with some certainty at the time of endoscopy, given the clinical context; however, for the majority of disorders, confirmation of the initial impression or definitive diagnosis is still dependent on biopsies and therefore on an active dialogue and close collaboration with a pathologist.

EROSIVE AND HEMORRHAGIC GASTRITIS OR GASTROPATHY

Most of these entities are diagnosed endoscopically, usually in patients presenting with GI bleeding. Because inflammation is not a feature of most hemorrhagic lesions, most conditions in this category are gastropathies. Biopsies are usually not required from erosive or hemorrhagic lesions. However, there are gastritides not in the erosive or hemorrhagic category that may present with erosions or hemorrhagic lesions; in these cases, biopsies are essential for the diagnosis (eg, *H. pylori*, Crohn disease, CMV, allergic gastritis).

"Stress" Gastropathy

This usually occurs within 24 hours of the onset of critical illness in which physiologic stress is present, such as shock, hypoxemia, acidosis, sepsis, burns, major surgery, multiorgan system failure, or head injury. These stressors cause reduction of gastric blood flow with subsequent mucosal ischemia[48] and breakdown of mucosal defenses.[49] Gastric acid is important in the pathogenesis of stress erosions, but actual hypersecretion is seen only in cases of

TABLE 29.2-2 CLASSIFICATION OF GASTRITIS AND GASTROPATHY IN CHILDREN*

Erosive and hemorrhagic gastritis or gastropathy
"Stress" gastropathy
Neonatal gastropathies
Traumatic gastropathy
Aspirin and other nonsteroidal anti-inflammatory drugs
Other drugs
Portal hypertensive gastropathy
Uremic gastropathy
Chronic varioliform gastritis
Bile gastropathy
Henoch-Schönlein gastropathy
Corrosive gastropathy
Exercise-induced gastropathy or gastritis
Radiation gastropathy
Nonerosive gastritis or gastropathy
"Nonspecific" gastritis
Helicobacter pylori gastritis
Crohn gastritis
Allergic gastritis
Proton pump inhibitor gastropathy
Celiac gastritis
Gastritis of chronic granulomatous disease
Cytomegalovirus gastritis
Eosinophilic gastritis
Collagenous gastritis
Graft-versus-host disease
Ménétrier disease
Pernicious anemia
Gastritis with autoimmune diseases
Plasmacytoma
Cancer
Gastric lymphoma (mucosa-associated lymphoid tissue lymphoma)
Other granulomatous gastritides
Cystinosis
Phlegmonous and emphysematous gastritis
Other infectious gastritides

Adapted from Dohil R et al.[47]

*Although some disorders can present as either erosive or nonerosive, each is classified by its most common presentation.

sepsis and central nervous system trauma. Risk factors for hemorrhage include gastric hypersecretion, mechanical ventilation, and use of corticosteroids.[49,50]

Stress erosions are typically asymptomatic and multiple and do not perforate, but when they do present, they do so with overt upper GI hemorrhage. Newborns and infants appear to be more prone to perforations.[51] Early lesions predominate in the fundus and proximal body, later spreading to the antrum to produce a diffuse erosive and hemorrhagic appearance. Antral involvement alone is uncommon.

Neonatal Gastropathies

Most neonatal gastropathies are due to physiologic stress, including prematurity, hypoxemia, prolonged ventilatory support, sepsis, and acid-base imbalance. Fatal hemorrhagic gastropathy has been reported in neonates treated with sulindac for patent ductus arteriosus[52] and dexamethasone for bronchopulmonary dysplasia.[53] A high prevalence of hemorrhagic gastropathy has been reported in sick neonates in the intensive care unit who had no upper GI symptoms or signs and underwent endoscopy under a research protocol.[54] Of note is that newborns without[54] and those with[55,56] upper GI symptoms or signs seem to have a high prevalence of hemorrhagic lesions described as esophagitis associated with gastropathy. These lesions are probably due to mechanical suctioning at the time of delivery or later. A retrospective study of 107 neonates who underwent upper GI endoscopy for irritability during feeds and hematemesis showed that 95% of those with hematemesis had endoscopically identifiable lesions.[56] However, in view of the known risk factors for upper GI bleeding and reflux in neonates and the usual good response to medical therapy, upper GI endoscopy is seldom likely to reveal specific lesions that alter the infant's supportive management or prognosis. In addition, endoscopy in sick, small infants is not without risk. More often than not, a conservative approach will better serve the patient.

Hemorrhagic gastropathy has also been reported in otherwise healthy full-term infants[57] presenting with severe upper GI hemorrhage and in one case as antenatal hemorrhage.[58] Endoscopy may be helpful in the rare instances of nonresponse to medical therapy, such as an actively or recurrently bleeding ulcer that may be amenable to endoscopic hemostasis,[59] to guide surgery, or for diagnosis and hemostasis in the extremely rare case of a gastric Dieulafoy lesion.[60]

An unusual gastropathy may occur in infants with congenital heart disease receiving prolonged infusions of prostaglandin E to maintain the patency of the ductus arteriosus. This appears as antral mucosal thickening or a focal mass consisting of foveolar cell hyperplasia, and it may present as gastric outlet obstruction.[61] This entity has also been described in a 6-week-old infant who received no medications.[62]

Traumatic Gastropathy

Forceful retching or vomiting produces typical subepithelial hemorrhages in the fundus and proximal body of the stomach. It is due to "knuckling" or trapping of the proximal stomach into the distal esophagus, resulting in vascular congestion, and is also known as prolapse gastropathy.[63,64] Mallory-Weiss tears immediately above or below the gastroesophageal junction also may occur. Although both prolapse gastropathy and tears tend to resolve quickly, they can result in significant blood loss. By a similar mechanism of trauma, linear erosions may occur in the herniated gastric mucosa of patients with large hiatal hernia, resulting in chronic blood loss anemia.[65] Suction through nasogastric tubes, especially in children who are receiving anticoagulants, can cause severe subepithelial hemorrhage and bleeding. Ingestion of foreign bodies, inflatable gastrostomy feeding devices, and endoscopic procedures such as diathermy[66–68] are also common causes of subepithelial hemorrhages, erosions, and ulcers.

Aspirin and Other NSAIDs

NSAIDs are the most commonly prescribed drugs in the world.[69,70] Although invaluable for treatment of many disorders, the usefulness of NSAIDs is limited largely by their adverse effects on the GI tract. They cause mucosal damage primarily in the stomach (gastropathy) but also in the duodenum, in a spectrum from only histologic changes to subepithelial hemorrhages, erosions, and ulcers. Gastroduodenal NSAID injury may be asymptomatic or result in life-threatening ulcer bleeding or perforation. Less frequent but well-recognized effects occur in the small and large bowel and esophagus.

NSAIDs exert their effects via inhibition of the cyclooxygenase (COX)-catalyzed conversion of arachidonic acid to prostaglandins.[71] Prostaglandins produced by the COX-1 pathway are largely constitutive, that is, responsible for mucosal integrity and hemostasis. Inhibition of COX-1 compromises mechanisms of mucosal protection, such as mucus and bicarbonate production, epithelial integrity and regenerative capacity, and microvascular supply. In contrast, prostaglandins produced by the COX-2 (inducible) pathway mediate pain, inflammation, and fever. There is overlap, however, and dual suppression of COX-1 and COX-2 is necessary for gastrointestinal mucosal damage to occur. Aspirin and other NSAIDS such as ibuprofen, naproxen, sulindac, diclofenac, indomethacin, mefenamic acid, and meloxicam are nonselective COX inhibitors, that is, they inhibit both pathways. Even a single low dose of an NSAID may cause petechial hemorrhages and ulceration within hours, and although early lesions may, on occasion, cause active bleeding, they are often asymptomatic and per se are not predictive of clinically significant ulcer formation.[72,73] Aspirin has an additional effect, that being the inhibition of thromboxane production by platelets.[74,75] Because platelets are anuclear, the effect is permanent for the life of the platelets. Although this property is of benefit in primary or secondary cardiovascular prophylaxis, it does enhance bleeding from the GI tract.

Although some adverse effects may result from topical action of ingested NSAID on the gastroduodenal mucosa, the systemic presence alone of an NSAID compromises mucosal integrity and may produce severe ulceration of the

mucosa. Although many patients report that enteric-coated (buffered) aspirin is associated with fewer symptoms, enteric coating of aspirin does not prevent complications.[71] Factors that place patients at higher risk for severe gastroduodenal ulceration and complications include a history of an ulcer complication, a history of an uncomplicated ulcer, drug dose, comcomitant use of aspirin and another NSAID, use of a corticosteroid, age over 65, use of an anticoagulant, and, possibly, *H. pylori* infection.[69,70,75,76] Although gastric acid does not appear to be a primary causative factor in NSAID mucosal injury, acid suppression with proton pump inhibitors (PPIs) does reduce the risk of gastroduodenal ulceration and bleeding. This suggests that some acid appears to be required for lesions to develop, but not much because NSAID lesions do occur in achlorhydric subjects.[71]

The newer (selective) COX-2 inhibitors or "coxibs," such as celecoxib, rofecoxib, and valdecoxib, produce fewer GI adverse effects in adults, but these drugs are expensive and not free of such effects. The rates of upper GI symptoms (dyspepsia) are only slightly lower on these drugs than on traditional nonselective NSAIDs. Combination of a coxib with aspirin—even at a low dose—substantially detracts from their otherwise improved GI safety profile.[77]

Although there are few data on the adverse GI effects of NSAIDs in children, these do occur.[78–84] As in adults, erosions and ulcers caused by NSAIDs may be single or multiple (Figure 29.2-4), and although the gastric antrum tends to be involved more than the body, they may involve any or all regions of the stomach. In young children, ulceration of the incisura presenting with upper GI bleeding is a typical NSAID lesion, and bleeding may occur after just one or two doses of drug or with more chronic use. The characteristic histologic NSAID lesion in adults and children is a reactive gastropathy, that is, epithelial hyperplasia, mucin depletion, enlarged (reactive) nuclei, fibromuscular (smooth muscle) hyperplasia, vascular ectasia, and edema (Table 29.2-3).[85] Less often, NSAIDs may cause a reactive gastritis. Reactive gastropathy or gastritis may be present at the edge of an erosion or ulcer or in endoscopically normal mucosa distant from such lesions, but it may also be absent even in the presence of severe NSAID lesions. Reactive gastropathy is not specific to NSAID injury; rather, it is a nonspecific feature of chemical injury of gastric mucosa (Table 29.2-4).[3,85]

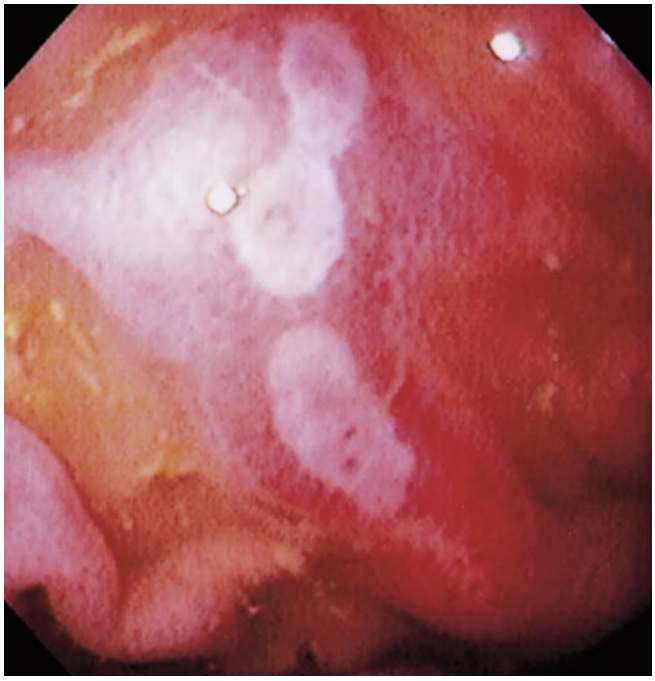

FIGURE 29.2-4 Gastric body erosions owing to a nonsteroidal anti-inflammatory drug in a 14-year-old girl presenting with hematemesis, epigastric pain, and anemia (8 g/dL). She took ibuprofen 400 mg three times daily for 2 days for severe menstrual discomfort. The endoscopic differential diagnosis includes Crohn disease and cytomegalovirus.

In children presenting with abdominal pain, blood loss, anemia, or upper GI lesions at endoscopy, it is particularly important to actively solicit a history of use of over-the-counter NSAIDs because parents and children often fail to mention nonprescription drugs in the medication history. For this reason, adverse effects of NSAIDs in children are likely underrecognized. Use of NSAIDs has increased among children (eg, for management of fever in infants). In a recent prospective randomized short-term clinical trial in children under 2 years of age treated for fever,[82] there was no significant difference in acute GI bleeding between ibuprofen (n = 17,938) and acetaminophen (n = 9,127). Only 7 of the children receiving ibuprofen (5 mg and 10 mg/kg/dose, 6 to 10 doses over 3 days) had symptoms of vomiting or hematemesis; bleeding occurred in 3 and was minor in all, not requiring transfusion, and related to forceful vomiting in some. Based on this study, short-term use in this age group appears to be relatively benign, but more data are required.

However, the main area of concern is long-term use of NSAIDs. Upper GI bleeding following NSAID ingestion in children has been well documented.[78–81] Naproxen is the most commonly used NSAID in pediatric rheumatologic practice.[84] In one study, 75% of children with juvenile rheumatoid arthritis who had taken one or more NSAIDs for over 2 months had endoscopic evidence of gastropathy, antral erosions, or ulcers[80]; of these, 64% had anemia and abdominal pain. Another study indicated that children taking NSAIDs were 4.8 times as likely to develop gastroduodenal injury as those not taking NSAIDs.[81] In that study, abdominal pain was present in 28% of patients taking NSAIDs versus 15% in those not taking them.

Because NSAIDs are protein bound, and hypoalbuminemia may occur in systemic juvenile rheumatoid

TABLE 29.2-3 MUCOSAL CHANGES OF REACTIVE GASTROPATHY OR GASTRITIS

Mucin depletion of surface and foveolar epithelium
Enlarged (reactive) nuclei
Foveolar hyperplasia
Fibromuscular (smooth muscle) hyperplasia
Vascular ectasia (vasodilatation)
Edema
Paucity of inflammatory cells (gastropathy)
Plasma cells
Neutrophils: may be found especially if erosion or ulcer is present

Adapted from DeNardi FG and Riddell RH.[85]

TABLE 29.2-4 CAUSES OF REACTIVE GASTROPATHY OR GASTRITIS

Duodenogastric reflux or bile reflux
Aspirin and other nonsteroidal anti-inflammatory drugs
Alcohol
Vascular disturbances (eg, shock, ischemia, stress)
Local trauma (eg, nasogastric tube)
Radiation and chemotherapy
Idiopathic

Adapted from DeNardi FG and Riddell RH.[85]

arthritis, with higher levels of free drug there is potential for greater NSAID toxicity.[84] This specific aspect has not been studied in children.

Strategies for minimization of risk in NSAID use include use of selective COX-2 inhibitors and/or concurrent use of PPIs or misoprostol.[70,71,75,76] Although both PPIs and misoprostol are very effective in prevention of morbidity, the adverse effects (abdominal cramps, diarrhea) of misoprostol limit its use, and PPI use is likely to be much better accepted in children, as it is in adults. At present, however, there are no data on this indication in children.

Risk reduction also involves consideration of *H. pylori* status. Although gastropathy induced by NSAIDs does not require the presence of *H. pylori* for its development,[86] there are conflicting and confusing data regarding the role and timing of *H. pylori* eradication in healing NSAID ulcers in adults. For example, the studies on omeprazole healing of NSAID ulcers showed higher healing rates with PPIs in *H. pylori*–positive than in *H. pylori*–negative patients.[87,88] Similar results were obtained from an analysis combining both of the US Food and Drug Administration pivotal trials for lansoprazole and NSAID ulcers.[89] This has led some to argue that *H. pylori* infection should not be treated until after the PPI has healed the ulcer; however, there is no consensus on this.

In *H. pylori*–infected adults with no ulcer disease, evidence for the effectiveness of *H. pylori* eradication as ulcer prophylaxis in chronic NSAID users is also contradictory.[90–92] Nevertheless, the weight of evidence suggests that when there is a history of *H. pylori* ulcer disease, eradication of *H. pylori* is indicated before instituting NSAID use.[75,90,92] Until data indicate otherwise, in general, it seems prudent to eradicate *H. pylori* in children requiring high-dose or long-term NSAIDs.

Use of NSAIDs in children will likely continue to increase, following the trends in adults, with the availability of the newer coxibs and for use in special groups such as those with premalignant intestinal polyposis syndromes and perhaps premalignant conditions such as chronic inflammatory bowel disease and Barrett esophagus. Data on NSAID use in children are very much needed.

Other Drugs

Although many drugs may cause nonulcer dyspepsia, erosive or hemorrhagic gastropathies have been described with valproic acid, dexamethasone, chemotherapeutic agents, alcohol, potassium chloride, and cysteamine.[53,93–101]

Portal Hypertensive Gastropathy

This congestive gastropathy occurs frequently in children with intra- or extrahepatic causes of portal hypertension.[102] The endoscopic findings vary from a mild gastropathy with a mosaic pattern of 2 to 5 mm erythematous patches separated by a fine white lattice to a severe gastropathy typified by the presence of cherry red spots or even a confluent hemorrhagic appearance.[102–104] The mosaic pattern is specific for portal hypertensive gastropathy (PHG) and was not found in any of 500 children without liver disease at endoscopy.[102] In adults, congestive gastropathy is more frequently associated with large gastroesophageal varices than with esophageal varices alone,[104,105] and sclerotherapy of esophageal varices may exacerbate PHG and gastric varices. In contrast, PHG, which has been reported to occur only after variceal obliteration therapy and not before, is more likely to be transient and less severe.[105,106] The histologic findings in PHG are ectasia of mucosal capillaries and venules and submucosal venous dilatation.[104] However, PHG is an endoscopic diagnosis; biopsy is not indicated and is potentially dangerous.

Uremic Gastropathy

In acute renal failure, gastropathy may be due to physiologic stress rather than to renal failure itself. When gastrointestinal bleeding occurs in acute renal failure, it is associated with erosions and/or ulcers in 71% of cases and with an increased risk of death and duration of hospital stay; additional factors that predispose the patient to bleeding are use of corticosteroids and other disease such as liver cirrhosis.[107]

Chronic renal failure (CRF) is associated with increased densities of parietal, chief, and gastrin-producing cells.[108] Despite this, gastric pH may be less acid than expected; this may reflect neutralization of gastric acid with ammonia, a breakdown product of urea that is very high in gastric juice in patients with CRF.[108,109] This may explain why patients are more likely to suffer acid-peptic complications after treatment for CRF, that is, lowering of the urea or gastric nitrogen levels may remove their neutralizing effect on gastric acid. Hypergastrinemia associated with CRF is likely to be secondary to the gastric acid neutralization as well as reduced gastrin clearance.[108–110]

There are few data on the effects of CRF on the stomach in children. In adults, whereas active peptic ulcer disease does not seem to be more common in CRF, hemorrhagic gastropathy is quite prevalent in patients receiving chronic hemodialysis.[109] In such patients, gastroduodenal lesions occur in up to 67%, the predominant lesion being antral gastropathy in some 50%.[110] When peptic ulcers do occur in CRF, their presentation is somewhat atypical; they are more often multiple, *H. pylori* negative, and less likely to present with pain; rather, they tend to be symptom free or present with bleeding.[110–112] Although angiodysplastic lesions in the stomach may account for some 13% of cases of upper GI bleeding in CRF, it is unclear whether they are more common in this population or simply more likely to bleed because of uremic platelet dysfunction or hemodialysis.[113,114] Endoscopic "gastritis"

was reported in 10 of 17 children with CRF, with only 4 having findings localized to the gastric antrum,[115] but the gastritis was not defined endoscopically or histologically.

Chronic Varioliform Gastritis

Also known as chronic erosive gastritis, CVG is an uncommon disorder of unknown etiology, described more commonly in Europe than in North America. Although CVG largely occurs in middle-aged and elderly men,[15,116–118] it has been reported in a few children presenting with variable combinations of upper GI symptoms, anemia, protein-losing enteropathy, peripheral eosinophilia, and elevated serum immunoglobulin (Ig)E levels.[16,17,119,120] Symptoms arise insidiously and often become subacute or chronic. Most striking endoscopically are the innumerable prominent nodules in the fundus and proximal body of the stomach (see Figure 29.2-2); in children, the antrum is less often involved. Typically, the gastric rugae are irregularly thickened, with nodules located on the crests of the folds. The nodules sometimes have an umbilicated central crater or erosion; the lesions are said to resemble the skin lesions of chickenpox—hence the name. Histologic features include edema, foveolar hyperplasia, active chronic inflammation, and eosinophilic infiltrates. Focal superficial subepithelial collagen deposition may represent fibrosis at points of previous surface erosions. We have observed variable degrees of collagen deposition with active inflammation and gland atrophy in three adolescents (see "Collagenous Gastritis" below). In adults, CVG is one cause of a "lymphocytic gastritis" in which the surface and foveolar epithelium is infiltrated with dark-staining T cells, as in celiac disease.[37,117,118] For other causes of lymphocytic gastritis, see Table 29.2-5. If the diminishing number of reports of CVG in the adult literature reflects prevalence, this disorder appears to be getting less common.

Bile Gastropathy

This is also known as alkaline gastropathy or duodenogastroesophageal reflux (DGER). Although it is well documented in the postoperative stomach,[121] reports of DGER in the intact stomach are confined mainly to the adult literature.[122,123] The mere finding of bile in the stomach at endoscopy is common and unlikely to be of any significance. Typical endoscopic features of DGER include "beefy" redness or erythema and, occasionally, erosions. Despite this, there is very little or no increase of cellular infiltrate in the lamina propria, the main histologic features being epithelial (ie, foveolar hyperplasia), occasionally with a corkscrew appearance, lamina propria edema, and venous congestion. These changes constitute the entity of a so-called reactive gastropathy.[9,85] Postoperatively, they are found more commonly in the stomach than at the stoma. Other features include stomal erosions, lipid islands, and mucosal cysts; the latter are sometimes grossly visible and are known as gastritis cystica profunda or polyposa. Some studies report a high prevalence of intestinal metaplasia, although this may reflect sampling from the stomal region, which normally reflects a mosaic of gastric and intestinal mucosa.

Fortunately, nowadays, there are hardly any indications for partial gastrectomy in children, and pyloroplasty in children[124] is seldom recognized to be attended by the above problems.

TABLE 29.2-5 CAUSES OF LYMPHOCYTIC GASTRITIS

Celiac disease
Ménétrier disease in adults
Cytomegalovirus
Chronic varioliform gastritis
Helicobacter pylori
Idiopathic

Henoch-Schönlein Gastritis

Henoch-Schönlein purpura (HSP), or Henoch-Schönlein disease, is a frequently recognized multisystem disorder attributable to an immune complex–mediated vasculitis. The systems involved are usually skin, the GI tract, kidneys, and joints. It manifests as a clinical syndrome of nonthrombocytopenic skin lesions, arthralgias and arthritis, renal disease, and colicky abdominal pain. GI symptoms and signs include abdominal pain, nausea and vomiting, and GI tract bleeding. Less common serious abdominal complications include intramural hematomas, intussusception, bowel infarction, bowel perforation, pancreatitis, appendicitis, and cholecystitis. In HSP, endoscopic findings in the stomach include erythematous or hemorrhagic swollen mucosa with erosions or ulcers. Raised blebs may be seen with punctate hemorrhages and often central erosions or ulcer with a yellow base.[18,19] The findings may be patchy, antral predominant, or diffuse.[125] Similar lesions are often present in the duodenum and jejunum. Although gastric mucosal biopsies are usually too superficial to show typical histologic changes, they may show a leukoclastic vasculitis similar to that seen in the skin.[18] Patients may have an elevated serum IgA and reduced factor XIII levels.[18,19] Endoscopy is seldom required for the diagnosis of this condition; however, it may be helpful in those children with persistent abdominal pain or vomiting who have not yet demonstrated the typical nonthrombocytopenic rash of HSP. A few may never develop the rash.[126–128] All children with hematemesis, even those with the HSP rash, should undergo endoscopy to diagnose complications or other causes of upper GI bleeding, such as duodenal ulcer disease.[125]

Corrosive Gastropathy

The most common ingestants affecting the stomach are acids, iron, and strong alkalis; the latter predominantly involve the esophagus but occasionally involve the stomach. When gastric injury does occur, the prepyloric area is particularly vulnerable,[129,130] probably because of pylorospasm and pooling of secretions. The presence of food may limit the degree of injury. Endoscopic findings range from mild friability and erythema to necrosis, ulcers, exudates, and hemorrhage, with rare perforation. Chronic cicatrization is relatively rare and may take several months to become apparent—hence the need for serial imaging studies. Transabdominal ultrasonography may prove useful in localizing injury, determining its

depth and the presence of peristalsis, and thereby reducing repeated radiation exposure.[131] Iron poisoning, especially with ferrous sulfate, is common in children in some areas of the world and may cause a corrosive gastropathy with stricture.[132] Therapeutic administration of oral ferrous sulfate may cause mild endoscopic abnormalities in the stomach, which are of uncertain clinical significance.[133] Pine oil cleaner ingestion may also cause gastric injury.[134]

Exercise-Induced Gastropathy or Gastritis

This is well recognized in long-distance runners. The condition usually presents with blood loss anemia with or without upper GI symptoms. Symptoms often occur post-exercise and include abdominal cramps or epigastric pain, nausea, and vomiting.[135] Both erosive gastropathy and nonerosive gastritis have been described, with mucosal lesions occurring almost equally in the gastric antrum, body, and fundus.[136,137] Gastritis is usually acute, with hemorrhagic inflammation on biopsy. Postulated mechanisms include splanchnic ischemia, with reports of up to 80% reduction in visceral blood flow compared with pre-exercise levels reported.[138] Strenuous exercise does not appreciably affect postprandial gastric secretion or gastric emptying.[139]

Cystinosis

This is an inherited lysosomal storage disorder characterized by the deposition of massive amounts of cystine within macrophages. To reduce the rate of development of renal deterioration with the need for transplant and also improve life expectancy, patients are required to take the drug cysteamine every day.[140] This drug is extremely ulcerogenic and has been used to induce duodenal ulcers in laboratory animals.[100] Cysteamine is a potent secretagogue, causing hypergastrinemia and gastric acid hypersecretion that occur for 1 to 2 hours after drug ingestion.[20,140] The additional ulcerogenic effects of cystinosis are due to delayed gastric emptying and inhibition of gastric bicarbonate and mucus production. At endoscopy, 2 of 11 children had a distinctive, sometimes erosive, diffuse fine nodular appearance throughout the stomach.[20] In contrast to the gastric nodularity seen in *H. pylori* gastritis, the nodules of cystinosis are much finer, and their distribution is pangastric. In some cases, crystalline structures are seen within lysosomes of macrophages of the lamina propria of gastric, duodenal, and esophageal biopsies.

Radiation Gastropathy

This condition, associated with abdominal irradiation given to patients with malignancy, causes erosions or ulcers particularly in the gastric antrum and prepyloric regions,[141] as well as severe diffuse hemorrhagic gastritis or gastropathy. Fibrosis and stricture formation may occur and lead to gastric outlet obstruction. A high total radiation dose and, perhaps more importantly, a high daily fraction appear to be the main risk factors, but onset of gastritis may not always be dose dependent.[142,143] Treatment may be difficult and sometimes includes surgical resection.[144,145] Radiation is often given together with chemotherapy, but the combined effects are similar to those of radiation alone.

NONEROSIVE GASTRITIS OR GASTROPATHY

In nonerosive gastritis, there is usually a poor correlation between endoscopic appearance and histologic findings, that is, the diagnosis is usually purely histologic. An exception is the nodular antrum of *H. pylori*–associated ulcer disease in children; however, nodularity persists even after eradication of *H. pylori*, so, in this case, the diagnosis is endoscopic and histologic. Furthermore, nodularity is not always present, so the diagnosis still ultimately depends on histology. Some of the entities in this section may also present as an erosive gastropathy or gastritis but are included here because they more commonly present without erosions.

Lymphocytic gastritis is a type of gastritis deserving of special mention; this may be seen in disorders as apparently diverse as celiac disease, CMV gastritis, Ménétrier disease, *H. pylori* infection, and CVG. Because ours is an endoscopic classification, lymphocytic gastritis is mentioned under each of those disease entities.

Nonspecific Gastritis

In our experience, a significant number of children have chronic gastritis for which no cause can be identified.[8] In these cases, the inflammation is chronic, lymphoplasma cellular, more focal than diffuse within the biopsy, and usually superficial. Although it appears to be more prevalent in the antrum than in the corpus, this may reflect sampling bias.

H. pylori Gastritis

This is addressed in Chapter 29.1.

Inflammatory Bowel Disease

Gastroduodenal involvement is relatively common, and in children, Crohn disease is the most common cause of granulomatous gastritis.[10] Such symptoms as may occur are similar to those of acid-peptic disease and of delayed gastric emptying, with hematemesis and melena occurring less frequently.[146–149] Macroscopic and/or histologic abnormalities are present in the esophagus, stomach, or duodenum in up to 80% of children with Crohn disease.[10,150] However, some of these changes are nonspecific. The figure becomes 30% if features specific to Crohn disease, such as giant cells and noncaseating granulomas, are considered[146]; if focal deep gastritis is included, the figure becomes about 50 to 100%.[10,151,152] As would be expected, the figures quoted for these studies will largely depend on the number of biopsy specimens taken and whether serial sections of those specimens are carefully examined. In one study of *H. pylori–negative* adults,[142] the focal gastritis in 80% of patients with Crohn disease was characterized by perifoveolar or periglandular accumulation of CD3+ lymphocytes and CD68+ and CD68R+ histiocytes, together with granulocytes. This characteristic gastritis was found in the antrum in 48% (36 of 75 patients) and in the body in 24% of cases.[142]

In the appropriate clinical context, the identification of noncaseating granuloma is diagnostic of Crohn disease, but differentiation from other granulomatous gastritides (Table 29.2-6) is important.[3,10] Endoscopic and/or histo-

logic evidence of Crohn disease of the stomach may occur in the absence of upper GI symptoms and sometimes precedes diagnostic features in the colon.

In our own experience, 67 of 229 (29%) patients with Crohn disease who underwent upper GI endoscopy had histologic evidence of gastritis[10,150]; only one-third of these had endoscopic features of loss of vascular pattern, mucosal swelling, aphthous ulcers, or luminal narrowing (Figure 29.2-5). Deep ulceration in the duodenum can also occur, and this can mimic primary peptic ulcer disease. Histologic features range from focal chronic active inflammation to more typical non-necrotizing granulomas. For both the endoscopic and histologic findings, the antrum is the most common repository of disease, but granulomas are also present in the corpus and the cardia. In our experience, gastric Crohn disease is second overall to *H. pylori* as an identifiable cause of gastritis in children.[150]

Not infrequently, histologic findings of chronic antral gastritis will result in a change of diagnosis from ulcerative colitis to Crohn disease, even in the absence of granuloma. However, mild chronic gastric inflammation is also seen in ulcerative colitis. Whether this represents a greater prevalence than in normal children is still unclear. In two reports in which 5 and 14 children, respectively, were said to have ulcerative colitis, gastric inflammation was typically chronic active and mild.[153,154] In the larger of the two studies, although none of the patients had moderate to severe gastritis, 69% of the patients did have mild antral gastritis. This, however, did not reach statistical significance when compared with "control" patients who were endoscoped for possible reflux esophagitis.[154] In another retrospective study, antral focal gastritis was reported in 60% (28 of 43) of children with Crohn disease, in 20.8% (5 of 24) with ulcerative colitis, and in 2.3% (3 of 129) of patients without inflammatory bowel disease. In this study, focal antral gastritis was not considered reliable in differentiating between the two conditions.[155]

Allergic Gastritis

This is the gastric component of allergic gastroenteritis. This condition usually presents in infancy but may be seen even in preterm infants.[156,157] Although allergic gastritis and eosinophilic gastritis (discussed below) have some features in common, in allergic gastroenteritis, the disease is always mucosal and not deeper, the endoscopic changes are milder, and it is a more benign disease, of limited duration. For the gastroenterologist, this condition is usually diagnosed through clinical suspicion, histologic findings, and the response to elimination diets. A temporal relationship between characteristic symptoms and the ingestion of certain foods is particularly helpful in establishing the diagnosis. Allergic gastritis is usually associated with a specific allergen; in children, cow's or soy milk protein, egg, and wheat are the most frequently identified antigens, usually causing symptoms such as irritability, vomiting, and growth failure within the first 6 to 12 months of life.[158] Vomiting may be due to allergen-induced gastric dysrhythmia and delayed gastric emptying in sensitized infants.[159] Unlike eosinophilic gastroenteropathy, in which some patients remain symptomatic into later childhood and even adulthood, in allergic gastritis, reintroduction of the antigen is almost always possible by 24 months of age and often earlier. The histologic features include an eosinophilic infiltrate in the lamina propria and the surface and foveolar epithelium, and, occasionally, lymphocytes, plasma cells, and neutrophils are present. Endoscopy may show normal mucosa or changes similar to those of eosinophilic gastritis but usually not as marked. However, erosions have been described in children.[160] Peripheral eosinophilia, elevated serum IgE levels, and positive radioallergosorbent testing for specific allergens may be detected.

TABLE 29.2-6 CAUSES OF GASTRIC GRANULOMAS

NONINFECTIOUS CAUSES
Crohn disease
Chronic granulomatous disease
Vasculitis associated
Sarcoidosis
INFECTIOUS CAUSES
Tuberculosis
Syphilis
Histoplasmosis
Parasites
Isolated granulomatous gastritis
Foreign body granulomas
Idiopathic

Adapted from Dohil R et al.[47]

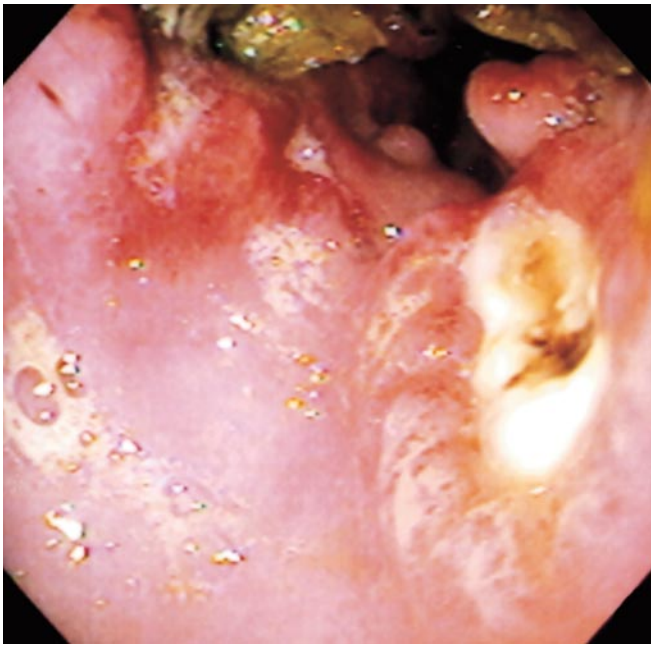

FIGURE 29.2-5 Crohn disease of the gastric antrum. Gastric erosions, ulcers, and pseudopolyps in a 16-year-old boy presenting with epigastric pain and early satiety; no other symptoms were present. Granulomas were present on biopsy.

PPI Gastropathy and Gastric Polyps

Long-term or high-dose PPI therapy often causes a characteristic hyperplasia of parietal cells, with a thickened parietal cell zone, and lingular pseudohypertrophy of individual parietal cells. Cystic changes often occur in the glands. In

some cases, benign fundic gland polyps may be present. The parietal cell changes regress to normal some weeks after cessation of acid suppression therapy.[161–165] In a study of patients on long-term (mean 7.9 years) PPI therapy, enterochromaffin-like cell hyperplasia was reported in over 50% of patients and was thought to be a trophic effect of the associated hypergastrinemia; no evidence of dysplasia was reported even after 10 years of therapy.[166]

Gastric polyps unrelated to PPI therapy are also uncommon in pediatrics.[46]

Celiac Gastritis

A lymphocytic gastritis has been described relatively recently in celiac disease.[167–171] In celiac disease and in *H. pylori*, it occurs in the presence of a usually normal gastroscopy. The gastritis in celiac disease is typified by the intraepithelial location of the lymphocytic infiltrate.

In one study, this gastritis was present in 10 of 22 (45%) adults with celiac sprue.[169] It was characterized by a striking mononuclear infiltrate (primarily T cells) mainly in the surface and pit epithelium of the antrum and body, with sparing of the deeper glandular epithelium; the lamina propria was expanded by an infiltrate of plasma cells, lymphocytes, and rare neutrophils. Children and adults with this pattern of gastritis have a mean of some 40 to 46 lymphocytes per 100 epithelial cells, compared with means of 3 to 5 in normal controls or those with the lymphocytic form of *H. pylori* gastritis.[169,170] In the latter, the infiltrate is predominantly in the lamina propria.[168] A milder lymphocytic gastritis was seen in another pediatric study.[171] The pattern of gastric lymphocytic inflammation in celiac disease resembles that seen in the small bowel and in the colon in that disease; this gastritis is associated with increased gastric permeability[172] and resolves in some patients following treatment of celiac disease.

In one pediatric study, 15 of 25 children with celiac disease had chronic gastritis; 9 of these had lymphocytic gastritis, and 6 had mild nonspecific inflammation.[170] A more recent study in children reported intraepithelial lymphocytic gastritis (more marked in antrum than body) in 29 of 33 children with untreated celiac disease; 15 of these 29 also had evidence of focal or diffuse chronic gastritis within the lamina propria.[171] Mucin depletion was often seen when increased intraepithelial lymphocytes were associated with chronic gastritis. None of the patients had endoscopic evidence of varioliform gastritis, mucosal swelling, or ulceration. The number of intraepithelial lymphocytes returned to normal on a gluten-free diet.[171] The variation in severity and prevalence of lymphocytic gastritis between studies may reflect varying amounts of dietary gluten intake as well as the lack of uniformity in the targeting of biopsies.[173] In one study, dyspeptic symptoms, such as epigastric pain and vomiting, were significantly more frequent in those celiac children with lymphocytic gastritis than without[170]; however, no such correlation was found in another study.[171] We have seen two childhood cases of celiac disease with multiple duodenal erosions, and a case of severe bleeding from multiple gastric ulcers has been described in an adult with celiac disease and lymphocytic gastritis.[174] It has been suggested that patients with *H. pylori*–negative antral-predominant lymphocytic gastritis should be evaluated for celiac disease.[173]

Chronic Granulomatous Disease

Chronic granulomatous disease is a rare X-linked recessive immunodeficiency disorder of boys in which granulomatous gastric wall involvement is common. When present, symptoms of delayed gastric emptying occur, with a narrowed, poorly mobile antrum on contrast radiography.[175,176] There are no specific endoscopic findings, but often the antral mucosa is pale, lustreless, and swollen. Histologic findings include focal, chronic, active inflammation in the antrum with granulomata or multinuclear giant cells. In our own experience of six cases, the diagnostic lipochrome-pigmented histiocytes were absent in gastric biopsies but were found in the lower gastrointestinal tract.[10]

Cytomegalovirus Gastritis

On those rare occasions when CMV infection occurs in immunocompetent children, it manifests as Ménétrier disease (see below); it is so uncommon in apparently immunocompetent adults[177] that its finding suggests an occult malignancy or early immunodeficiency.[178] Conversely, CMV infection is so common in immunosuppressed patients (such as those with acquired immune deficiency syndrome [AIDS] or following solid organ or bone marrow transplant[179]) that, in some cases, it is difficult to know whether it is a pathogen or a commensal. In such patients, this compounds the diagnostic difficulty in distinguishing between gross or histologic lesions caused by infection, graft-versus-host disease (GVHD), physiologic stress, or chemotherapy. However, if the highly distinctive pattern of injury is present, it is more likely that CMV is the cause. The infection tends to occur in the gastric fundus and body and may cause wall thickening, ulceration, hemorrhage, and perforation.[180,181] Histologic findings include active acute and chronic inflammation with edema, necrosis, and cytomegalic inclusion bodies in epithelial and endothelial cells, as well as in ulcer bases and mucosa adjacent to ulcers.[182] In contrast to herpes virus infection, which tends to be superficial, CMV usually affects deeper portions of the mucosa, and the active inflammation may be focal or panmucosal. The diagnostic yield is increased by viral culture of mucosal biopsies and by immunohistochemical detection of CMV early antigen. Treatment with ganciclovir may be beneficial in immunosuppressed patients, but, otherwise, spontaneous recovery usually occurs within 1 to 2 months.

Eosinophilic Gastritis

This is the gastric component of eosinophilic gastroenteritis; the term *gastroenteritis* is somewhat misleading because, in addition to the stomach and small bowel, the colon and esophagus may also be involved in this disorder. This condition usually presents in infancy but has also been reported in preterm infants.[156] It is a chronic, severe disease, of unknown etiology, characterized by the presence of upper GI symptoms and signs, as well as poor

growth, gastrointestinal bleeding, and, often, diarrhea. Iron deficiency anemia and hypoproteinemia with protein-losing enteropathy are commonly present.[157,183–185] In most, but not all, patients, serum IgE is elevated, and peripheral eosinophilia is present.[184] All layers of the gastric wall may be involved; the eosinophilic infiltrate may be patchy, and there may be selective predominance of eosinophilic infiltrates in the mucosa, muscle layer, or subserosa.[183] Therefore, diagnosis by endoscopy with biopsies may not always be possible; sometimes, surgical full-thickness biopsy is necessary. When present, gastroscopic features are nonspecific and include friability and erythema, erosions, swollen mucosal folds, and scattered mucosal blebs or nodular lesions, particularly in the gastric antrum. When present, these nodules differ from those associated with *H. pylori* gastritis in that they are scattered, few in number, and not of uniform size. Even when the mucosa is normal at endoscopy, biopsies often reveal a striking eosinophilic infiltrate through the lamina propria into the epithelium; occasionally, small numbers of lymphocytes and plasma cells are present. Eosinophilic gastritis has also been described as a manifestation of collagen vascular disease such as scleroderma[186] and also with acute infections with the parasite *Anisakis*. The latter is most likely due to an allergic reaction occurring in sensitized individuals.[187]

Collagenous Gastritis

This rare entity, which is characterized by subepithelial collagen deposition and an associated gastritis, may not itself comprise a distinct disorder but, rather, a consequence of inflammation or a local immune response in the stomach or as one histologic feature of a more diffuse disease process. For example, it has been described in association with the histologically similar conditions of collagenous sprue and collagenous colitis, lymphocytic colitis, and celiac disease.[188–193] In some of these reports, it appears to be a "stand-alone" disorder. It has also been described as a prominent histologic feature in some children with the typical endoscopic features of CVG, including diffuse gastric erythema, erosions, and hemorrhage.[17,194] The pattern of mucosal fibrosis in collagenous gastritis, colitis, or sprue is subepithelial in the lamina propria and quite different from the much deeper (usually circular muscle) involvement seen in scleroderma.[195] In children, collagenous gastritis most often presents with upper abdominal pain, gastrointestinal bleeding, and anemia.[17,194,196,197] None of these children had endoscopic or histologic improvement at follow-up, although symptoms may resolve with acid suppression treatment. Adults more often present with diarrhea, and this most likely represents associated lymphocytic or collagenous colitis or even celiac disease. Symptomatic improvement has been reported with therapies including gluten-free diet, corticosteroids, and acetylsalicylic acid preparations.[190–192]

Graft-versus-Host Disease

Acute GVHD occurs 3 to 4 weeks after transplant, with varying degrees of mucositis, dermatitis, enteritis, and hepatic dysfunction.[141] Upper GI symptoms are also commonly seen. More recently, the stomach has been shown to be an important area for the histologic diagnosis of gastrointestinal GVHD, even when diarrhea is the main symptom[198,199]; the gastric endoscopic and histologic findings may underestimate the severity of GVHD elsewhere in the gut. Endoscopy with biopsies is not routinely required for the diagnosis of GVHD, but when performed for investigation of abdominal pain, for bleeding, or to exclude opportunistic infection, the findings vary considerably. These range from normal or subtle changes, even when most or all of the epithelium is lost, to patchy erythema with erosions, to extensive mucosal sloughing; the early biopsy findings are unique to GVHD, consisting of crypt epithelial cell apoptosis and dropout. In more severe cases, whole crypts may drop out. There is variable lymphocytic infiltration of the epithelium and lamina propria. In advanced cases, there may be ulceration, edema, fibrosis, and perforation. When acute GVHD is suspected, the duodenum and esophagus should be biopsied, in addition to the proximal and distal stomach, but with recognition that duodenal biopsy carries higher risk in these patients.[198–200] Histologic distinction between GVHD, CMV infection, human immunodeficiency virus (HIV), and other immunodeficiencies may be difficult.[201] Chronic GVHD rarely involves the stomach.

Ménétrier Disease

Ménétrier disease (hypoproteinemic hypertrophic gastropathy) is a rare, acquired disorder of the stomach that is premalignant or may even present with malignancy in adults but is generally a clinically benign, self-resolving disorder in children. It is characterized by large folds that most often involve the fundus, excess mucus secretion, decreased acid secretion (hypochlorhydria), and hypoproteinemia secondary to selective loss of serum proteins across the gastric mucosa.

The childhood form of this rare disorder may follow a viral prodrome and includes epigastric pain, anorexia, vomiting, edema, hypoproteinemia, and raised IgE levels in some.[202] Full-thickness gastric biopsy at laparotomy or even partial gastric resection for diagnosis has become obsolete in children with the advent of pediatric endoscopy, although other "thick fold" diseases are in the differential diagnosis and may require this approach (see "Multizone Biopsy Sampling" above). The combination of endoscopic and histologic findings is diagnostic. Endoscopy shows swollen convoluted rugae sometimes with polypoid or nodular configuration. The histology typically shows elongated, tortuous foveolae, with reduction of chief and parietal cell glands and often with cystic dilatations that may extend into the muscularis mucosae and submucosa. The lamina propria is edematous with increased eosinophils, lymphocytes, and round cells, and the muscularis mucosa may be hyperplastic with extensions into the mucosa. Gastric wall thickening as determined by ultrasonography is not diagnostic of Ménétrier disease, but serial studies may be helpful in monitoring the course of the disease.[203] Endoscopic ultrasonography has also been used to diagnose hypertrophic gastric folds,

which may reach up to 20 mm in diameter in adults.[204] Ménétrier disease has been strongly associated with CMV infection in immunocompetent children, and elevated CMV IgM levels, positive CMV testing by polymerase chain reaction, or positive culture of gastric tissue may be helpful in confirming the diagnosis.[202,205] The cause of adult Ménétrier disease is unknown. Reports of CMV-associated gastric fold thickening in adults are rare.[206] A genetic predisposition was suggested in a report of three affected generations in one family,[207] and cure of *H. pylori* infection has resulted in resolution of adult Ménétrier disease.[208] Increased signaling of the epidermal growth factor receptor has been implicated in the pathogenesis of adult Ménétrier disease.[209]

Although this condition is reported from the neonatal period onward, the mean age at onset in children is 4.7 years.[210] In children, the natural history is of self-resolution within weeks or months.[202,210,211] In contrast, the adult disease is usually chronic, and evidence of the benefits of anticholinergic drugs, acid suppression, octreotide, and eradication of *H. pylori* is inconsistent; occasionally, partial gastrectomy has been required to alleviate persistent abdominal symptoms, hypoproteinemia, and blood loss. Dramatic resolution of many manifestations in adults has been reported with the use of antiepidermal growth factor receptor antibody.[212] A lymphocytic gastritis has been described in the adult form.[213]

Pernicious Anemia

Although megaloblastic anemia may result from dietary, malabsorptive, or other causes of vitamin B_{12} (or folate) deficiency, the term *pernicious anemia* (PA) is applied to the anemia and condition that result from a deficiency of intrinsic factor.[214] The classic or adult form of PA occurs also in children and is due to an autoimmune process with autoantibodies to parietal cell components, including the proton pump and intrinsic factor. This results in absolute achlorhydria and megaloblastic anemia secondary to vitamin B_{12} deficiency. Conditions associated with PA include endocrinopathies such as autoimmune thyroid disease and diabetes mellitus, vitiligo, selective IgA deficiency, abnormal cellular immunity, chronic candidiasis, and collagen vascular disease.[215–217] The typical finding at endoscopy is thin rugae of the gastric body, sometimes with blood vessels visible through the mucosa. Histology shows severe atrophic fundic gland gastritis with absence of parietal cells. Adenocarcinoma of the stomach occurs as a complication of PA. Although gastric adenocarcinoma is very rare in children, it does occur,[218–220] and endoscopic surveillance of PA is indicated.

A separate category entirely is so-called childhood or juvenile PA. This is a heterogeneous group of conditions that can be considered as metabolic rather than autoimmune. There is no gastric atrophy, but megaloblastic anemia and hypo- or achlorhydria are present.[221] Recently, secretion of abnormal intrinsic factor or abnormalities of secretion of intrinsic factor have been found as the cause of juvenile PA.[222] A congenital anomaly of vitamin B_{12} metabolism (cobalamin *C* disease) occurs very rarely; it is accompanied by striking cystic dysplastic changes in gastric mucosa and total absence of parietal and chief cells.[223]

Gastritis Associated with Autoimmune Diseases

Gastritis with and without atrophy has been seen in children with autoimmune thyroiditis and nongoitrous juvenile hypothyroidism, some with achlorhydria and gastric parietal cell antibodies.[215] Autoimmune atrophic gastritis has also been described in 15% of adults with vitiligo.[216]

In children and adults with connective tissue diseases, a mast cell gastritis and a combination of mast cell and eosinophilic gastritis has been described.[186,224] We have seen atrophic gastritis in a teenage girl with scleroderma. Gastrointestinal bleeding in patients with systemic sclerosis and CREST syndrome has been reported and is most often due to mucosal telangiectasia, although peptic ulcers and erosive gastritis are also described.[225]

In a large group of children with insulin-dependent diabetes mellitus, 7% had upper GI symptoms for which endoscopy was performed[226]; 48% had evidence of erosions and ulcers, and 35% had evidence of delayed gastric emptying. Histologic gastritis was reported in 25 of 27 children in whom biopsies were taken; all were negative for *H. pylori*.

Other Granulomatous Gastritides

Granulomatous gastritis other than that attributable to Crohn disease is rare. The differential diagnosis includes foreign body reaction, tuberculosis, histoplasmosis, and Wegener disease, among other disorders (see Table 29.2-6).[227–229]

Idiopathic isolated granulomatous gastritis is a rare condition of a chronic granulomatous reaction limited to the stomach and a diagnosis of exclusion. Primarily reported in adults, it has also been reported in a 14-year-old who responded to steroids.[227] However, in most cases of idiopathic granulomatous gastritis, an etiology of Crohn disease or sarcoidosis can be established.[228]

Langerhans cell histiocytosis (histiocytosis X), a rare condition in which organs are infiltrated by proliferating histiocytes, can cause granulomatous gastritis[230] and gastric polyps.[231] Sarcoidosis is very rarely encountered in the gastrointestinal tract, and reported cases are confined to the adult literature.[232–234]

Phlegmonous Gastritis and Emphysematous Gastritis

Phlegmonous gastritis is a rare, life-threatening condition in which a rapidly progressive bacterial inflammation of the gastric submucosa results in necrosis and gangrene.[235] Most cases are due to α-hemolytic streptococci, *Staphylococcus aureus*, *Escherichia coli*, and *Clostridium welchii*, but other organisms, such as *Candida albicans* and *Mucor*, may be involved.[236] Patients may have infections elsewhere in the body or be immunocompromised.

Acute emphysematous gastritis is a complication of phlegmonous gastritis in which gastric wall infection is due to gas-forming bacteria.[237–240] This often fatal condition is characterized by severe abdominal pain and systemic toxic-

ity, with radiologic evidence of gas bubbles and thickening of the gastric wall. Predisposing factors include ingestion of caustic agents and abdominal surgery. It has also been reported in a leukemic child,[239] in a child with a phytobezoar,[237] in a patient who ingested large volumes of a carbonated beverage,[240] and in hepatic cirrhosis owing to chronic alcoholism, Indian childhood cirrhosis, and Budd-Chiari syndrome.[241] Treatment should be prompt if mortality is to be avoided and may involve gastrectomy, drainage of localized intramural collections that can be identified by computed tomography or endoscopic ultrasonography, and the use of broad-spectrum antibiotics.[242] Emphysematous gastritis must be distinguished from two other entities that cause gas to be present in the gastric wall, gastric emphysema and cystic pneumatosis. These usually follow instrumentation or gastric outlet obstruction and in and of themselves are not clinically significant.[239,240]

Other Infectious Gastritides

Giardia lamblia is said to be the most common of all gastrointestinal parasites and occurs worldwide.[243] It is characteristically a small bowel parasite. Gastric colonization with *Giardia* has been reported in 41 (0.37%) of some 11,000 patients who underwent gastroscopy and biopsies over a 5-year period at one institution.[244] All 41 had chronic atrophic gastritis, and most had intestinal metaplasia with or without *H. pylori* infection. *Giardia* trophozoites were found on the surface epithelium and at the base of pits; they were always present in the antrum, never in association with fundic-type mucosa. They had presented with symptoms including dyspepsia, epigastric pain, and abdominal distention. Some patients had received acid-suppressing drugs for peptic ulcer disease or partial gastrectomy. Hypochlorhydria was a likely prerequisite for the organisms to infect the stomach. In all 9 of the 41 who had concurrent duodenal biopsies, *Giardia* was present. In larger numbers of patients with duodenal *Giardia*, none who had normal or near-normal gastric mucosa had gastric *Giardia*. In another study of 252 *Giardia*-positive cases, trophozoites were found within the gastric antrum in about 9% of those who had gastric biopsies taken, and non–*H. pylori* chronic active gastritis was reported in only 2.9%.[245] This study also showed that *Giardia* does not cause atrophic gastritis. Given the evidence, *Giardia* may be a pathogen in the stomach in some patients and may be a regurgitant contaminant from the duodenum in others.

Helicobacter heilmanii (previously *Gastrospirillum hominis*) is probably transmitted from cats and dogs[246,247] and may cause chronic active gastritis similar to that of *H. pylori* but with less severe inflammation, which is focal and usually restricted to the antrum.[247–250] Gastric ulceration has been reported in one teenager and antral nodularity in another.[246,248] However, as yet, a definite association between *H. heilmanii* infection and ulcer disease has not been established.[250] Associated gastritis responds to therapy.[251]

Herpes simplex is a rare cause of gastritis and erosions in immunosuppressed patients, with biopsy showing the characteristic intranuclear inclusion bodies.[251,252] Evidence of herpes simplex virus type 1 was identified in 4 of 22 gastric or duodenal ulcers using immunohistochemistry and molecular probes.[253] The herpes varicella-zoster virus is a rare cause of gastritis in adults and possibly children.[254,255]

Influenza A is a rare cause of bleeding from hemorrhagic gastropathy in children and is sometimes fatal.[256] Serology was positive in all cases, but gastric biopsies were negative for virus. This may have been a stress gastropathy owing to a severe systemic illness rather than directly attributable to virus.

A gastropathy with hypertrophic gastric folds and protein-losing enteropathy has been described in a 3-year-old with a rising titer of IgM to *Mycoplasma pneumoniae* and no evidence of recent CMV infection.[257]

Mycobacterium tuberculosis involvement of the stomach is very rare and usually associated with tuberculosis elsewhere or with immunodeficiency.[258–260] Syphilis involving the stomach is very rare.[261]

Fungal infections of the stomach, such as *C. albicans*, histoplasmosis, and mucormycosis, may occur, especially in sick neonates, malnourished children, and those with burns or immunodeficiencies.[262–267] If gastric ulceration is seen in immunodeficient patients, fungal infection should be sought and, if present, should be treated, along with peptic ulcer therapy.

Infection with fungi of the Mucoraceae family (*Rhizopus*, *Mucor*, and *Absidia*) can cause the systemic disease mucormycosis, which is fatal in malnourished or immunosuppressed children and preterm neonates.[268,269] Mucoracea are ubiquitous organisms occurring in bread, fruit, and decaying material. Bleeding, gastric ulcers, and perforation may occur in the rare involvement of the stomach .

Fungal infection of the stomach with histoplasmosis and aspergillosis or the parasite *Strongyloides stercoralis* occurs rarely.[270]

Acute gastric anisakiasis simplex infection occurs frequently in Japan and in areas of high consumption of raw fish. Gastric symptoms may occur within 3 hours of ingestion, and in sensitized people, systemic allergic symptoms may arise within 5 hours of ingestion. Peripheral leukocytosis and eosinophilia may also occur. Endoscopy shows one or more worms protruding into the lumen a couple of millimeters off the gastric mucosa, surrounded by a ring of intense erythema, mucosal swelling, and sometimes gastric erosions. They can be in the antrum or body but tend to favor the greater curvature of the stomach. Early endoscopy is diagnostic and therapeutic, allowing for removal of the worm and relief of symptoms.[187,271–273]

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CHAPTER 30

ESOPHAGEAL AND GASTRIC NEOPLASMS

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ESOPHAGUS

Three tumor types occur with any frequency in the esophagus: small benign mucosal leiomyoma, adenocarcinoma arising in Barrett esophagus, and squamous cell carcinoma. Unusual even in adulthood, all are reported in children. The latest US cancer surveillance data from the Surveillance, Epidemiology, and End Results (SEER) program show a measurable but small incidence of esophageal malignancies in the 10- to 14- and 14- to 18-year age groups. Other tumors, benign or malignant, including papillomas, granular cell tumors, and esophageal sarcomas, are unusual in adults and even more so in childhood. A specific pediatric issue related to tumors of the esophagus is the recognition of pathologies in childhood that may predispose the patient to adult malignancy. These are summarized in Table 30-1 and are discussed in the specific sections on tumor types. Childhood mediastinal malignancies treated with irradiation may result in various esophageal solid tumors in early adult life. Human papillomavirus (HPV) infections have been linked to both esophageal papilloma[1] and, less certainly, adult squamous cell carcinoma. Nutritional factors and genetic disorders may manifest as benign pediatric esophageal pathology, predisposing the patient to adult malignancy. Pediatric esophageal tumors may also occur as part of an inherited or genetic syndrome or systemic disorder (those relevant to this chapter are listed in Table 30-2).

TABLE 30-1 PEDIATRIC ESOPHAGEAL PATHOLOGY ASSOCIATED WITH ADULT MALIGNANCY

PATHOLOGY	ETIOLOGY	TUMOR
Squamous papilloma	Viral	SCC
GERD	BE	ACA
Tylosis	Genetic	SCC
Achalasia	Inflammatory	SCC
TEF	Inflammatory	SCC
Chemoradiation	Oxidative stress	Sarcoma/SCC/ACA
Lye	Corrosive	SCC
Esophagitis*	Various†	SCC

ACA = adenocarcinoma; BE = Barrett esophagus; GERD = gastroesophageal reflux disease; SCC = squamous cell carcinoma; TEF = tracheoesophageal fistula.
*In geographic areas of high squamous cell carcinoma incidence.
†Including vitamin and mineral deficiency, dietary carcinogens, and cooking methods.

Clinical Symptomatology, Diagnosis, and Therapy

Most benign tumors of the esophagus are small, more than 50% are asymptomatic, and they are discovered incidentally during upper endoscopy performed for unrelated problems. Dysphagia occurs with larger tumors. In infants, feeding and respiratory difficulty may be the presenting problem.[2] Bleeding, weight loss, and vomiting are less common and indicate a larger or malignant tumor. A mediastinal mass discovered on chest radiography is rare.[2,3] Esophageal tumors are usually investigated using upper gastrointestinal endoscopy, with or without barium esophagraphy; computed tomography, magnetic resonance imaging, and endoscopic ultrasonography help define the depth of invasion and degree of spread. Biopsy diagnosis is usually possible in most cases, and endoscopic procedures may also be curative for small lesions. Surgical resection is the treatment of choice for the remainder, but minimally invasive surgery has decreased morbidity for such resections. Data on adjuvant chemo- and radiotherapy for esophageal malignancy in children are not readily available because malignant tumors are rare.[4]

Benign Epithelial Esophageal Tumors

A summary of benign epithelial tumors of the pediatric esophagus is provided in Table 30-3.

TABLE 30-2 ESOPHAGEAL PATHOLOGY ASSOCIATED WITH EXTRAESOPHAGEAL DISEASE

ESOPHAGEAL PATHOLOGY	EXTRAESOPHAGEAL DISORDER
Cysts	Vertebral anomalies, malrotations, and TEF
Glycogen acanthosis	Cowden disease
Leiomyomatosis	Alport syndrome
Webs	Plummer-Vinson syndrome
SCC	Celiac disease
SCC	Keratodermatoses

SCC = squamous cell carcinoma; TEF = tracheoesophageal fistula.

TABLE 30-3 BENIGN TUMORS OF THE PEDIATRIC ESOPHAGUS

RELATIVELY FREQUENT	RARE	NOT REPORTED IN CHILDHOOD
Leiomyoma	Pseudodiverticulosis	Inflammatory fibroid polyp
Papilloma		
Granular cell tumor		
Duplication cysts		

Esophageal Squamous Papilloma. The esophageal squamous papilloma is a benign polypoid tumor usually found in the lower esophagus. The reported incidence rates vary, from more than 0.1 to 0.4%, based on adult autopsy studies.[5] Because this polyp is most often asymptomatic and regenerative in nature, these rates may be a large underestimate. Esophageal squamous papilloma is reported in childhood, with similar demographic distribution as in adults,[6,7] and has also been described in a case of Cowden disease.

Two main categories of etiologic factors have been implicated in the development of these tumors: physical trauma (including acid reflux, radiation, irritation from chemicals or foreign bodies) and infection with HPV infection, which remains controversial. In the lower esophagus, most papillomas are associated with acid reflux and hiatal hernia.[8] In the mid- and upper esophagus, and with multiple esophageal squamous papillomas, HPV (subtypes 16–11) is found in a variable percentage.[8,9]

At endoscopy, esophageal squamous papillomas are usually solitary mucosal protrusions without a true stalk. The overlying squamous mucosa is normal in appearance or roughened and white owing to mucosal keratinization. Most are small (< 5 mm), although "giant" esophageal squamous papilloma (as large as 23 cm in maximum dimension) has been reported, mainly within the adult population.[8]

Pathology. Esophageal squamous papilloma consists of a central fibrovascular core covered by benign mature squamous epithelium (Figure 30-1). Inflammation and reactive epithelial atypia are common. Cytoplasmic clearing and nuclear irregularity (koilocytosis), when seen, may indicate the presence of HPV.[8]

Papillomatosis, the presence of multiple esophageal squamous papillomas distributed throughout the esophagus, is rare but occurs predominantly in the pediatric age group.[10] HPV is suspected but only occasionally found.[11] A single case report links these esophageal squamous papillomas to laryngotracheal papillomatosis.[12] Multiple esophageal squamous papillomas are reported with Goltz syndrome (focal dermal hypoplasia).[13]

Malignant Transformation. Most small esophageal squamous papillomas are reactive rather than truly neoplastic and will not recur or progress. Large or multiple esophageal squamous papillomas arising in association with HPV subtypes 16 and 18 have been reported to show more aggressive behavior in some instances. Some reports of esophageal squamous papilloma with carcinoma may represent papillary carcinoma. The association between HPV and esophageal carcinoma is also debated.[14,15] No cases of malignant esophageal squamous papilloma have been reported in the pediatric literature.[5,15]

Esophageal Cysts and Reduplications. Gastrointestinal reduplication with resulting cysts and tubules occurs throughout the gastrointestinal tract. The esophagus is involved in 19% of cases, where spherical and cystic forms are more common. Resulting from a possible abnormality of the notochord, they are usually seen in the lower third and toward the right side. Usually single, they may extend the entire esophageal length or communicate with a subdiaphragmatic gastric duplication. Intramural and extramural forms occur, with associated vertebral anomalies (in the form of clefts or fusion) in the extramural form. Most cases present within the first year of life associated with respiratory difficulty; with increasing age, dysphagia or feeding problems become more common.[2,16] The mucosal lining of the cyst may be ciliated columnar (developmental form), gastric, or esophageal squamous. The wall contains

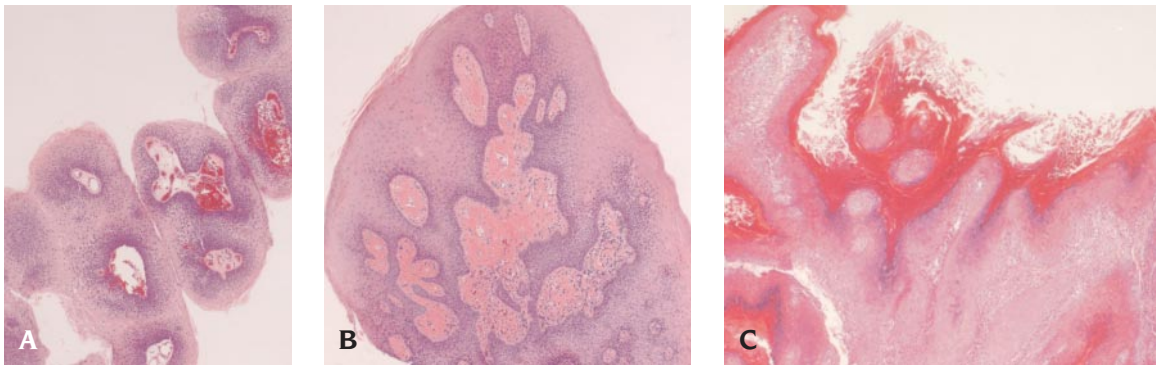

FIGURE 30-1 Esophageal squamous papillomas. *A*, The exophytic form has a branching fibrovascular core, with the "branches" covered by mature, sometimes thin squamous mucosa (hematoxylin and eosin; ×40 original magnification). *B*, Endophytic papilloma is a sessile polyp with smooth benign squamous epithelium overlying a fibrovascular core into which the deep squamous "rete" extend. It is also occasionally called a "fibroepithelial polyp" (hematoxylin and eosin; ×100 original magnification). *C*, The "spiked" exophytic esophageal squamous papilloma, least frequent, is characterized by keratohyaline granules within the superficial keratinocytes and a thick layer of orthokeratin forming hyperkeratotic "spikes" (hematoxylin and eosin; ×40 original magnification).

smooth muscle, nerves, and blood vessels forming a muscularis propria–like appearance in the wall that may be shared with the adjacent intestinal wall.[17,18] Intramural cysts without muscular components are considered esophageal cysts if lined by squamous mucosa and "bronchogenic" if the lining is ciliated columnar and there is cartilage within the cyst wall. The presence of muscularis propria indicates an intestinal reduplication (Figure 30-2). Communication with a gastric reduplication is relatively common, but only 10% of the cysts communicate with the esophageal lumen.[17]

Treatment is by surgical resection. Extramural forms separate easily from the adjacent esophageal muscularis propria; intramural forms tend to share the muscle wall with the esophagus. Complete excision is recommended, which may be possible by thoracoscopy.[19,20] There is a small risk of malignant transformation in adulthood of the epithelial lining of untreated cysts.[21,22]

Pseudodiverticulosis. In this unusual condition, the duct orifices of the esophageal submucosal glands become dilated to form multiple intramural cysts. Achalasia may be present, and esophageal dysmotility is the presumptive cause.[23] The pseudodiverticula may involve a segment, usually in the upper esophagus, or be diffuse. Endoscopically small pit-like openings may be seen on these mural protrusions, but radiologic studies may suggest a cystic neoplastic process, particularly in localized disease. Biopsy reveals a squamous lining, commonly with candidal superinfection.

Glycogen Acanthosis. Glycogen acanthosis appears as a white patch of esophageal mucosa at upper endoscopy. Glycogen acanthosis is flat and, although not neoplastic, may be confused with leukoplakia. Biopsy reveals esophageal squamous mucosa with enlarged superficial keratinocytes. The cleared-out appearance of the cytoplasm is due to the accumulation of glycogen within the cells. Glycogen acanthosis is usually sporadic, but multiple glycogen acanthoses in childhood may indicate the presence of hamartomatous polyposis (Cowden disease and Lhermitte-Duclos syndrome)[24] related to germline mutation in the *PTEN* gene and associated with thyroid and breast malignancy.

Benign Nonepithelial Esophageal Tumors

Esophageal Leiomyoma. Small mucosal leiomyomas derived from the muscularis mucosa are considered the most common benign neoplasms of the esophagus. Autopsy studies suggest a steady increase in incidence with longevity.[25] Accordingly, esophageal leiomyoma is an unusual tumor in childhood.[26]

Esophageal leiomyoma has been reported in children as young as 4 years old,[26] but most are reported in the teenage years. Unlike adults, in childhood they appear more commonly in females (female-to-male ratio 1.7:1), and diffuse or multiple forms are more common, with isolated single esophageal leiomyoma representing only 9% of cases.[26]

Esophageal leiomyomas may occur as part of Alport syndrome (nephropathy and sensorineural hearing loss).[26] Gastroesophageal reflux disease (GERD) has been reported as an association, possibly reflecting the frequency of GERD, with coincidental esophageal leiomyoma. Esophageal diverticulum has been reported with esophageal leiomyoma, an association of uncertain significance. Rare cases of congenital stricture with esophageal leiomyoma may represent the development of stromal tumors rather than esophageal leiomyoma in families with inherited mutations of the gastrointestinal stromal tumor–associated gene *KIT*.[27] Esophageal leiomyoma has also been seen in patients with multiple endocrine neoplasia type I. The characteristic gene mutation at chromosome 11q13 was demonstrable within these tumors but does not appear to be responsible for sporadic leiomyoma.[28]

Endoscopic Appearance. Esophageal leiomyomas appear as submucosal bumps or sessile polyps at endoscopy. The overlying mucosa is intact.

Pathology. Most esophageal leiomyomas are intramural (97%), with 1% presenting as polyps and 2% as extramural mediastinal tumors.[29] The most common sites are the distal esophagus and gastroesophageal junction, with decreasing numbers aborally. Esophageal leiomyomas form well-circumscribed, noninfiltrative nodules. The characteristic whorled white-yellow cut surface is due to intersecting fascicles of spindle smooth muscle cells. Calcification and cystic degeneration are common in larger forms. Rarely, lesions grow circumferentially around the esophagus, forming a stricture.[29,30] In small esophageal leiomyomas, an origin from the muscularis mucosa is often apparent. Treatment is by enucleation for single tumors and surgical resection for multiple esophageal leiomyomas.[31]

Leiomyomatosis. Multiple esophageal leiomyomas, when confluent, are sometimes referred to as "diffuse leiomyomatosis." This term has also been used to indicate diffuse hyperplasia of one of the layers of the muscularis propria. These two processes should be clearly delineated

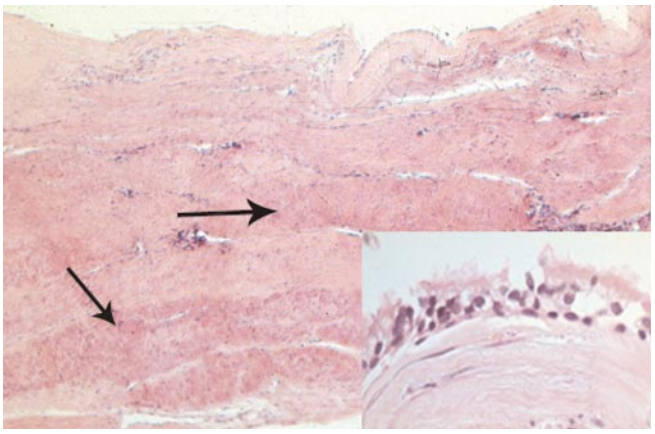

FIGURE 30-2 Esophageal reduplication cyst. The partially denuded cyst wall contains muscle layers (*arrows*) reminiscent of muscularis propria (hematoxylin and eosin; ×20 original magnification). *Inset:* Intact cyst lining has a ciliated columnar appearance (hematoxylin and eosin; ×200 original magnification).

because esophageal leiomyoma, although benign, is a neoplasm. The etiopathogenesis of the hyperplastic process is unknown.[32] The proliferative form is seen in Alport syndrome and familial leiomyomatosis that formed 22% of esophageal leiomyomas in a recent pediatric review.[26]

Alport Syndrome and Familial Leiomyomatosis. Alport syndrome, or sensorineural hearing loss with nephropathy, is reported in children as young as $2^1/_2$ to 5 years old.[26,33] The genetic basis is a mutation in genes responsible for the α_5 chain of type 4 collagen (*COL4A5*). Type 4 collagen is essential for basement membrane synthesis[34] and may play an important role in cell-matrix interactions. Similar mutations have been found in both Alport syndrome and a familial form of diffuse leiomyomatosis without Alport syndrome features.[34,35]

Granular Cell Tumor. The esophagus is the main site of occurrence of the granular cell tumor in the gastrointestinal tract. This unusual mesenchymal tumor is reported in the pediatric population[36] and even in the newborn.[37] Most are small and are found incidentally at upper endoscopy, where a sessile, yellow-white firm nodule is found. The overlying mucosa is intact, and most tumors are 2 cm or less. The lower esophagus is a more common location than the upper esophagus, and males and blacks are more likely to be affected. Occasional reports have linked granular cell tumor to neurofibromatosis.[36]

The tumor is composed of epithelioid or plump-spindled cells with abundant coarsely granular eosinophilic cytoplasm and small round nuclei without obvious nucleoli. The cytoplasm of the cells is characteristically positive with periodic acid–Schiff stain, and both cytoplasm and nucleus are S-100 positive by immunohistochemistry, consistent with schwannian differentiation.[38] Short fascicles of tumor cells diffusely infiltrate the lamina propria of the esophagus. The overlying squamous mucosa is often markedly hyperplastic, with tongues of atypical-appearing squamous epithelium extending deeply into the granular cell tumor. This pseudoepitheliomatous hyperplasia is a close mimic of invasive squamous cell carcinoma and is a trap for unwary pathologists (Figure 30-3). Small granular cell tumor has been reported to remain stable in size, but some large granular cell tumors require resection for symptoms.[39] Malignant forms, although exceedingly rare, are reported,[40] although not so far in the pediatric age group.

Vascular Tumors. Hemangioma and lymphangioma have both been reported in the esophagus of children, albeit rarely.[3,41,42] Both are composed of benign vascular channels, and the former may present with bleeding. The lesions are usually apparent at endoscopy.

Malignant Esophageal Tumors

The esophagus and proximal stomach are the sites of cancer with the largest and most rapid increase in incidence in the last two decades.[4,43] Most of these are adenocarcinoma. In the same time period, the incidence of esophageal squamous cell carcinoma has been falling. Squamous cell carcinoma is still more common than adenocarcinoma of the esophagus, but the rise in adenocarcinoma in older white males is alarming, and its cause is not understood. Given the time course of evolution from preneoplasia to overt carcinoma, many of these tumors may trace their origin to childhood esophageal pathology.

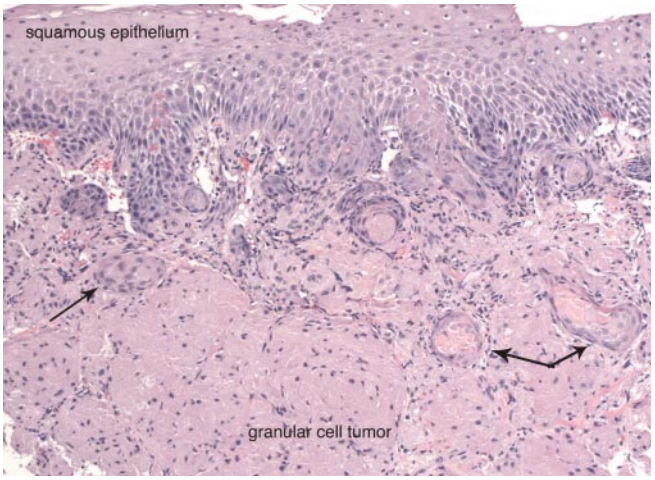

FIGURE 30-3 Granular cell tumor. Esophageal squamous epithelium overlies neoplastic cells with abundant amphophilic cytoplasm and small nuclei. Trapped by or infiltrating into the granular cells are tongues of squamous epithelium with dysmaturation (pseudoepitheliomatous hyperplasia) (*arrows*) (hematoxylin and eosin; ×200 original magnification).

Malignant Epithelial Tumors

GERD, Barrett esophagus, and adenocarcinoma arising in Barrett esophagus are the most significant causes of neoplasia of the pediatric esophagus (Table 30-4).

GERD and Barrett Esophagus. Barrett esophagus represents glandular mucosa lining the lumen of the esophagus. Barrett esophagus is an acquired defect, the result of chronic mucosal injury, usually from acid reflux, but also

TABLE 30-4 PEDIATRIC ISSUES RELATING TO BARRETT ESOPHAGUS

GERD is common in childhood and increasing in incidence.
GERD is an accepted risk factor for the development of BE in childhood (2.5–13% incidence).
BE increases the risk of adenocarcinoma of the esophagus 40- to 125-fold.
The reversibility of metaplasia is not well established.
Some reports suggest that the endoscopic appearance of pediatric BE may not be classic.
Approximately 50% of pediatric BE occurs in the setting of significant other disease, including neurologic impairment, where GERD-related symptoms may be absent.
Esophageal and proximal gastric adenocarcinoma incidence rates are rising more rapidly over the last two decades than any other carcinoma; both tumors are linked statistically to BE.
Extrapolation from adult data suggests a 30% incidence of adenocarcinoma in pediatric patients with BE if followed for 50 years.
Relatively good survival has been reported with early surgical intervention for esophageal adenocarcinoma.

BE = Barrett esophagus; GERD = gastroesophageal reflux disease.

possibly from bile, alkali (lye), and other physicochemical causes. Gastroesophageal acid reflux into the esophagus results in active chronic esophagitis; epithelial injury often results in ulceration. Healing occurs by ingrowth by epithelium from the ulcer borders. These ingrowing epithelial progenitor cells are of uncertain and disputed derivation. The possibilities include a stem cell from the basal cell region or residual mucosa, which, in dividing, rapidly becomes less differentiated. In the stem cell theory, repair after a single episode of damage may be by normal squamous epithelium. Repeated injury and repair in the continuing presence of acid, pepsinogen, or in combination with alkali may result in differentiation of the covering mucosa to a more resistant type, glandular mucosa. The second possibility, regrowth from nearby residual mucosa, requires the repairing mucosa to be glandular in type; origin from the ducts of esophageal mucosal glands has been posited for this pathway. Both of these would explain the cardiac-type nature of "columnar-lined esophagus." Barrett esophagus occurs in only 10% of patients with GERD, implying that other factors, genetic or environmental, play an undetermined role. Familial Barrett esophagus is reported, and a study of these families should elucidate associated genetic abnormalities.[44,45]

Significant comorbidity is common in childhood Barrett esophagus, and a high prevalence of neurologic disease or autism is found. This can make the medical management of these cases harder.[46] The development of intestinal metaplasia is increasingly accepted as a preneoplastic step in a sequence through dysplasia to adenocarcinoma arising in the esophagus. In the adult population, a continuum from GERD to Barrett esophagus through dysplasia to adenocarcinoma is relatively well documented, but this pathway is less clear in childhood.[47] GERD is a common phenomenon in children, but because adenocarcinoma is rare, the issue of risk for dysplasia and screening is unclear.[48–51] Management of acid reflux with medical therapy and/or surgery may not be adequate to reverse the metaplastic process.[52]

Initial reports of pediatric Barrett esophagus have little documentation of the presence of goblet cells in the mucosa and recently have been considered "columnar-lined esophagus."[51] Intestinal metaplasia in GERD or columnar-lined esophagus with the appearance of goblet cells within the mucosa increases with age and time; recent studies suggest that documentable goblet cells in columnar-lined esophagus begin to occur at the age of 7 years.[51,53] Progression from intestinal metaplasia through dysplasia to carcinoma has been documented to require approximately 20 years.[54]

The scattered case reports of adenocarcinoma in reflux-associated Barrett esophagus in children display similar pathologic features of malignancy in a setting of dysplastic and metaplastic Barrett esophagus, suggesting that the pathway of GERD to Barrett esophagus to adenocarcinoma is similar to that in adults.[55,56]

Endoscopic Appearance. The presentation of Barrett esophagus is similar to that in adults, with the exception that strictures appear more common in childhood, and malignancy is less frequent.[57] Barrett esophagus is grossly apparent at endoscopy as velvety red tongues extending up the esophagus from the proximal gastric fold at the gastroesophageal junction. Within an area of Barrett esophagus, there may be islands of residual white squamous mucosa (Figure 30-4). Other endoscopic changes include ulceration and nodularity or friability.[54,58] All of these may be related to peptic injury and regeneration but should alert the endoscopist to biopsy generously because these features also occur with dysplasia and adenocarcinoma.[51,59]

Pathology. Historically (pre-endoscopy), Barrett esophagus was recognized as glandular mucosa extending for more than 3 cm from the gastroesophageal junction. Nomenclature issues related to recognition of anatomic landmarks at endoscopy have been the source of controversy in the adult literature. Many adult pathologists will not diagnose Barrett esophagus now without the presence of goblet cells to indicate the intestinal rather than the gastric phenotype.[54] The remainder of cases are called columnar-lined esophagus. This relates to the recognition that the presence of goblet cells (indicating intestinal metaplasia) is generally indicative of progression of the pathologic process, and their presence suggests that screening for dysplasia should be instituted. The presence of intestinal metaplasia between the proximal gastric fold and 3 cm into the tubular esophagus (in the adult literature, termed short-segment Barrett esophagus) is associated with an increased risk of adenocarcinoma, although not as high as for standard or long-segment Barrett esophagus.[58]

Classic Long-Segment Barrett Esophagus. The mucosa in Barrett esophagus may show a gastric phenotype with antral or body- or fundic-type glands and foveolar surface epithelium. Rarely is the mucosa the well-organized body or fundic type. The presence of hiatal hernia should be suspected if pure body or fundic mucosa is seen. Focal goblet cells are present, resulting in incomplete intestinal metaplasia. In the absence of goblet cells, the presence of a villiform surface appearance is most suggestive of Barrett esophagus (Figure 30-5).[60,61] The presence of submucosal glands with

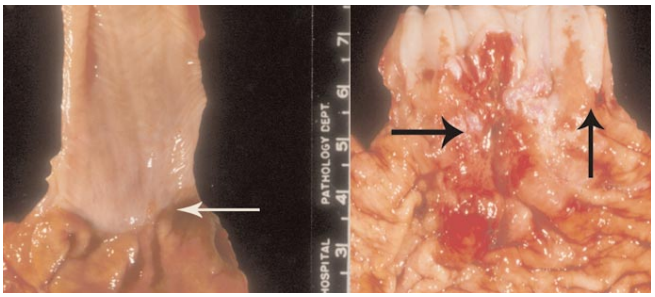

FIGURE 30-4 Normal gastroesophageal junction (GEJ) and Barrett esophagus (BE) with adenocarcinoma. The esophagogastrectomy specimen on the left shows the normal white appearance of squamous esophageal mucosa with a *white arrow* at the squamocolumnar junction. The specimen on the right shows a GEJ obscured by a carcinoma on the left (*black horizontal arrow*) with ulceration and raised edges. On the right, the normal white mucosa is partially replaced by a tongue of "salmon-pink" BE extending proximally from the GEJ (*black vertical arrow*) (see CD-ROM for color image).

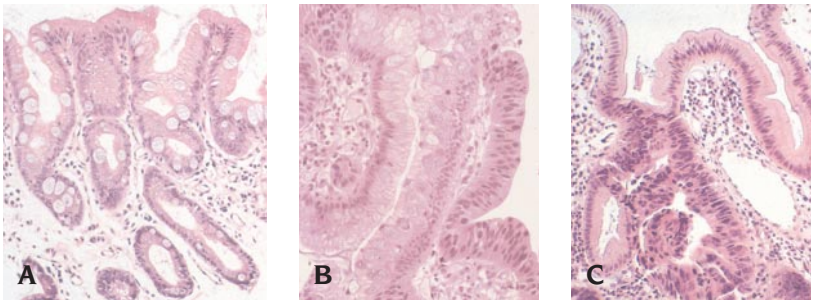

FIGURE 30-5 Barrett mucosa and dysplasia. *A*, Barrett esophagus: villiform mucosa with foveolar-type cells alternating with goblet cells in "incomplete metaplasia." *B*, Low-grade dysplasia: metaplastic mucosa is partially replaced by an abrupt focus characterized by cell crowding, pseudostratification, and nuclear hyperchromatism. *C*, High-grade dysplasia: the focal area shows increased atypia; nuclei are large with nucleoli. Individual cell necrosis or apoptosis is present. Architectural changes with back-back glands or cribriforming (like a sieve with the gland lumina as the holes) are apparent (hematoxylin and eosin; ×200 original magnification).

mucosal ducts lined by transitional-type mucosa is very good evidence for anatomic localization of the specimen from the tubular esophagus. Because goblet cells are often sporadic, and studies have shown that goblet cells "yield" increases with the number of biopsies taken[47] and that goblet cells tend to evolve after the first decade in children,[53] it is hard to be dogmatic about the pathologic definition of Barrett esophagus. Dysplasia refers to early neoplastic change in the mucosa, recognized as changes resembling colonic adenoma formation in the surface epithelium (see Figure 30-5), and is graded as low or high grade. High-grade dysplasia is associated with a high rate of invasive adenocarcinoma within 1 year. Photodynamic therapy is being used to ablate dysplastic mucosa in adult patients; no data are available in children.

Adenocarcinoma. Adenocarcinoma arising in Barrett esophagus usually occurs in the distal esophagus. The reported cases in childhood have shown the presence of surrounding areas of dysplasia and residual Barrett esophagus (Figure 30-6).[55,56] Survival is related to stage. Long-term survival is possible with mucosal carcinoma. Submucosal invasion and lymph node metastasis are associated with rapidly diminishing survival.[43]

Squamous Cell Carcinoma. Squamous cell carcinoma is a rare tumor in the esophagus in children.[4,62,63] It is more common in blacks than in whites and in males than in females (ratio 3.7:1). The major predisposing factors in the United States are alcohol and tobacco use, but in other regions, where the incidence of squamous cell carcinoma is very high, such as Linxian in Henan province in China, Northern Iran, South Africa, and areas of India, factors such as the ingestion of hot foods, vitamin-deficient diets, mineral deficiencies (zinc), and other dietary problems (foods high in nitrosamines such as pickled vegetables, diets low in fresh foods, high intake of benzo[a]pyrenes from coal smoke) are key. In these areas, investigation of children in their teens has shown the presence of chronic esophagitis in families with squamous cell carcinoma at double the rate of control families.[64] It is unclear as yet the relative importance of genetic over environmental forces in these populations. Some data suggest regression of the

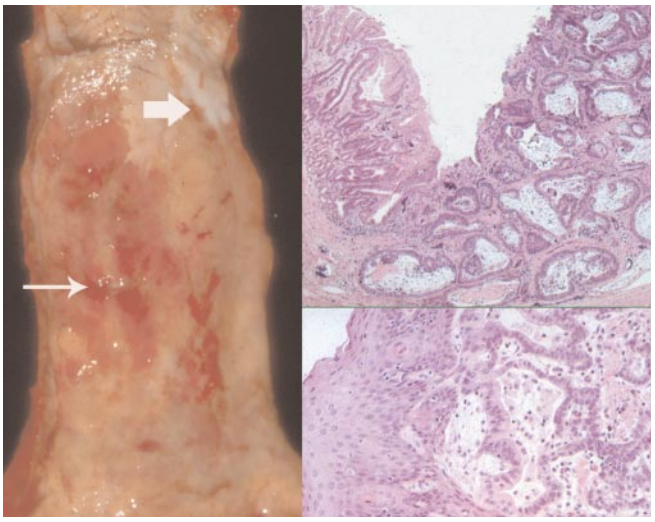

FIGURE 30-6 Barrett esophagus (BE) with early adenocarcinoma. The resection specimen shows a long segment of metaplastic mucosa with the squamocolumnar junction now in proximal esophagus (*thick arrow*). The pink BE mucosa is irregular with areas of reddening and nodularity (*thin arrow*) corresponding with dysplastic and malignant changes. The upper photomicrograph shows a benign BE on the left, with intramucosal adenocarcinoma on the right half of the figure. The malignant glands are dilated and invade into but not through the muscularis mucosa (hematoxylin and eosin; ×100 original magnification). The lower photomicrograph shows intact squamous mucosa with infiltrating adenocarcinoma within the lamina propria beneath (hematoxylin and eosin; ×100 original magnification) (see CD-ROM for color image).

inflammatory changes with vitamin A and zinc supplementation, at least in laboratory animals.[65]

Inherited or sporadic genetic disorders, mainly affecting keratin synthesis, including palmoplantar keratosis and tylosis, may be associated with very high rates of esophageal squamous cell carcinoma. These conditions often manifest in childhood, usually with cutaneous lesions. Mutations in keratin genes or linkage to the tylosis esophageal carcinoma gene (*TEC*) on 17q25.1 has been documented.[66]

Celiac disease is associated with a significant increased risk of esophageal squamous cell carcinoma. The pathogenesis is unclear, and the tumor so far has not been reported in childhood.[67]

Caustic ingestion is another pediatric problem associated with squamous cell carcinoma of the esophagus. The latency between ingestion and neoplasm is around 40 years, although cases as short as 13 years have been reported.[68–69]

The presence of stricture of the esophagus, by increasing food impaction, irritation, inflammation, ulceration, and repair, is associated with squamous cell carcinoma formation in the esophagus. Achalasia, dysmotility owing to the absence or destruction of the distal innervation of the esophagus, whether congenital or acquired, is associated with squamous dysplasia and squamous cell carcinoma. Squamous cell carcinoma has also been reported owing to postoperative strictures related to early childhood surgery. Childhood malignancies leading to mediastinal irradiation may also predispose the patient to early squamous cell carcinoma.[69]

Endoscopic Appearance. Squamous pre- and early neoplastic lesions form white plaques, nodules, and occasional polyps. Granular surface or ulceration is often associated with higher grades of dysplasia (Figure 30-7). The mid- and lower esophagus is most often affected. Lye-associated neoplasias are usually seen at the site of tracheal bifurcation, where the stricture is located. Although, occasionally, squamous cell carcinoma may be grossly papillary, benign esophageal squamous papilloma is not associated with a significant rate of malignant progression in the esophagus.

Pathology. Biopsy of a nodule or plaque may show thickened squamous mucosa with layers of keratin on the surface: benign hyperplasia (see Figure 30-7). Inflammation, both chronic within the lamina propria and acute within the epithelium, is common, and there may be prominent small blood vessels. As dysplasia occurs, maturation to the flat squamous phenotype is progressively delayed; when no maturation is seen, carcinoma in situ is present (see Figure 30-7). Ultimately, stromal invasion occurs, and early squamous cell carcinoma has developed. The lesion may still have the appearance of a plaque, but as the tumor grows, central ulceration with raised edges or annular stenosing growth may occur. Early invasion into mediastinal soft tissue occurs, with metastasis to regional nodes.

Prognosis and Therapy. The prognosis is grim for advanced tumors, regardless of age. Three-year survival with mucosal carcinoma is more than 80%. This drops with submucosal invasion to 45% and with lymph node metastasis to 17%.[70,71] Death is usually related to local disease.

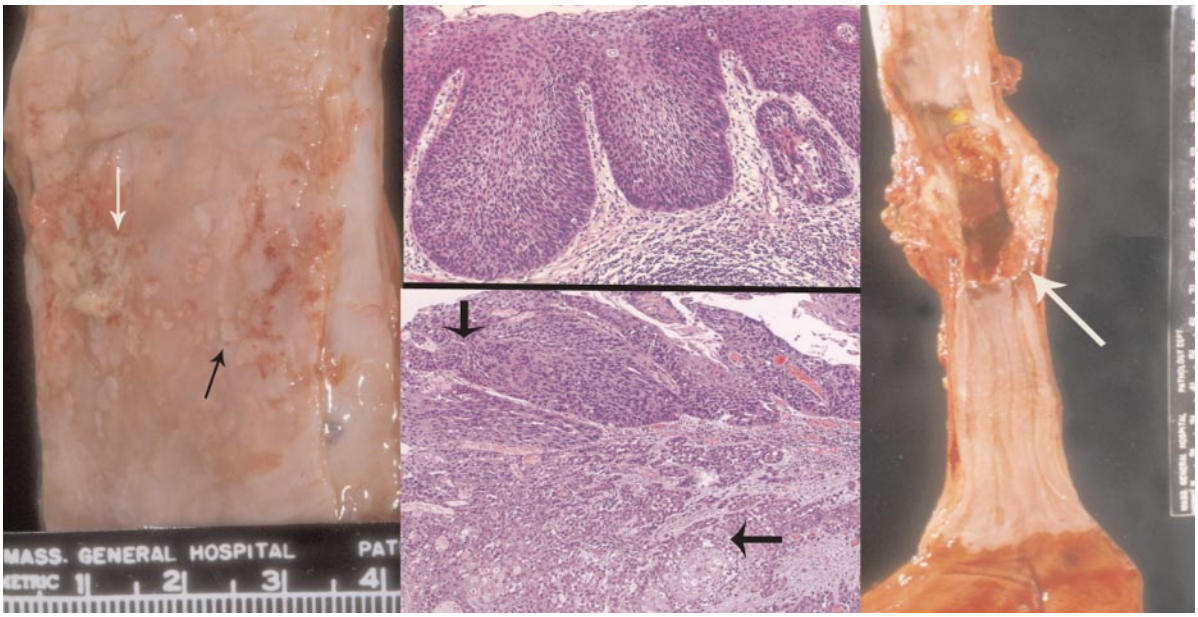

FIGURE 30-7 Squamous cell carcinoma of the esophagus. The esophagectomy specimen on the left shows esophageal squamous mucsoa with loss of the normal folds and with areas of nodularity and redness (*white arrow*) indicating areas of dysplasia and a raised irregular area (*black arrow*) of early carcinoma. The top photomicrograph shows severe squamous dysplasia with increased cellularity, nuclear enlargement, and loss of maturation (hematoxylin and eosin; ×200 original magnification). The lower photomicrograph shows intraepithelial carcinoma on the right half (*vertical arrow*), with invasive intramucosal carcinoma on the left (*horizontal arrow*) (hematoxylin and eosin; ×100 original magnification). The resection specimen on the right shows advanced squamous cell carcinoma of the midesophagus forming a deeply penetrating ulcer extending through the wall of the esophagus (*arrow* at lower margin of tumor edge) (see CD-ROM for color image).

Fistulization into the tracheobronchial tree and hemorrhage occur. Distant spread is a late phenomenon. Surgical resection is often now delayed until after chemoirradiation, although the results are inconclusive thus far.[62,72]

Malignant Melanoma. Melanocytes are normally found in small numbers in the esophagus, more numerous distally, and malignant melanoma of the esophagus is reported in young adults.[73] A single case report of malignant melanoma in an 8-year-old boy revealed an aggressive melanin-producing tumor.[74] Whether this case represented a melanoma deriving from mature melanocytes or a more primitive precursor, such as a primitive neuroectodermal tumor with melanocytic differentiation, is not entirely clear. Melanoma presents with dysphagia and usually forms a sessile polyp in the lower esophagus. The surrounding esophageal squamous mucosa may be heavily pigmented (melanosis). The tumors are often at a late stage at diagnosis, and curative therapy is rare. Metastasis from cutaneous melanoma to the esophagus must be considered, although the ileum is the most common site for secondary melanoma.

Nonepithelial Esophageal Malignant Tumors

Esophageal Sarcomas. These are very rare; most are the focus of case reports or small series, occasionally arising in the radiation field after treatment of mediastinal hematopoietic malignancy (Figure 30-8). Rhabdomyosarcoma, malignant schwannoma, leiomyosarcoma, and carcinosarcoma are reported. Most present with dysphagia, and a large submucosal mass, often with ulceration and necrosis, is found on endoscopy.[75,76]

Gastrointestinal Stromal Tumor. This tumor, arising from the interstitial cells of Cajal and uniformly expressing the KIT protein (a tyrosine kinase receptor) (CD117), has not been reported in the pediatric age group. However, germline mutations of the *KIT* gene are associated with a younger age of gastrointestinal stromal tumor; in these families, there is a theoretic risk of gastrointestinal stromal tumor in the pediatric group.[27]

Leiomyosarcoma. This tumor has not been reported in the pediatric age group. In adults, this is the rarest of the esophageal soft tissue tumors.[76] Uniformly aggressive and bulky, these tumors express smooth muscle markers (smooth muscle actin [SMA]), not CD117.[31,77]

Conclusion

Although tumors of the pediatric esophagus are rare, they are likely to increase in number if the current increase in esophageal adenocarcinoma continues. Guidelines for assessing pediatric patients with GERD for Barrett esophagus may emerge, as may consensus regarding surveillance for dysplasia. Benign tumors show different patterns than in the adult esophagus, and underlying pathophysiologic processes are more likely to be apparent.

STOMACH

Although more frequent than their esophageal counterparts, gastric neoplasms are uncommon in the pediatric population. Reviewing the charts of 4,547 pediatric cancer patients admitted over 44 years, Bethel and colleagues reported only three neoplasms arising in the stomach, making the relative incidence of primary gastric neoplasms 0.06%.[78] Although malignancies, particularly lymphomas and sarcomas, are the most frequent neoplasms, a number of benign tumors can occur, either inflammatory or malformative in nature.[78–81] Because they belong to the differential diagnosis of gastric neoplasms, these tumor-forming lesions are also reviewed in this chapter.

Benign Tumors of the Stomach

Although uncommon, benign gastric neoplasms, either epithelial or stromal in nature, can be observed in children.

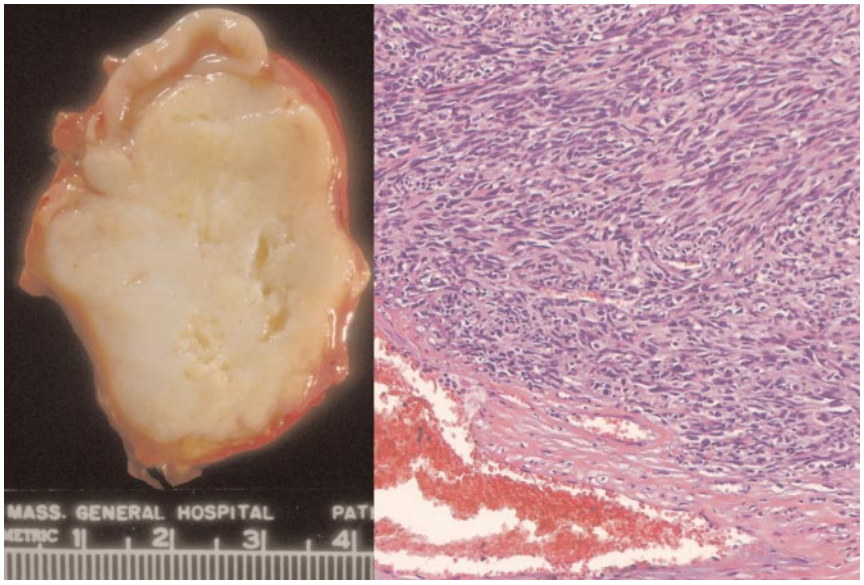

FIGURE 30-8 Esophageal sarcoma. Fibrosarcoma following irradiation forms a rubbery, well-circumscribed mass deep in the wall of the esophagus, with intact overlying mucosa. The photomicrograph shows a cellular spindle cell proliferation without anaplasia with a pushing rather than an infiltrative edge (hematoxylin and eosin; ×100 original magnification).

Gastric Polyps. Sporadic or more common manifestations of a polyposis syndrome, polyps are found less frequently in the stomach than in the lower gastrointestinal tract. They can be of several different types: adenomatous, fundic gland type, juvenile, or hamartomatous. Familial adenomatous polyposis is the most common polyposis syndrome of childhood.[82] The patients frequently develop multiple gastric polyps. Fundic gland–type polyps are found more frequently than adenomatous polyps. Fundic gland polyps have recently been shown to be neoplastic in nature, with frequent somatic mutations of the adenomatous polyposis coli (*APC*) gene.[83] However, the development of carcinoma in these lesions remains rare and usually occurs beyond the pediatric age range. However, one case of adenocarcinoma has been diagnosed in a 16-year-old familial adenomatous polyposis coli (FAPC) patient.[84–88] Gastric adenomas should be completely excised, and the entire stomach should be examined carefully. Endoscopic follow-up should be initiated because there is a relatively high risk of developing new adenomas. Compared with sporadic cases, fundic gland polyps and adenomas associated with FAPC occur at a much younger age. They increase in prevalence with increasing age. Given the risk, regular surveillance is indicated in patients with FAPC.

Peutz-Jeghers Syndrome. Forty percent of Peutz-Jeghers patients present with gastric polyps.[82] These hamartomatous polyps are usually silent, although rare presentations may occur, such as antral obstruction or, in one case, autoamputation. The polyps preferentially affect the antrum. Histologically, the polyps display an arborizing framework of smooth muscle covered by hyperplastic fundic– or antral-type mucosa with elongation and cystic change of foveolar epithelium with frequent atrophy of the underlying glands.[89] The risk of malignancy, via the development of dysplasia, is low but not nonexistent, with gastric adenocarcinomas reported as early as the second decade.[82,90,91]

Juvenile Polyposis. This type of polyposis is distinguished by its early clinical manifestations, with three-quarters of the affected patients presenting during childhood.[82,91] Among the different forms of juvenile polyposis, the stomach is involved in generalized juvenile polyposis (with a reported involvement of 13.6% of the cases) and in the usually fatal juvenile polyposis of infancy typified by the diffuse involvement of the gastrointestinal tract, with patients developing severe diarrhea, hemorrhage, and protein-losing enteropathy.[90–92] Juvenile polyposis of the stomach is limited to that site. Most sessile hamartomatous polyps measure between 5 and 40 mm. They are characterized by large cystic spaces lined by foveolar epithelium and embedded in the lamina propria with mixed inflammatory infiltrate.[89,92] The risk of malignant transformation in the stomach is lower than in the colon.[93] Dysplasia can sometimes be found. It is therefore reasonable to suggest that patients with juvenile polyposis should undergo periodic upper endoscopy. In addition to the colon and stomach, patients with juvenile polyposis have an increased risk of developing adenocarcinomas in the biliary tract and pancreas.

Gastric Teratoma. This rare tumor is composed of mesodermal, endodermal, and ectodermal elements and occurs almost exclusively in the pediatric population. The pathogenesis of gastric teratomas is unknown, but they are thought to arise from pluripotential cells. Nearly all of the patients are male, and most are either infants or neonates less than 2 years of age.[94,95] The patients usually present with large intra-abdominal masses that may lead to obstruction, whereas younger patients may present with oratory insufficiency because of the limitation of movements of the diaphragm secondary to tumor displacement.[95] Upper gastrointestinal bleeding, resulting from ulceration of the overlying mucosa, can also be observed. Characteristically, preoperative imaging studies may demonstrate calcifications corresponding to teeth or bone structures developed within the tumor. The histology demonstrates intermixed mature tissue elements such as skin, smooth muscle, bone, cartilage, and adipose and neural tissue. With the exception of a single case reported in an adult, no malignant transformation is available in the pediatric literature, and excision is curative.[94]

Malignant Tumors of the Stomach

Adenocarcinoma. Between 2 and 10% of gastric carcinomas are diagnosed in patients younger than 40 years old, and cases seen in the pediatric population are extremely rare.[96,97] Also, the marked variations in the incidence of cancer found in adults between different regions with high rates (Asia, Central and Eastern Europe, South America) and Western nations are not encountered in the pediatric population either.

In a series of 501 gastric cancers in individuals younger than 31 years of age, only 0.4% of cases occurred in children 10 years or younger, 3.4% occurred in children between 11 and 15 years, and 8% occurred in children between 16 and 20 years.[98] A study of 3,079 cases of gastric carcinoma diagnosed in British Columbia over a 10-year period revealed that only 65 cases occurred in younger individuals (under 40 years of age) and that the youngest patient was 24 years old. No pediatric cases were reported in this series. Interestingly, the younger patients exhibited more adverse clinical and pathologic features compared with older patients.[99] In fact, most publications are case reports, with a case diagnosed as early as 20 months of age.[96,97, 100]

In contrast to the adult population, the risk factors for the development of gastric cancers in childhood are not well established. Approximately 10 to 25% of young gastric cancer patients have a positive family history, suggesting the role of genetic factors.[101] To the best of our knowledge, the cases associated with the familial diffuse gastric carcinoma syndrome associated with germline mutations in the E-cadherin/*CDH1* gene have been diagnosed in young adults but not in pediatric patients.[102–105] *Helicobacter pylori* infection associated with the development of adult gastric cancer is apparently not reported in pediatric cases. Other precursor lesions, such as intestinal metaplasia, pernicious anemia, and hypertrophic gastropathy, as well as previous gastrectomy, are also rare in this population.

However, several conditions that occur in infancy and childhood have been linked with gastric cancer. These include immunoglobulin A deficiency, common variable immunodeficiency syndrome, and ataxia telangiectasia.[106–108] A rare association with Rothmund-Thomson syndrome (characterized by short stature, cataracts, pigmentation of skin, baldness, and abnormalities of bones, nails, and teeth) has also been reported.[109] Polyposis syndrome, that is, familial polyposis coli, and Peutz-Jeghers syndrome are associated with the development of adenocarcinoma, although it usually develops later in life. Finally, two cases of gastric adenocarcinoma have also been reported as a late complication in individuals previously treated (3.5 years and 10 years earlier) for abdominal lymphoma by irradiation and chemotherapy.[110]

In many instances, the presenting symptoms are not different from those of older patients.[100] The most common presenting complaints include pain and vomiting, followed by anorexia and weight loss.[101,111] Abdominal distention is also reported.[100,112] A mass can be palpated in 70% of the patients according to one series.[98] A rare association with tumor-thrombotic microangiopathy has been reported.[113]

Although the histologic pattern of gastric cancer in childhood can be similar to that in the cases reported in adults, some authors report a predominance of mucinous and signet ring cell carcinoma (Figure 30-9).

Because there are no large series of children with gastric carcinoma, it is not possible to draw significant conclusion with regard to prognosis. Despite anecdotal reports of cure, the evidence provided suggests that a delay in diagnosis usually contributes to limited patient survival.[101] Various protocols, usually based on experience in adult patients, include surgery and various chemotherapy protocols with or without radiotherapy. Lymphatic, vascular, direct extension and seeding of peritoneal surface have been reported.[81,100,101,112–114]

Hematopoietic Neoplasms. ***Gastric Lymphoma.*** In the adult population, the stomach is the most common site of gastrointestinal lymphomas, comprising about 40% of the cases, followed by the small bowel in 27% of cases.[115] Conversely, in children, the small bowel is the predominant site, accounting for 40 to 45% of cases, whereas gastric lymphoma represents between 2.5 and 17% of gastrointestinal non-Hodgkin lymphomas.[115–118] The reported increase in the annual incidence of gastrointestinal lymphoma observed in adults has not been observed in the pediatric population.[115]

Predisposing conditions for gastric lymphoma include primary immunodeficiency such as severe combined immunodeficiency, X-linked agammaglobulinemia, common variable immunodeficiency disease, Wiskott-Aldrich syndrome, and ataxia telangiectasia.[119] Also, B-cell lymphomas, which account for the most frequent neoplasias in human immunodeficiency virus (HIV)-infected patients, also frequently involve the stomach.[120]

The mode of clinical presentation may include nonspecific symptoms such as abdominal pain, a palpable abdominal mass, or gastrointestinal bleeding. Gastric outlet obstruction by a lymphoma has been reported (Figure 30-10).[121] Most gastric lymphoma tumors are cytologically high-grade malignancies, either the lymphoblastic or large cell anaplastic type.[115,116] Primary treatment includes

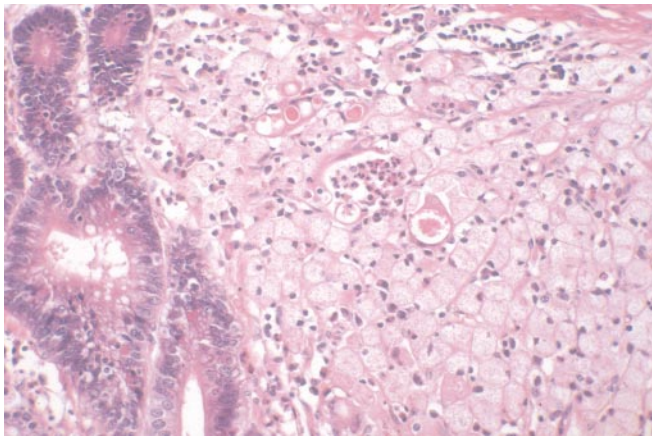

FIGURE 30-9 Diffuse-type gastric carcinoma in a 17-year-old patient. The tumor is composed of single cells expanding the lamina propria. Rare signet ring cells can be seen (hematoxylin-eosin stain; ×400 original magnification).

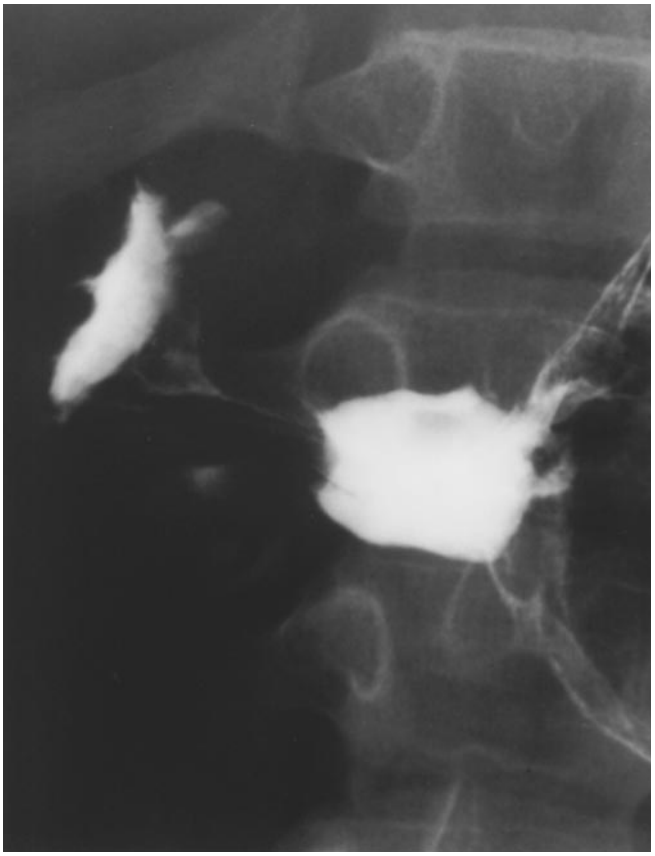

FIGURE 30-10 Selective view of the antrum from an upper gastrointestinal series in a 15-year-old boy who presented with hematemesis, weight loss, and right upper quadrant abdominal pain. A mass is seen encircling the antrum and almost completely obstructing it. Endoscopic biopsies revealed a large cell immunoblastic lymphoma. Courtesy of the Teaching Collection, Department of Radiology, The Children's Hospital, Boston.

resection of the tumor, followed by postoperative chemotherapy and/or radiotherapy. Careful medical and surgical management following prompt diagnosis have been shown to offer long-term survival.[116]

Langerhans Cell Histiocytosis. Langerhans cell histiocytosis of the stomach has been reported in a 14-year-old girl.[122] Besides its rarity, the diffuse antral and fundic polyposis and the granulomatous pattern displayed by the histiocytosis are noticeable.[122] To date, only a handful of cases with gastric involvement have been reported, all but one in adults.[123–125] In the case in point, the patient remained asymptomatic at 10 months after diagnosis.[122]

Mesenchymal Neoplasms. ***Gastrointestinal Stromal Tumor.*** Gastrointestinal stromal tumors are rare, accounting for less than 1% of all gastrointestinal malignancies. They are uncommon before middle age and extremely rare in children.[126,127] Nowadays, gastrointestinal stromal tumors are defined as spindle and/or epithelioid mesenchymal neoplasms that usually express CD117 and do not have diagnostic features of any other type of mesenchymal tumors.[126,128] Most neoplasms now included in this category would have been previously diagnosed as smooth muscle tumors (leiomyoma, leiomyoblastoma, leiomyosarcoma, fibromatosis, and schwannomas). However, the massive amount of information collected on gastrointestinal stromal tumors has been based on the adult experience. Whether it is true for the pediatric population is unknown. Bates and colleagues recently reported that congenital stromal tumors of the stomach in children were morphologically similar to those in adults but did not express CD117 and carried a favorable prognosis.[129] However, other cases reported in the pre-CD117 era have followed a course similar to that seen in adults.[109]

Although, in most cases, they occur sporadically, some gastrointestinal stromal tumors have been described as a component of the Carney triad (gastric epithelioid stromal sarcoma, functioning extra-adrenal paraganglioma, and pulmonary chondroma).[130] Rare myogenic tumors have been reported in acquired immune deficiency syndrome (AIDS) patients. Their pathogenesis is unknown, but the initiating role of HIV, as well as that of Epstein-Barr virus, has been entertained.[120,131]

It has been shown that gastrointestinal stromal tumors share phenotypic similarities with the interstitial cells of Cajal, including the expression of KIT and CD34.[126,132] These pacemaker cells are now considered to represent the origin of these rare neoplasms. Another important biologic breakthrough has been the demonstration that most gastrointestinal stromal tumors have oncogenic mutations of the KIT gene. This has been translated clinically by the use of an inhibitor of the tyrosine kinase activity of Kit (ST-571), which has shown significant promise in treating patients with metastatic disease.

Most gastrointestinal stromal tumors are diagnosed in the stomach (60–70% of the cases). The majority measure between 3 and 15 cm, although tumors as small as a few millimeters and as large as 30 to 40 cm can be observed. Gastrointestinal stromal tumors may present as intraluminal or subserosal masses that may compress regional adjacent organs (Figure 30-11).[128] Depending on their size, various nonspecific clinical presentations (ie, nausea, vomiting, abdominal pain, bleeding) may be observed. The most common presenting symptoms of gastrointestinal stromal tumors in children are gastrointestinal bleeding (with frank hematemesis in about 40% of patients and occult bleeding in up to 80%) and symptoms of intestinal obstruction (in about 50% of patients). Abdominal pain, weight loss, fever, and abdominal masses also are reported.[126–129,133] Gastrointestinal stromal tumors present as firm, tan, well-circumscribed masses (Figure 30-12). Hemorrhage, necrosis, and cystification are seen in large tumors. They are variably cellular and composed of spindle cells and/or epithelioid cells. The spindle cells can be organized in fascicles, storiform and herringbone arrangements, or palisading or organoid groupings (Figure 30-13).[133]

The microscopic characteristics of gastrointestinal stromal tumors are poor indicators of their clinical behavior. A recent workshop sponsored by the National Institutes of Health concluded that the benign and malignant behavior of a tumor could not be predicted on the basis of morphology alone. It is now recommended that all gastrointestinal stromal tumors be considered potentially malignant and be classified according to their risk of aggressive behavior based on the size and the number of mitoses in 50 high-power fields.[128]

Miscellaneous. ***Gastric Hemangioma.*** Visceral hemangiomas are rare outside the liver. In the stomach, although

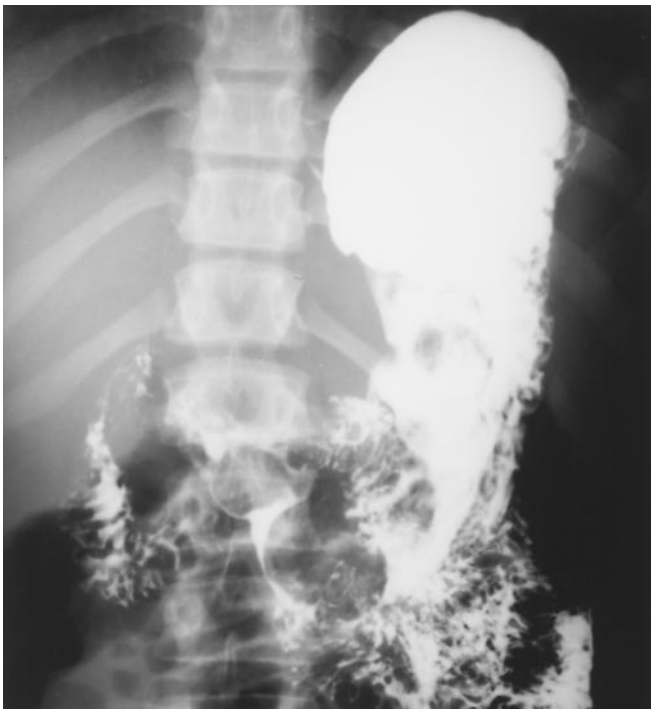

FIGURE 30-11 Upper gastrointestinal series in a 10-year-old girl with severe anemia and tarry stools. A large nodular leiomyosarcoma is seen in the gastric antrum. Courtesy of the Teaching Collection, Department of Radiology, The Children's Hospital, Boston.

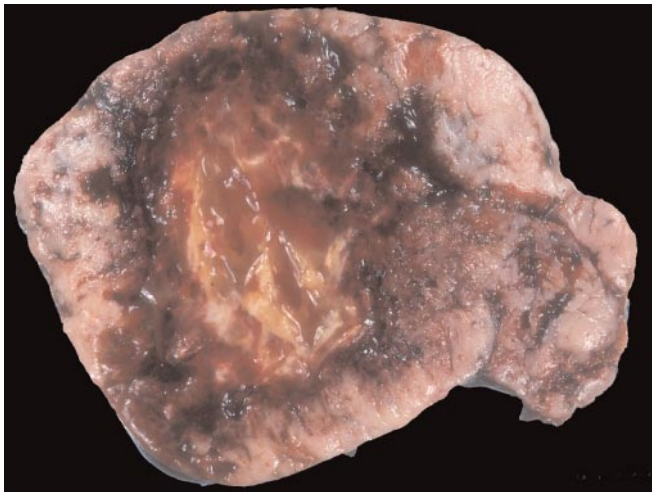

FIGURE 30-12 Gastric gastrointestinal stromal tumor. The tumor is well circumscribed but not encapsulated. Note the central necrosis, characteristic of a malignant biologic behavior.

hemangiomas can be isolated, they are frequently associated with vascular lesions of the skin and intestine.[134–136] The cases associated with gastrointestinal hemangiomatosis tend to occur at an earlier age, with cases discovered in the neonatal period.[136] Hematemesis is a frequent initial symptom. Despite their benign nature, these sometimes large hemorrhagic masses require surgical therapy, ranging from wedge excision to partial and even total gastrectomy.[136]

Gastric Lipoma. Composed of lobules of mature adipose tissue, gastric lipomas are slow-growing tumors. They frequently originate from the submucosa, whereas others are centered on the subserosa. The common antral location of these usually sessile and polypoid lesions accounts for the reports of intussusceptions into the pylorus or duodenum.[137] Mucosal ulceration with chronic blood loss but also hemorrhage has been reported.[138] On barium swallow, gastric lipomas change shape with peristalsis and show a preserved mucosal profile, both characteristics of a benign process.

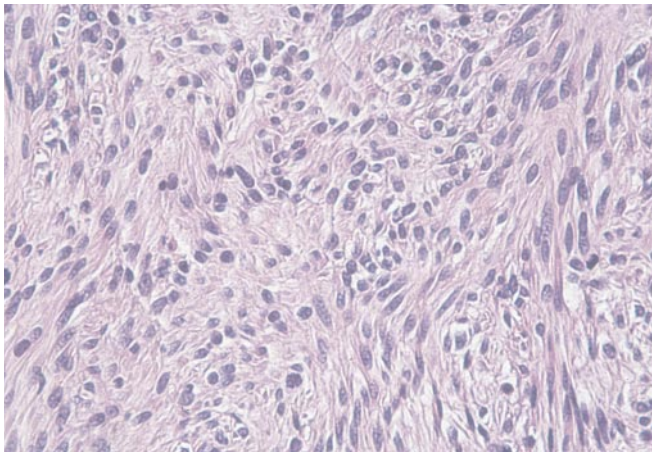

FIGURE 30-13 Gastric gastrointestinal stromal tumor. Photomicrograph with typical fascicular arrangement of spindle cells. Scattered mitoses can be seen (hematoxylin and eosin; ×400 original magnification).

Magnetic resonance imaging shows, on T_1-weighted images, a solid hyperintense lesion with a signal corresponding to fat. Finally, on endoscopy, the typical "sinking impression" is felt when the mass is poked with forceps.[137] If small, gastric lipomas can be removed by polypectomy, but larger lesions may necessitate a laparotomy.[138]

Inflammatory Myofibroblastic Tumor. Despite much interest in the pathology literature, the pathogenesis of this unusual process, also known as inflammatory pseudotumor and plasma cell granuloma, remains indeterminate.[139] It preferentially affects children and young adults, and most cases are reported in the lungs, whereas only rare cases have been seen in the stomach. Microscopically, these tumors show a mixed pattern composed of a variable number of spindle-shaped myofibroblasts embedded in a usually collagenized stroma with intermixed chronic inflammatory cells in which plasma cells may be numerous. The spindle cells are CD117 negative and usually positive for SMA.[139] Although usually benign, large-size tumors and invasion into surrounding tissues have characterized the aggressive nature of rare cases. Such cases may require several surgical resections.[140]

Gastric Hamartoma. Hamartomas of the gastric wall are benign lesions showing considerable histologic variation. They are composed of abnormal admixture of components of the gastric wall, including hypertrophied bands of muscularis mucosa branching out and dissecting through the mucosa that shows misplaced and cystically distended glands.[141] They should be differentiated from the adenomyomatous variant of ectopic pancreas, which is formed of cystic pancreatobiliary type ducts.[118,142]

Gastric Leiomyosarcoma. Leiomyomas and leiomyosarcomas have been reported in association with Alport syndrome, AIDS, pulmonary osteoarthropathy, and Carney triad.

Rare Mesenchymal Lesions of the Stomach. A distinctive malignant gastric mesenchymal tumor sharing some features with clear cell sarcoma of soft part has been reported in a 13-year-old boy.[143] This type of tumor, also found in the small bowel, displays a distinct nesting pattern formed by medium-sized tumor cells with a clear to acidophilic cytoplasm admixed with osteoclast-like multinucleated cells. Strong positive immunohistochemistry for S-100 protein and t(12;22)(q13;q12) also supports the similarities with clear sarcoma of soft part.[143] A case of gastric rhabdomyosarcoma with disseminated metastases and death within $2^1/_2$ months following diagnosis has been reported.[81] Only a few cases of hemangiopericytoma have been observed in the stomach. The younger patient was 2 days old when he developed hematemesis and had surgery at day 12. Although these uncommon vascular tumors have the potential to behave in a malignant fashion, metastasis of gastric neoplasms seems to be rare, perhaps because of the usual early diagnosis and treatment.[144] Although less frequent than in adults, Kaposi sarcoma has also been reported to affect the stomach of pediatric AIDS patients.[120]

Conclusion

Given their rarity, gastric tumors in childhood are almost always unexpected findings. Nevertheless, the differential

diagnosis of upper gastrointestinal symptoms in childhood must include these rare lesions. Their diagnosis is of obvious clinical importance because, in many cases, timely treatment is the only hope for good prognosis.

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CHAPTER 31

MOTOR DISORDERS INCLUDING PYLORIC STENOSIS

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The movement of bowel contents from one specialized region of the gut to another occurs as a result of the coordinated contraction of the smooth muscle coats. In this chapter, the present state of knowledge of motor activity of the stomach and duodenum in the infant and child is summarized and conditions in which motor activity is disordered are discussed. Despite investigation spanning the last century, knowledge derived from systematic scientific observation in this area remains scarce, and our understanding of the pathophysiology of human gastroduodenal motility in the infant and child is far from complete. However, the practical application of such knowledge as exists is beginning to affect the practice of clinical pediatric medicine.

NORMAL MOTOR ACTIVITY OF THE STOMACH AND DUODENUM

The pattern of contractions that occurs in a particular region of the gut is related to and integrated with the function of that region of the gastrointestinal (GI) tract. In the case of the stomach, the organ can be divided into two areas. The proximal half acts as a reservoir, exhibiting mainly tonic activity, which allows for large changes in intragastric volume. The process of digestion is initiated here with the secretion of acid and pepsin. The distal region includes the lower half of the body, the antrum and the pylorus. Contraction here is phasic and organized to segment and break up food into small particles or to impart movement. To empty food from the stomach, gastric and duodenal contractions are coordinated, resulting in movement of luminal contents in an aboral direction. When digestible food has been emptied from the stomach, the digestive phase ends and the fasting or interdigestive phase begins. Any remaining gastric contents are swept into the duodenum by bursts of forceful, rhythmic contractions, phase III of the fasting state, in the antrum and the duodenum.

The process of digestion is continued in the duodenum, where pancreatic and biliary secretions are added to the chyme. Two patterns of motor activity occur: (1) when the duodenum is receiving chyme after feeding, continuous segmenting activity occurs to ensure maximal mixing of the intraluminal contents and exposure to the mucosa; (2) in the fasting or interdigestive state, when the lumen is devoid of nutrients, a band of forceful contractions is propagated in an aboral direction, sweeping exhausted chyme down the gut (Figure 31-1).

SPECIFIC PATTERNS OF GASTRODUODENAL MOTILITY

Proximal Stomach

Proximal Receptive Relaxation. Little contractile activity occurs in proximal regions of the stomach, where the predominant activity is receptive relaxation[1]; on swallowing, the proximal muscle relaxes to accommodate the ingested food. This mechanism is so efficient that the average full-term infant can accommodate 60 to 70 mL of feed with a rise in intragastric pressure of only 5 mm Hg. Receptive relaxation is mediated by a vagal reflex. A number of putative neurotransmitters have been studied, and most recent evidence favors vasoactive inhibitory polypeptide and nitric oxide (NO) as the nonadrenergic, noncholinergic inhibitory transmitters in this region of the stomach.[2]

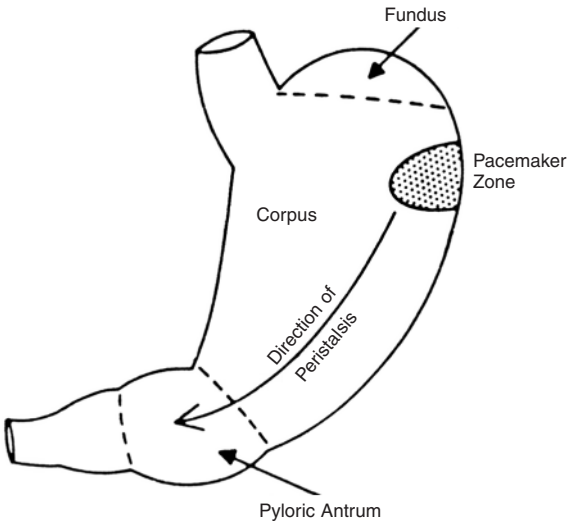

FIGURE 31-1 Regions of the stomach.

Tonic Contraction. Following ingestion of a meal, the proximal region of the stomach exhibits weak tonic contractions, which may play a role in passing intragastric contents to the antrum and pylorus. Vagal and intrinsic cholinergic factors are believed to be important in stimulating such a response.

Distal Stomach and Duodenum

Postprandial or Digestive Activity. The caudal or distal region of the stomach behaves quite differently from the proximal region, and, together with the duodenum, a separate pattern of activity occurs according to whether a meal has just been eaten or the individual is fasting. After a meal, rhythmic, phasic contractions that begin near the middle of the stomach are propagated toward the duodenum. The antral waves (about three per minute in humans) are constant in terms of frequency and propagation velocity and are determined by a pacemaker region on the greater curvature of the stomach (see Figure 31-1). The timing and spread of the contractions are regulated by the slow-wave activity of the smooth muscle cells and the pacemaking activity of the interstitial cells of Cajal.[3] Action potentials spread from the midstomach to the duodenum but not to the fundal region of the stomach, ensuring that propagation of contractions with a maximum frequency and velocity of the action potentials occurs only in the aboral direction. The occurrence, timing, amplitude, and spread of gastric contractions depend on the integrity of the gastric muscle, its innervation, the activity of the interstitial cells of Cajal, and the humoral environment of the smooth muscle cells.

These seemingly simple peristaltic contractions of the gastric body and antrum cause fairly complex movements of the intragastric contents, which result in mixing of the contents, mechanical breakdown of food particles, and gastric emptying. The antrum and pylorus are the sites of regulation for the emptying of solids. When a mixed meal is consumed, liquids empty more rapidly than solids, which are retained by antral contractile activity and passed through the pylorus only when they have been broken down into particles of approximately 1 mm.[4,5]

When contraction begins, gastric contents are propelled toward the duodenum, but only a small amount enters the duodenum. As a consequence, pressure rises in the antrum and provides a continued driving force across the pylorus. As the pressure falls, retropulsion may occur, and the consequent mixing and shearing of flows that take place cause food particles to be broken down. Gastric emptying also occurs during the contractions, but the amount emptied depends on the peristaltic contractions, tone of the proximal stomach, and contractile activity of the pylorus and duodenum. In the digestive phase, contraction of the pylorus and proximal duodenum is highly coordinated, up to 70% of antral contractions being linked to groups of one to three sequential duodenal contractions separated by periods of relative quiescence. This pattern of activity seems to be important for gastric emptying and is modulated not only by neural factors but also by nutrients via paracrine and endocrine polypeptide hormones.[1] That both the nature of a meal and polypeptide hormones influence gastric emptying seems to be beyond doubt from a number of experimental studies in animals[1] and the human infant.[6,7] In the infant, it is clear that increasing caloric density of feeds, with either carbohydrate in the form of glucose polymers versus monosaccharides or lipids in the form of long-chain triglycerides or medium-chain triglycerides, results in slower emptying.

Fasting or Interdigestive Activity. Toward the end of fed activity, contractions become more forceful and propel any remaining undigested food into the duodenum, leaving the stomach empty. The pattern of activity then changes to that seen in the fasted state in both the stomach and duodenum. In the interdigestive period, the motor activity of several regions of the GI tract has a characteristic, specific, integrated cyclic pattern. This cyclic pattern of fasting activity was first shown in dogs by Szurszewski in 1969,[8] and, for the most part, coordination of contractions between the antrum and the duodenum does not occur. The pattern is characterized by what he termed a "migrating myoelectric complex" (MMC).

It was not until 1975 that the first demonstration of the MMC in humans was published.[9] At intervals of approximately 100 minutes, brief periods (about 5 minutes) of rhythmic contraction recur at the frequency of the slow wave of 3 cycles per minute in the antrum and 11 cycles per minute in the duodenum. These episodes of rhythmic contraction, phase III activity, are preceded by a period of irregular contraction (phase II activity) in which about 10% of antral contractions are coordinated with those in the duodenum and are followed by a period of quiescence (phase I activity). Phase III activity migrates relatively slowly down the entire small intestine from the antrum and duodenum to the terminal ileum, taking about 90 to 120 minutes to migrate; a new complex usually arises proximally as one dies away distally. However, some cycles may begin distal to the duodenum, and others may fail to progress down the complete length of the small intestine. Fenton and colleagues demonstrated similar patterns in children[10]; these differ from those in adults only in the very young, in whom propagation velocity is slower and the duration of phase III activity is longer.[11] The purpose of the MMC is to prevent the aboral migration of colonic bacteria[12] and to cleanse the intestine of cellular debris, residual food, and secretions. These effects are aided by the increased gastric acid, biliary, and pancreatic secretion at the start of phase III activity. Studies in both humans and experimental animals have shown phase III activity to be highly propulsive.

Like fed activity, fasting activity requires the integrity of smooth muscle interstitial cells of Cajal, innervation, and humoral secretion. In the stomach, extrinsic innervation seems to be much more important than in the jejunum or ileum, and motilin surges are associated with phase III activity.[13]

DISORDERS OF GASTRODUODENAL MOTOR ACTIVITY

Motility disorders result in alterations of motor activity that are clinically significant and interfere with normal

receptive relaxation[14] and propulsive functions of the stomach and duodenum. These disorders, which alter the motor control mechanisms of the gut, may affect the muscle coats themselves, the intrinsic and extrinsic gut nerves, or the humoral environment of the neuromusculature. Most often no obvious anatomic abnormality is present, but hypertrophic pyloric stenosis clearly is an exception. Some disorders may be acute owing to transient metabolic changes or immunologic responses: in this chapter, only chronic conditions are considered. Motor dysfunction may be restricted to the stomach or duodenum or may be part of a more diffuse disease. Often the clinical presentation is regional (eg, recurrent vomiting), yet investigation reveals dysfunction in other regions of the gut.

Clinical Manifestations of Disordered Gastroduodenal Motility

Vomiting and epigastric discomfort are common symptoms of disordered foregut motility in children. Despite careful investigation, a diagnosis of organic disease is often not made. Table 31-1 lists the symptoms that may present, but none are very specific. In most cases, a diagnosis cannot be made on clinical history alone. The symptoms of delayed and accelerated gastric emptying have much in common, and the typical symptoms of dumping owing to hypovolemia and hypoglycemia occur in only a minority of cases. It is only when food from the previous day is vomited that seriously delayed gastric emptying is certain. Thus, gastroduodenal motor disorders often present with nonspecific clinical signs and symptoms. The obvious exception is hypertrophic pyloric stenosis, in which the character of the vomiting, the presence of visible gastric peristalsis, and a palpable pyloric tumor (see below) enable the clinician to diagnose the condition with sufficient certainty for surgery to be undertaken in a large proportion of patients.

Emesis

Surface electrogastrography has proven useful to differentiate vomiting owing to activation of the emetic reflex and that owing to gastroesophageal reflux (GER).[15–17] Activation of the emetic reflux results in an output that consists of two phases: a pre-ejection phase and an ejection phase. The pre-ejection phase is marked by a variety of autonomic responses, including nausea, skin pallor, sweating, and tachycardia. Nausea is due to a dysrhythmia of the gastric antrum and duodenum, which results in cessation of gastric emptying and bloating of the stomach. The pre-ejection phase is followed by ejection of the gastric contents by forcible contraction of the abdominal, diaphragmatic, and intercostal muscles. Activation of the emetic reflex may be initiated by a variety of different inputs to the central processing apparatus of the reflex, which includes the dorsal vagal nuclei and the chemoreceptor trigger zone in the area postema in the floor of the fourth ventricle. Inputs may be metabolic, as occurs with cytotoxic drugs, urea cycle defects, or lactic acidosis, or peripheral from the gut, such as in gastritis or enteropathies, visual flicker effects, and vestibular and auditory stimuli.[16,17] When recurrent activation of the emetic reflex occurs, the term cyclic vomiting is often used.

TABLE 31-1 SYMPTOMS OF DISORDERED GASTRODUODENAL MOTILITY

RAPID EMPTYING/DUMPING	DELAYED EMPTYING
Nausea, vomiting	Epigastric fullness
Abdominal cramps, diarrhea	Early satiety
Early satiety	Nausea, vomiting
Epigastric fullness	Pyrosis
Faintness	Belching
Pallor	
Sweating	

Rapid Gastric Emptying. Rapid emptying of liquids most frequently occurs following surgical procedures and in some patients with peptic ulcer. After both proximal and distal gastric resection, increased liquid emptying rates are seen. After proximal resection, the accommodating reservoir is lost, and after a meal, intragastric pressure rises. Following distal resection, resistance from the antropyloric region is lost. Vagotomy is rarely performed in childhood, but damage to the vagus may occur following esophageal surgery and have similar effects, which are discussed below.

Delayed Gastric Emptying. There are many causes of delayed gastric emptying, as shown in Table 31-2. Of course, it is always important to exclude anatomic causes such as hypertrophic pyloric stenosis, duodenal stenosis (both intrinsic and extrinsic), duodenal web, and malrotation of the small intestine causing duodenal obstruction. Some of these conditions are associated with disordered motor activity other than that caused by the obstruction in which either the smooth muscle or the innervation is diseased. Alternatively, the neuromusculature operates in an abnormal humoral environment usually created by an associated inflammatory process.

Duodenogastric Reflux. Bile-stained vomiting occurs in conditions in which there is obstruction beyond the second part of the duodenum and in conditions causing pseudo-obstruction of the duodenum and upper jejunum. Usually, when there is severe bilious vomiting and no anatomic obstruction is found, the pseudo-obstruction is neuropathic in origin.

DISEASES OF THE STOMACH AND DUODENUM

Hypertrophic Pyloric Stenosis

The grossly thickened circular muscle of hypertrophic pyloric stenosis results in a well-recognized clinical presentation of projectile vomiting and failure to thrive. This muscular hypertrophy is usually not present at birth but starts in the first few weeks of life.[14]

Incidence and Inheritance. This condition occurs 2.5 times more commonly in whites than in other ethnic groups. The incidence rose from 1 to 2 per 1,000 live births

TABLE 31-2 CAUSES OF DELAYED GASTRIC EMPTYING

Anatomic obstruction	Pyloric stenosis, duodenal stenosis, duodenal web
Metabolic or electrolyte disorder	Hypokalemia, acidosis, hypothyroidism
Drugs	Opioids, anticholinergics
Neuronal dysfunction	Central nervous system disease, vagotomy, intestinal pseudo-obstruction
Muscle disease	Visceral myopathy, systemic lupus erythematosus, myotonic dystrophy
Infection	Viral, microbial toxins (eg, *Rotavirus*, *Parvovirus*)
Idiopathic	Slow-wave arrhythmias

in the early 1950s to 6 to 8 per 1,000 live births some 30 years later. The cause for this increase in incidence is not clear, but in the United Kingdom, it correlates positively with a 30% increase in breastfeeding.[18] The natural history of the condition is for vomiting to continue into the third month of life, after which survivors recover. Pyloric stenosis is more common in firstborn children. Males are affected five times more frequently than females. Inheritance is polygenic, and both siblings and the offspring of affected children are at increased risk. The risk is highest (approximately 20%) in the firstborn male of a mother who herself was affected.[19]

Etiology and Pathogenesis. Although the etiology is not known and the nature of the pathologic process is not clear, it is now clear that the stenotic pylorus is not present congenitally.[20] There is an increasing body of evidence that the local enteric innervation is involved[21] and that primarily argyrophilic NO synthase–containing neurons[22,23] are affected.

Study of neuronal NO synthase knockout mice implicates the genetic control of neuronal NO synthase in the etiology of pyloric stenosis. Transcriptional control of neuronal NO synthase is complex, with nine first exons.[24] Exon 1c is particularly important in the gut,[25] and it appears that in pyloric stenosis, there is overexpression of exon 1c (H. D. Allescher, personal communication, 2003). Another study suggested that there is also profound abnormality of development of the network of interstitial cells of Cajal within the muscle layers in pyloric stenosis.[26] Stem cell factor also appears to be defective. Taken together, these findings suggest that it is more likely that the developmental abnormality of the circular muscle layer occurs as a consequence of the defective nitrergic innervation, although how this comes about is still unknown, but bone morphogenetic protein 4 appears to be involved.[27] In most cases, hypertrophic pyloric stenosis can be thought of as an isolated pseudo-obstructive lesion. In some situations, however, it is part of a more generalized pseudo-obstructive condition and is associated with bowel malrotation and short small intestine.[28] Pyloric stenosis is increasingly recognized in association with other gut anomalies, such as esophageal atresia, duodenal atresia, anorectal anomalies, Cornelia de Lange syndrome, Smith-Lemli-Opitz syndrome, and Zellweger syndrome. In about 15% of cases of pyloric stenosis, a hiatal hernia and GER are present.[29] Whether this abnormality is a consequence of the obstruction or whether the neural abnormality is not restricted to the pylorus is not clear.

Clinical Features. These patients present with vomiting, which is never bile stained but frequently contains stale milk. Vomiting usually begins in the second or third week of life, but it may present earlier or much later. The vomiting becomes increasingly forceful and copious until it is projectile. Initially, the infant is irritable and hungry, with increasing malnutrition; however, the baby becomes miserable and lethargic. Characteristically, the early part of the feed is taken eagerly, but as the stomach fills, the baby starts to become anxious and fretful, visible peristalsis may be easily seen (it is presumed that the baby perceives this prior to vomiting), and then vomiting occurs, which is often projectile in nature. A pyloric tumor is then usually easily palpable immediately before the vomiting. The other clinical features are the consequence of gastric outlet obstruction with loss of gastric secretions leading to constipation, dehydration, and metabolic alkalosis.

The profuse and continuous loss of acidic gastric contents results in the loss of protons and chloride and hypochloremic alkalosis. When proton loss becomes severe, hypokalemia may also occur. Jaundice occurs in association with pyloric stenosis in 2 to 5% of cases, and, as in some infants with neonatal bowel obstruction, there is an unconjugated hyperbilirubinemia in up to 50%.[30] This jaundice has been associated with low levels of glucuronyl transferase.[30]

When there is doubt about the diagnosis, radiologic or ultrasonographic studies should be undertaken. A plain film often shows pyloric hold-up and a dilated stomach with absent gas distal to the pylorus. Ultrasonographic studies confirm the presence of a pyloric tumor and barium studies the long, narrow pyloric canal. The presence of GER should be sought because it may influence postoperative management.

It has been suggested that ultrasonography is the diagnostic imaging of choice. Diagnostic criteria have been developed and include a muscle thickness of more than 4 mm and a pyloric length of more than 16 mm. Using these criteria, ultrasonography has a specificity of 100%, a sensitivity of 97%, and positive and negative predictive values of 100% and 98%, respectively.[31]

Management and Prognosis. Virtually all major centers advocate surgical treatment for pyloric stenosis. Pyloromyotomy is the operative intervention of choice. Most infants are referred for surgery before marked biochemical disturbances have occurred and require no special preparation other than a gastric washout 2 to 3 hours prior to operation. However, if dehydration and metabolic alkalosis are present, they must be corrected first and surgery delayed for 24

to 48 hours. Infants with moderate to severe fluid and electrolyte depletion with elevated serum bicarbonate levels should be given 5% dextrose in 0.45% saline initially as a bolus of 10 to 20 mL/kg and then at one to two times maintenance as required until serum electrolytes approach normal and dehydration is corrected. Twenty-five to 40 mmol of potassium chloride should be added to each liter of fluid once the infant is passing urine. Correction of alkalosis prior to surgery is essential to avoid postoperative apnea owing to alkalemic respiratory depression.

The surgical treatment of pyloric stenosis has a morbidity of between 1% and 5%, a recurrence rate of 1 to 3%, and a mortality of less than 0.5%.[29] There are two well-recognized postoperative problems: continued signs of pyloric obstruction owing to an incomplete myotomy and wound dehiscence.[29] The former frequently settles with conservative management, so several days should elapse before re-exploration. The latter is probably due to malnutrition rather than surgical technique. In about 15% of patients, vomiting continues owing to the associated GER; usually, only a small proportion require further surgery and fundoplication.[29]

Idiopathic Gastroparesis

Gastroparesis may develop in apparently healthy children without evidence of systemic disease. The symptoms may be acute, may be preceded by a flu-like illness, or may be part of a gastroenteric infection. The abnormality may be confined to the antrum, with impairment of emptying of solids but not liquids. There have also been several case reports of patients with arrhythmias of gastric electrical control activity (ECA) and idiopathic gastroparesis. Symptoms may be mild, with early satiety, nausea, and occasional vomiting, or severe, with uncontrollable nausea and vomiting.

Gastric Antral Dysrhythmias

Telander and colleagues and others described an unusual case of persistent vomiting in an infant associated with marked impairment of gastric emptying.[32,33] A series of elegant investigations showed marked dysfunction of antral smooth muscle owing to a disturbance of slow-wave activity.[32] The slow wave increased from its customary frequency of three cycles/min to six cycles/min. The authors coined the term "tachygastria."[32] In this patient, normal depolarization frequencies occurred in vitro when indomethacin was added to the bathing medium, suggesting that an abnormality of the local synthesis of prostaglandins was involved. In another infant, antrectomy was curative.[33] You and colleagues found that 50% of a group of patients with functional upper abdominal discomfort had abnormalities of the antral slow wave.[16] Diagnosis of these infants, however, relied on highly invasive and difficult methods for recording intraluminal myoelectric activity. More recently, attempts to measure antral slow-wave activity by surface electrodes have been more successful owing to the application of sophisticated signal processing techniques, and now a noninvasive way of investigating these patients exists. In a group of patients with intestinal pseudo-obstruction investigated using surface electrogastrography, a variety of dysrhythmias were found.[34] If the dysrhythmias were correlated with histopathologically proven enteric neuromuscular disease, those with neuropathic disease had very obvious tachygastria, as shown in Figure 31-2. In contrast, in myopathic conditions, no dominant frequency could be demonstrated.

Migraine and Nonulcer Dyspepsia

Nausea and vomiting are frequent symptoms in classic migraine and nonulcer dyspepsia. The Rome II Paediatric Working Group on Functional Gastrointestinal Disorders divided functional or nonulcer dyspepsia into three subgroups[35]: ulcer-like dyspepsia, which is predominantly pain central in the upper abdomen; dysmotility-like dyspepsia, in which nonpainful sensations, including early satiety, bloating, and nausea, occurs; and nonspecific dyspepsia in those patients whose symptoms did not fulfill the above two criteria. It should be noted that these conditions have not been rigorously defined in children. Activation of the emetic reflux results in delayed gastric emptying in both migraine and nonulcer dyspepsia. Clinically, it may be difficult to differentiate patients with migraine and nonulcer dyspepsia and idiopathic gastroparesis from those with psychogenic vomiting and bulimia nervosa.

Lesions of the Extrinsic Innervation

Central Nervous System. Local lesions of the vagal and vestibular nuclei and of the labyrinth, as well as raised intracranial pressure, may result in disordered gastroduodenal motility and vomiting. Tumors and congenital abnormalities are the most common chronic disorders and interfere either directly with the dorsal vagal nuclei or, more likely in the case of raised pressure, by stimulating the chemoreceptor trigger zone in the area postrema in the floor of the fourth ventricle.

Infectious, Metabolic, and Degenerative Disorders. A number of infectious organisms, including varicella[36] and Epstein-Barr virus,[37] have been reported to cause pseudo-obstruction. A recent study has shown that varicella zoster may be latent in the enteric nervous system, just as occurs in dorsal root ganglia of the spinal cord.[36] Automatic dysfunction also occurs in Guillain-Barré syndrome, where it may be unrelated to the degree of sensory and motor disturbances.

The common metabolic causes of gastroparesis in adults—diabetes mellitus and amyloidosis—occur only extremely rarely in childhood. However, degenerative conditions clearly occur and may be familial. The most common of these is the pandysautonomia of Riley-Day syndrome, in which there are abnormalities of both cholinergic and nonadrenergic, noncholinergic nerves.[38] Vomiting is very common and may be associated with constipation, internal ophthalmoplegia, lack of tears and sweating, and orthostatic hypotension. With the exception of orthostatic hypotension, these features were also present in four children reported to have postganglionic cholinergic dysautonomia.[39] In one of these patients with GI dysmotility, on the basis of in vitro studies of antral muscle, the authors

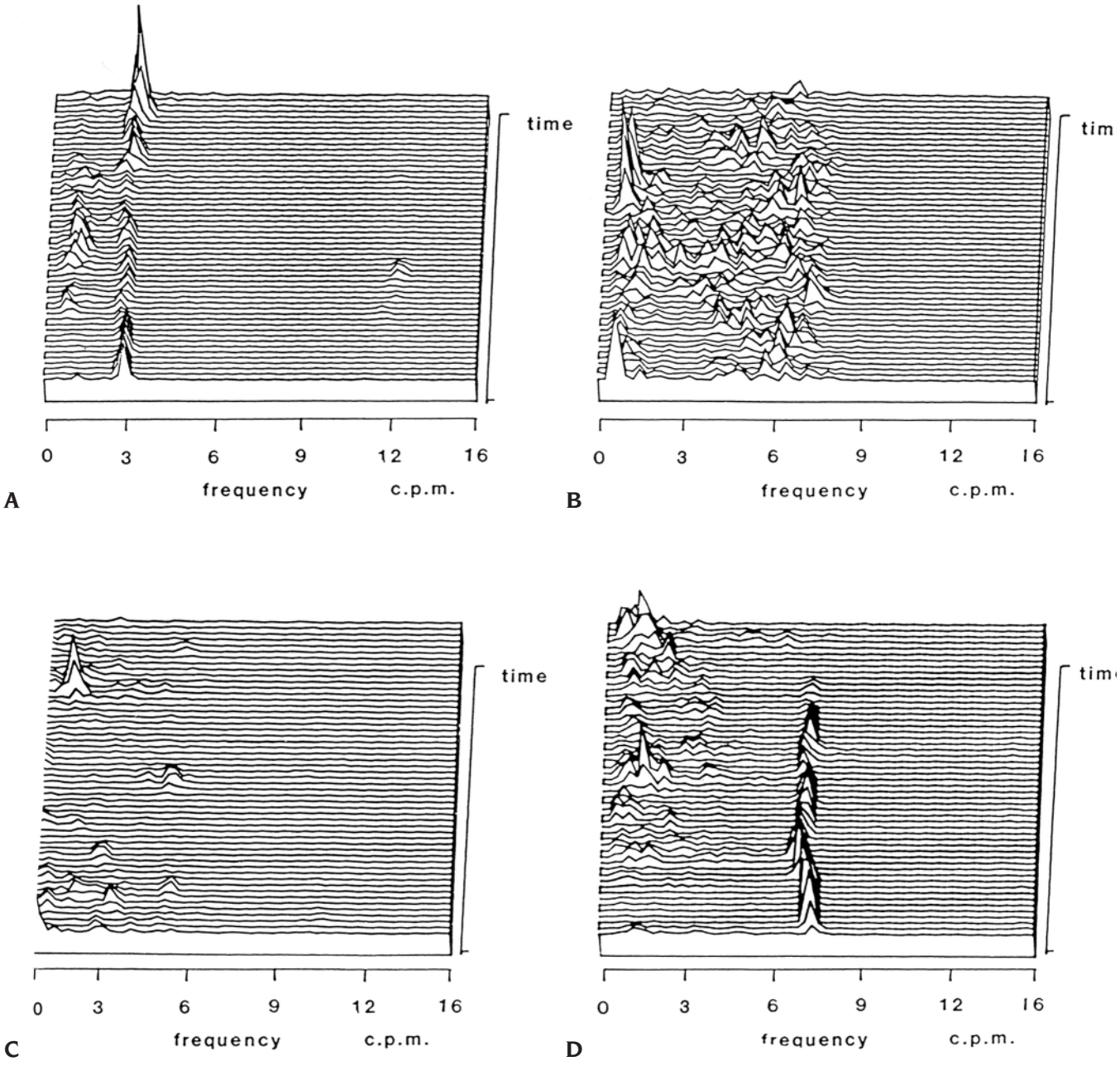

FIGURE 31-2 Pseudo–three-dimensional plots of a running spectral analysis of surface electrogastrograms of *A*, control children; *B* and *C*, myopathic pseudo-obstruction and hollow visceral myopathy; and *D*, neuronal pseudo-obstruction.

demonstrated defects proximal to smooth muscle and enteric nerves and speculated that the condition was due to a failure of nonadrenergic inhibitory innervation. It is now clear that degeneration of sensory and autonomic nerves occurs owing to mutations of the *IKBKAP* kinase gene[40] in Riley-Day syndrome.

Vagotomy. Although vagotomy is not commonly a purposeful operation in children, it has been used in the treatment of severe recurrent peptic ulcer disease and may occur as an unintended consequence of difficult surgery for congenital esophageal anomalies. After a highly selective vagotomy, relaxation of the proximal stomach is impaired.[41] As a consequence, rapid initial emptying of liquids may occur, with about 25% of patients developing symptoms of early satiety and epigastric fullness. These symptoms tend to improve with time.[41] With truncal and total vagotomy, the entire stomach loses its vagal innervation. In addition to impaired relaxation of the proximal stomach, disturbances of ECA occur more frequently after these types of interventions, and there is, thus, defective antral motility and delayed emptying of solids.[42]

Interdigestive motor complexes are less regular and less frequent, which may also contribute to the stasis of solids. The combination of very fast liquid and slow solid emptying can be extremely difficult to manage. The use of uncooked starch has been proposed to control the dumping, which is very troublesome in infants.[43] Drainage procedures such as pyloroplasty, although helpful in controlling the stasis of solids, do nothing to control the dumping and diarrhea.

Enteric Nervous System

Primary disorders of gut motor activity owing to either systemic disease or disorders of the enteric nervous system may be either diffuse or regional in their presentation. In this section, we are concerned with those disorders that result in abnormal gastroduodenal motility and present with early satiety, postprandial epigastric fullness, nausea, vomiting, and failure to thrive. The etiology of these conditions remains obscure. Some cases are congenital and may be inherited, whereas others are acquired and potentially reversible. It is likely that the so-called superior mesenteric artery syndrome, is, in fact, a duodenal pseudo-obstructive disorder rather than a mechanical obstruction.[44]

When the primary disease appears to be of the stomach and/or duodenum, the terms "idiopathic gastroparesis" and "duodenal pseudo-obstruction" are used. Such conditions are uncommon but usually present during the first few years of life. It is in this age group that there is the highest mortality rate.[44–49] In the majority, distinctive abnormalities can be found either in the smooth muscle or in the myenteric plexus.

Disease of enteric nerves may be familial and limited entirely to the gut, as in congenital absence of argyrophil nerves (which is inherited as an X-linked or perhaps an autosomal recessive trait) and familial megaduodenum,[46] or as part of a familial visceral neuropathy. Sporadic cases have also been reported with a peripheral neuropathy in which enteric nerves have been involved. The pathogenesis of the neural disease is not known.

Disorders Affecting Gastroduodenal Smooth Muscle

In adult life, most gastroduodenal muscle diseases occur secondary to a number of different conditions, including dystrophia myotonica, progressive systemic sclerosis, Ehlers-Danlos syndrome, dermatomyositis, and systemic lupus erythematosus. However, smooth muscle disease restricted to the gut is rare. The reverse is true in children; smooth muscle disease as part of a systemic disease occurs extremely rarely, and the majority suffer from two syndromes: hollow visceral myopathy[47,48] and megacystis microcolon hypoperistalsis syndrome.[49] The pathogenesis of these conditions is not understood and are described in detail in Chapter 46.2, "Dysmotilities," and Chapter 46.4, "Chronic Intestinal Pseudo-obstruction Syndrome."

Drugs Affecting Gut Motility

A variety of drugs have been found to affect gastroduodenal motility, and a number of others may be expected to do so. These include cholinergic agents, adrenergic, dopaminergic, and chemotherapeutic compounds. Opioids and Ca^{+} channel blockers may also impact on normal gut motility.[50–53]

Gastroduodenal Motility in Other Gastrointestinal Disorders

Gastroesophageal Reflux. Although the motor mechanisms of GER are relatively well described in children, the association with disease elsewhere in the GI tract is less well defined. It is clear that GER may occur with obstructive lesions (see above) such as pyloric stenosis and malrotation or may be part of a generalized pseudo-obstructive disease that also involves the stomach and duodenum. However, it is not clear whether gastric emptying is affected in those who present with GER alone.

In patients treated surgically by fundoplication, a proportion may develop acute gas-bloat syndrome or a more chronic syndrome of early satiety, bloating, nausea, recurrent retching, and vomiting.[15] These complications occur most frequently in children with severe neurologic disorders. Electrogastrographic studies show that there is increased sensitivity of the emetic reflex.[54] The nature of this is ill understood, but a study of fundoplication in an animal model suggests that inflammation and fibrosis around vagal nerve fibers induced by surgery result in abnormal vagal nerve function.[55]

Small Intestinal Malrotation. The majority of children who develop symptoms related to malrotation do so within the neonatal period with features of complete or incomplete upper intestinal obstruction. A proportion of children postoperatively have prolonged feeding difficulties and recurrent vomiting. Investigation of such children shows aberrant antroduodenal dysmotility, which is compatible with a neuropathic pseudo-obstruction.[56] It is of interest that malrotation is a common feature of pseudo-obstructive disorders. Devane and colleagues speculate that the underlying disease process is responsible for the disordered movement of the intestine around the superior mesenteric artery during embryologic development.[56]

DIAGNOSTIC TECHNIQUES

To understand the gastroparetic or pseudo-obstructive disorder and plan rational treatment, the involved areas must be defined and the physiology and pathology of the affected areas studied.

Radiology and Transit Studies

Conventional contrast radiography is used to delineate anatomic abnormalities, and, together with studies of gut transit, it provides a limited description of the disease but no clues to the underlying nature of the disorder.

Manometry

Studies of motility are helpful in delineating both the nature and extent of the disease. In patients with suspected pseudo-obstruction, at least three areas of the GI tract—the esophagus, the upper small intestine, and the rectosigmoid colon—should be studied because the disease may not be restricted to the stomach and duodenum. Swallow-induced peristalsis, fasting activity, and the gastrocolonic response to food each can be used as tests of the integrity of the enteric nervous system and of the contractility of the smooth muscle.[57] In addition, postprandial activity provides information regarding the humorally mediated response to food and whether enteroenteric reflexes are intact.

In the esophagus, swallow-induced peristalsis and the associated relaxation of the lower esophageal sphincter can be studied using a Dent sleeve assembly modified for use

in infants and younger children.[58] Particular attention should be paid to the nature of the primary peristaltic sequence, to determining whether secondary peristalsis occurs in response to reflux, and to the presence of tertiary contractions. The amplitude and form of the contractile waves are also noted.

The cyclic nature of the fasting gastric and duodenal motor activity is determined by the inherent activity of the enteric nervous system. This relationship can be used to test whether the enteric nervous system is intact and whether extrinsic nervous modulation is present. Observation of the disruption of fasting activity and the establishment of postprandial activity provide information regarding the hormonally mediated responses to food and clarify whether enteroenteric reflexes are intact. To test these motor functions, a standardized, age-appropriate meal is administered after observing three cycles of fasting activity.[57]

Manometric studies show that myopathic processes produce low-amplitude, poorly propagated contractions,[57] whereas neuropathies are associated with contractions of normal amplitude that are often bizarre in waveform, abnormally propagated, and, in phase III activity, ill formed.[59] Disturbance of the neuroendocrine environment may be signaled by increased slow-wave frequency in diseases in which catecholamines are secreted in excess (such as hyperthyroidism, pheochromocytoma, and ganglioneuroma) and decreased slow-wave frequency in preterm infants and in hypothyroidism.

Gastric Emptying

Methods used to measure gastric emptying in infants previously involved invasive intubation studies or the use of radioisotopes. Until recently, these methods have been most used: the serial test meal or a modified version of the George method.[60] The George method and its modifications[60] allow a single meal to be followed.

For those with access to a gamma camera, emptying of milk or solids[61] can be studied by labeling a test meal with indium diethylenetriamine pentaacetic acid for the fluid phase or technetium 99m attached to a number of different stable substances, such as aggregated ferrous hydroxide or tin colloid, which are neither adsorbed onto the gastric mucosa nor absorbed systemically. Successive 90-second images over a 60- to 90-minute period are then obtained, the counts in each image are computed, and emptying curves and half-empty times are calculated. This method suffers from the difficulty of relating planar changes to a three-dimensional organ; in small infants especially, superimposition of parts of the stomach over the small intestine makes separation of emptied and retained meal difficult.[61]

In more recent years, noninvasive methods using real-time ultrasonography, electrical impedance tomography, and C^{13} octanoic acid breath testing each have been developed.[62] However, scintiscanning currently remains the gold standard.[62]

Electrogastrography

Electrogastrography[63] is defined as the recording of myoelectric activity of the smooth muscle of the stomach by means of electrodes attached to the abdominal skin. The first electrogastrogram was recorded by Alvarez in 1921, but the advent of powerful personal microcomputers has revived interest in this diagnostic modality. This test has the great advantage of permitting the study of gastric myoelectric activity in both the fasted and fed state totally noninvasively; moreover, it readily detects disturbances of slow waves.[63]

Histology

Usually, when no microscopic lesion of nerve or muscle has been reported in cases of pseudo-obstruction, adequate full-thickness biopsies of an appropriate region of the gut have not been taken or have not been rigorously examined. To demonstrate disease of smooth muscle or enteric nerves in the majority of patients with pseudo-obstructive disorders, paraffin-embedded material stained with hematoxylin and eosin or a trichrome stain is inadequate. Only if there is widespread severe fibrosis can a defect of a muscle be detected in this way. Ultrastructural studies are necessary for both muscle and nerve. In addition to electron microscopy, enteric nerves must be studied by silver staining and by immunocytochemistry and histochemistry.[64,65] Such studies define the nature and the extent of the disease and, in some instances, are helpful in understanding disease etiology.

TREATMENT

Operative Management

In patients in whom there is no anatomic abnormality, it is usually not possible to treat a primary motility disorder by surgical means. This discussion should dispel the naive view that all problems of delayed gastric emptying can be resolved by cutting the pylorus.

In those patients with an isolated tachygastria, antrectomy has been successful.[32,33] However, this step should be considered only after a thorough evaluation to eliminate the diagnostic possibility of a generalized pseudo-obstructive disorder. In some children with the superior mesenteric artery syndrome or duodenal pseudo-obstruction, a gastroenterostomy has proved beneficial.

In those with generalized pseudo-obstructive disease, adhesional obstruction often occurs after laparotomy, and the risk increases with repeated laparotomy. Adhesional obstruction should be treated conservatively, with surgery being employed only when there are localizing signs, incipient peritonitis, or bowel necrosis.

Medical Management

Little can be done directly to treat these underlying disease processes, even when they are acquired, but much can be done to treat the consequence of the secondary effects of malnutrition and primary disease exacerbation. The majority of patients who die from gastroduodenal motor disorders do so from malnutrition and its consequence or serious electrolyte imbalance.

Primary Disease Exacerbation. Although the nature of the pathologic process is often not known, it is common

for exacerbation to be associated with infection, anesthetics, drugs, and procedures involving gut handling that may normally adversely affect gut motility.

Malnutrition. Many patients with motor disorders die from malnutrition. These deaths are totally avoidable with the judicious use of parenteral nutrition. Many episodes subside completely provided that nutrition is maintained. Only the most severely affected need to be considered for home parenteral nutrition. Others tolerate modern enteral formulas but not normal food.

Pharmacologic Agents. Most attempts at treatment with prokinetic agents (metoclopramide, domperidone, or cisapride) or motilin agonists (erythromycin) in patients with neuromuscular disease of the gut are unsuccessful, yet, occasionally, one of these agents is helpful. Some tachygastrias have been associated with disturbed prostaglandin metabolism.[32] In such patients, treatment with a prostaglandin synthetase inhibitor such as indomethacin may be successful. Until an understanding of the disease processes is available, drugs will provide only marginal relief at best.

PROGNOSIS

The prognosis for patients with uncomplicated pyloric stenosis or malrotation is excellent, and only a success rate approaching 100% is acceptable. However, in those patients in whom the stomach and duodenum are involved in a pseudo-obstructive disorder, either restricted to that region of the gut or as part of a more generalized disease of the GI tract, the prognosis is more uncertain. In conditions in which the extrinsic innervation is affected, the prognosis is that of the underlying condition. In those patients in whom there is intrinsic neuromuscular disease, particularly when it is part of a diffuse condition of the gut, the overall mortality rate may be as high as 25%,[45] with the highest rate in the first few years of life. The majority of these patients die from sepsis, malnutrition, electrolyte imbalance, or aspiration.

Only in the last 20 years have many of the diseases that cause gastroduodenal motor disorders been recognized. However, effective treatment for many will ensue only when a much greater understanding of the disorders is achieved.

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CHAPTER 32

CONGENITAL ANOMALIES

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EMBRYOLOGY

The primitive gut develops during the fourth week of gestation by division of the primitive yolk sac into primitive gut and yolk sac proper. These two structures remain in continuity through the vitelline (omphalomesenteric) duct until the duct obliterates during the seventh week. Most of the epithelial attachments to the gut, including the liver and the pancreas, arise from the endoderm of the primitive gut. Connective tissue elements of the gut, such as smooth muscle, are of splanchnic mesenchymal origin. The primitive gut is divided into three parts: foregut, midgut, and hindgut.

The foregut gives rise to the pharynx, lower respiratory system, esophagus, stomach, and proximal duodenum down to the level of the bile duct, liver, pancreas, and biliary system. The foregut enteric nervous system is derived from migration of somatic neural crest cells. The blood supply is derived from the foregut artery, which later becomes the celiac artery.

The midgut gives rise to the small intestine beyond the opening of the bile duct, cecum, appendix, and ascending and proximal transverse colon. The foregut and hindgut enteric nervous systems are derived from migration of neural crest cells from the vagal region of the hindbrain into the developing esophagus and their subsequent caudal migration. This process is complete by 12 weeks gestation. The midgut blood supply is from the midgut artery, which subsequently becomes the superior mesenteric artery. Between the sixth and twelfth weeks of gestation, the midgut herniates into the umbilical cord and by a complex series of rotational movements, probably reflecting differential growth, returns into the peritoneal cavity and assumes the postnatal position.

The hindgut derivatives are the distal third of the transverse colon, descending colon, sigmoid colon, rectum, and rostral portion of the anal canal. Organs derived from the hindgut are supplied by the inferior mesenteric artery. The distal end of the hindgut ends in the cloaca. This is separated from the ectoderm of the anal canal by the cloacal membrane. As the hindgut differentiates, a sheet of mesenchyme, the urogenital septum, divides the distal hindgut into dorsal and ventral parts. When separation is complete, the ventral component forms the urogenital sinus and the dorsal component forms the anorectal canal. The epithelium of the anal canal is derived from the endoderm of the hindgut rostrally and ectoderm caudally, as demarcated by the pectinate line.

RECOGNIZING CONGENITAL ANOMALIES

Prenatal ultrasonography will identify the major abdominal wall defects, exomphalos and gastroschisis, and gastric or small bowel distention suggestive of intestinal obstruction. The distinctive "double bubble" characteristic of duodenal atresia may be seen, and associated anomalies may be present, notably cardiac. Polyhydramnios raises the possibility of esophageal atresia and upper gastrointestinal obstruction. The abdominal wall defects may be associated with elevated maternal serum α-fetoprotein levels. Fetal karyotyping may be considered in abnormalities with a high risk of chromosomal disorders, such as exomphalos. Prenatal diagnosis provides the opportunity for parental counseling and allows arrangements to be made for prompt postnatal surgical care.[1] However, prenatal diagnosis may not be accurate, and confirmation of the diagnosis after birth is important.

ABDOMINAL WALL DEFECTS

Exomphalos and Gastroschisis

Exomphalos is a midline developmental defect of the anterior abdominal wall, as a result of which some of the abdominal organs lie outside the abdominal cavity (Figure 32-1). The eviscerated abdominal organs are contained within a membranous sac derived from the amniotic membrane. Depending on the size of the lesion, the sac will contain stomach, intestine, liver, and spleen. More complex lesions include upper abdominal midline defects in which exomphalos coexists with defects of the diaphragm, sternum, pericardium, and heart (pentalogy of Cantrell) and lower midline defects associated with bladder exstrophy. Up to 75% of infants have major associated structural abnormalities, of which congenital cardiac defects are the most common, or chromosomal anomalies, notably trisomy 13, 18, or 21, which may determine the outcome.[2] In Beckwith-Wiedemann syndrome, which includes exomphalos, macroglossia, and hypoglycemia associated with pancreatic islet cell hyperplasia, early recognition is essential to prevent complications of hypoglycemia.[3]

With gastroschisis, the stomach and intestine are eviscerated through a defect to the right of the base of the umbilical cord (Figure 32-2). There is no enveloping sac, and evisceration of other organs is rare. The likely mechanism, supported by evidence from serial sonograms, is prenatal rupture of a small hernia of the base of the umbilical cord at the site of the obliterated fetal right umbilical vein.[4] Abnormalities of the intestine, notably atresia, occur in up to 10% of infants, but chromosomal and extra-abdominal anomalies are rare.[5]

Accurate diagnosis is possible with antenatal ultrasonography.[2] Amniocentesis and karyotyping may be appropriate for exomphalos, with the high risk of associated anomalies.[2] Delivery should take place in a specialist unit. Stable infants with a gastroschisis or a small exomphalos may be safely delivered per vaginam, and cesarean section is recommended for most infants with exomphalos.

Preoperative correction of hypovolemia is essential, particularly with gastroschisis, in which boluses of saline or albumin may be required to replace the protein-rich fluid losses (see the section "Intravenous Fluids"). With an intact exomphalos, there is no urgency to remove the sac if it remains intact and infection is not a concern. For gastroschisis or ruptured exomphalos, primary closure of the defect may be possible, depending on the volume of the herniated viscera relative to the abdominal cavity; postoperative ventilation is required because of the resultant abdominal distention. Alternatively, the eviscerated organs are placed in an artificial bag (silo) fashioned from a sheet of reinforced Silastic and sutured to the abdominal wall.[5] This is progressively reduced in size each day, allowing secondary closure of the abdomen by 7 to 10 days. A commercial silo has been developed that is introduced into the defect without the need for anesthesia or sutures.[6] Infection is a major cause of morbidity, and antiseptic care and prophylactic antibiotics are important. Establishment of gastrointestinal function is slow, and intravenous feeding is required. Overall survival for gastroschisis is greater than 90%; postoperative problems include adhesive obstruction and short-gut syndrome. For exomphalos, the outcome is dependent on the associated abnormalities; in the absence of these, most infants survive to lead a normal life.[7]

Umbilical Granuloma

Umbilical granuloma is a mass of pink granulation tissue at the umbilicus caused by low-grade infection of the stump of the umbilical cord. It must be distinguished from an omphalomesenteric duct mucosal remnant. Topical treatment suffices, with local cleansing and applications of either topical steroids or silver nitrate. The latter carries a risk of damage to the adjacent skin, which must be protected.

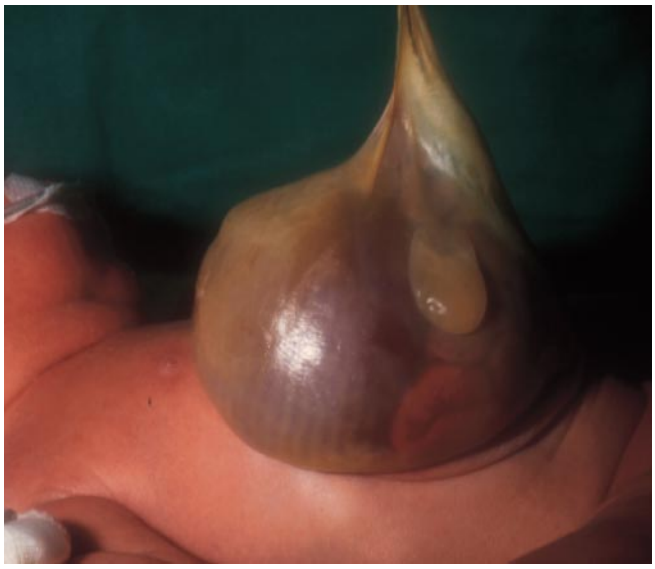

FIGURE 32-1 A large exomphalos. The sac is intact and contains intestine and liver; typically, the umbilical cord is attached to the apex of the sac.

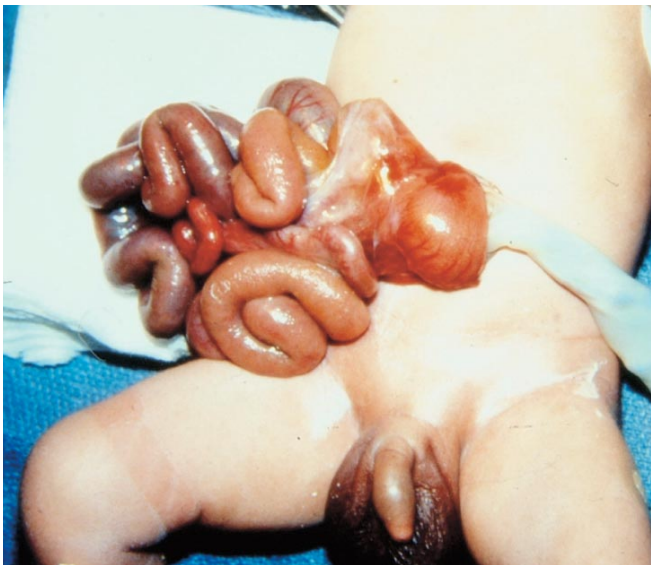

FIGURE 32-2 Gastroschisis. The abdominal wall defect is to the right of the umbilical cord, and there is no sac. Only the stomach (lying to the left) and intestine are prolapsed, not the solid organs.

Omphalomesenteric Duct Remnants

Failure of regression of the omphalomesenteric duct[8] results in a fistula that presents with a persistent umbilical discharge, often with ectopic intestinal mucosal at the umbilicus (Figure 32-3). The diagnosis is confirmed by passing a nasogastric tube through the fistula and aspirating small bowel content or injecting radiopaque contrast. The entire fistula is resected through a subumbilical incision. The omphalomesenteric duct may obliterate but persist as a band between the umbilicus and small intestine.

The most common remnant of the omphalomesenteric duct is persistence of the enteral end as a Meckel diverticulum, which is lined by ileal mucosa but may contain ectopic gastric mucosa.[9] Complications include Meckel diverticulitis, which is clinically indistinguishable from acute appendicitis. Ectopic gastric mucosa within the diverticulum may lead to local ulceration with bleeding or perforation. Ectopic gastric tissue causing mucosal ulceration and bleeding may be identified by 99m technetium scanning (Figure 32-4), which has an 85% sensitivity and a 95% specificity.[9] Intestinal obstruction may result from intussusception of the diverticulum or small bowel volvulus around a connecting band to the umbilicus (Meckel band). In all situations, the diverticulum is resected with the adjacent segment of ileum. There is no sound evidence to support routine resection of an asymptomatic diverticulum encountered incidentally at operation.

An isolated mucosal remnant at the umbilicus must be distinguished from an umbilical granuloma and is suspected when the "granuloma" fails to respond to topical treatment. Treatment is excision of the ectopic mucosa. A limited exploration beneath the umbilicus to exclude an omphalomesenteric (Meckel) band is important; if present, it is resected.

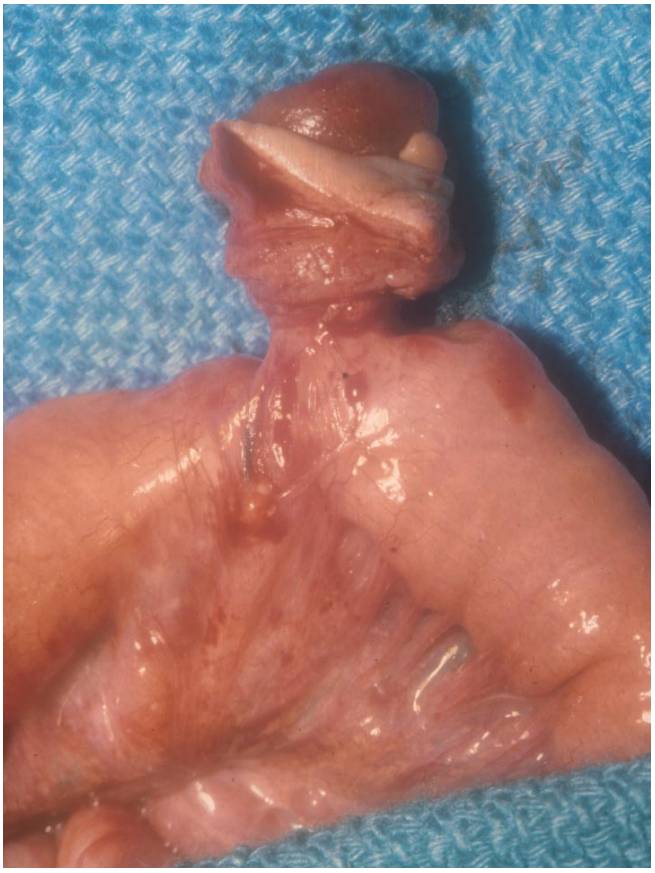

FIGURE 32-3 A patent omphalomesenteric duct excised intact. At the top of the specimen, note the ectopic mucosa at the umbilicus. Below this, the fistula joins the small intestine.

Congenital Hernia and Hydrocele

Congenital inguinal hernia and hydrocele are common abnormalities of childhood, with a peak incidence in the neonatal period. They result from persistent patency of the processus vaginalis, an extension of the peritoneal cavity that passes through the inguinal canal within the spermatic cord in boys and along the round ligament in girls. Normally, the processus vaginalis begins to obliterate once testicular descent is complete; it follows that obliteration does not occur when the testis is undescended. It is important to distinguish between congenital hernia and hydrocele because the natural history and management of each are radically different in the newborn period.

Congenital inguinal hernia is the presence of an abdominal viscus in the processus vaginalis (hernia sac), usually the small intestine but occasionally an ovary, which presents as a firm mobile inguinal mass and may be confused with a lymph node. Spontaneous resolution does not occur, and because of the high risk of incarceration during the first months of life, prompt operation is advised (see Chapter 36, "The Surgical Abdomen").

Congenital hydrocele is associated with a narrow patent processus vaginalis that becomes distended by peritoneal fluid. Most hydroceles will spontaneously resolve in the first 6 months of life, and because there is no risk of incarceration, treatment is expectant. After 2 years of age, a hydrocele is not likely to close spontaneously, and operative closure of the processus vaginalis is recommended.

INTESTINAL OBSTRUCTION IN THE NEWBORN INFANT

Congenital intestinal abnormalities present most commonly with intestinal obstruction. The following general observations are fundamental to the diagnosis and management of these infants.

Clinical Features

Vomiting. Bile-stained (green) vomiting is a characteristic of intestinal obstruction distal to the ampulla of Vater, and, if present, obstruction must be excluded. Non–bile-stained vomiting may be due to obstruction at the pylorus or in the proximal duodenum and must be distinguished from gastroesophageal reflux.

Abdominal Distention. This will depend on the level of obstruction. It is most marked in the case of large bowel obstruction and least apparent with duodenal atresia, where the distention is confined to the epigastrium. Visible peristalsis may be apparent. Abdominal colic is not a characteristic of congenital intestinal obstruction.

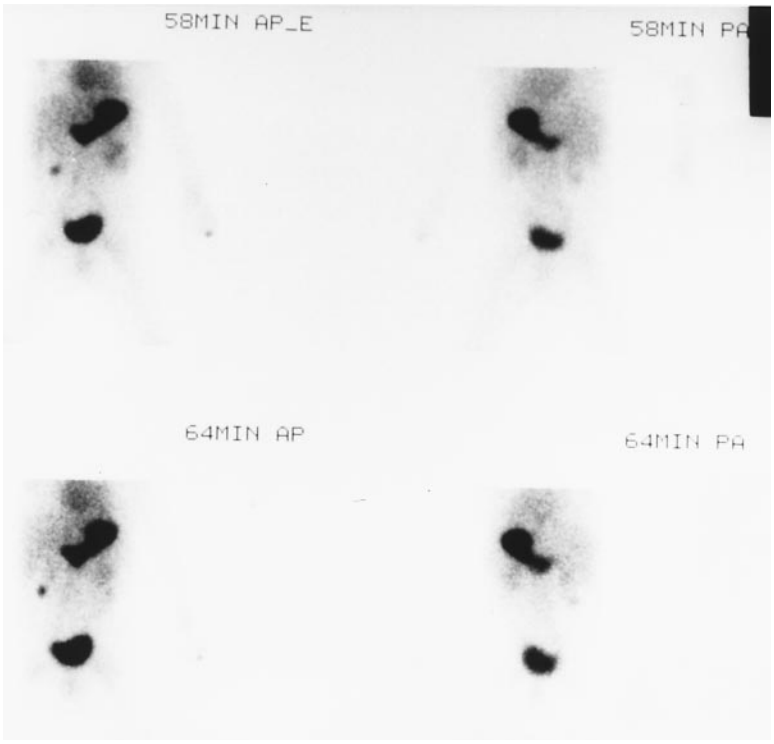

FIGURE 32-4 99m Technetium scan showing uptake by ectopic gastric mucosa in a Meckel diverticulum in the right lower abdomen. Anteroposterior images on the left, posteroanterior images on the right, at 58 minutes (above) and 54 minutes (below).

Failure to Pass Stools. The normal infant should pass meconium within 36 hours of birth. Failure to pass stool in association with abdominal distention suggests colonic, rectal, or anal obstruction. The passage of stool does not exclude a proximal congenital obstruction such as an ileal atresia because meconium already in the colon at the time that the obstruction develops will be evacuated, after which no further stools will be seen.

Diagnosis

A plain abdominal radiograph will show distended bowel loops characteristic of obstruction in most cases. This may be less obvious when the bowel is filled with fluid or meconium, as with meconium ileus. Contrast studies and ultrasonography have specific diagnostic roles.

Management

Gastric Drainage. The largest comfortable nasogastric tube, usually 8 to 10 French, is inserted to drain the stomach to prevent vomiting and aspiration and minimize gastric secretions. Ventilation will be optimized by reduced abdominal distention, which restricts diaphragmatic movement. The newborn infant is an obligate nasal breather, and although a nasogastric tube has the advantage that it can be firmly secured to the face, it does reduce the nasal airway by 50%, and in the premature infant with increased oxygen requirements, an orogastric tube may be preferred. The tube must be left on continuous drainage and regularly flushed with air or water and aspirated to confirm that it is functional.

Intravenous Fluids. The use of 10% dextrose solutions reduces the risk of hypoglycemia, but regular monitoring of the blood sugar level is important. Gastric aspirates and third-space fluid loss in the obstructed intestine will increase sodium, potassium, and chloride requirements, which cannot be accurately measured and are not fully compensated for by standard "maintenance" solutions containing 0.18% sodium chloride. In anticipation of these additional requirements, we use 0.45% sodium chloride (half normal) with potassium chloride 10 to 20 mmol/L. Fluid volume requirements are increased for the same reason, and a starting rate of 4 to 5 mL/kg/h for full-term infants and 5 to 6 mL/kg/h for premature infants is recommended, depending on the degree of dehydration of the infant. With severe hypovolemia, as occurs with gastroschisis, 10 to 20 mL/kg boluses of crystalloid or albumin may be required in addition. Measured gastric aspirates are replaced with 0.9% sodium chloride (normal saline). Hydration is monitored by clinical assessment of the peripheral circulation, skin turgor, and anterior fontanel tension and by accurately monitoring the urine volume (normal in a neonate is 2 mL/kg/h) and concentration (ideal specific gravity is 1008–1012). Serum electrolytes and acid-base balance are monitored, and urine sodium estimations are useful for interpreting renal function. Based on these findings, the volume of intravenous fluid is increased or decreased every 4 to 8 hours, the actual frequency of assessment depending on the individual clinical situation.

Vomiting in the Newborn Infant

Causes of congenital intestinal obstruction are shown in Table 32-1. Nonsurgical causes of vomiting must be excluded, including feeding difficulties (under- or overfeeding), systemic infection, urinary tract infection, raised intracranial pressure, food allergy, and adrenogenital syndrome.

TABLE 32-1 CONGENITAL CAUSES OF GASTROINTESTINAL OBSTRUCTION

VOMITING IN THE NEWBORN INFANT
Duodenal atresia
Malrotation
Jejunoileal atresia
Meconium ileus
Duplication cyst
Incarcerated inguinal hernia
FAILURE TO PASS STOOL
Anorectal malformation
Hirschsprung disease
Meconium plug syndrome
Colonic atresia

Duodenal Atresia. The site of obstruction is typically at the level of the ampulla of Vater and may take the form of complete obstruction owing to atresia or an intact membrane or partial obstruction owing to stenosis or a fenestrated membrane. The common bile duct usually opens on the membrane and is vulnerable if an attempt is made to excise the membrane. At the level of the atresia, there is a marked decrease in the caliber of the distal duodenum. Occasionally, an intact membrane will bulge distally, forming the windsock anomaly, as a result of which the site of obstruction appears to be more distal than it actually is.

The incidence of duodenal atresia ia about 1 in 5,000 live births.[10] Prematurity is common, and associated anomalies include trisomy 21 (Down syndrome), congenital cardiac disease, esophageal atresia, anorectal abnormalities, and malrotation. The cause of the anomaly is not understood; its occurrence at a complex site of development of the duodenum, pancreas, and biliary and pancreatic ducts and the association with trisomy 21 suggest a genetic origin.[11]

The diagnosis may be suspected prenatally when the fetal sonogram shows hydramnios and a distended stomach. Because of the association with trisomy 21, these findings may prompt fetal karyotyping, particularly if termination of pregnancy is a consideration.[12] Following birth, the typical presentation is with bilious vomiting and epigastric distention, but vomiting may be nonbilious if the ampulla of Vater opens distal to the atresia. A plain abdominal radiograph showing the characteristic double bubble representing the distended stomach and proximal duodenum, but no gas distal to this, is diagnostic (Figure 32-5). When there is gas distal to the dilated proximal duodenum, malrotation must be distinguished from duodenal stenosis by ultrasonography or a contrast study. Partial duodenal obstruction may not be recognized for months or years until persistent postprandial vomiting prompts a contrast study or endoscopy.

Preoperative management includes gastric decompression and correction of fluid and electrolyte abnormalities. The duodenum is approached through a supraumbilical right transverse incision. The gastric tube is pushed distally into the duodenum to define the site of obstruction and exclude a "windsock" abnormality. An annular pancreas may encircle the duodenum at the point of atresia. Because of the risk of injury to the ampulla of Vater, the preferred procedure for duodenal atresia is a duodenoduodenal anastomosis, bypassing the obstruction. The "diamond" incision described by Kimura and colleagues uses a transverse incision in the dilated upper pouch and a longitudinal incision in the narrow distal duodenum.[13] Postoperative ileus may be prolonged, and intravenous feeding is often needed; if this is not available, a transanastomotic nasoduodenal feeding tube is passed at operation under direct vision to enable early postoperative enteral feeding. For duodenal stenosis or a fenestrated diaphragm, the proximal duodenum is entered through a longitudinal incision that is then extended across the stenosis, taking care to avoid the ampulla of Vater by placing the incision anterolaterally. The incision is closed transversally. Survival rates for duodenal atresia and stenosis are over 90% and depend largely on the influence of associated anomalies.[14]

Midgut Malrotation. Malrotation describes a situation in which the intestine does not lie in a normal position. The common form is midgut malrotation in which there are two components: the third part of the duodenum lies to the right of the vertebral column instead of curving across to the left, and the cecum lies in the upper abdomen to the left of the duodenum. As a result, the mesentery of the midgut (jejunum to mid–transverse colon) is not attached across the posterior abdominal wall but is narrow and confined to the base of the superior mesenteric artery. As a result, the midgut is liable to twist around this narrow pedicle at any time. Torsion of more than 270° may lead to potentially fatal irreversible midgut ischemia (Figure 32-6). Traditionally, malrotation is attributed to failure of the intestine to

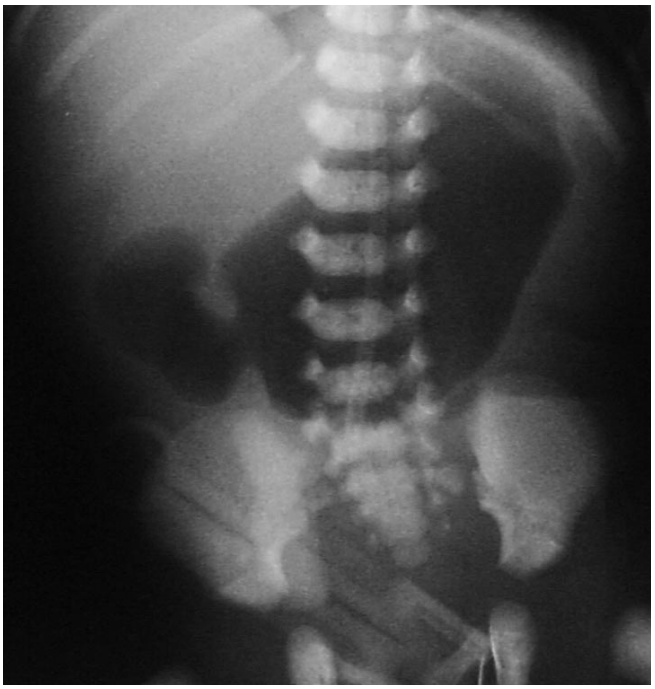

FIGURE 32-5 Abdominal radiograph showing the characteristic "double bubble" sign owing to gas in the distended stomach and duodenum proximal to a duodenal atresia.

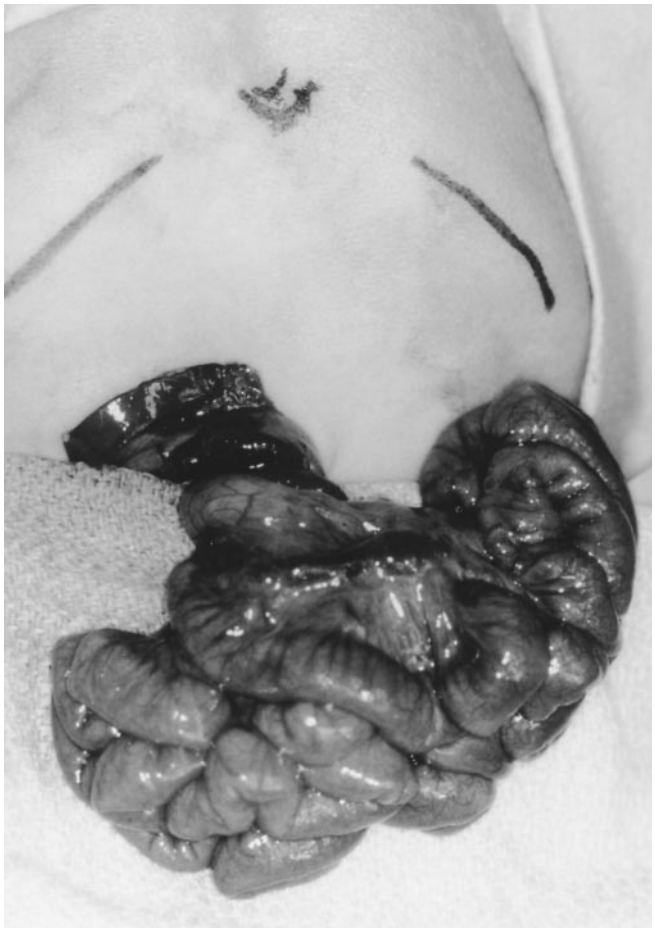

FIGURE 32-6 Midgut malrotation with volvulus. The midgut (small bowel, cecum, and right half of the colon) has twisted in a clockwise direction around the pedicle of the superior mesenteric vessels. In this example, the intestine is still viable.

"rotate" to its normal position when it moves into the peritoneal cavity from the normal umbilical cord hernia during the first month of gestation. This is probably an oversimplication, and the true mechanism is not understood.[15]

The risk of acute volvulus is highest during the neonatal period, but the true incidence of malrotation is not known because it may remain asymptomatic throughout life. Recurrent volvulus may present at any age with a history of chronic intermittent abdominal pain with or without vomiting. A contrast radiograph will show the characteristic features of malrotation (Figure 32-7).

In the infant, acute midgut volvulus presents with bilious vomiting, abdominal pain, and progressive abdominal distention and tenderness. Stools may be passed in the early stages; the passage of blood suggests intestinal ischemia. With progressive midgut ischemia, the infant rapidly deteriorates with hypovolemia and persistent metabolic acidosis. A plain abdominal radiograph may show signs of duodenal obstruction; beyond this, the bowel may contain more fluid than air, with resultant opacification of the abdominal cavity.

Urgent laparotomy is required after rapid correction of fluid and electrolyte abnormalities and nasogastric drainage. A generous supraumbilical transverse incision is used to enable easy delivery and inspection of the small and large bowel. The volvulus is derotated, usually in an anticlockwise direction, to allow restoration of the mesenteric circulation and reveal the cecum lying adjacent to the duodenum. The peritoneal folds extending from the cecum across the duodenum (Ladd bands) are divided to enable the cecum to be mobilized toward the left, thus widening the base of the mesenteric pedicle and exposing the superior mesenteric vessels. If the intestine is viable, the cecum is replaced on the left side of the abdominal cavity and the small intestine on the right side. The appendix may be removed to avoid future diagnostic confusion. When the viability of the bowel is uncertain, obvious nonviable bowel is resected, stomas are made, and the abdomen is closed; the bowel is re-examined by laparotomy after an interval of 24 to 36 hours. If the entire volvulus clearly is not viable, resection will inevitably result in severe short-gut syndrome and the inevitable consequences of long-term intravenous feeding.[16]

Jejunoileal Atresia. Congenital obstruction of the jejunum or ileum due to atresia or stenosis develops as a result of ischemic infarction of a segment of fetal intestine. This was first demonstrated experimentally in a fetal dog model in 1955 by Louw and Barnard.[17]

Patterns of small bowel atresia are atresia in continuity (type I), atresia with connecting band and mesenteric defect (type II), atresia with adjacent mesesnteric defect (type

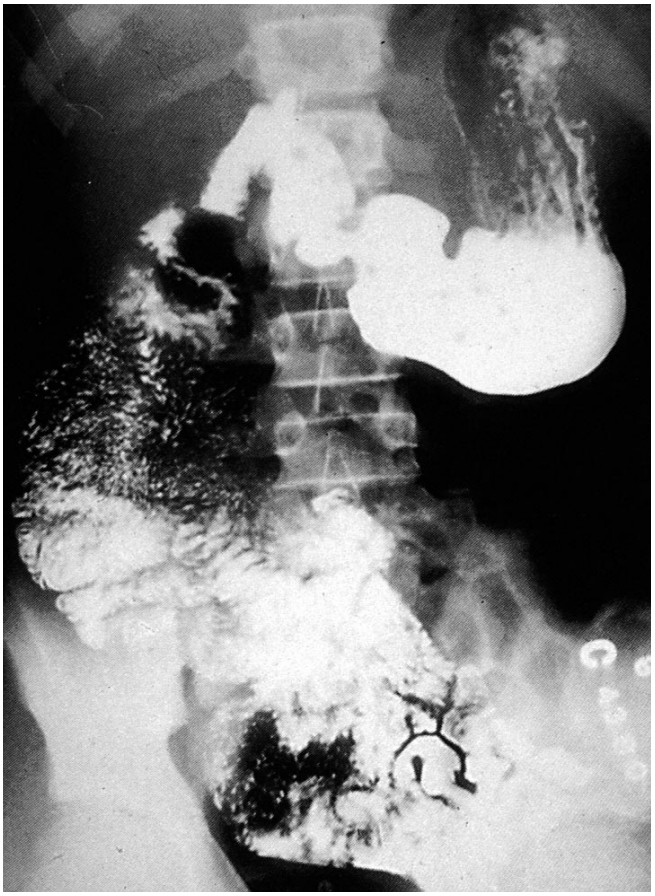

FIGURE 32-7 Midgut malrotation: an upper gastrointestinal contrast study showing the duodenum and small intestine lying to the right of the vertebral column.

IIIA) or extensive mesenteric defect and "apple peel" bowel configuration (type IIIB; Figure 32-8), and multiple atresias (type IV). Presentation is with bilious vomiting and abdominal distention. Meconium stool already in the colon may be passed, confirming that the obstruction occurs after the secretion of bile into the embryonic gastrointestinal tract. The diagnosis is confirmed by plain abdominal radiography, which shows multiple dilated loops of intestine with air fluid levels. Distal ileal atresia must be distinguished from meconium ileus and long-segment Hirschsprung disease; a contrast enema may be required.

Preoperative preparation is as described above. At operation, the site of obstruction is easily identified by the abrupt change in caliber from the dilated proximal intestine to the empty narrow distal small bowel. In type IIIA atresia, there is a defect in the adjacent mesentery. The dilated proximal bowel is resected, and continuity is restored by end-to-end or end-to-back anastomosis. In the case of multiple atresias (type IV), as many segments as possible should be salvaged to avoid short-gut syndrome. Postoperative recovery may be prolonged, and parenteral nutrition may be required. Survival rates over the past two decades have ranged from 78 to 100%.[18] Postoperative complications include anastomotic leak or stenosis and functional intestinal obstruction at the anastomosis. Extensive loss of small bowel attributable to multiple atresias (type IV) or a precarious blood supply (type IIIB) may result in short-bowel syndrome, requiring prolonged intravenous nutrition.

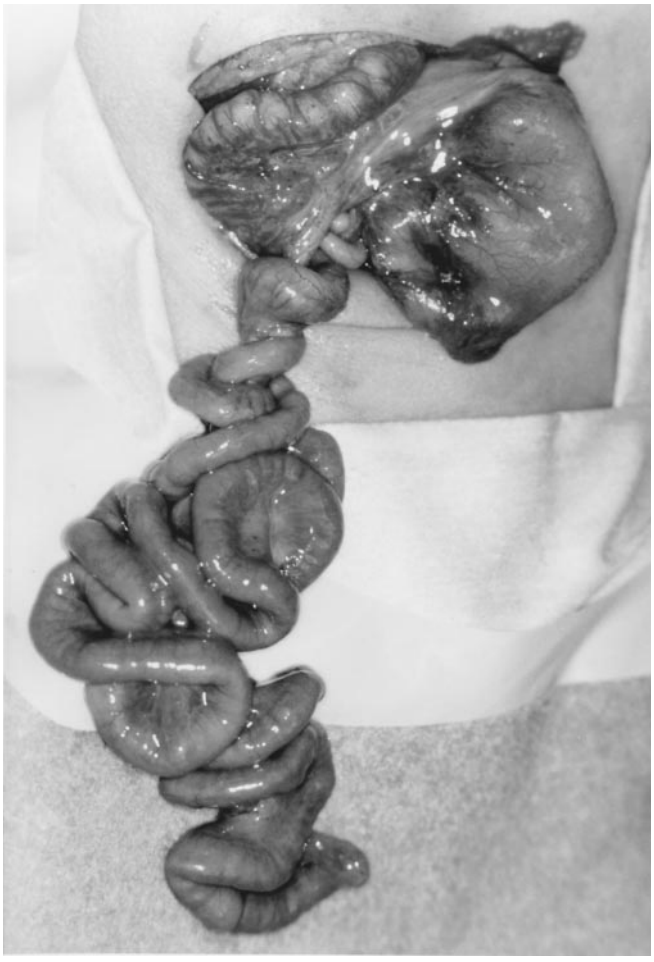

FIGURE 32-8 Jejunoileal atresia type IIIB: there is an extensive mesenteric defect and an "apple peel" bowel coiled around the long marginal artery. There is a risk of the bowel twisting and obstructing the tenuous mesenteric blood supply.

Where there are concerns about the safety of the anastomosis, either because of impaired vascularity or possible distal obstruction, stomas may be created but at the expense of high fluid and electrolyte loss. This may be alleviated by refeeding the effluent into the distal stoma. This also has the additional advantage of promoting growth of the distal intestine.[19] With proximal jejunal atresia, resection of the dilated bowel is not possible, and tapering by antimesenteric excision or inversion of the proximal jejunum facilitates anastomosis and may enhance postoperative transit.

Meconium Ileus. This is a misnomer because the obstruction is mechanical. As a result of pancreatic enzyme deficiency, the distal ileum is plugged by viscid and often nonpigmented meconium with a high albumin content. The deficiency of pancreatic enzymes is, with rare exceptions, associated with cystic fibrosis. Up to 20% of newborn infants with cystic fibrosis present with meconium ileus. The diagnosis is suspected when there is a family history of cystic fibrosis and is confirmed by chromosomal analysis, which in 85% of patients will demonstrate the ΔF508 mutation, a three-basepair deletion from chromosome 7.[20] Mutations result in defective chloride transport in the apical membrane of epithelial cells and an abnormally high excretion of chloride from the skin.[21] This can be measured by iontophoresis (the sweat test), an alternative diagnostic test in mature infants.

The characteristic abnormality in meconium ileus is a narrow terminal ileum obstructed by multiple pellets of meconium, with an abrupt transition proximally to dilated ileum containing meconium that is abnormally tenacious and adherent to the intestinal mucosa. The colon is empty and contracted (microcolon). Presentation is with distal ileal obstruction, which must be distinguished from ileal atresia and long-segment Hirschsprung disease. Abdominal radiographs show air-fluid levels in the proximal small bowel. The meconium-filled ileum appears opaque, whereas in the right iliac fossa, the dilated meconium-filled distal ileum contains multiple translucencies owing to entrapped fat globules, the "soap bubble" appearance. Meconium ileus may be complicated by volvulus of a dilated meconium-filled loop of distal ileum, which may result in atresia or may perforate, leading to meconium peritonitis. The latter is seen on abdominal radiography as multiple calcifications in the peritoneal cavity.

Following nasogastric decompression and correction of fluid and electrolyte abnormalities, the diagnosis is confirmed by water-soluble contrast enema, which will show the microcolon, the meconium pellets in the distal ileum, and the dilated proximal dilated ileum (Figure 32-9). Switching to Gastrografin enema, it is possible to clear the obstructing meconium in over 50% of cases.[22] Gastrografin has an osmolality of approximately 1,700 mOsm/L, and great care must be taken to anticipate and replace fluid

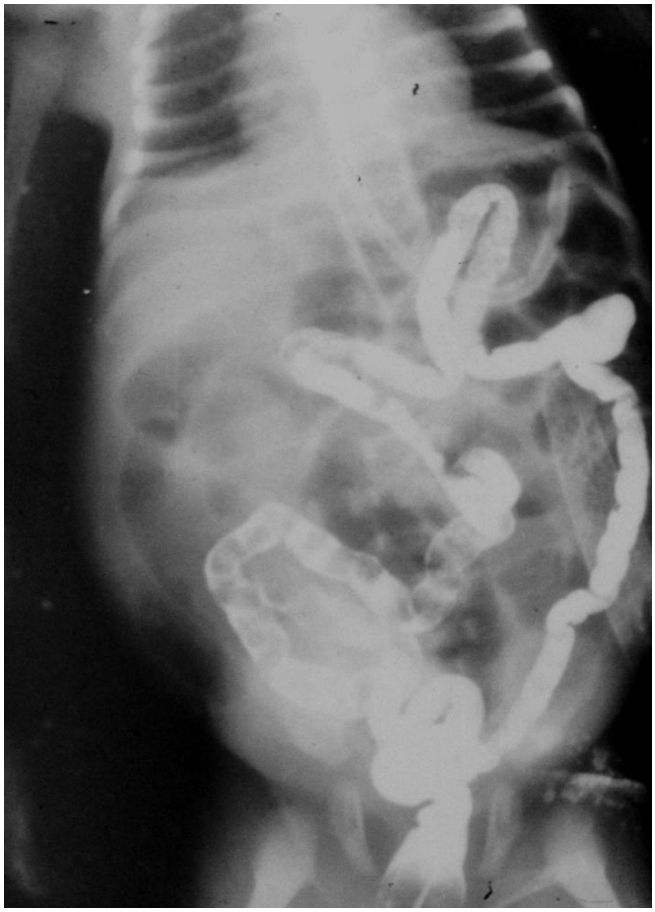

FIGURE 32-9 Meconium ileus. The contrast enema shows the typical microcolon, displaced cecum, and filling defects representing meconium pellets in the terminal ileum.

losses into the intestine. At operation for uncomplicated meconium ileus, the proximal dilated ileum is opened and the meconium is removed using saline irrigation. Simple enterostomy and resection of the dilated intestine with primary anastomosis are safe when the bowel wall is healthy and the distal obstruction can be cleared with certainty. Alternatively, proximal and distal stomas should be created.

The survival rate for meconium ileus has improved from 30% in the 1960s to over 90%.[23] This is attributable to the overall improvement in perioperative respiratory care and increasingly successful nonoperative management and avoidance of stomas. In older children, episodic obstruction due to inspissated meconium may occur (meconium ileus equivalent, distal intestinal obstruction syndrome), at times associated with underhydration and altered enzyme replacement needs. Presentation is with pain, tenderness, and possibly a mass in the right iliac fossa, which must be distinguished from an appendix mass, ovarian tumor, and inflammatory bowel disease. In most children, oral Gastrografin will relieve the obstruction.[24]

Duplication Cysts. Duplication cysts are congenital tubular or spherical cysts attached to the alimentary canal anywhere between the mouth and the anus, most commonly in the ileocecal region. The cysts have a muscle layer and an epithelial lining that usually resembles the adjacent part of the gastrointestinal tract. In the abdomen, nodules of ectopic gastric mucosa may be present. The blood supply is shared with the adjacent normal structure. Thoracic duplications may communicate through the diaphragm with the intra-abdominal gastrointestinal tract, from which the blood supply is derived (a potential pitfall for the unwary surgeon). Intestinal duplications lie on the mesenteric side of the small intestine and the antimesenteric side of the large bowel. Tubular duplications may communicate with the intestinal lumen.[25]

Presentation may be with an abdominal mass or obstruction of the adjacent intestinal lumen. In a tubular cyst, peptic ulceration secondary to acid secretion from ectopic gastric mucosa may lead to rectal bleeding or perforation. The diagnosis may be suspected on a prenatal sonogram.

For diagnosis, ultrasonography and plain or contrast radiography may be helpful, depending on the site of the lesion. Computed tomography or magnetic resonance imaging will help to distinguish the duplication from an ovarian cyst. Complete excision is the treatment of choice. Where this is not possible, partial excision and removal of mucosal lining from the residual cyst or resection of the duplication and adjacent intestinal are options.

Failure to Pass Stool in the Newborn Infant

Causes of failure to pass stool are shown in Table 32-1.

Anorectal Anomalies. Anorectal anomalies (imperforate anus) occur in approximately 1 in 2,500 live births.[10,26,27] The embryologic events surrounding hindgut development remain poorly understood. The cloaca forms at approximately 21 days gestation and is a cavity into which hindgut, tailgut, allantois, and mesonephric ducts open. By 6 weeks, a combination of programmed cell death and differential growth results in the formation of an anterior urogenital cavity and a posterior anorectal cavity. When this process is disrupted, an anorectal malformation may result. The anatomic findings in children with anorectal malformations are considerable, and a number of complex classification systems have been proposed. The simplified classification of Pena and Hong (Table 32-2) conveniently summarizes most commonly encountered variants.[28]

The cause of anorectal anomalies is unknown. Although prenatal dosing of rats with the antimitotic agent doxorubicin can cause anorectal malformations,[29] there is little evidence for environmental factors playing a major causative role in humans.

Anorectal anomalies are most often detected during routine postnatal examination, although with some anomalies, the perineum may appear relatively normal to casual inspection. Anatomically, the lesion is usually associated with a fistulous communication between the rectum and either the genitourinary tract or perineum; the spectrum of abnormalities ranges from simple anterior malposition of the anus to complex anal agenesis, in which, typically, the anus is absent or represented by a shallow pit, the infants' buttocks are flattened, and the sacrum and anorectal innervation are deficient. The degree of abdominal distention is variable, and intestinal perforation is rare.

TABLE 32-2 CLASSIFICATION OF ANORECTAL ANOMALIES

MALES	FEMALES
Cutaneous fistula, bucket handle malformation, anal stenosis, anal membrane	Cutaneous (perineal) fistula
Rectourethral fistula (bulbar or prostatic)	Vestibular fistula
Rectovesical fistula (bladder neck)	Persistent cloaca
Imperforate anus without fistula	Imperforate anus without fistula
Rectal atresia/stenosis	Rectal atresia/stenosis

About 60% of infants will have malformations affecting other organ systems, most commonly cardiac, gastrointestinal, genitourinary, or vertebral in origin.[26,27] These may coexist as the VACTERL (vertebral, anorectal, cardiac, tracheoesophageal, renal, limb) sequence of anomalies.[27] A structured approach to the assessment of these infants needs to be adopted. Thorough physical examination is essential. Echocardiography, renal tract and spinal ultrasonography, plain spinal radiography, and karyotyping all need to be performed. Neonates with multiple anomalies may require assessment by a range of specialists, including clinical geneticists, neonatologists, orthopedic surgeons, otolaryngologists, neurologists, and neurosurgeons. Children should be maintained on urinary tract antibiotic prophylaxis until micturating cystourethrography, often performed following definitive reconstructive surgery, has excluded vesicoureteric reflux.

The principles underlying the initial management of infants with imperforate anus are relief of the distal bowel obstruction and protection of the anal sphincter mechanism. Initial resuscitation should include passage of a nasogastric tube (this will also exclude esophageal atresia) and establishment of intravenous access. Surgical options are to form a colostomy or to perform definitive reconstruction. Only low lesions such as those presenting with rectocutaneous fistula in which the anomaly is distal to the anal sphincter are amenable to relatively straightforward perineal reconstructive procedures without diverting a colostomy. The rectocutaneous fistula discharges meconium and can occur anywhere in the midline anterior to the presumptive site of the anus, including the scrotum and penis (Figure 32-10). Occasionally, it is recognizable as a chain of whitish pearl-like nodules. In all other cases, or when there is doubt, it is advisable to perform a temporary diverting colostomy followed by a staged reconstructive procedure. A sigmoid colostomy is suitable for most cases of imperforate anus, but for a cloacal anomaly, a transverse colostomy is recommended.

Most anorectal anomalies are amenable to reconstruction via a posterior sagittal approach, although a laparotomy is often required in boys with rectovesical fistula and girls with cloacal anomaly. The two main steps in the reconstruction are, first, mobilization and ligation of the fistula and, second, creation of the neoanus. In girls with a cloacal anomaly, genitourinary reconstruction is also required. Meticulous surgical technique is essential to optimize the long-term outcome. More recently, laparoscopic anorectal reconstruction has been advocated, and long-term outcome data are awaited.[31] Following reconstruction, the parents are taught to dilate their infant's anus to avoid stenosis of the infant's neoanus.

The long-term outcome in children with anorectal malformations is variable and to a large extent dependent on the initial anatomy and subsequent clinical management. Children with "high" malformations tend to have a poorer outcome, with higher rates of fecal incontinence.[28] Troublesome constipation can occur in children with "low" lesions. Careful follow-up and parental support are necessary to ensure that constipation and development of a dilated megarectum are avoided. The psychological effects of incontinence and constipation in this group of children and their families as they grow up are considerable.[32] In the last decade, the antegrade continence enema procedure has been found to be useful in establishing independent continence.[33] In later life, females will need specialist obstetric assessment and advice in choosing the most appropriate form of delivery.

Hirschsprung Disease and Related Disorders. Hirschsprung disease affects 1 in 5,000 newborns and is defined as an absence of ganglion cells (aganglionosis) in a variable length of distal bowel.[34,35] In 80% of infants, the aganglionosis is confined to the rectum and sigmoid, includ-

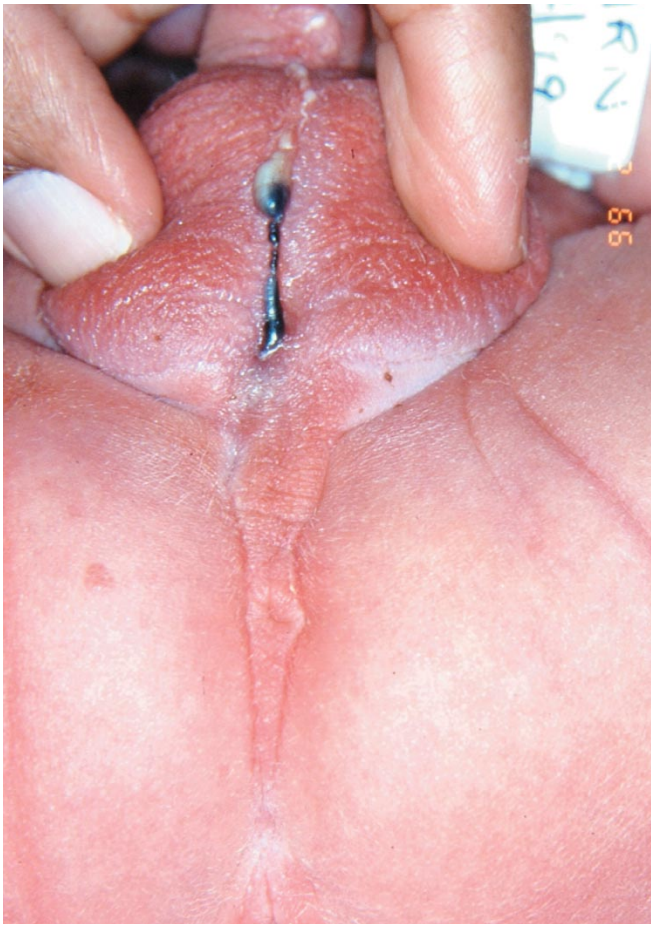

FIGURE 32-10 Anorectal malformation: cutaneous fistula. The anus is covered by a skin cap from which meconium is tracking along the fistula anteriorly in the midline onto the scrotum.

ing the internal sphincter (short-segment disease), but it may extend to encompass the entire colon (total colonic) or rarely affect the entire intestine. Although macroscopically normal, the affected gut is unable to relax, causing a functional bowel obstruction with dilatation of the proximal intestine.

Ganglion cells of the enteric nervous system are derived from the vagal neural crest. During the first trimester, neural crest cells migrate from the vagal neural crest into the esophagus and then colonize the developing gut in a craniocaudal direction. Aganglionosis can result from a failure of migration, differentiation, or survival of these cells. Mutations in several genes can cause aganglionosis. The receptor tyrosine kinase gene *RET* is the most common gene in which a mutation may be found.[36] Significantly, mutations in *RET* have been found in multiple endocrine neoplasia syndrome types IIA and IIB and familial medullary thyroid carcinoma; a careful family history should therefore be taken and genetic counseling offered to families at risk.[37] Hirschsprung disease is also more common in children with trisomy 21 (Down syndrome).

Hirschsprung disease should be suspected in all infants who have not passed meconium within 48 hours of birth and all infants presenting with signs of bowel obstruction. Constipation and abdominal distention are the most common presenting signs, although affected children may also present with bile-stained vomiting, failure to thrive, or cardiovascular collapse owing to Hirschsprung enterocolitis. A minority of children will present later in life with chronic constipation. Plain abdominal radiographs will show distended loops of bowel, although in neonates, often it is impossible to distinguish large from small bowel obstruction. Initial management should be directed at resuscitation, establishment of intravenous access, and passage of a nasogastric tube. When the abdomen is very distended, gentle anal dilatation and rectal washouts with 0.9% saline solution can often result in dramatic decompression and improvement in the physical condition of the child.

Diagnosis is made by suction rectal biopsy of the submucosal plexus, a procedure that can be performed using custom-made biopsy forceps on the ward, with minimum discomfort and distress to the infant. Multiple biopsies should be obtained at 2 and 4 cm above the anal verge. Histopathologic examination will reveal an absence of ganglion cells and thickened nerve trunks that often extend into the lamina propria. Increased acetylcholinesterase staining is also seen and can aid diagnosis. Availability of an experienced pediatric pathologist is essential. Where there is doubt, repeat biopsies should be obtained and the need for a full-thickness biopsy that includes the myenteric plexus considered. A contrast enema may be useful in determining the extent of aganglionosis if a clear transition zone from ganglionic to aganglionic colon is seen; however, false-positives can occur, and Hirschsprung disease should not be diagnosed on the basis of a contrast enema alone. Radiologically, Hirschsprung disease must be distinguished from meconium plug syndrome, small left colon syndrome, colonic atresia, and distal ileal atresia. Anorectal manometry can also be diagnostic in older children on the basis of an absent rectoanal inhibitory reflex, but this cannot be reliably performed in neonates.

The principles of definitive surgical reconstruction are excision of the aganglionic colon and "pull-through" of ganglionic colon with a coloanal anastomosis. The traditional operative approach is to perform a colostomy in the neonatal period at the distal limit of ganglionic bowel (confirmed histologically) followed by staged surgical procedures over 3 to 9 months. Increasingly, infants are initially managed by rectal washouts until definitive surgical treatment. Initial data would suggest little difference in eventual outcome from either approach.[38] Recent advances have been adoption of a laparoscopically assisted or purely transanal approach.[39] Frozen section biopsies are used to determine the presence of ganglion cells at the level of resection.

Following definitive operation for Hirschsprung disease, fecal incontinence has been reported in about 60% of patients compared with age-matched controls,[40] and the incidence was higher when evaluation was carried out by a psychologist.[41] Most patients have constipation with soiling that appears to improve with age, but 9 to 40% are severely incontinent.[40–42] The reasons for these poor results are multifactorial. Incomplete excision of the aganglionic segment, internal sphincter dysfunction, external sphincter damage, and dysmotility of the apparently normal ganglionic bowel may all play a role, and children need careful follow-up into adulthood.

Hirschsprung enterocolitis, characterized by malaise, pyrexia, abdominal distention, constipation, or diarrhea, is a potentially life-threatening complication that can occur before or following surgery. The pathologic basis of Hirschsprung entercolitis is poorly understood and may represent alterations of bacterial flora, relative gut stasis, and impaired mucosal or neuronal immunity. Current treatment is empiric, consisting of rectal washouts, antibiotics (vancomycin or metronidazole), probiotics,[43] and sodium cromoglycate.[44] Chemical (botulinum toxin[45] or topical glyceryl trinitrate[46]) or surgical internal sphincterotomy may be of benefit. Occasionally, it is necessary to perform an urgent colostomy.

There is a further small group of children who present with symptoms of Hirschsprung disease but who have ganglion cells on rectal biopsy. Often their symptoms are transient. Some investigators have found histopathologic features such as altered numbers of ganglion cells and increased acetylcholinesterase activity and labeled this intestinal neuronal dysplasia.[47,48] Others feel that such findings are part of the spectrum of normality in infants and neonates. Other children continue to have pseudo-obstructive symptoms throughout life. It is difficult to define whether histopathologic abnormalities are responsible for the obstructive symptoms or secondary to them.

Meconium Plug Syndrome. Occasionally, when a rectal examination or contrast enema is performed in neonates with symptoms suggestive of Hirschsprung disease, a whitish "plug" of epithelial cells is expressed followed by brisk passage of meconium and flatus with relief of symptoms (Figure 32-11). This condition is called meconium plug syndrome and is common in premature infants, possibly owing to relative immaturity of their ganglion cell

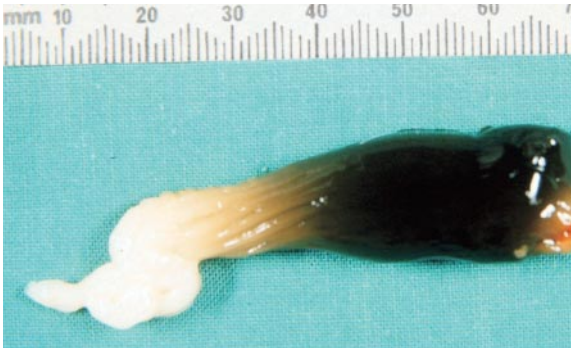

FIGURE 32-11 A meconium plug consists of thick meconium proximally and pale epithelial cells distally. Presentation is with distal colonic obstruction, which is relieved by a contrast enema.

TABLE 32-3 KEY POINTS FOR NEONATAL TRANSFER

Trained nursing and, if necessary, medical staff must accompany the patient.
Temperature control using a transport incubator to prevent hypothermia
Intubation and ventilation must be established before transfer of infants with respiratory distress.
Nasogastric drainage is essential for abdominal disorders, with the tube on open drainage and regular aspiration during transport.
Intravenous fluids appropriate for the needs of the infant
Send with patient:
Maternal blood sample for cross-matching
Documentation of medications given, notably vitamin K and antibiotics
Results of investigations and copies of radiographs
Written consent by the mother for operation where appropriate.

development. Cystic fibrosis and Hirschsprung disease should be positively excluded in children with meconium plug syndrome.

Colonic Atresia. This rare cause of distal intestinal obstruction presents with abdominal distention, failure to pass stool, and vomiting. A contrast enema will distinguish it from distal ileal atresia, with which it may coexist, and Hirschsprung disease. Management is local excision of the atretic segment.

TRANSPORTING THE SURGICAL NEWBORN INFANT

Ideally, infants known from prenatal screening to have a major surgical anomaly should be delivered in a specialist center. Postnatal transfer is safe provided that attention is paid to the key points in Table 32-3.[49]

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CHAPTER 33

HERNIAS

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The term "hernia" (from the Latin) means "bud" or "bulge" and is extensively used in medical practice to address the various defects of the walls of the abdominal or other body spaces. In this chapter, an apparently heterogeneous group of hernias that may involve the gastrointestinal tract is addressed. Particular attention is paid to the digestive symptoms that may appear before and after the treatment of such conditions. These hernias are summarized in Table 33-1.

OMPHALOCELE (EXOMPHALOS)

"Omphalos" (from the Greek "ομφαλος") means "navel" and "cele" (from the Greek "κηλη") means tumor. Therefore, omphalocele means umbilical swelling. This anomaly is also referred to as "exomphalos" (in Greek "εξ," meaning "out") or prominent umbilicus. In fact, these terms describe a congenital defect of the umbilical region in which the umbilical cord is replaced by a sac, formed by Wharton jelly and peritoneum, that contains bowel loops and sometimes part of the liver and that is implanted on the rim of a more or less large parietal defect.

The prevalence of omphalocele ranges between 1 in 5,000[1] and 1 in 20,000[2] live births, but it is probably much higher if stillborns and aborted fetuses are considered. It is more frequent in boys than in girls, and there are no racial differences in incidence.

This developmental defect has an early origin during intrauterine life. Its causes are unknown, but chromosomal defects,[3] particularly trisomy 18,[4,5] are detected in these patients in proportions ranging from 10[2] to 40%.[1,6] Its association with other genetic disorders, such as Beckwith-Wiedemann syndrome, and the observation of some familial cases[7] support an embryonal origin for this malformation. However, it could be plainly derived from the arrest of the process of abdominal wall closure by growth of the ectodermal and mesodermal structures that surround the implantation of the umbilical stalk during the period in which the midgut reintegrates into the abdomen from the "physiologic" hernia into the umbilical stalk. During this phase, the bowel grows rapidly in length, and part of the jejunum, ileum, and proximal colon migrate into the umbilical stalk until the abdomen is large enough to accommodate them. During reintegration, the midgut undergoes a process of rotation in which we can distinguish two components that take place simultaneously: the duodenojejunal junction, originally located on the right side, rotates counterclockwise downward and leftward for a total of 180° passing underneath the vitelline (superior mesenteric) artery to end up on the left side to form the angle of Treitz. The cecum, in turn, rotates counterclockwise upward and rightward for another 180° in front of the artery from its original lower left position to its final right iliac position. Once rotation is completed, the bowel becomes fixed by several attachments from the bowel to the abdominal walls. When the process of intestinal repositioning is interrupted, the abdominal space remains empty, and rotation of the gut is incomplete.

Nowadays, omphalocele is generally diagnosed on prenatal ultrasonography between the second and third trimesters of pregnancy because it is very visible as a more or less enlarged umbilical stalk containing bowel loops and surrounded by amniotic fluid.[8] Cell karyotype from fluid obtained by amniocentesis is mandatory in these cases because of the rather high incidence of associated chromosomal diseases, particularly when the sac is small and the liver is intracorporeal.[3,9,10] In cases with trisomy 18, exomphalos is frequent, but there are several craniofacial, limb, and visceral malformations that are incompatible with life. The same happens with trisomy 13, and these diagnoses allow for the well-counseled parents to decide on continuation or termination of gestation.[11,12]

If pregnancy proceeds to term, patients with omphalocele often have neonatal weights appropriate for their gestational age and are immediately diagnosed because the malformation is very prominent: there is an abdominal wall defect of variable width centered at the umbilicus with herniation of bowel loops and sometimes the liver depending on the size of the defect. The viscera are covered by a gelatinous, translucent sac on top of which the umbilical cord enters the fetal body (Figure 33-1A). The umbilical arteries

TABLE 33-1 TYPES OF HERNIAS IN CHILDREN

Omphalocele (exomphalos)
Cloacal exstrophy
Gastroschisis (laparoschisis)
Congenital diaphragmatic hernia
Hernia of Morgagni
Inguinal hernia
Femoral hernia
Umbilical hernia
Epigastric hernia

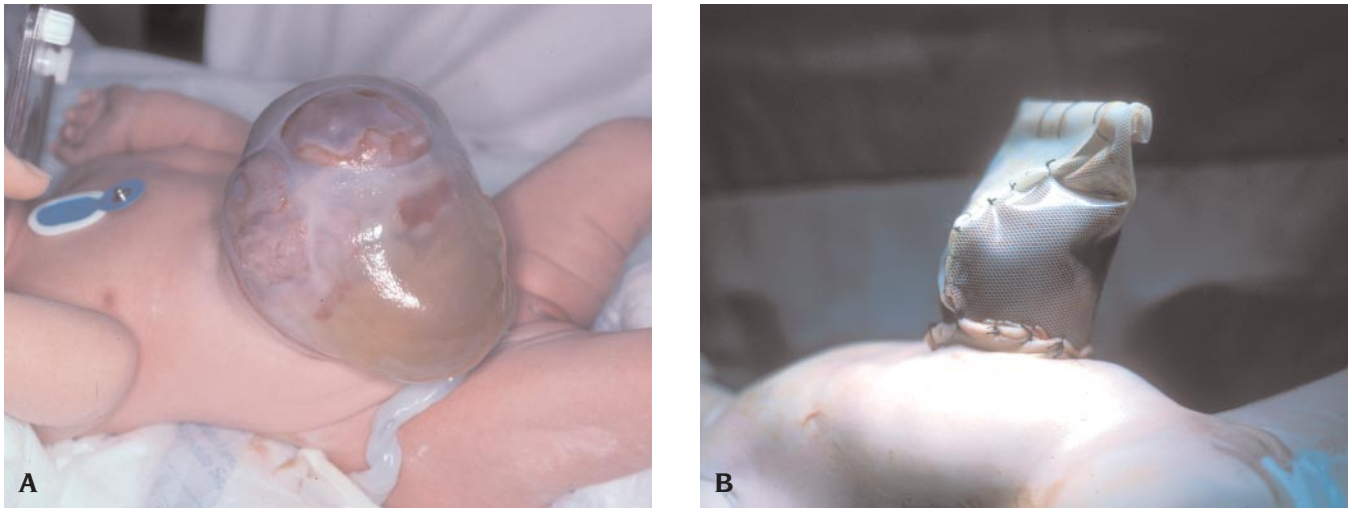


FIGURE 33-1 Omphalocele. *A*, Huge herniation of intra-abdominal organs (intestine and liver) into a sac inserted on a large anterior abdominal orifice. This female fetus had normal birthweight. *B*, Silastic chimney or silo used for staged reduction of the omphalocele when primary closure is not possible.

run downward along the inferior wall of the sac to reach the abdominal wall. The urachal stump is located between them. The umbilical vein enters the abdomen on the superior side of the stalk. The abdomen may be remarkably small owing to the lack of content during fetal life, and this represents one of the main therapeutic problems.

The phenotype of the newborn may display the anomalies seen in either trisomy 18 or trisomy 13, but it may also be normal. Internal anomalies, particularly heart defects,[13] should be sought as soon as possible to tailor the timing of treatment to the clinical situation. Associated malformations are present in half[2] to three-quarters[1] of patients with omphalocele.

Termination of pregnancy may be indicated in fetuses with trisomy,[11,12] but it cannot be recommended in all other cases because successful treatment is possible in most of them. There is no evidence of the need for cesarean section on the basis of the incidence of neonatal infection or of the results of treatment. However, the convenience of starting the treatment as soon as possible after birth prompted many institutions to adopt a policy of induction of the labor or cesarean section.

After looking for associated anomalies that might contraindicate or delay surgery at this stage, repair of the defect is performed early after birth. This usually consists of excision of the membrane, reintegration of the viscera into the abdomen without or with stretching of the wall, and closure under bearable tension. Primary closure is possible only in some instances, and the risks of creating excessive intra-abdominal pressures capable of impairing arterial blood flow to the organs and venous return to the heart (compartment syndrome), as well as difficulties in ventilation, prompt the use of temporary coverage of the viscera with a Silastic chimney or "silo," as proposed originally by Schuster (Figure 33-1B).[14] The silo can be progessively squeezed in the ensuing few days to allow for progressive bowel reintegration without excessive intra-abdominal pressure.

The difficult bowel reduction, the prolonged contact of the bowel with the silo, and the daily manipulations usually interfere with the introduction of enteral feeding at this stage. Some surgeons advocate the use of a temporary gastrostomy for decompression and step-by-step feeding. This tube may facilitate the measurement of intra-abdominal pressure during surgery to avoid compartment syndrome.[15] The use of total parenteral nutrition for as many days as required greatly facilitates the treatment of omphalocele.

These patients may develop normally once the defect has been closed and the gastrointestinal function is resumed, but some of them may undergo gastrointestinal symptoms that should be recognized by the pediatrician or the pediatric gastroenterologist.

Early intestinal necrosis owing to closure of the abdominal defect under tension may cause short bowel and require long-term parenteral support.[16] Incomplete or abnormal rotation is not corrected in most cases during the neonatal operation because at that time, the priorities are visceral repositioning without excessive tension and secure wall closure. Malrotation does not cause problems in most cases, but it may become symptomatic as well.[17] Rarely is volvulus a problem, but this may happen with all of the risks involved. Repeated, often bilious, vomiting should direct the attention to the position of the bowel. Barium meal helps in defining the anatomic arrangement of the duodenum and the duodenojejunal angle. Barium enema is rarely necessary to depict the position of the colon if upper tract malposition is found in upper series, but it might be otherwise necessary. Surgical correction of malrotation might be eventually required for alleviation of the symptoms.[18]

Gastroesophageal reflux with esophagitis is detected in almost half of these children and may manifest itself early after abdominal wall closure.[19] The hiatus is abnormally located in them with a more or less marked anterior displacement, and the intra-abdominal pressure is increased after closure under tension. This creates an increased abdominothoracic gradient that facilitates reflux.[20] Some

patients may require fundoplication,[21] and it is obvious that gastric emptying should be assessed and malrotation ruled out prior to operation. The surgical approach to the esophagogastric region by laparoscopy or laparotomy may be difficult in these cases, and a thoracic approach has been considered advisable.[22]

If patients with chromosomal anomalies are not considered, the results in this group of patients are good in terms of survival and quality of life. Most patients live normal lives as adults, with occasional problems related to the scar or relatively mild gastrointestinal dysfunctions.[23]

CLOACAL EXSTROPHY (VESICOINTESTINAL FISSURE)

This is a major defect of the abdominal wall in which an omphalocele, a bladder exstrophy, and an ileal prolapse in the middle of a colonic plate replace the anteroinferior wall of the trunk. The anus is absent, and there are many other malformations.

Cloacal exstrophy is very rare, and its prevalence is probably below 1 in 200,000 or 1 in 400,000 live births. It may be seen in both genders, but it is slightly more frequent in males.

It has been traditionally attributed to a grossly defective closure of the abdominal wall folds, but many aspects of the malformation are not explained by this simple interpretation. In addition, there is prenatal ultrasonographic evidence in some fetuses of an intact lower abdominal wall that only secondarily ruptures.[24,25] Nevertheless, the association with other malformations suggests a profound disturbance of organogenesis that can occur only during early intrauterine life.[26]

The two halves of the exstrophied bladder are located on both sides of an intestinal mucosal surface, corresponding to the ileocecal region, in which two or more orifices can be recognized: the proximal ileal one is often prolapsed, whereas the distal colonic one ends in a blind pelvic pouch (Figure 33-2A). Other orifices corresponding to one or two appendices can eventually be seen on both sides. The external genitalia are also split below and on both sides of the exstrophied bladder halves. The genital tubercles are generally difficult to identify because the corpora of the penis or clitoris, implanted on the widely separated pubic bones, are not fused. The genital folds are also rudimentary, and their scrotal or labial nature is difficult to ascertain, particularly because the testes are usually undescended. The vagina may be absent. Gender assignment is therefore difficult at birth on visual inspection of the genitalia alone. There is an omphalocele containing intestines and liver on top of the exstrophied structures, the anus is absent, the perineum is generally flat, and the sacrum may be split by an open or covered spina bifida (Figure 33-2B). The vertebral bodies are often malformed, and ultrasonography and magnetic resonance imaging may demonstrate tethered cord, accounting for innervation deficits.[27,28] The colon ends

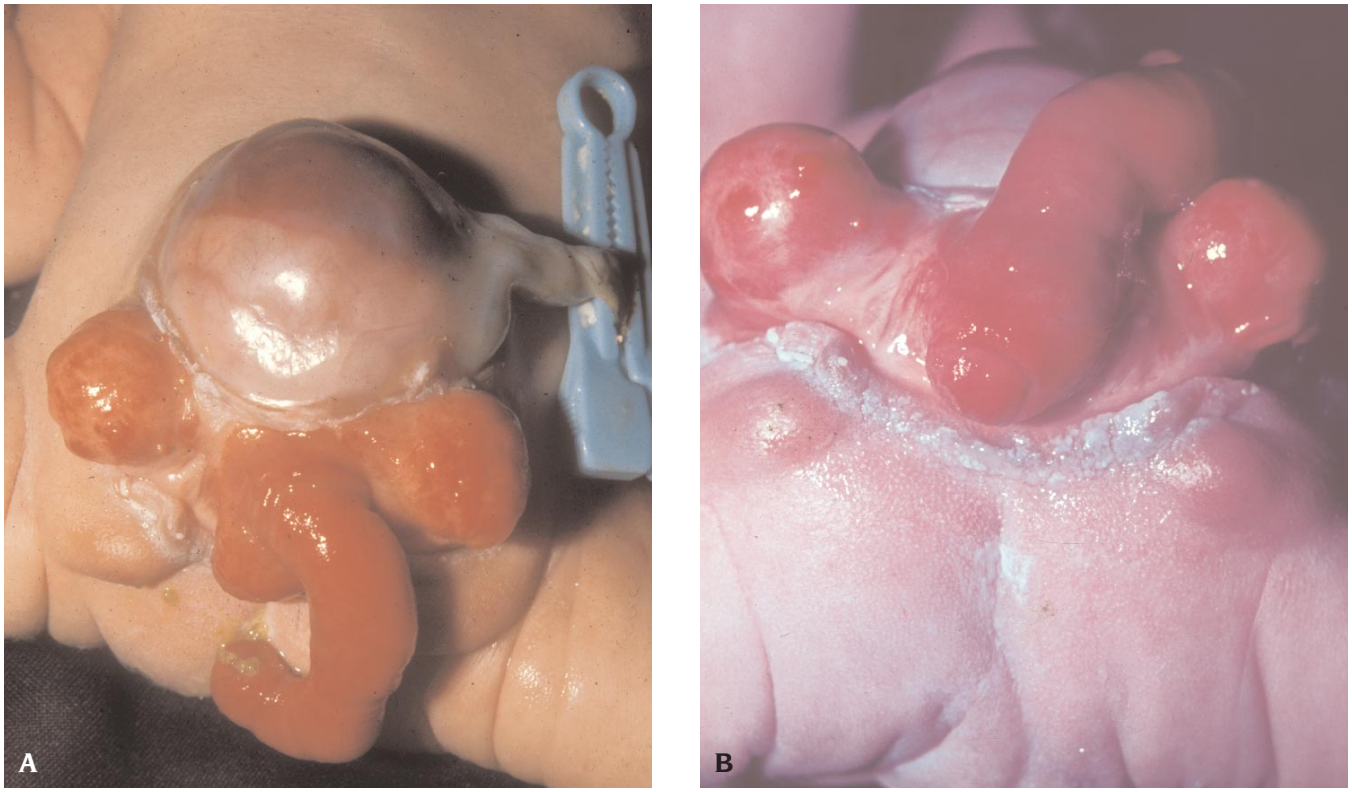


FIGURE 33-2 Cloacal exstrophy. *A*, Gross defect of the abdominal wall with a midsize omphalocele, an ileocecal plate with prolapse of the ileum, and two hemibladders on both sides of the plate. *B*, Details of the perineal region in which the anus was absent and the genitalia were uncertain in this male patient in whom the corpora were very small and the testes undescended. He also had a neural tube defect, which is not shown in the picture.

blindly, and it is generally shortened and limited to a terminal blind-ending pouch. The small bowel can also be somewhat shortened. Both ureters open at the exstrophied bladder halves and drain usually normal kidneys.

The aims of treatment will be preservation of life and renal function, to obtain continence of bladder and intestine, and maintenance of a positive self-image, allowing adequate integration into society,[28–31] and it is obvious that they are extremely difficult to reach. Gender assignment at birth must take into account the possibilities of reconstructing a functional phallus with the available material, and for this reason, some of these babies are raised as females in spite of all of the multiple problems and uncertainties that this decision involves. Closure of the omphalocele with reconstruction of the colon preserving its entire available length and diversion as a colostomy is followed by anastomosis of the two bladder halves to form a closed reservoir, which may later require augmentation with bowel. A continent urinary diversion using a Mitrofanoff appendicostomy is generally performed at a later stage. Colonic pull-through is generally possible and should be attempted[30,31] in spite of the grim expectations of continence owing to the anatomy of the region and to the neurologic impairment. A program of daily washouts may be very helpful. Neurosurgical, orthopedic, and other problems may require attention for life. Nowadays, most patients survive, but they endure multiple operations and face difficult choices and adaptative efforts.

Fecal incontinence or permanent stomas are often unavoidable in these patients. Short bowel may exist from the beginning, and it is eventually aggravated by the use of intestinal segments for reconstruction of the urinary tract, vagina, or the continent stomas.[28]

GASTROSCHISIS OR LAPAROSCHISIS

These terms are derived from the Greek ("σχισις," meaning fissure; "γαστρης," meaning stomach; and "λαπαρα," meaning abdomen) and describe an abdominal wall defect consisting of an orifice, generally quite narrow, not more than 2 or 3 cm, located on the right side of the umbilical cord insertion, that allows evisceration of part of the abdominal contents, small bowel, large bowel, stomach, and, sometimes, the gonads. These organs are matted in a more or less thick "peel" that makes anatomic boundaries difficult to define.

Gastroschisis is a condition clearly different from ruptured omphalocele with which it was confused in the past. The etiology is unknown, but the scarcity of associated malformations and the relative ease with which identical lesions can be reproduced in laboratory animals by fetal operations are consistent with the interpretation of an unknown agent acting during early fetal life as the origin of the orifice. The explanation for its almost constant location on the right side is obscure, but it has been suggested that fetal occlusion of the omphalomesenteric artery could account for it.[32] Gastroschisis is becoming relatively frequent in industrialized countries because its prevalence, which ranges from 1 in 3,000 to 1 in 20,000 gestations,[2,6,33] has increased in the last 30 years. It is certainly higher (1 in 3,000 to 1 in 6,000)[6,34] if stillborns and terminated fetuses are taken into account.[35] Gender distribution is roughly balanced. The prevalence increased almost three times in the last 30 years in developed countries,[2,33,36] in parallel with the increased use of tobacco and other drugs[2,37] by progressively younger, often primiparous mothers.[33] These gestational agents might account for the defect itself, whereas the remaining lesions are caused by prenatal evisceration. Prolonged exposure of the intestinal walls to the amniotic fluid accounts for the intestinal shortening, serosal thickening, and matting of the loops and can be reproduced in chicks,[38–40] rats,[41] rabbits,[42–44] and lambs[45–47] after fetal operations, which expose the bowel to this irritant fluid for more or less prolonged periods of time. The eviscerated bowel may undergo ischemia during gestation owing to the narrowness of the orifice, and this may cause fetal distress,[48] intestinal atresia,[49] or even fetal demise.[11] On the other hand, fluid, proteins, immunoglobulins, and electrolytes are exchanged between the fetal internal environment and the amniotic fluid,[50] causing fetal malnutrition and protein losses[51] that may impair healing or immune defense and interfere with the response of the patient to neonatal treatment.

Prenatal diagnosis is possible on ultrasonographic screening in about 60 to 80% of cases[52,53] from the twentieth week on,[52] but it can be done well before this date. The bowel loops floating into the amniotic fluid are identified on the right side of the umbilicus, and the distended loops, sometimes with thickened walls, can be seen quite early.[52] Amniocentesis is probably unnecessary in gastroschisis because of the lack of association with chromosomal diseases.[8,54] However, close ultrasound monitoring is mandatory because it allows assessment of fetal growth and wall thickness and Doppler imaging of intestinal blood flow.[55] Echographic signs of fetal distress may be helpful for deciding on early delivery.[56]

In contrast to those with omphalocele, patients with gastroschisis are usually small for gestational age because of fetal malnutrition related to the above-mentioned reasons.[37] At birth, they are generally normal, except for the narrow orifice, which is almost invariably located on the right side of the base of the umbilical cord. The root of the usually prominent mass of eviscerated bowel is more or less constricted, and its aspect ranges from quite normal to matted in a thick, reddish, inflammatory mass in which the different parts of the bowel are difficult to discern (Figure 33-3). Like in patients with exomphalos (omphalocele), the abdomen is generally small owing to its emptiness during gestation.

There is some debate about the convenience of acting "in utero" on the gastroschisis fetus. Because some of the local and general changes observed in these babies are related to the duration of the contact of the eviscerated bowel with the irritant components of the amniotic fluid, shortening this contact by preterm delivery might be beneficial. This would additionally reduce the effects of bowel constriction at the orifice and prevent loop distention and later paralysis.[57] On the other hand, there is evidence of the

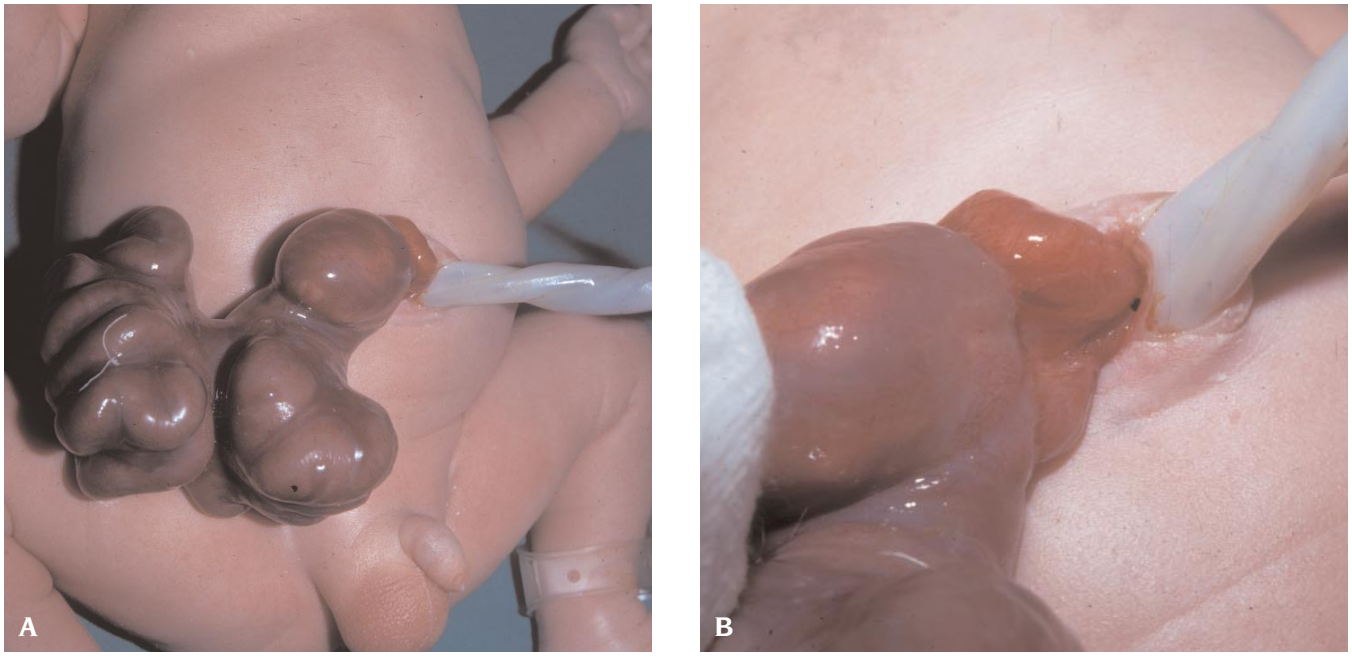


FIGURE 33-3 Gastroschisis. *A*, Massive prolapse of the intestine through a small paraumbilical orifice. The bowel loops are matted into a thick peel that makes anatomic definition of the underlying structures very difficult. *B*, Detail of the umbilicus depicting the intact cord with a right-sided and quite narrow orifice.

detrimental effect of compression on the intestinal wall during delivery, and to avoid it, premature, prelabor cesarean section has been advocated.[58] Cesarean section is indicated anyway whenever fetal distress is detected on frequent monitoring,[56,59] but, in other cases, there is not much evidence of the benefits of cesarean section over induction of labor when the rationale of prelabor delivery is not adopted.

Other prenatal approaches aimed at preventing the bowel lesions are possible: repeated amniotic fluid exchange or amnioinfusion to dilute the amount of offending solutes has been tried in the experimental[60–62] and clinical settings.[63,64] The use of prenatal anti-inflammatory agents such as dexamethasone has been shown to be beneficial in animal experiments.[65,66]

After birth, repositioning of the eviscerated bowel into the abdomen is urgently needed because excessive heat irradiation, fluid depletion, and bacterial contamination are rapidly progressive. Careful coverage of the lesions, adequate fluid and electrolyte replacement, and prompt surgery are usually performed. Some authors proposed treating these infants in the delivery room itself,[67] and it is generally accepted that referring the mothers for delivery in specialized neonatal surgical centers is beneficial. If adequate bowel coverage and intensive care are provided over an interim time period of some hours, delaying definitive treatment does not seem to decrease the chances of survival in these babies.

The rationale of surgical treatment of gastroschisis is basically similar to that of omphalocele: reintroduction of the bowel into the abdominal space after enlarging the orifice when necessary, abdominal wall stretching, meconium expression to reduce the bulk of the colon, and primary closure if possible. If the pressures into the abdomen become unbearable, silo-staged closure[68–70] or skin-only closure and late repair of the ventral hernia[71] can be lifesaving. Gastrostomy is preferred by some surgeons to facilitate postoperative handling.[72] An increasing proportion of patients can be primarily closed, and, in the last few years, careful reduction of the eviscerated bowel in the incubator by manual compression without anesthesia has allowed primary closure with excellent cosmetic results in a growing number of patients.[73]

Babies with gastroschisis may have associated intestinal atresia owing to vascular flow interruption to a segment of the bowel during gestation. The lesions may be masked by the thick peel covering the bowel loops at birth, and it is a wise policy to leave the treatment (and even the diagnosis) of this relatively rare associated condition for a second-look operation performed if the symptoms of intestinal obstruction do not subside after a few weeks.[74] The peel disappears, and the bowel lengthens when it is repositioned into the abdomen.[75] The anatomic definition of the atresia and its repair are definitely easier at that moment.

The development of abdominal "compartment" syndrome during primary repair owing to excessively increased intra-abdominal pressure and vascular compromise may lead to necrosis and dramatic amputation of the gut.[16] This can be prevented by careful assessment of the intra-abdominal pressure[15] and the use of silo whenever necessary. Even if these precautions are taken, secondary necrotizing enterocolitis may intervene in these patients,[58] with all of the additional risks of necrosis and bowel loss involved. It should be taken into account that gastroschisis is one of the leading causes of end-stage gastrointestinal failure referred for transplant.[76]

Owing to bowel wall thickening with abnormal collagen and muscle,[45–47] to focal ischemic changes,[39] or to disturbed neuromediator secretion,[77] these children almost

invariably suffer intestinal dysmotility that causes prolonged ileus and makes enteral feeding impossible for more or less prolonged periods of time. The intrinsic innervation of the bowel has been found to be intact in some experimental studies,[38,39,78] although others described reduced density of ganglia in the intermuscular or submucosal plexuses.[66] In addition to the motor dysfunction, some difficulties for absorption of nutrients that might further impair nutrition in gastroschisis have been investigated in experimental animals.[77,79,80] Total parenteral nutrition allows adequate caloric and plastic inputs for days or weeks until intestinal motility recovers, and it is one of the cornerstones of treatment.

Malrotation is almost constant in gastroschisis survivors because usually no attempt is made at correcting this malposition during the early phase of treatment. Nonrotation without obstruction is probably the more frequent situation, but incomplete, obstructing malrotation should be ruled out when prolonged ileus extends for too much time in these infants.[17,18,81,82] Volvulus is another cause of short bowel consecutive to gastroschisis and should be thought of when symptoms of strangulation are present.[73]

The other problem that may be encountered in these patients later in life, like in survivors of neonatal exomphalos repair, is gastroesophageal reflux.[18,19,83] The same anatomic factors play a role in the failure of the antireflux barrier together with prolonged intestinal obstruction. In addition, the conditions of abdominal wall closure increase the pressure gradient between the abdomen and the thorax that is one of the reflux driving forces.[20]

CONGENITAL DIAPHRAGMATIC HERNIA

Congenital diaphragmatic hernia (CDH) is a developmental defect in which intra-abdominal organs are displaced into the thorax through a posterolateral diaphragmatic orifice. Babies with CDH also bear lung hypoplasia and immaturity, as well as other associated malformations that make neonatal treatment very difficult and cause a high mortality.

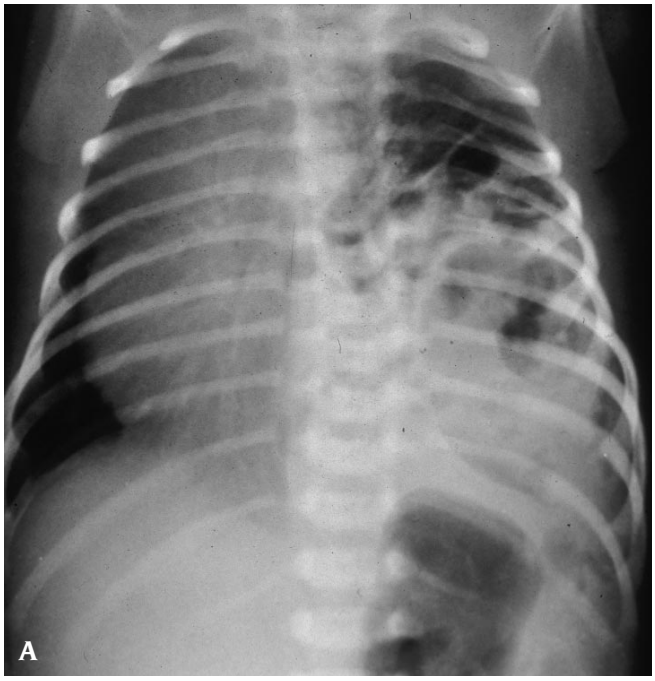
CDH is observed in 1 in 4,000 live births, without gender or racial differences, but, again, this prevalence can be much higher if stillborns and aborted fetuses are accounted for.[84] Harrison and colleagues pointed out in the past the importance of the "hidden mortality" in this particular condition,[85] and although the refinements of prenatal diagnosis have reduced the hidden proportion of fetuses with CDH, this effect still plays a role.

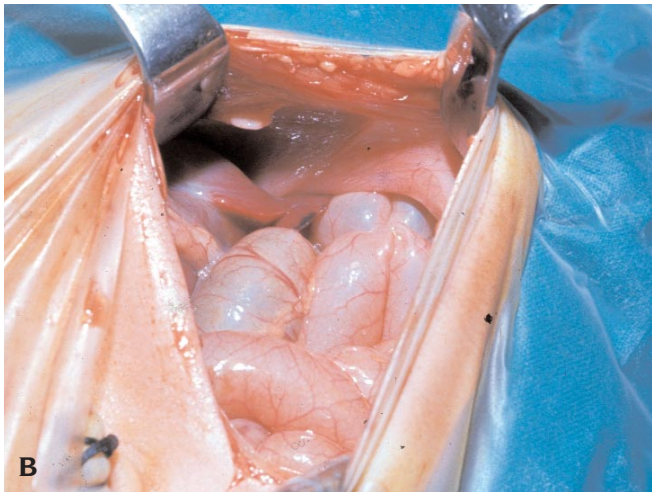
CDH is probably caused by abnormal molecular signaling in the early phases of development. This explains the multiorgan involvement and the striking similarities with animal conditions induced experimentally by genetic manipulation. The name of Bochdalek has been associated with CDH for a long time because this author was one of the first to describe it, but his report involved herniation through the lumbocostal posterolateral orifice in the diaphragm.[86] For a long time, it was thought that CDH was caused by incomplete closure of the posterolateral pleuroperitoneal canal, which communicates the pleural and the abdominal compartments of the celomic cavity. In fact, experimental studies have shown that the diaphragmatic orifice is due to incomplete growth of the posthepatic mesenchymal plate prior to pleuroperitoneal canal closure.[87] On the other hand, it has been shown that lung hypoplasia is primary because it is already present before herniation of intra-abdominal organs into the thorax.[88,89] Compression of the lungs later in gestation would play a secondary but important role in the lung lesions.[90] There is evidence of underexpression of several transcription and growth factors in the lungs of animal models of CDH, and this is interpreted as an indication of a molecular dysregulatory origin.[91–93]

The diaphragmatic orifice is usually located on the posterolateral area of the left hemidiaphragm, which may eventually be absent. In these cases, the hiatus may be practically nonexistent, and the esophagus enters the abdomen through the same orifice. The small and large bowel, spleen, and left lobe of the liver are often into the thorax in direct contact with the lung and the mediastinum because these hernias rarely have a sac. When the orifice is located on the right side, the corresponding lobe of the liver is herniated into the thorax. Very rarely are these hernias bilateral. As a consequence of compression or owing to a primary maldevelopment, there is a marked lung hypoplasia that is more severe on the side of the defect.[94] The lungs are small, with reduced bronchoalveolar ramification and excessive muscularization of the terminal arterioles.[95] Owing to their early displacement into the thorax, the intestines are not normally rotated and attached.[96] More often, they adopt the nonrotation position, but sometimes there are also Ladd bands between an upward displaced right colon and the right abdominal wall or the gallbladder.[97,98]

In addition to lung hypoplasia, the scant and small air spaces left cannot expand because of the immaturity of the surfactant system or simply because of the lack of alveolar surface for the surfactant to exert its action.[99] Some degree of heart hypoplasia, particularly of the left side, has been observed, together with several other heart malformations that may interfere with the transition from the fetal to the extrauterine patterns of circulation in these patients.[100–103] At birth, appropriate ventilation is often impossible, and hypoxia, acidosis, and the abnormal arteriolar shaping cause pulmonary hypertension without enough flow into the pulmonary vascular bed. The blood is shifted to the aorta through the ductus arteriosus and the oval orifice, perpetuating a fetal pattern of circulation that is rapidly lethal in patients in whom pulmonary hypertension persists.[104]

In some cases, polyhydramnios prompts ultrasonographic detection early during pregnancy, but the malformation may be found as well on routine screening.[105–107] At birth, these babies have a hollow abdomen with displacement of the heart bruits toward the contralateral side, and the majority of them show symptoms of severe respiratory insufficiency. Plain radiographs of the thorax depict displacement of the abdominal viscera into the thorax with major restriction of the lung space on the side of the hernial orifice and mediastinal shift toward the contralateral side (Figure 33-4A). Hypoxia, hypercapnia, and acidosis are progressive, and unless vigorous treatment is instituted, patients succumb to the disease.





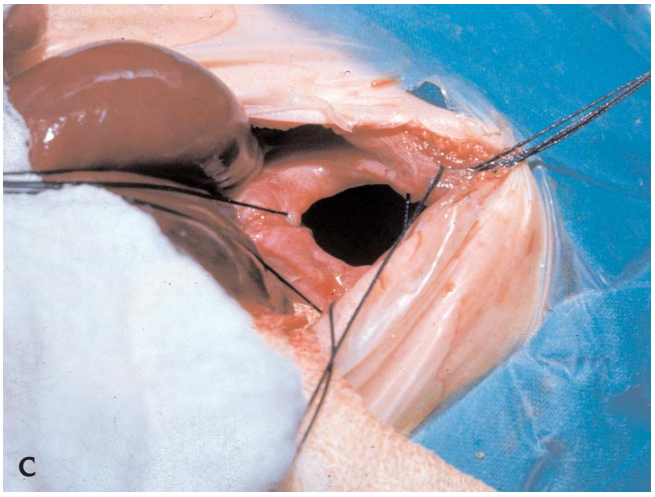


FIGURE 33-4 Left congenital diaphragmatic hernia in a newborn. *A*, Radiograph of the thorax shows herniation of intestinal contents into the left hemithorax, shifting of the mediastinum to the right, and reduction of the lung space. *B*, Detail of the left diaphragmatic orifice during operation. The bowel loops are seen entering the thorax. *C*, The orifice after extracting the bowel and the left lobe of the liver, which are now in the abdomen. The orifice will be straightforwardly closed.

Less often, the clinical picture is milder. In some cases, the disease manifests itself more subtly, and the diagnosis is delayed beyond the first days of life and may be made even much later.[97,108] These patients more often have a hernial sac, and their prognosis is good.

Nowadays, most mothers bearing fetuses with CDH are referred to specialized centers for perinatal treatment, which may even involve endoscopic fetal surgery consisting of reversible tracheal plugging,[109–112] prenatal corticosteroids,[113,114] and neonatal instillation of surfactant[115] followed after birth by a wide range of ventilatory techniques (conventional, high-frequency, oscillatory, liquid ventilation with perfluorocarbons) and extracorporeal membrane oxygenation (ECMO) in cases in which a minimally adequate gas exchange cannot be established and persistent pulmonary hypertension cannot be reverted.[116] With appropriate ventilatory support, vasodilator medication, and, eventually, ECMO, the first phase of the disease can be overcome by two-thirds to three-quarters of the patients. The abdominal viscera may then be surgically repositioned into the abdomen and the diaphragmatic orifice closed (Figure 33-4B and 33-4C). In some patients, a gastrostomy is placed at that moment to facilitate enteral feeding later. Usually, no attempt is made at correcting malrotation because the priorities at that time are the reconstruction of the diaphragm with relocation of the abdominal viscera below it and closure of the abdominal wall. This may be difficult, such as in exomphalos or gastroschisis, owing to the smallness of an abdominal space that remained partially empty during gestation.

Thanks to the vigorous efforts directed at improving the prognosis of this highly lethal disease, more patients survive in the last few years after often very sophisticated treatments. Many of them suffer chronic respiratory insufficiency, sensorineural sequelae, pectus excavatum, and gastrointestinal symptoms.[117]

Gastroesophageal reflux is particularly frequent in these children for several reasons.[118,119] First, the hiatus is mal-

formed or distorted by the repair of the hernia, which always involves some degree of tension on the left diaphragm. Second, the lung hypoplasia contributes to create very high intrathoracic negative pressures during inspiration after closure of the defect. This, together with increased intra-abdominal pressures owing to the forceful reintegration of the intestine and other organs into the abdomen, creates a very unfavorable abdominothoracic pressure gradient that facilitates reflux.[120] Third, esophageal motility in these patients is probably abnormal because the organ is often dilated and inert.[121,122] Finally, gastroduodenal emptying may be delayed owing to malrotation.[123] All of these causes create an environment favorable for reflux, and this explains why many of these patients, already burdened by a failing respiratory function, require fundoplication.[124]

Malrotation may cause other problems, such as duodenal obstruction or, more rarely, volvulus.[123] The former can be suspected at the time of withdrawing a gastrostomy tube eventually inserted during surgery when the gastric fistula, sealed spontaneously in most cases after a few days, persists. It is imperative that volvulus be ruled out whenever one of these patients suffers a clinical picture of intestinal obstruction with shock.

Necrotizing enterocolitis is another possible gastrointestinal complication of CDH.[125] Perinatal and neonatal hypoxia, formula feeding, and increased intra-abdominal pressure after intestinal reposition during hernia repair contribute to provide an environment prone to this disease.

HERNIA OF MORGAGNI

Herniation of abdominal viscera through an anterior, retrosternal orifice of the diaphragm was described by Morgani and bears his name. This type of hernia is quite rare, and its prevalence is higher in patients with trisomy 21.[126] It is due to delayed or failed closure of the anterior part on the diaphragm during septation of the celomic space. The hernia generally has a sac constituted by peritoneum, which is in close contact with the pericardium, although both spaces may communicate. This sac may contain the transverse colon or the stomach, as well as part of the liver, which adopts the shape of the inner surface of the defect. The stomach may undergo volvulus in this abnormal position.

Hernia of Morgagni is often asymptomatic, but it may sometimes induce repeated respiratory infections, abdominal pain owing to the colonic entrapment into the sac, or, eventually, vomiting because of the gastric malposition or intestinal malrotation.[127,128] Sometimes there are other associated malformations.[126]

Surgical repair of the hernia is advisable once the diagnosis is made. This consists of generally straightforward transabdominal excision of the sac with closure of the defect. The operation can be performed laparoscopically.[129]

INGUINAL HERNIA

When the fetal communication between the peritoneal and the vaginal celomic compartments remains partially patent for a part (hernial sac) or for the entire length of the peritoneovaginal canal after birth, it is possible for intestinal loops to pass from the abdominal cavity to the inguinal region or into the scrotum (Figure 33-5). This is known as inguinal hernia or "indirect" inguinal hernia, as opposed to the "direct" hernia, rare in childhood, in which a weak posterior wall in the inguinal canal permits the bulge. Hernia also occurs in females because, although extra-abdominal displacement of the gonads does not occur in them, the round ligament of the uterus has its lower insertion in the labia and exits the abdomen through the inguinal canal accompanied by an evagination of the peritoneal lining, which constitutes the sac. Inguinal hernia is, in this respect, a developmental defect rather than an acquired condition owing to weakness of the wall.

The inguinal canal contains the spermatic cord with its elements: the vas, the spermatic vessels, and the cremaster muscle in males and the round ligament in females. The canal runs obliquely between the laterally located internal inguinal orifice and the medially located external ring. In older children and adults, both orifices are separated by several centimeters, and the canal itself has a floor and clearly defined walls. In contrast, in young infants, it is so short that both orifices almost overlap, facilitating the bulging of the intestine into the hernia.

There are other varieties of the same developmental defect. A hydrocele (from the Greek "υδρος," meaning water, and "κηλη," meaning bulge) is a cystic swelling of the vaginal space of the testicle that is full of fluid and may have a large volume. Often it is the result of the persistence of a thin communication between the peritoneal and the vaginal spaces, allowing the fluid to accumulate during the day and disappearing during the night after a few hours in the recumbent position. This is a "communicating hydrocele," as opposed to the "congenital hydrocele" of the newborn, which is usually noncommunicating and generally resolves spontaneously in the first months of life. When the peritoneovaginal canal closes, leaving a fluid-filled cyst isolated midway between the abdomen and the vaginal spaces, the result is a cyst or hydrocele of the cord. The equivalent in females is the cyst of the canal of Nuck.

The hernia is "incarcerated" when a loop of intestine is blocked into the narrow sac and becomes difficult to reduce. The hernia is "strangulated" when the loop is not only blocked in the canal but also compressed to the point of having its blood flow compromised and its integrity threatened. In girls, the ovary may be incarcerated or strangulated as well, and it may undergo torsion in the sac.[130]

Inguinal hernia is extremely frequent because it is observed in 1 in 50 boys and 1 in 500 girls.[131] It is considerably more frequent in premature infants for reasons that are explained later. Two-thirds of inguinal hernias appear on the right side in boys, whereas laterality in girls is even. Bilateral hernias are less frequent in boys than in girls, in whom they may attain a proportion of 50%.[131]

The closure of the peritoneovaginal canal is a late phenomenon during intrauterine life because the descent of the gonads into the scrotum takes place in the seventh month of gestation, with the left testis arriving first to its final location. Respiratory tract disease increases intra-

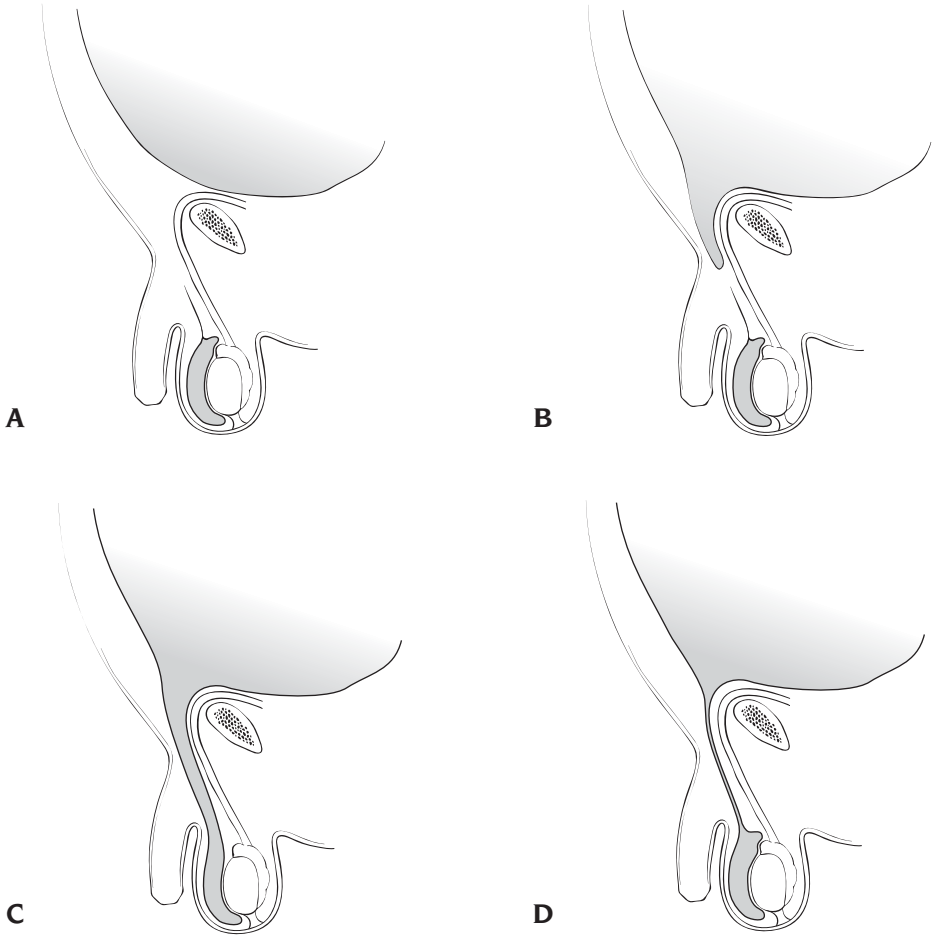


FIGURE 33-5 Schematic drawing of the inguinal region in a male baby. *A*, In a normal situation, the peritoneal and the vaginal spaces are widely separated. *B*, In inguinal hernia, the sac protrudes into the inguinal region above the pubic bone. *C*, In the inguinoscrotal hernia, the peritoneovaginal canal is widely patent, leaving ample passage into the scrotum. *D*, In the communicating hydrocele, the canal is very narrow, allowing only the passage of peritoneal fluid in both directions.

abdominal pressure, facilitating the clinical expression of hernia. This is particularly true for premature babies, but also for cystic fibrosis children and adult chronic respiratory patients (see below).[132] Closure is probably influenced by the local action of neuropeptides such as calcitonin gene–related peptide or growth factors such as hepatocyte growth factor during late gestation.[133] The different timing of gonadal descent on each side explains why hernias are more frequent on the right side than on the left.

Hernias are particularly frequent in association with diseases in which intra-abdominal pressures are increased (chronic respiratory disease,[134] cystic fibrosis[135]), in the presence of abundant peritoneal fluid (after ventriculoperitoneal shunting[132] or during peritoneal dialysis[136]), or in some diseases of the connective tissue (Ehlers-Danlos syndrome,[137] mucopolysaccharidoses[138]).

The main symptom is the appearance of a bulge in the groin that only in some cases reaches the scrotum and reduces itself spontaneously most of the time (Figure 33-6A). This swelling is not particularly painful, but it can bother young infants considerably, in whom it is frequent for the herniated bowel loop to be partially blocked, interfering with the passage of liquids or gases. The hernial bulge may be permanent or appear and disappear intermittently. It generally reduces itself or becomes inapparent during sleep. On physical examination, the inguinal canal is occupied when the bowel is herniated. If not, it is not unusual to perceive a thickened spermatic cord, and, sometimes, well-trained individuals can perceive a sensation of smooth friction, described as the "silk glove sign," on displacement of the examining finger transversely to the axis of the cord. An enlarged inguinal orifice may be palpated when exploring purposefully the inguinal region directly or invaginating the scrotal skin into the canal with the finger. In cases of communicating hydrocele, the scrotum is full of fluid, and it can be easily transilluminated with a pocket lamp. In these cases, the cord is usually not enlarged. Cysts of the cord are palpated as elastic fluid-filled masses located midway between the inguinal orifice and the scrotum.

In girls, the hernial swelling is similar to that seen in boys, although it rarely extends itself down to the inferior part of the labia (Figure 33-6B). Sometimes, particularly in young female infants in whom the pelvic space is small, an almond-shaped mass corresponding to the ovary can be palpated in the groin, and it may be difficult to reduce.

When the hernia is incarcerated, the swelling becomes painful, intestinal transit may be slowed or arrested, and patients manifest their discomfort rather explicitly. However, if the inguinal regions are not purposefully examined, the diagnosis may be missed, and ill-oriented tests may be requested.

In cases of strangulation, the clinical picture is one of intestinal obstruction with more or less marked signs of vascular suffering of the intestine. This picture rarely progresses

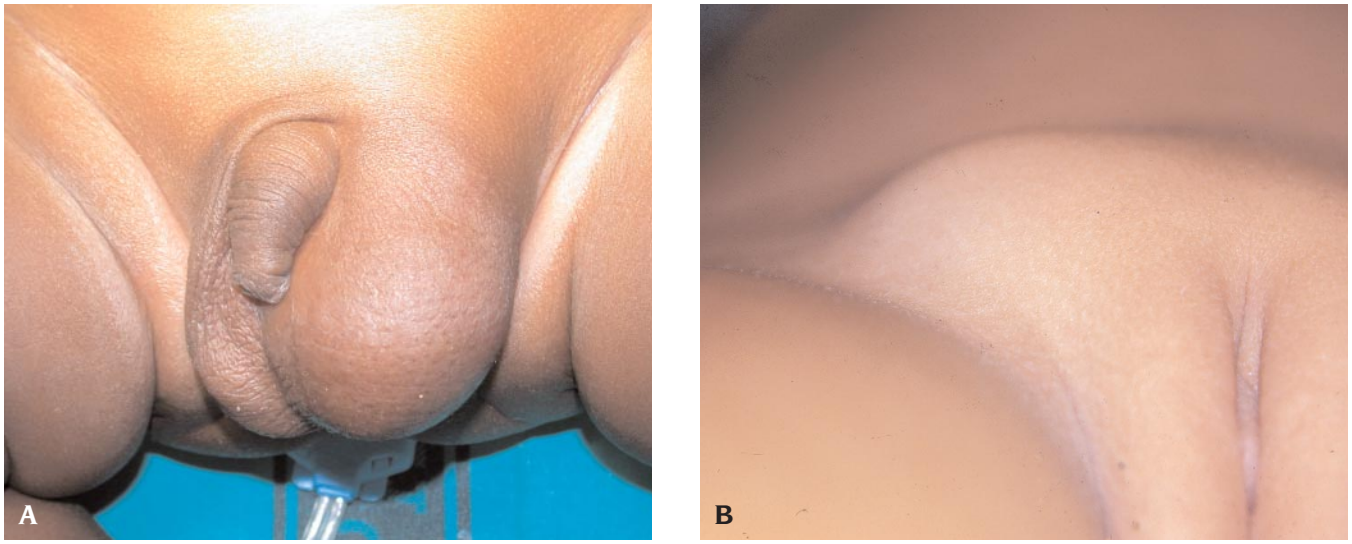


FIGURE 33-6 Inguinal hernia. *A*, Left inguinoscrotal hernia in a boy. *B*, right inguinal hernia in a girl.

to one of frank obstruction because the inguinal region is swollen and painful and because all efforts are made to reduce or treat the ailment, but it may happen that omission of groin examination interferes with prompt diagnosis and treatment. In girls, a strangulated ovary is also very painful, but the mass is less conspicuous on inspection, making diagnosis more difficult if the groins are not palpated. A rare event is strangulation of Meckel diverticulum into an inguinal hernia. This condition is known as Littre hernia, after the person who first described it.[139] In this case, the strangulation may or may not obstruct the small bowel, but it may also cause ischemia and necrosis of the diverticulum.

Treatment of inguinal hernia must be surgical and is one one of the most frequent operations of pediatric surgery. It aims at the interruption of the peritoneovaginal canal or to high ligation and excision of the hernial sac. In opposition to adults, in whom the inguinal wall weakness plays a major role, inguinal hernia in children is approached more as a developmental delay that left the sac open than as a parietal problem requiring surgical reinforcement. Therefore, simple closure of the hernial sac or peritoneovaginal canal heals the hernia, allowing the wall to reinforce itself and to regain a normal anatomy. The operation, often performed in toddlers through the external inguinal orifice without opening the inguinal canal, is usually done as day surgery and has excellent results, with a minimal risk of recurrence. However, when the same operation is performed in premature infants, it is technically more difficult owing to the thinness of the sac, and both the anesthesia and the postoperative periods may be burdened by respiratory complications, particularly in individuals who previously had lung diseases and required assisted ventilation in the neonatal intensive care unit. In this particular group of ex-premature infants, it is wise to delay the operation until the second semester of life or, if that is impossible, to admit them into the hospital to be operated on with all of the necessary precautions. The risks of damaging the vas or the spermatic vessels during surgery are smaller in the hands of trained pediatric surgeons. In girls, the rationale of the operation is similar, but the inguinal orifices can be closed safely if preferred. There are also considerable risks for the testicle when the hernia incarcerates or strangulates. Some gonads may undergo atrophy as a consequence of ischemia during strangulation, and this has to be explained to the parents after every episode.

An interesting and hotly debated issue is whether contralateral inguinal exploration of an asymptomatic hernia should be performed simultaneously at the time of surgery for a symptomatic unilateral one. The higher incidence of bilaterality in girls would make routine bilateral exploration more attractive in them, and the higher incidence of righ-sided hernias in boys could invite the same attitude when the hernia is on the left. Some surgeons have advocated routine bilateral exploration in all cases to decrease the risks of repeating anesthesia, but, on the other hand, the risks to the integrity of the vas or the spermatic vessels invite treatment of the hernia only after it is clinically patent.[140] In the past, introduction of some radiologic contrast into the peritoneum (peritoneography or herniography) allowed preoperative diagnosis of the patency of the peritoneovaginal canal,[141] but although this patency is constant in all inguinal hernias, not all patent canals involve real hernias. A high proportion of asymptomatic individuals have some degree of patency on necropsy.

Laparoscopy has modified the attitudes of some surgeons on the management of the asymptomatic contralateral side at the time of repair of a symptomatic hernia. Visual inspection of the inner inguinal ring after introducing a laparoscopic telescope through the hernial sac during an open operation is a relatively simple manipulation that allows diagnosis of otherwise inapparent contralateral hernias,[142,143] but the meaning of the patency of the canal in the absence of hernia will remain unclear in this setting. It is presently recommended to operate only in clinically manifest hernia, except perhaps in girls, in premature infants, after incarceration, or in individuals with comorbid conditions.[144]

The inguinal hernia repair itself may be performed by laparoscopy,[145,146] but this is probably hard to justify

because standard herniotomy in infants is practically as minimally invasive, brief, and painless as a two- or three-port laparoscopy. However, this approach, consisting of closure with a purse-string suture of the peritoneal orifice leading into the sac from inside, may be useful in recurrent hernias in which inguinal canal scarring makes open dissection difficult.[147–150]

Incarcerated hernia can usually be reduced by trained persons avoiding unwanted operations in an emergency setting. Sedation, bottle feeding, or a warm bath may facilitate careful manipulation (taxis) of the herniated bowel (or the ovary) back into the abdomen. Strangulated hernia is a different matter. Taxis may be successful if strangulation is very recent, but it is otherwise discouraged when it has evolved for some hours. The risks of reducing damaged bowel into the abdomen make it advisable to proceed with operation as soon as possible to assess the viability of the herniated bowel, to resect it if necessary, and subsequently to repair the hernia. This may be particularly difficult in these cases owing to the swelling, hemorrhage, and edema of the inguinal structures. Testicular atrophy happens in some patients after inguinal hernia strangulation.[151]

Irreducible ovaries should benefit from a prompt operation as well.[152]

Recurrence is rare and is more often related to comorbid conditions such as prematurity, ventriculoperitoneal shunts, peritoneal dialysis, or strangulation.[153]

FEMORAL HERNIAS

The passage of a hernial sac that may contain bowel through an enlarged femoral orifice is known as femoral or crural hernia. The femoral orifice is located underneath the inguinal ligament (and therefore below the inguinal canal) and allows passage of the femoral vein, artery, and nerve from the pelvis to the thigh. This enlargement is always medial, and the sac is therefore in close contact with the femoral vein.

Femoral hernias are rare in children. Most series collect only a few cases among hundreds of inguinal hernias. Both genders and all races are equally affected.

The diagnosis is based on the observation of a groin swelling located underneath the external inguinal orifice. However, this location is easily missed[154,155] because, unless the bulge is visible on examination, relatives and doctors will first interpret its appearance as the expression of an inguinal hernia. This explains why half of these patients are operated on for inguinal hernia and why only when the sac is not found, exploration of the femoral area allows diagnosis and repair.[156,157] Furthermore, the femoral defect can be missed even during inguinal exploration in a consistent proportion of these patients, and the correct diagnosis is made in them only when the bulge persists after the alleged inguinal hernia has been "repaired."

A well-directed operation allows excision of the sac and adequate closure of the enlarged orifice without damaging the femoral vein. This is more often performed through an inguinal approach, displacing forward the inguinal ligament to treat the femoral region from above. A mesh-plug technique has been recently adopted by some surgeons for this purpose,[158] and a laparoscopic approach might be particularly indicated in these cases of apparently "recurrent" inguinal hernia.[150,159]

UMBILICAL HERNIA

Failed closure of the fascial orifice of the umbilicus shortly after birth causes umbilical hernia in which there is a parietal defect centered at the umbilical level, covered externally by skin, and lined internally by peritoneum. It may contain bowel loops or epiploon and sometimes may attain considerable dimensions.

Umbilical hernias are frequent in newborns, particularly in premature infants, and they are observed more often in black babies. Both genders are equally affected. The natural history of the hernia is favorable at large because the fascial orifice reduces spontaneously with time and eventually closes itself. Most hernias disappear during infancy or early childhood, and only a minimal proportion of them persist beyond the fourth year. This proportion is certainly higher when the orifice is not concentric with the umbilical scar because supraumbilical hernias do not tend to heal spontaneously.[160] Umbilical hernia is particularly frequent in individuals with Ehlers-Danlos[161] or Beckwith-Wiedemann[161] syndrome, mucopolysaccharidoses,[162] hypothyroidism,[163] or trisomy 18, and the expectation of spontaneous resolution in them is certainly reduced. It is also more frequent when chronic peritoneal dialysis is used.[164,165]

During intrauterine life, the umbilical stalk houses the vessels (umbilical vein and arteries and vitelline artery) connecting the embryo and the fetus with the placenta. The process of closure of the abdominal wall, consisting of ectodermal (skin) and mesodermal (peritoneum, muscle and fascia) growth and differentiation, proceeds concentrically around the umbilical stalk, and, at birth, after the interruption of these vascular connections, it is completed by further closure, leaving the umbilical scar and a fibrous area in which the peritoneal and fascial layers fuse. This process may be incomplete, and in such cases, there is a fascial orifice leaving the peritoneum and the skin in contact with and closely related to the umbilical arteries, the round ligament derived from the umbilical vein, and the urachus. Intra-abdominal pressure pushes the hernial layers outward, creating a hernial space in which bowel loops or epiploon can be located.

Umbilical hernia is immediately visible because of the prominence of the umbilicus (Figure 33-7). It may be very huge, particularly in black infants, in whom it may adopt the shape of an elephant's trunk.[166] On palpation, the content is almost always easily reduced, and the borders of the fascial defects can be palpated through the skin. Only very rarely, umbilical hernias incarcerate[167,168] or strangulate[169]; even more rarely, they rupture.[170] The swelling may be painful, particularly when the epiploon is involved in the content or as a symptom of intra-abdominal sepsis.[171] Sometimes, particularly in young infants, the frequent presence of bowel loops in the hernia interferes with adequate feeding and intestinal transit.

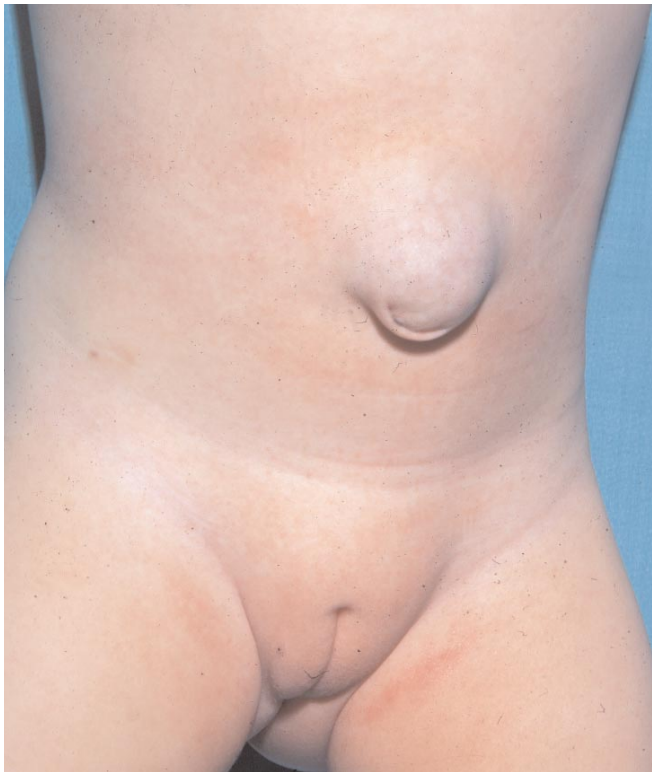


FIGURE 33-7 Umbilical hernia in a young girl.

In view of the favorable natural history of umbilical hernia, surgical indications are restricted to those located slightly above the umbilicus; to those persisting beyond the age of 3 or 4 years, particularly in girls, in whom future pregnancies will enlarge the orifice; and to the above-mentioned associated conditions.

EPIGASTRIC HERNIA

Defects of the linea alba, where the fascial layers of the anterior abdominal wall fuse, may allow protrusion of the intra-abdominal contents. This condition is known as epigastric hernia. This hernia is quite frequent, but it is asymptomatic in most cases, and its prevalence is certainly underestimated. Slim individuals in whom there is a wide diastasis between the rectus muscles of the abdomen are more often affected. It is usually asymptomatic, but it may cause pain, sometimes quite disturbing, particularly when the preperitoneal fat pad and eventually the peritoneum and some of the epiploon are herniated into the defect. In these cases, intra-abdominal causes for pain may be mistakenly investigated. On physical examination, the defect can be palpated in the midline together with its small fat content.[172] Epigastric hernias are straightforwardly closed by surgical reinforcement of defect in the linea alba.

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CHAPTER 34

PERITONITIS

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Peritonitis is an inflammation of the peritoneum in reaction to contamination by microorganisms or chemical irritation by organic fluids (intestinal fluids, blood, bile, urine). Among infectious peritonitis, there is primary peritonitis and secondary peritonitis, which is caused by bowel perforation. The latter is more common. In children, the incidence, pathophysiology, and etiology of peritonitis vary according to age.

PATHOPHYSIOLOGY

The peritoneum is a functional membrane that lines the intra-abdominal wall and the viscera contained within the peritoneal cavity. Under normal circumstances, the peritoneal cavity is a sterile environment, and the peritoneum provides a smooth lubricated surface within which the intestines can move freely. It contains serous fluid, which is an ultrafiltrate of plasma and a small number of cells (less than 300 cells/mm^3). Almost half of normal peritoneal cells are macrophages, 44% are lymphocytes, 2% are dendritic cells, and a few are eosinophils and mast cells.[1–3] The basic structure of peritoneum is a smooth layer of mesothelial cells resting on a basement membrane with a deeper layer of vascularized loose connective tissue. By electron microscopy, mesothelium appears as a continuous cellular surface covered by numerous microvilli. Mesothelium is capable of extensive secretory activity, which is evident from the large nuclei present in mesothelial cells, the abundance of rough endoplasmic reticulum, and the prominence of Golgi complexes.

When bacteria reach the peritoneal cavity, a local peritoneal and systemic response is initiated to eradicate the invading pathogens. This response is characterized by hyperemia; exudation of protein-rich fluid containing fibrinogen, albumin, opsonins, and complement; and a marked influx of neutrophils into the peritoneal cavity, for example, peritonitis.[1,2] Mesothelial cells play an active role through secretion of cytokines and up-regulation of adhesion receptors that stimulate the transmigration of leukocytes across the mesothelium. Interaction between bacteria and mesothelium and its influence on inflammation, coagulation, and fibrinolysis are especially important processes that may induce permanent adhesions.

Bacterial adherence to mesothelial cells is the initial step in contact between mesothelial cells and bacteria, resulting in activation of mesothelial cells and causing peritonitis. Adherence to mesothelial cells is made possible by the presence of cell wall substances and opsonization or ingestion, resulting in sequestration of the bacteria protecting them from antibiotics and neutrophils, allowing them to persist and cause relapsing episodes of peritonitis (by *Staphylococcus aureus*).[4] Adherence of *Bacteroides fragilis* to peritoneal mesothelial cells is firm, and extended saline lavage fails to significantly reduce the mesothelial microbial population.[5]

Mesothelial cells produce cytokines after stimulation. Cytokines are proteins capable of regulating immune and inflammatory responses. Peritoneal mesothelial cells can produce several cytokines, such as interleukin (IL)-6 and IL-8 after stimulation with IL-1β or tumor necrosis factor (TNF)-α.[6]

IL-6 has the ability to stimulate T-cell, macrophage, and B-cell differentiation.[7–9] IL-8 is a chemoattractant that is highly selective for polymorphonuclear leukocytes and is secreted in a polarized way, preferentially oriented toward the apical side of the cell layer, creating a gradient.[10] *Escherichia coli* has an activating effect on IL-8 secretion by mesothelial cells, with a role for lipopolysaccharide or peptidoglycans. The latter has the same effect after interaction with gram-positive bacteria.

Neutrophil influx into the peritoneal cavity is one of the most important host defense mechanisms. The greater omentum is a source of exudative neutrophils.[11] The bacterial concentration decreases within the first 3 hours after bacterial penetration into the peritoneal cavity.

Bacteria can have synergistic activity, which increases the pathogen effect. Synergistic activity exists between *E. coli* and *B. fragilis* caused by a heat-stable factor.[12]

ETIOLOGY AND MANAGEMENT

Antenatal Peritonitis

Prenatal peritonitis is defined by the presence of meconium in the peritoneal cavity. It occurs after prenatal intestinal perforation, and the most common cause is meconium ileus associated with cystic fibrosis. The other causes are intestinal atresia, appendicular perforation associated with Hirschsprung disease, antenatal appendicitis, and intrauterine parvovirus B19 infection.[13]

Meconium peritonitis is a chemical fibroadhesive peritonitis[14] because meconium is sterile within the prenatal period. Digestive enzymes in meconium cause a peritoneal inflammatory reaction (Figure 34-1).

Diagnosis is made by prenatal ultrasonographic examination showing ascites and/or calcifications in the bowel wall and peritoneum.[15] Free ascites develops when the bowel perforation occurs just before birth. This should be distinguished from urinary ascites.

There are several types of meconium peritonitis: generalized, localized, and cystic.[16] Meconium peritonitis is localized near the intestinal perforation when atresia occurs. The generalized type is severe, and its surgical treatment is often difficult.

Prenatal diagnosis should include genetic counseling to determine if cystic fibrosis exists. A blood test is carried out in the two parents. When parents are heterozygotes, genetic tests to detect cystic fibrosis are performed on the fetus.

At birth, management depends on the clinical and radiologic presentation. When abdominal distention is extensive after birth, surgery is performed after abdominal decompression to improve the respiratory condition of the newborn. When the newborn is asymptomatic, plain abdominal radiography is performed to look for intra-abdominal (Figure 34-2) or scrotal calcifications.[17,18] When the clinical status is stable, without evidence of obstruction or pneumoperitoneum, the digestive perforation may have healed spontaneously, and surveillance is reasonable. When radiographic examination shows evidence of intestinal obstruction or perforation (pneumoperitoneum), a laparotomy should be performed.

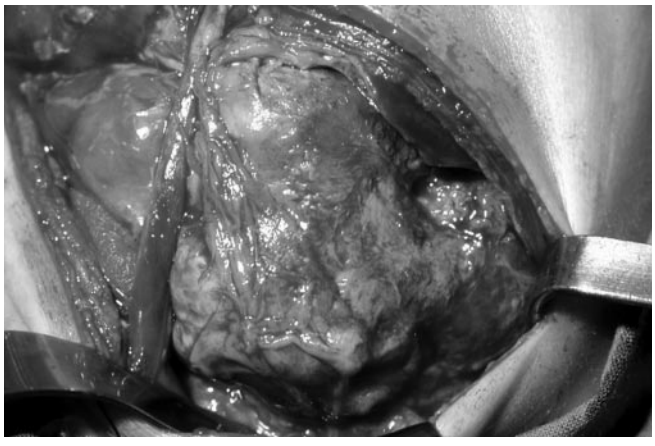

FIGURE 34-1 Meconium peritonitis, an operative view.

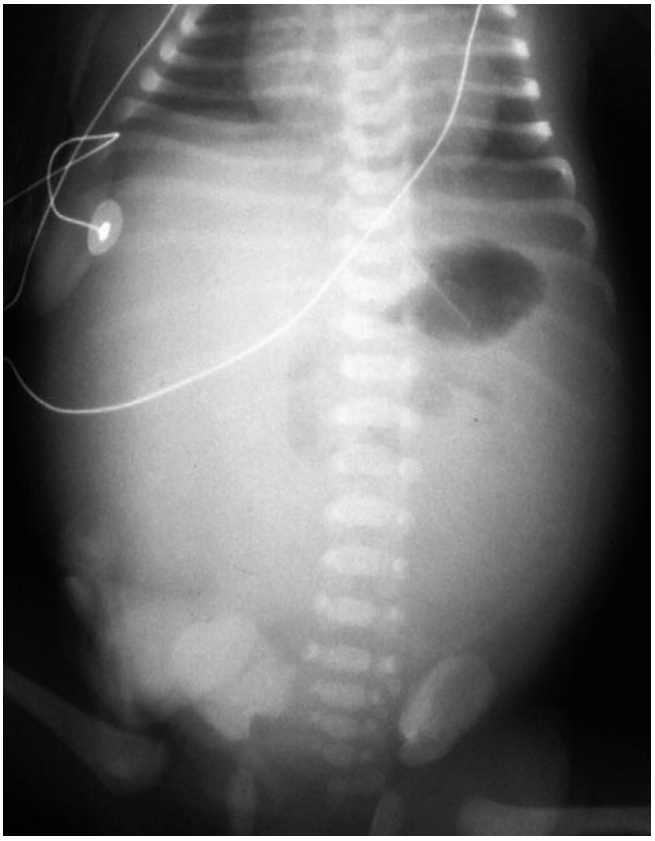

FIGURE 34-2 Meconium peritonitis, anteroposterior radiograph. Calcification on the right flank.

When surgical exploration is performed, laparotomy is done through a transverse umbilical or midline incision. The intestinal perforation is removed by segmental resection, and anastomosis is performed if the bowel wall is not inflamed. If the peritoneum is too inflamed, a stoma must be created. The appendix can be removed to be examined for ganglia cells. Surgery can be difficult, and hemorrhage can occur.

Neonatal Peritonitis

The clinical presentation of peritonitis in neonates is marked by bilious vomiting with fever. The general signs (tachycardia, oliguria, dyspnea, lethargy) can be extensive and lead to septic shock with multiorgan failure. Locally, the abdomen is tender and edematous, and the wall is glistening and marked by a venous distention pattern. The biologic assessment suggests an infectious syndrome with leukocytosis and increased C-reactive protein. Leukopenia is possible, as well as thrombocytopenia. Acidosis and hyperkalemia are suggestive of digestive failure. The radiographic assessment should include plain abdominal radiography and ultrasonography. Anteroposterior and lateral radiographs in the supine position can show an ileus and can lead to a diagnosis of pneumoperitoneum. Ultrasonography can be done at the newborn's bedside. This approach makes possible the capacity to show intraperitoneal disruption and helps contribute to the etiologic diagnosis of peritonitis. In the event of ischemic digestive failure, ultrasonography can reveal liquid in connection

with the presence of blood or pus, a peritoneal cavity filled with liquid, and a thick and aperistaltic bowel wall. Treatment consists of fluid re-equilibration and insertion of a nasogastric tube before undertaking surgery, the nature of which is determined by the etiology of the peritonitis. Surgery will be carried out with intravenous antibiotics appropriate to the diagnosed bacteria and is usually included on the administration of ceftriaxone, aminoside, and metronidazole for 10 days following surgery.

Etiology

Necrotizing Enterocolitis. Necrotizing enterocolitis is the most common surgical emergency in newborns, especially premature infants (90% of cases). Some cases are described in full-term infants with low birth weight and cardiac malformations.[19] The etiologic factors are low blood flow in the mesenteric vessels, enteral feeding, and infection.[20] Inflammatory factors such as platelet activating factor and TNF-α contribute to the development of the initial histologic lesion represented by coagulating necrosis of the digestive mucosa, which can extend the full thickness of the digestive wall. This lesion is the result of the production of metalloproteinase[21] enzymes that cause destruction of the extracellular matrix of the intestinal mucous membrane. The digestive wall is then invaded by monocytes, macrophages, and neutrophils.[22] The lesions can be located at any place in the digestive tract but are generally located in the terminal ileum, right colon, and left colonic angle. Clinically, enterocolitis occurs in the first 3 weeks of life, with a septic syndrome, abnormal gastric residues during enteral feeding, abdominal distention with tenderness, and bloody stools. Some authors ascribe importance to an increased fecal calprotectin,[23] a marker of inflammation of the digestive mucosa in newborns that is increased in necrotizing enterocolitis. Plain abdominal radiography has an important role in the diagnosis, showing a pneumatosis intestinalis (Figure 34-3) that, when localized, would be a better prognostic factor. Radiography searches for portal venous gas, which is easier to visualize on ultrasonography and is pathognomonic of necrotizing enterocolitis but associated with a poor prognosis. Radiography can visualize pneumoperitoneum when intestinal perforation occurs.[24]

The treatment is initially medical: for example, discontinuation of enteral feeding and continuous gastric aspiration, parenteral nutrition, and intravenous antibiotics with broad-spectrum medications. Surgery is limited to digestive tract perforation with pneumoperitoneum or when plastron occurs.[25] Other surgical methods are debatable, including peritoneal drainage, which is indicated for the newborn premature infant with a very low birth weight (< 1,000 g) and represents a unique treatment in 30% of cases,[26] and laparotomy with intestinal resection and ileostomy. Morbidity is estimated at 47% in connection with sepsis, digestive stenosis, short-bowel syndrome, and complications of parenteral nutrition. Mortality reaches 50% of cases when sepsis is associated with necrotizing enterocolitis.[27] See Chapter 42, "Necrotizing Enterocolitis," for a comprehensive review of this topic.

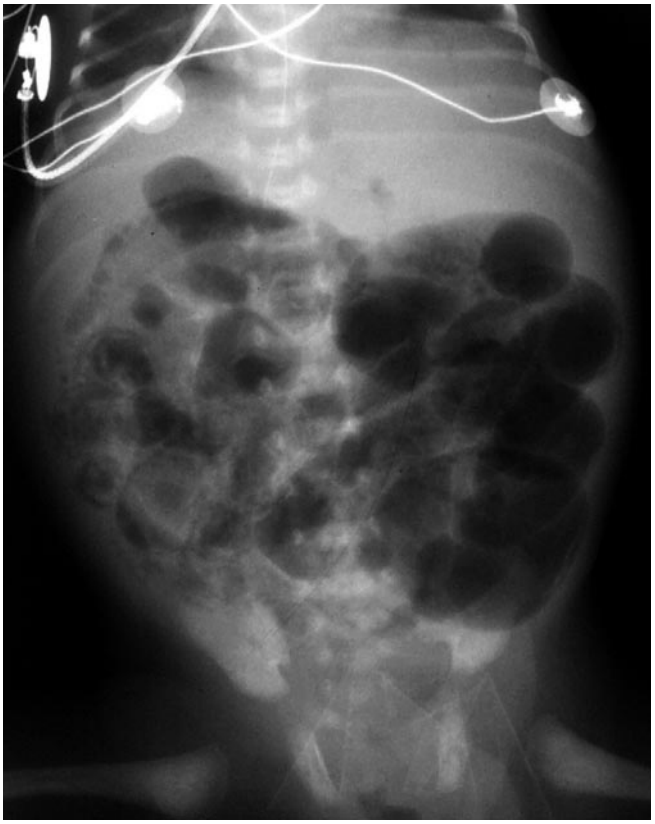

FIGURE 34-3 Anteroposterior radiograph. Necrotizing enterocolitis with pneumatosis intestinalis.

Idiopathic Gastrointestinal Perforation. Described by Siebold in 1825,[28] this perforation occurs in premature infants with a low birth weight. Perforation is located in the terminal ileum or jejunum in an antimesenteric position. Spontaneous neonatal gastric perforation is possible but rare, occurring in 1 in 2,900 live births.[29] Gastric perforation is 4 times more frequent in boys and is found in 85 to 95% of cases in the anterior part of the greater curvature. Perforation generally occurs earlier than enterocolitis (eg, in the first week of life) and has an incidence of about 6% in premature infants with a very low birth weight. It occurs at one-tenth the frequency of necrotizing enterocolitis. Many factors can contribute to this perforation: twin pregnancy, neonatal ventilation, an umbilical artery catheter,[30] administration of nonsteroidal anti-inflammatory drugs and corticosteroids, *Staphylococcus epidermidis* infection, and candidiasis.[31] Histologically, there is a discontinuous absence of the internal layer of the muscularis propria or muscularis mucosae.[32] In cases of gastric perforation, zones without Cajal cells have been found, suggesting a disorder of gastric motility.

Newborns have extensive abdominal distention with bluish discoloration of the abdomen. The general health status is otherwise preserved, except when gastric perforation occurs in relation to pneumoperitoneum. Anteroposterior and lateral abdominal radiographs reveal pneumoperitoneum (Figure 34-4).[33] Initially, pneumoperitoneum can be deflated with a needle[34] to ameliorate ventilation and to try to obtain healing of the intestine because of peritoneal

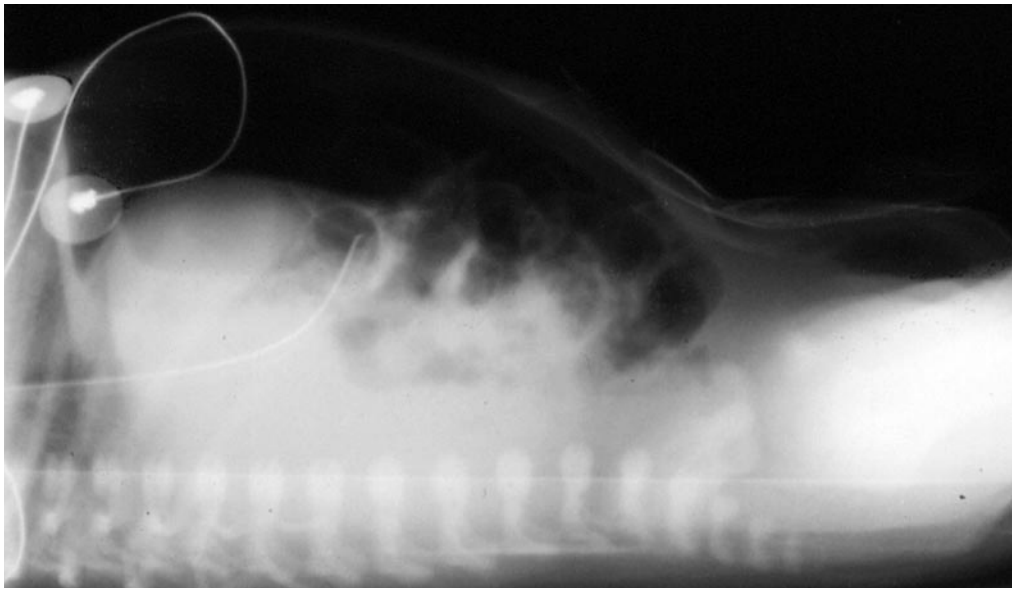

FIGURE 34-4 Idiopathic gastrointestinal perforation. Lateral abdominal radiograph. Visualization of the pneumoperitoneum.

adhesion. The effectiveness of the treatment is based on the absence of pneumoperitoneum recurrence on repeated abdominal radiographs. If pneumoperitoneum recurs after two or three punctures, laparotomy is indicated to suture the perforation or to carry out a segmental resection with concomitant anastomosis.[35] Enterostomy is rarely necessary. When gastric perforation occurs, the stomach must be explored completely because the perforations can be multiple. The prognosis is good when adapted treatment is undertaken.[36]

Diastatic Perforation in Hirschsprung Disease. Hirschsprung disease can present with an intestinal perforation in the newborn. The appendix is usually involved. It occurs with a long segment of Hirschsprung disease, and appendix perforation suggests Hirschsprung disease. With the sealing of the Bauhin valve, the appendix is likely to perforate, particularly at the level of its base.[37] During surgery, the histologic appendix is removed and examined immediately. If ganglia cells are present, a piece of sigmoid wall is then obtained for analysis. If there are ganglia cells, a stoma in the sigmoid colon is created. If the sigmoid colon does not have ganglia cells, the colonic wall is resected until ganglia cells are present to create a stoma in normal tissue. If there are no ganglia cells in the appendix, an ileostomy is performed.[38] See Chapter 46.3, "Hirschsprung Disease," for more details.

Spontaneous Biliary Perforation. Spontaneous biliary perforation is the second most common cause of jaundice in infancy after biliary atresia. The incidence occurs between the first week and 3 months of age. The etiology is unclear. In the majority of cases, perforation is localized to the junction of the cystic and common hepatic ducts and is thought to be due to embryologic mural weakness. It has been suggested that the posterolateral arterial blood supply of the bile duct has a susceptibility to ischemia of its anterior wall, which may cause a weak point that is prone to perforation. Stones or duct stenosis as a cause of distal obstruction and perforation of the bile duct are rarely encountered.[39] Antenatal perforation of the biliary system has been reported with fetal ascites on ultrasonography.[40]

Clinically, the newborn has abdominal distention, vomiting, and fever. Abdominal ultrasonography shows fluid located preferentially around the gallbladder and porta hepatis without dilatation of the bile duct.

Liver function tests may differentiate this condition from biliary atresia and neonatal hepatitis because with perforation of the bile duct, serum enzymes are not elevated significantly. The bilirubin level in the ascitic fluid is greater than the serum level.

Preoperative fluid resuscitation is mandatory because of malnutrition, dehydration, and electrolyte imbalance, especially hyponatremia. Currently, the surgical recommendation for perforation of the bile duct includes simple peritoneal drainage. Bile duct stenosis is the most common complication after simple drainage for bilomas, pseudocyst, and portal vein thrombosis. If the site of perforation is apparent, suture repair can be performed. Biliary intestinal anastomosis is needed if there is a distal biliary tract obstruction or stenosis on the operative cholangiogram. Cholecystotomy may sometimes be helpful for better decompression and for evaluation of the biliary tree.[41]

Omphalitis. Peritonitis is one of the complications of omphalitis in neonates (16% total complication with a 6% mortality rate).[42] It is a common problem in neonates in developing countries and occurs as a result of poor cord care practices in these countries. Peritonitis is the result of direct spread of infection to the peritoneal cavity. Peritonitis should be suspected if the ileus fails to respond to appropriate treatment.[43] If abdominal ultrasonography shows localized intraperitoneal abscesses, laparotomy and drainage are indicated. When the umbilical arteries contain pus, excision may be necessary to control infection.[44] Early recognition and prompt treatment of the peritonitis can avoid significant morbidity and mortality.

Perforation of the Urachal Cyst. The urachus exists generally as a fibrous cord extending from the dome of the bladder to the umbilicus. It occupies a midline space between the peritoneum and the transversalis fascia. Disorders of the urachus are usually due to its incomplete regression.[45] Urachal cysts can become infected, the most common organism being *S. aureus*. Peritonitis can occur when the urachal infected cyst perforates into the peritoneal cavity. Clinically, there is a medially located palpable inflammatory mass with peritoneal irritation. Diagnosis can be suspected during surgery because of a medially located perforated abscess with peritonitis. Abdominal ultrasonography and computed tomography (CT) are useful for a diagnosis. Treatment is surgical. The abscess must be drained first, and then it must be resected with part of the bladder dome to avoid the long-term complications of adenocarcinoma, transitional cell carcinoma, or sarcoma.[46]

PERITONITIS IN CHILDREN

Appendicular Peritonitis

Appendicular peritonitis is the most frequent form of peritonitis in children. It occurs when the appendix has perforated or when, in the absence of overt perforation, a diffusion of bacteria through an inflamed appendix wall occurs. The most common microorganisms are *E. coli*, *Enterococcus*, *Bacteroides*, and *Pseudomonas aeruginosa*, which are found in 10% of cases. Mortality is estimated at about 1%; morbidity is higher and results from deep parietal abscess and obstruction of the bowel. Adhesions increase the risk of tubar sterility by five times (personal data). The clinical diagnosis includes abdominal pain, which can initially be located in the epigastrium and then in the right iliac fossa.

Abdominal pain is associated with vomiting, fever, and deterioration of the generalized clinical status. The clinical presentation can also be intestinal obstruction with fever.

Clinical examination shows a swollen, rigid abdomen with cutaneous hyperesthesia. Palpation reveals an abdominal contracture that is painful, rigid, and continuous. A recrudescence of the pain in the right iliac fossa with a rigid abdomen suggests appendicitis as the origin of the peritonitis.

The white blood cell count reveals increased leukocytes, and C-reactive protein is increased. Abdominal radiographs can show ileus and appendiceal stercolith. Abdominal ultrasonography[47] highlights intraperitoneal masses and signs of appendicitis (eg, thickening of the appendix wall and infiltration of mesenteric fat). However, the abdominal examination can also be normal and therefore misleading. Treatment consists of appendectomy after fluid therapy and the installation of a gastric tube. The peritoneal cavity is rinsed. Drainage of the peritoneal cavity is not essential in the majority of cases. The intervention is performed by a McBurney incision when peritonitis is localized or by laparotomy if the peritonitis is diffuse. Laparoscopy plays an important role in the treatment of peritonitis; it allows sampling of intraperitoneal liquid for bacterial analysis. The peritoneal cavity should also be rinsed, and an appendectomy should be performed. However, laparoscopy is contraindicated when abdominal distention owing to intestinal obstruction occurs or when sepsis exists. However, laparoscopy's esthetic benefit is greater than that of laparotomy. The postoperative period requires parenteral antibiotics[48] for 10 days with ceftriaxone, aminoside, and metronidazole. Antibiotics are then continued for 8 days per os. See Chapter 37, "Appendicitis," for more details.

Perforation of Meckel Diverticulum

Meckel diverticulum is the result of a persisting omphalomesenteric duct. Its incidence is estimated at 2%. Complications occur in 4% of Meckel diverticula, with intestinal obstruction, gastrointestinal bleeding, or perforation. Perforation occurs in 14.5% of cases.[49] Infection of the gastric mucosa by *Helicobacter pylori* plays a minor role in this complication of Meckel diverticulum.[50] Complications occur before the age of 10 years in 89.5% of cases and before the age of 2 years in 47% of cases.[51] In newborns, nonsteroidal anti-inflammatory drugs can contribute to a Meckel perforation when neonatal ischemia is involved. Exceptional antenatal cases have been reported and discovered with surgery for inflammatory hydroceles at birth.[52] Perforation is due to ectopic gastric mucosa occurring in the small intestine. Perforation can be a complication of ileoileal intussusception. A preoperative diagnosis is very difficult also because perforation of Meckel diverticulum is usually discovered at the time of surgery (Figure 34-5). Treatment is based on surgery with segmentary resection of the intestine and an end-to-end anastomosis of the small intestine.

Gastric Ulcer Perforation

Gastric ulcer perforation is rare in children.[53] It occurs in situations of stress, such as neurosurgical intervention.

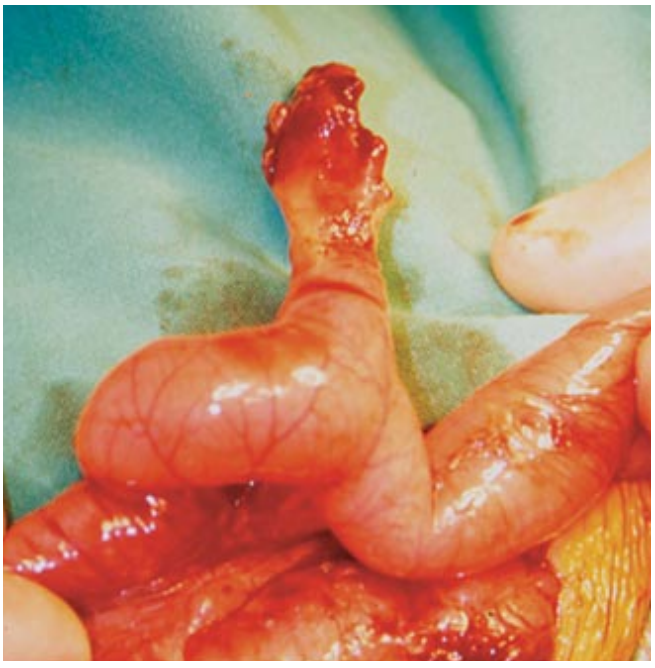

FIGURE 34-5 Perforation of a Meckel diverticulum.

Symptoms include intense abdominal pain, hemorrhage, and vomiting. Abdominal radiographs show a pneumoperitoneum. Treatment begins with installation of a nasogastric tube, and then a laparoscopy is performed if the perforation is recent.[54] Laparoscopy can be used to suture the perforation; protect the suture with a epiploic patch and wash the peritoneal cavity because chemical peritonitis predisposes the patient to infectious peritonitis. Drainage and laparotomy are justified in cases of peritonitis with abdominal distention owing to intestinal obstruction or septic shock.[55] Treatment with a proton pump inhibitor is indicated for 1 month after surgery.

Traumatic Perforation of the Intestine

Perforation of the intestine in children often has a traumatic etiology (eg, a traffic accident when the child is attached to the back seat with a two-point seat belt, a fall from a bicycle, Silverman syndrome). These intestinal lesions are associated in 30 to 60% of cases with either additional intra- or extra-abdominal lesions.[56] The mechanisms are a direct crushing of organs, a dramatic increase in the intraluminal pressure, and a shearing movement between fixed organs. Perforation occurs in 65% of post-traumatic bowel lesions. The intestinal perforation can be secondary to an initial mesenteric lesion and can thus occur secondarily. It is usually localized at the junction of a fixed and mobile bowel segment (eg, Treitz angle and ileocecal junction). The intestinal lesion can be associated with a lumbar fracture, characteristic of the "seat-belt" syndrome,[57] which is frequent with the two-point fixed belts. The diagnosis remains difficult and must be determined as soon as possible to avoid an increased morbidity and to allow early surgical intervention. A lesion of the digestive tract must always come to mind when blunt abdominal trauma in a child occurs. The diagnosis is difficult when hemoperitoneum is associated with fever and ileus. The clinical signs of perforation are the presence of abdominal tenderness, tachycardia, and fever. Abdominal radiographs rarely show pneumoperitoneum. Abdominal ultrasonography is not helpful in the diagnosis. CT, when hemodynamic status is stabilized, is the best procedure to detect intestinal lesions.[58] It can reveal the presence of pneumoperitoneum (eg, bubbles of air in an extradigestive sector). It shows a thickening of the bowel wall associated with a localized intraperitoneal effusion. Analysis of the density of an intraperitoneal effusion suggests, in some cases, a digestive origin. In case of doubt about a perforation, it can be helpful to repeat the CT scan a few hours later, or when the doubt persists (eg, when the CT scan appears normal), lavage of the peritoneum or a laparoscopy can be carried out.[59] Treatment consists of a laparotomy and suturing of a small intestinal lesion. When the lesions are more severe, a segmentary resection should be performed with anastomosis to the small intestine. The surgical procedure ends with a peritoneal washing. When the colon is perforated, a primary suture should be performed with parenteral antibiotics. However, when the lesion is seen too late, a colostomy must be done.

Neutropenic Colitis

Neutropenic colitis is an inflammatory process involving the colon, most frequently the cecum, which is associated with immunocompromised patients. The pathogenesis is most likely related to multiple factors, such as immunocompromised status, neoplastic infiltration of the bowel, intestinal ischemia, and bacterial overgrowth. The preferential location within the cecum is in connection to its distensibility, its existent high bacterial count, its predisposition to mucosal ischemia, and the relative stasis of its contents. Neutropenic colitis is most common in children who develop acute leukemia or lymphoma or are post–renal transplant. Involved bowel loops have edema, ulceration, and hemorrhage. Transmural necrosis may also occur. The prognosis is very poor because the mortality rate is between 50 and 100%.[60] Children present with diarrhea, abdominal pain, gastrointestinal hemorrhage with vomiting, and fever. They can also present with an acute abdomen if a perforation has already occurred. Abdominal radiographs can show pneumatosis intestinalis. A CT scan demonstrates bowel wall thickening and dilated loops of bowel. Pneumoperitoneum is a sign of perforation. Typically, there is also pericolonic inflammation. Therapy of neutropenic colitis usually includes high-dose intravenous antibiotics and antifungal agents, as well as complete bowel rest with parenteral nutrition. Surgery may be required if there is perforation, development of an abscess, gastrointestinal hemorrhage in spite of a correction of the neutropenia, thrombocytopenia, or hemostasis.[61] When sepsis is not controlled or worsens, the general status of the child deteriorates.[62] Surgical treatment consists of resecting all necrotic scars with a right hemicolectomy and ileostomy.[63]

Tuberculosis Peritonitis

Tuberculosis peritonitis is one of different types of abdominal tuberculosis that can involve the mesentery, the digestive tract itself, or abdominal solid organs (eg, spleen and liver). The diagnosis is difficult and has to be distinguished from lymphoma itself. Two historical elements are important to consider in the child: contact with adult pulmonary tuberculosis and documented weight loss.[64] Normal chest radiographs do not eliminate the diagnosis of tuberculosis. Approximately 40% of patients have a normal chest radiograph, and 50% have a normal abdominal radiograph.[64] Abdominal ultrasonography and CT can detect lymphadenopathy, ascites, thickening of the bowel wall, and omental masses. Paracentesis may reveal the presence of *Mycobacterium tuberculosis*, but culture may take 4 to 6 weeks. An advance in the diagnosis of tuberculosis is the determination of adenosine deaminase activity. This enzyme converts adenosine to inosine in the T cells activated by mycobacterial antigens. When the diagnosis is suspected, a laparoscopy can be performed to obtain a peritoneal biopsy.

Salpingitis

Especially in children, the mode of contamination can occur by two routes: ascending and hematogenous. It is part of the differential diagnosis of appendicular peritoni-

tis and is a perioperational discovery. Appendicular peritonitis can also drain into the female genital tract and simulate salpingitis. Diagnosis and treatment benefit from laparoscopy, which allows purulent liquid to be taken for bacterial analysis as well as washing out of the peritoneal cavity. This local treatment should be accompanied by parenteral antibiotics.

Primary Peritonitis

Primary peritonitis is defined as an infection of the peritoneal cavity without an anatomic break in the continuity of the intestinal lumen. Pathogens may reach the peritoneum from the bloodstream, from the lymphatics, or by ascension from the vagina by translocation from the intestinal lumen or through foreign bodies inserted into the peritoneal cavity.[1,65,66] Primary peritonitis characteristically develops in patients with an impaired ability to clear intraperitoneal bacteria, especially children with nephrotic syndrome. In nephrotic syndrome, the most frequent organism found is *Streptococcus pneumoniae*. In addition, a child with a level of blood albumin lower than 1.5 g/dL is more likely to develop primary peritonitis.[67]

When the diagnosis is made according to the treatment protocol, parenteral antibiotic therapy is implemented after paracentesis. When surgery is performed, a diagnosis is made operatively with the presence of purulent liquid in the peritoneal cavity in the presence of a normal bowel. Surgical intervention can be performed by laparoscopy, which allows access to the peritoneal cavity.

CONCLUSION

Peritonitis is a serious pathologic state in children. It requires early diagnosis based on the age of the child. Its treatment is surgical after fluid resuscitation, and it benefits from laparoscopy, especially in older children.

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CHAPTER 35

BENIGN PERIANAL LESIONS

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Perianal disease in children is common and encompasses a broad spectrum of pathologic processes, including fissures, fistulae, abscesses, hemorrhoids, rectal prolapse, pilonidal sinus, and pruritus ani. Occasionally, systemic illnesses such as inflammatory bowel disease may coexist. This chapter provides a diagnostic and therapeutic guide to perianal lesions based on an understanding of the underlying anatomy and the specific pathogenesis of each entity.

ANATOMY

The diagnosis and treatment of perianal disease can be confusing if the anatomy of the pelvic floor and sphincter muscle complex is not understood. Thus, as a preface to the discussion of perianal lesions, it is useful to review the anatomy of the rectum and anus (Figure 35-1).

The rectum is a continuation of the sigmoid colon that starts approximately at the sacral prominence. It can be distinguished from the colon by its lack of taeniae bands. In their place, it has a complete covering of longitudinal muscle fibers. The luminal pattern of the rectum also differs from the colon because it has two to three lateral curves that form mucosal folds known as the valves of Houston. The posterior rectum is free of peritoneum, and the most distal third of the rectum is devoid of peritoneum circumferentially.

The rectum terminates in the anal canal, which is composed of that portion of bowel that passes through the levator ani muscles and opens onto the anal verge. The external and internal sphincter muscles form an important continence mechanism in association with the anal canal.

The internal anal sphincter is a continuation of the circular muscle layer of the rectum. As this muscle layer enters the anal canal, it becomes thickened. Both the puborectalis muscle and external sphincter muscle then wrap the anal canal, suspending the rectum and facilitating contraction of the anus, thereby assisting with anal continence.

On examination with anoscopy, the dentate line can be visualized marking the transition from the columnar epithelium of the rectum to the squamous epithelium of the anal canal. The dentate line is 1 to 2 cm proximal to the external orifice of the anus. The longitudinal folds of mucosa at the dentate line are known as the columns of Morgagni. The internal and external hemorrhoidal plexus of veins accomplish venous drainage of the anus and rectum.

ANAL FISSURE

An anal fissure is a tear in the epithelium and superficial tissues of the anal canal. In children, the tear is usually linear, extending from just below the dentate line to the anal verge (Figure 35-2). Fissures can be classified as either acute or chronic and can be further subdivided into primary or secondary processes. When first formed, a fissure is a simple crack in the anoderm; however, with infection and poor healing, a chronic fissure can develop with a "sentinel tag" of skin, fibrotic edges, and exposure of the internal sphincter. Primary fissures are not associated with underlying systemic pathology, whereas secondary fissures are the consequence of illnesses such as Crohn disease. Although fissures occur in all age groups, they are most common in children. The majority of anal fissures are located in the posterior midline (90%),[1] with the next most frequent location being the anterior midline.

PATHOGENESIS

Anal fissures are thought to be traumatically induced by overstretching of the anoderm because most cases are asso-

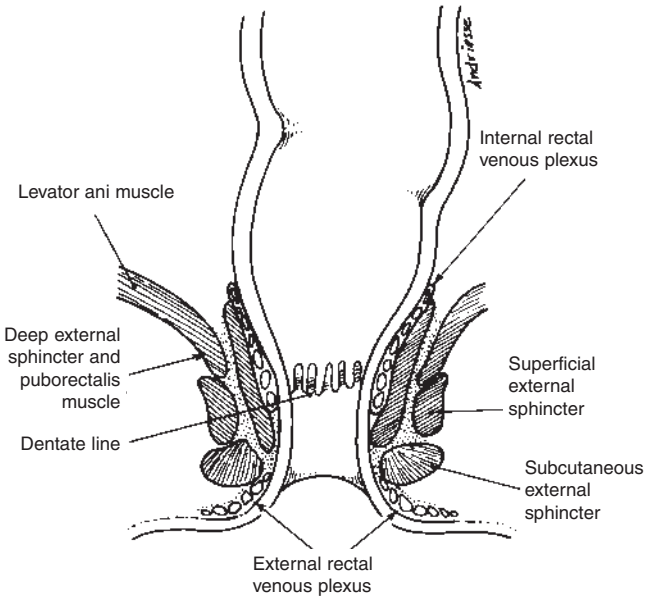

FIGURE 35-1 The anatomy of the rectum and anus is illustrated in coronal section.

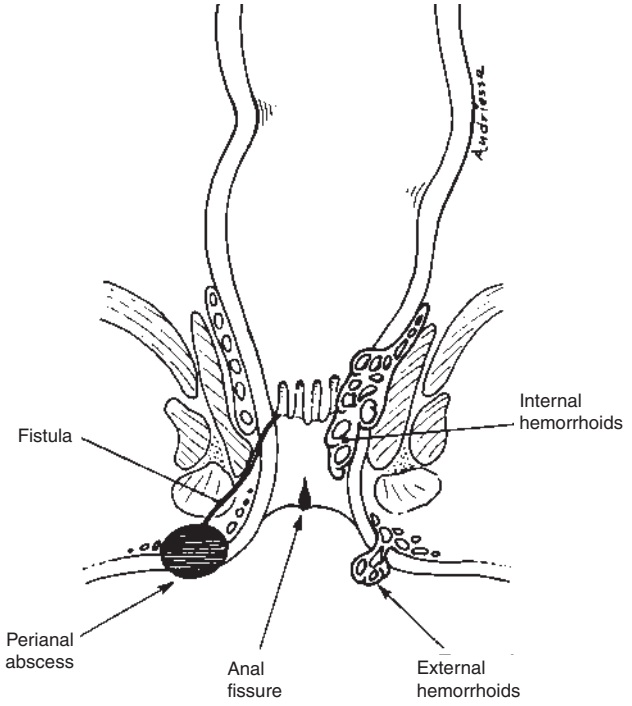

FIGURE 35-2 A diagrammatic representation of perianal lesions and their anatomic relationships is depicted in coronal section.

ciated with constipation and because the appearance of fissures is often noted after the passage of a large, hard stool.[2] Parents can, at times, describe which bowel movement resulted in the superficial tear of the anoderm because it is a painful event for the child. Pain with the next bowel movement leads to hesitancy and avoidance of bowel movements on the child's part. This fecal retention leads to more hardened stools and a persistent, cyclic problem.

In addition to the traumatic insult to the anoderm, the pathogenesis of anal fissures appears to be closely related to two predisposing factors: hypertonicity of the anal sphincter and poor perfusion of the anoderm at the posterior midline.

Angiograms show compromised blood flow through the inferior rectal artery to the posterior midline.[3] Manometric studies done in patients with chronic fissures demonstrate hypertonicity of both internal and external sphincters.[4] These higher pressures also correlate with a further decrease in perfusion.[5] The poorly perfused tissue is thus predisposed to poor wound healing.

Therefore, the etiology of anal fissures is trauma in an environment predisposed to poor wound healing. It is unclear whether constipation and anal hypertonicity are primary causal factors or a consequence of pain, but by the time a patient develops a symptomatic fissure, sphincter hypertonicity is present and is certainly a factor in the persistence of the fissure.

Clinical Presentation and Diagnosis

The initial symptom of an anal fissure is pain at defecation, which may last for minutes to hours afterwards. A small amount of red blood per rectum is common. Anal fissure is the most frequent cause of rectal bleeding in the first 2 years of life. The diagnosis can be made after inspection of the anal canal. If a fissure is not visualized on superficial examination, an anoscope can be helpful in identifying the lesion. Acute fissures are usually small, with no signs of chronicity. Chronic fissures are associated with hypertrophy of the anal papilla, fibrosis, or a distal skin tag. A large fissure associated with bruising (as well as other signs of injury) should raise the suspicion of child abuse. Crohn disease and leukemic infiltration are underlying conditions that require further investigation if a fissure persists after standard treatment.

Treatment

Given the previously described etiologic factors of anal trauma, anal hypertonicity, and poor perianal perfusion, successful therapy should accomplish one or more of the following: a decrease in trauma associated with stooling, a reduction in resting anal tone, and an increase in anal blood flow.[6,7] Initially, treatment is directed at the associated constipation by the use of stool softeners, lubricants (eg, mineral oil), or fiber supplementation. Additionally, warm baths have been shown to reduce anal tone. Results from these measures are good, with a cure in over 80% of acute fissures. Topical steroid and topical or injected anesthetic have not been shown to improve healing rates.

It is important to maintain good anal hygiene in the care of fissures. Failure to adequately clean the anus after each bowel movement may result in the continued presence of fecal matter within the fissure, consequent inhibition of healing, persistent pain, and anal hypertonicity.

The treatment of chronic fissures presents a more difficult problem. A chronic fissure is defined by symptoms that persist longer than 6 weeks after treatment and the presence of fibrosis at the base of the fissure. Fortunately, this is a relatively rare problem in children. Chronic fissures are unlikely to heal with a high-fiber diet and warm baths alone. Thus, the focus of treatment must be directed at the reduction of resting anal pressure.

Nitric oxide is an agent that will lower resting anal sphincter pressure and increase anal blood flow. A 92% cure rate of chronic anal fissures has been reported with the topical application of 0.2% glyceryl trinitrate ointment three times per day.[8] Two randomized trials using the local application of nitric oxide donor compounds in patients with chronic fissures also report good healing rates (70–90%) and no long-term complications such as incontinence.[9] Recurrence rates were 7 to 8%. Although this treatment is promising, it has not as yet been evaluated in children.

Botulinum toxin injection also reduces resting anal tone and appears to be more effective than glyceryl trinitrate. A 95% healing rate for chronic anal fissures has been reported with botulinum toxin injection.[10] However, it should be noted that in the pediatric population, botulinum toxin injection usually requires sedation or general anesthesia. As with glyceryl trinitrate application, botulinum toxin injection for chronic anal fissures has not been well studied in children.

In infants, a standard treatment for chronic fissures is gentle anal dilatation. Parents can be instructed to perform daily anal dilatations. This will help break the cycle of anal spasm and pain and thus assist wound healing. In infants

and young children, anal dilatation generally has good results, and complications such as incontinence are rare.

Very infrequently, when treatment fails despite dietary regulation, hygiene, and anal dilatation, it may be necessary to operate under general anesthesia on infants with chronic fissures. This approach allows for a satisfactory examination, possible further anal dilatation, and complete excision of the chronic anal fissure. In older children or young adults, the fissure is not removed; rather, lateral internal sphincterotomy is the procedure of choice. Lateral internal sphincterotomy achieves healing within several weeks. Studies comparing open versus closed lateral internal sphincterotomy show that both have excellent rates of healing (95%). Differences may exist, however, in long-term continence, with slightly better results achieved in patients undergoing closed lateral internal sphincterotomy.[10,11]

Special Considerations

Rarely, patients develop fissures as a consequence of systemic diseases. Fissures that result from group A β-hemolytic streptococcus infection have been reported. Patients may also evolve multiple, chronic, and difficult to manage fissures as an early manifestation of Crohn disease.

PERIRECTAL ABSCESS AND FISTULA IN ANO

A perirectal abscess is a localized, purulent fluid collection in the surrounding perirectal tissues (see Figure 35-2). Perirectal abscesses are usually classified by their location relative to the levator and sphincteric muscles of the pelvic floor. In order of frequency, abscesses are located in the perianal, ischioanal, intersphincteric, and supralevator locations. Clinical presentation and treatment interventions vary according to site.

A fistula in ano is the result of the spontaneous drainage of a perirectal abscess. This forms a chronic infected tract from the dentate line to the skin.

Pathogenesis

Perianal infections are often encountered in infants in diapers. Usually, there is no specific inciting event, but, occasionally, there is an accompanying diaper rash. In these cases, infection may be the result of an inward spread from the skin. Group A β-hemolytic streptococcal infection of the perianal tissue is also sometimes associated.

More commonly, perirectal abscesses are thought to result from abnormal columns of Morgagni. The crypts tend to trap bacteria, initiating a subsequent cryptitis that, if persistent, will become a perianal abscess. This hypothesis is supported by the presence of columnar, transitional, and stratified squamous epithelium (entrapped migratory cells from urogenital sinus development) lining the tract of a fistula in ano. In 90% of cases of perirectal abscess, the source of the abscess can be traced to an infection occurring in the crypts of Morgagni.[12]

Clinical Presentation and Diagnosis

The presentation of a perianal abscess and associated fistula may vary greatly in the pediatric population. In infants, perirectal abscess and fistula present more frequently in males.[13]

A child with a perirectal abscess will usually experience persistent rectal pain. Occasionally, diarrheal illness or anal fissure precedes the abscess. More infrequently, cryptoglandular infections are secondary to diabetes mellitus, Crohn disease, tuberculosis, or acquired immune deficiency syndrome (AIDS).

Perirectal pain is present in 98% of patients who can communicate their discomfort. The onset can be acute, and often there is no prior history of perirectal pain, inflammation, or trauma. The pain of a perirectal abscess is constant, whereas the pain associated with anal fissure is transient.

The earliest sign of perianal abscess is an indurated, tender area of the perianal skin, with or without erythema, which may occur in any location around the anus. External perianal and digital rectal examinations identify the abscess in 95% of patients.[14] In children and infants who are unable to communicate, the parent will bring the child to the clinic with complaints of crying or irritability that is worse at diaper change. Examination reveals painful perirectal swelling and possibly a fever. If the abscess ruptures and progresses to a fistula, persistent drainage and recurrent abscess formation are seen.

In children, fistula in ano usually occurs during the first year of life, when it is evident as a single cutaneous external orifice. This fistula communicates directly with the rectum at the level of the crypts of Morgagni (see Figure 35-2). It often passes through the lowest fibers of the internal sphincter. This type of fistula is thus termed "low" owing to its lack of compromise of the sphincter complex. Unlike a fistula in adults (in whom the internal opening of the fistula is usually posterior or midline in location with a circular tract following Goodsall law), the internal opening of a perianal fistula in children is usually located radially opposite to its external opening.

Treatment

Different treatment options exist depending on the age and presentation of the patient. Antibiotics have little place in the care of an established perirectal abscess. Incision and drainage with topical anesthesia are the standard of care in infants because abscesses respond poorly to antibiotics, and untreated abscesses will form chronic fistulae in ano in 40 to 60% of patients.

A nonoperative approach was reported in a group of 97 infants with perianal abscess or fistula. During their first year of life, most of these infants recovered spontaneously, but they had a high rate of recurrence. In infants, neither surgery nor antibiotics are routinely indicated. [15]

In older children, perirectal abscesses tend to extend into deeper tissues; thus, the primary treatment is surgical drainage, usually under general anesthesia. Antibiotics have no role in the primary treatment of these abscesses (although they may be useful adjuncts in complicated abscesses). In the operating room, under general anesthesia, the abscess can be drained quickly and painlessly, and a full examination is facilitated to identify concurrent abscesses or associated fistulae.

Persistence of an anal fistula is generally considered an indication for surgery. Chronic fistulae, in addition to troubling persistent drainage, are associated with recurrent abscesses. Anal fistulae are best managed with fistulectomy (excision) or fistulotomy (opening and curetting).[16] Rarely, the use of a seton loop is necessary. This loop is placed through the fistula and slowly tightened, thus allowing for division and fibrosis of higher tracts that may encompass both sphincters. The goal of treatment of a fistula is complete eradication of the fistula while preserving fecal continence.

Special Considerations

Recurrences of perirectal abscesses and fistulae are not uncommon, and treatment may be protracted.[17] Additionally, with recurrent perianal disease, one must always consider associated diseases. Older children with perianal abscess may have other undiagnosed medical problems, including drug-induced or autoimmune neutropenia, leukemia, human immunodeficiency virus (HIV), side effects from the use of immunosuppressive drugs, diabetes mellitus, and Crohn disease. For example, up to 15% of children with Crohn disease present with recurrent anal fistulae. Additionally, rare diseases, such as rectal duplication, may be mistaken for an anal fistula.[18] In general, with persistent and multiple fistulae, further investigation should be considered.[19]

HEMORRHOIDS

Hemorrhoids are generally classified into two subtypes, external and internal (see Figure 35-2). A hemorrhoid is a varicose vein from either the internal or external rectal plexus of veins. External hemorrhoids involve the skin of the anoderm external to the dentate line, and their nerve supply is cutaneously derived. Hence, newly thrombosed external hemorrhoids are associated with acute pain.

An internal hemorrhoid is also a swollen blood vessel (specifically a varicosity of the tributaries of the superior rectal vein). Internal hemorrhoids differ from external hemorrhoids in that they are located under the lining of the rectum. Patients who are suspected of having hemorrhoids should undergo a visual and digital examination to determine the type and grade of hemorrhoid. Internal hemorrhoids are graded on an ascending scale of severity from 1 to 4. With a first-degree hemorrhoid, anal cushions are present on rectal examination. Second-degree hemorrhoids may prolapse below the dentate line but reduce spontaneously. Third-degree hemorrhoids must be manually reduced. Fourth-degree hemorrhoids are irreducible.

Pathogenesis

A bout of constipation, straining, or diarrhea is often associated with a thrombosed external hemorrhoid. Chronic constipation and excessive straining are also implicated in the development of internal hemorrhoids. However, internal hemorrhoids may also be associated with chronic liver disease and portal hypertension.[20]

Clinical Presentation and Diagnosis

External hemorrhoids rarely cause symptoms. If an external hemorrhoid becomes very large, it can become difficult to clean after bowel movements. Occasionally, sudden pain occurs when a clot develops within the external hemorrhoid and becomes thrombosed. A painful, grape-like protrusion is noted on examination. This pain usually does not persist for more than a few days because the natural history of the clot is to drain spontaneously.

In children, primary internal hemorrhoids are virtually unknown. Their presence should raise questions regarding possible portal vein obstruction. Internal hemorrhoids present with either a protruding rectal mass or rectal bleeding. They are a common cause of rectal bleeding in older patients, but not in children, in whom anal fissures are much more common. Internal hemorrhoids do not cause pain. Classically, they manifest in three positions around the anus: the left lateral, right posterolateral, and right anterolateral. They can enlarge to the point that they protrude out of the rectum and may have to be pushed back in after straining. In advanced cases, they protrude enough so that they cannot be pushed back inside, resulting in chronic drainage and spotting of blood.

Treatment

Generally, an external hemorrhoid does not require surgery. External hemorrhoids are of no potential danger even if left untreated because they do not develop into cancers or other serious conditions.

The thrombosed external hemorrhoid is the only hemorrhoidal condition that is actually painful and, as such, may require surgical intervention during the acute thrombotic episode. Simple incision and drainage can promptly alleviate the pain of a thrombosed external hemorrhoid.

Treatment of internal hemorrhoids can vary from simple alterations in the diet to surgical removal. Internal hemorrhoids should first be treated with stool softeners and bulk agents (eg, psyllium). This approach is generally adequate treatment for hemorrhoids of degrees 1 to 3. Fourth-degree hemorrhoids usually require surgical intervention; banding is effective. Having said this about treatment, it must be re-emphasized that primary internal hemorrhoids are so uncommon in children that their presence should raise questions as to their etiology. A full investigation is necessary prior to treatment because hemorrhoidectomy in the face of portal hypertension can cause exsanguination.

RECTAL PROLAPSE

Rectal prolapse refers to either a mucosal or full-thickness herniation of the rectum through the anus. This diagnosis can be confused with chronic prolapsed internal hemorrhoids. Because rectal prolapse is a protrusion of the entire circumference of the rectal mucosa, concentric rings of mucosa are seen on examination. In fourth-degree hemorrhoids, the protrusion occurs only in a defined sector of the anus (usually lateral).

Pathogenesis

Rectal prolapse may be attributed to various causes and should be viewed as a sign of an underlying condition rather than a specific disease in itself. Potential etiologies include increased intra-abdominal pressure, diarrheal and neoplastic diseases, malnutrition, and conditions predisposing to pelvic floor weakness (such as prior surgery).[21] The most common cause is chronic constipation that may or may not be associated with neurologic or anatomic abnormalities. Acute diarrheal disease is the next most frequent etiology, followed by cystic fibrosis. Frequently, an extensive workup fails to elucidate any underlying factor.

Clinical Presentation and Diagnosis

Rectal prolapse is not uncommon in children, occurring more often in boys than in girls and tending to appear during the period of toilet training. The condition is usually self-limited.[22]

In infancy, there are two types of rectal prolapse.[23] The first is less pronounced and intermittent; the other is more pronounced and occurs with most bowel movements over a period of several weeks or months. Patients with rectal prolapse usually present with a history of prolonged straining at defecation. Rectal prolapse may also be associated with an acute diarrheal episode, cystic fibrosis, or a neurologic or anatomic anomaly. Although seen very rarely in developed countries, severe malnutrition or parasitosis can also cause rectal prolapse.

Children with unexplained or recurrent rectal prolapse should have a sweat chloride test to rule out cystic fibrosis. If an anatomic abnormality can be identified as a cause of rectal prolapse, a sweat chloride test is not usually indicated.[24]

Treatment

In general, the treatment of rectal prolapse is nonoperative and directed at the underlying condition predisposing the patient to prolapse. Initial management consists of manual reduction and the administration of bulk laxatives or stool softeners.

If prolapse is a persistent problem, surgical intervention may be required. Injection sclerotherapy with D50W (dextrose 50% in water) or hypertonic saline is an effective treatment with few complications.[25] Sclerotherapy can be accomplished by injecting no more than 1 cc/kg of D50W submucosally or submuscularly above the dentate line.

More aggressive surgical efforts may be needed for prolapse that fails sclerotherapy and in children with pelvic anatomic distortion caused by previous surgery. A variety of surgical procedures have been developed to treat rectal prolapse, but there is no consensus on the operation of choice. Surgery may be relatively simple, such as encircling the anus with a suture,[26] or involve complex operations such as posterior repair and suspension,[27] transsacral rectopexy,[23] transabdominal rectopexy with resection,[28] and posterior sagittal anorectoplasty.[29] Recent series advocate for the laparoscopic treatment of rectal prolapse. This minimally invasive technique allows for either a simple rectopexy or a more elaborate rectosigmoid resection for the treatment of prolapse. Regardless of the approach, the prognosis is generally good.

PILONIDAL DISEASE

A pilonidal abscess is an inflammatory cavity overlying the sacrococcygeal region in the midline and is often accompanied by multiple draining sinus tracts (Figure 35-3).

Pathogenesis

A pilonidal sinus begins in the natal cleft at the site of an ingrown hair follicle. It commonly presents 1 or 2 inches above the anus and leads into a cavity underlying the skin. The result is a pilonidal cyst that may drain spontaneously. If the superficial opening of the tract is occluded, a pilonidal abscess will form.

Clinical Presentation and Diagnosis

A pilonidal abscess presents with persistent pain in the region of the sacrum accompanied by a boil located in the midline just above the anus. The diagnosis is made on physical examination that includes a rectal examination. A pilonidal abscess that drains spontaneously often results in a chronic pilonidal sinus. A pilonidal sinus tract is usually a slightly sore spot and can be a source of bloodstained or cloudy drainage that soils the underclothes.

Treatment

If a pilonidal abscess or fistula is found, the patient should be referred for operative management. Primary treatment involves incision and drainage of any acute abscess. After resolution of the abscess, the traditional procedure of choice involves the subsequent wide excision of the underlying cyst and fistulous tracts, with the wound left to close by secondary intent. More recent experience has shown that excision of the cyst and primary closure of the wound can be attempted in most cases provided that there is careful follow-up. When successful, this latter technique allows for rapid healing and less discomfort to the patient.

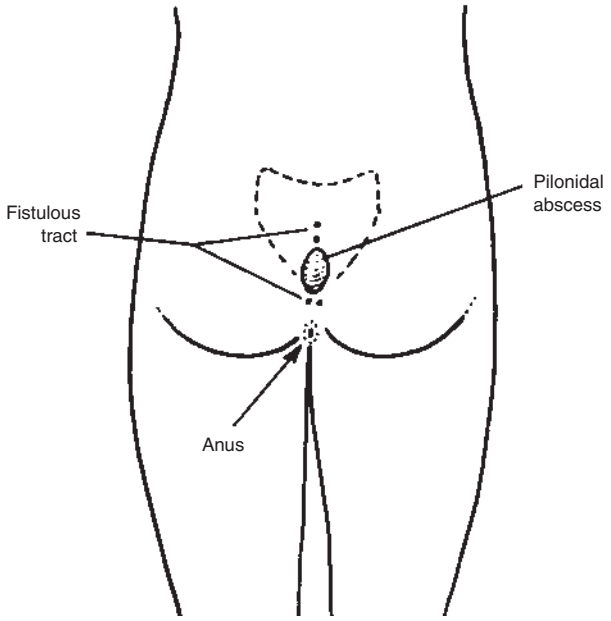

FIGURE 35-3 A pilonidal abscess and its associated tracts are shown in relation to the sacrum.

PRURITUS ANI

Pruritus ani simply implies persistent anal itching. It is a common skin condition and very frequently misdiagnosed as "hemorrhoids." Pruritus can be grouped into either primary or secondary etiologies.

Pathogenesis

Most cases of pruritus ani are secondary in nature, with primary pruritus ani being a diagnosis of exclusion. Secondary pruritus is usually the result of chronic moisture exposure, pinworm (*Enterobius vermicularis*) infection, or excessive use of soaps and detergents. Caffeine intake, which reduces anal pressure, is the most common cause of pruritus ani in older subjects. Aggressive use of soaps and detergents perpetuates the problem of pruritus ani by changing cutaneous pH and stripping the natural oils of the skin.

Pinworm is a contagious intestinal parasite infestation that is common in children. It is found throughout the United States and is especially prevalent in urban areas and day-care settings. Approximately 20% of children in the United States will develop pinworm at some point in their lives. The parasite is easily spread through a fecal-oral route. The adult parasite lives in the cecum and colon and lays its eggs outside the anus during the night.

Clinical Presentation and Diagnosis

Patients typically present with persistent anal itching that is unrelenting. Other symptoms include irritability, sleep disturbance, decreased appetite, excoriation around the anus from constant scratching, and vaginal irritation.[30] Adults and siblings exposed to children with pinworm may also note symptoms shortly after exposure. Pinworm can be easily diagnosed by applying transparent adhesive tape to the skin around the anus before bathing or using the toilet in the morning. The tape is then transferred to a slide, where the presence of pinworm eggs may be confirmed by microscopy.

Treatment

Because most causes of anal pruritus are secondary, treatment should be directed at the underlying etiology. This is not to imply that all pruritus ani is easily treated because it can be the result of a self-perpetuating cycle. A complete anal and rectal examination should be performed to look for specific causes, especially pinworm. A thorough history of dietary and hygiene habits is also helpful.

For pinworm infestation, initial treatment consists of patient and family education about hygiene and fecal-oral spread.[31] General measures, such as hand washing, keeping fingernails short and clean, laundering all bed linens twice weekly, and cleaning toilet seats daily, can usually eliminate the problem in 1 to 2 weeks. If this fails, all family members must be treated with pyrantel pamoate or mebendazole.[32] To be successful, drug therapy needs to be repeated in 2 to 3 weeks.

For pruritus ani not attributable to pinworm reassurance, patient education and follow-up are key elements of treatment. Stopping scratching, although difficult to do, can often break the itching cycle. Excessive anal hygiene habits and use of ointments, steroids, and anesthetic agents should be discouraged. Food sensitivities may exist to spices, coffee, milk, and chocolates. The aim of treatment is to achieve clean, dry, intact skin. In selected cases, some medications may also be of assistance, such as psyllium (promotes complete evacuation) and loperamide (increases resting anal pressure).

Although disturbed sphincter function has been proposed as causative (primary pruritus ani), it is an infrequent etiology. Generally, surgical intervention should be discouraged. When extensive tags or prolapsed tissue appears to contribute to poor hygiene, an operation may be considered.

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CHAPTER 36

THE SURGICAL ABDOMEN

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The term "surgical abdomen" describes a constellation of symptoms and signs indicative of an intra-abdominal process that may require surgery. A degree of urgency is usually implied. The processes underlying the surgical abdomen include intestinal obstruction and peritonitis owing to inflammatory conditions such as appendicitis. This chapter reviews the more common causes of these processes and their differential diagnoses, some of which are addressed in greater detail in other chapters. Congenital intestinal conditions requiring surgical management are discussed in Chapter 32, "Congenital Anomalies."

INTESTINAL OBSTRUCTION

General Features and Principles of Management

The cardinal features of intestinal obstruction are bile-stained (green) vomiting, abdominal distention, failure to pass stool, and colicky abdominal pain. The clinical picture depends on the level of obstruction and the presence of complications, notably strangulation. With proximal obstruction, vomiting and colicky pains occur early, and distention is confined to the epigastrium. In more distal small bowel obstruction, the vomiting may be less frequent, and as a result of stasis and bacterial overgrowth, the vomitus becomes foul smelling and feculent. The abdomen becomes distended, and loops of bowel may be palpated. Visible peristalsis may coincide with episodes of abdominal colic. With a complete obstruction, no stools will be passed once the distal bowel has evacuated, and, typically, the rectum is empty. The finding of abdominal tenderness in a child with features of obstruction may indicate progressive intestinal ischemia. Colonic obstruction leads to marked abdominal distention, cramping pains, and cessation of stools; vomiting is a later feature.

Imaging. Plain abdominal radiography is the most commonly used imaging method for confirming the diagnosis of intestinal obstruction, and, in many cases, the level and possibly the cause of obstruction may be identified. In most cases, the supine abdominal view showing dilated proximal bowel and absence of gas distally will suffice (Figure 36-1A); erect abdominal radiographs will show air-fluid levels but are rarely needed for diagnosis and can be omitted (Figure 36-1B). Contrast x-ray examinations are useful in specific situations and should be planned in consultation with a radiologist. For example, an upper gastrointestinal study is useful for identifying duodenal malrotation with or without volvulus, whereas an air or contrast enema is helpful for suspected intussusception. In general, low-osmolality water-soluble contrast materials are used. Ultrasonography has a limited role in the diagnosis of intestinal obstruction but may be of value in malrotation by identifying the relative positions of the superior mesenteric artery and vein and in the diagnosis of intussusception.

Management. The general principles of management include nasogastric drainage, intravenous fluids, and pain control. A large nasogastric tube is passed to prevent vomiting and reduce gastric secretions and is firmly secured to the face. In small infants, an orogastric tube may be preferred. The tube is left on continuous drainage and is regularly flushed with air or water and aspirated to confirm that it is functional.

There are two phases to intravenous fluid therapy: resuscitation and maintenance. The initial fluid deficit is corrected using 0.9% (normal) saline or Ringer lactate at 3 to 6 mL/kg/h, depending on age and weight, and supplemented, if indicated, by a 20 mL/kg bolus of the same solution, repeated as required. In newborn infants, care must be taken not to overload the circulation with fluid or sodium. This is followed by maintenance infusion of Ringer lactate or 0.45% (half normal) saline with potassium chloride 10 to 20 mmol/L at 2 to 5 mL/kg/h to provide for maintenance requirements, plus anticipated ongoing hidden fluid losses into the obstructed bowel. Dextrose 5 or 10% is added to prevent hypoglycemia. Gastric aspirates are replaced with equal volumes of normal saline. Hydration is monitored by clinical assessment of the peripheral circulation and by accurate monitoring of the urine volume (normal = 1 to 2 mL/kg/h depending on age) and specific gravity (normal 1.008 to 1.012). Serum electrolytes and acid-base balance are monitored. Urine sodium estimations are useful for interpreting renal function. Based on these findings, the volume of intravenous fluid is increased or decreased every 4 to 8 hours, the frequency of assessment depending on the individual clinical situation.

With few exceptions, the definitive management of intestinal obstruction requires urgent operation. This should

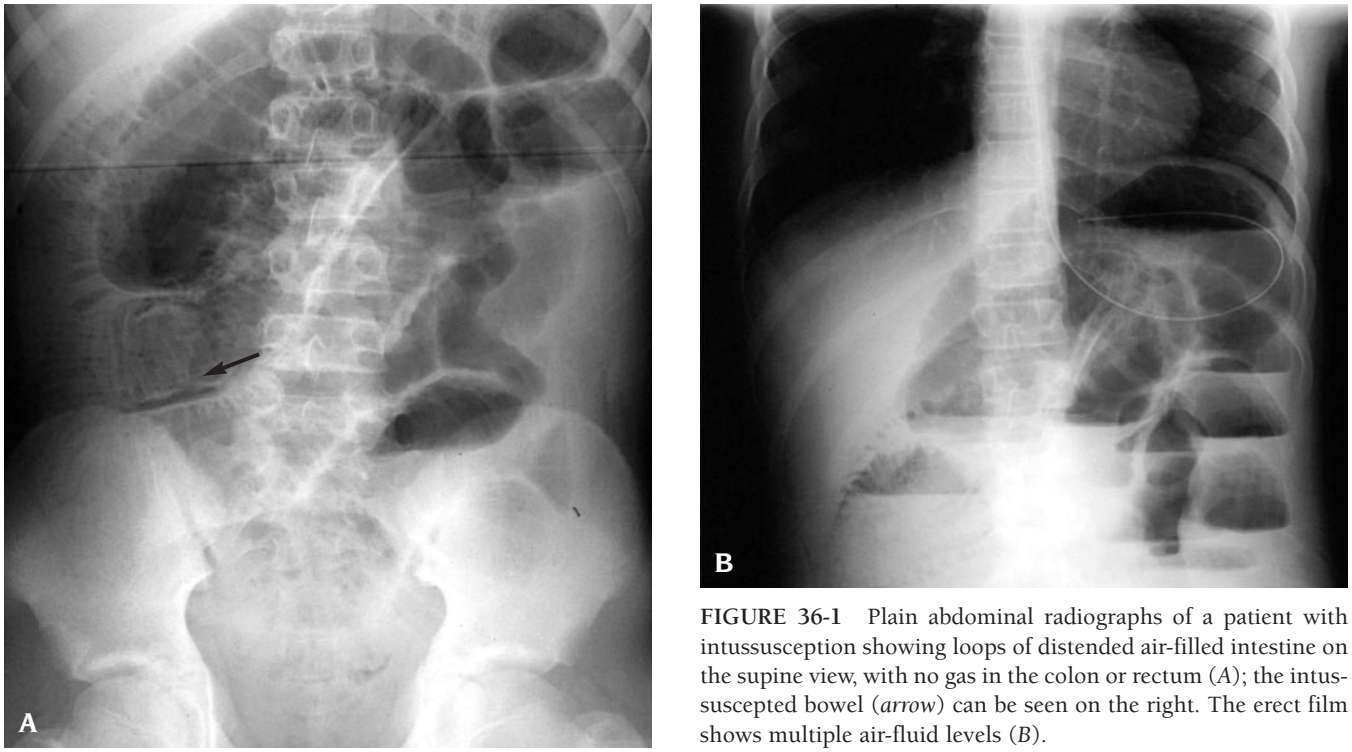

FIGURE 36-1 Plain abdominal radiographs of a patient with intussusception showing loops of distended air-filled intestine on the supine view, with no gas in the colon or rectum (*A*); the intussuscepted bowel (*arrow*) can be seen on the right. The erect film shows multiple air-fluid levels (*B*).

be undertaken only after the patient has been adequately resuscitated with correction of fluid, electrolyte, and acid-base abnormalities. The urgency for operation depends on whether there is clinical evidence that intestinal viability is being compromised, as in the case of an incarcerated hernia, complicated intussusception, or intestinal volvulus. Operation may be delayed when the diagnosis is clear and there is a possibility that the obstruction will resolve spontaneously, for example, with postoperative adhesive obstruction or intramural hematoma of the intestine.

For simple (uncomplicated) intestinal obstruction, nonoperative therapy with nasogastric drainage and intravenous fluids may be continued as long as there is evidence of progressive resolution of the symptoms. If there is no improvement by 12 to 24 hours, operative intervention generally is advisable, depending on the individual situation, including the presence of coexisting disease. Intravenous feeding should be considered in situations in which intravenous therapy and the period of enteral starvation are likely to be prolonged.

Simple obstruction may progress to complicated obstruction if the viability of the intestine becomes compromised owing to strangulation or volvulus. In this situation, there will be evidence of local or generalized peritonitis, notably abdominal distention with tenderness and guarding. There is a progessive deterioration in the condition of the patient with increasing oxygen requirement, tachycardia, hypotension, peripheral vasoconstriction, and oliguria owing to a combination of intraluminal fluid loss and septicemia caused by translocated enteric bacteria and toxins. Urgent laparotomy is required after full resuscitation and administration of broad-spectrum antibiotics with activity against aerobic and anaerobic bacteria. Causes of intestinal obstruction are summarized in Table 36-1.

Incarcerated or Strangulated Inguinal Hernia

Congenital inguinal hernia is the presence of an abdominal viscus in the processus vaginalis (hernia sac), usually the small intestine but occasionally the ovary. Spontaneous resolution does not occur, and because of the high risk (10–28%) of incarceration during the first 3 months of life, and hence strangulation, prompt operation is advised.[1] If possible, this is done within 7 days of diagnosis, provided that the infant is fit for general anesthesia.

The infant with an incarcerated hernia is admitted to hospital and sedated; the hernia is then gently reduced. The hernia is repaired after an interval of 2 days to allow tissue swelling to resolve. If reduction by taxis is not easily achieved, the attempt is abandoned, and urgent operation is undertaken to reduce and repair the hernia. When strangulation has occurred, the infant is ill and shows features of intestinal obstruction. The hernia is tender, and the overlying skin may be inflamed. Urgent resuscitation is followed by emergency repair of the hernia, potentially an extremely difficult operation. In addition to causing intestinal ischemia, a strangulated hernia may compress

TABLE 36-1 CAUSES OF INTESTINAL OBSTRUCTION

Incarcerated/strangulated inguinal hernia
Meckel band obstruction
Intussusception
Peritoneal adhesions
Hypertrophic pyloric stenosis
Meconium ileus equivalent
Milk inspissation syndrome
Foreign body ingestion
Superior mesenteric artery syndrome
Paralytic ileus
Chronic idiopathic intestinal pseudo-obstruction

the spermatic cord, resulting in testicular ischemia and, subsequently, atrophy.[2]

Meckel Band Obstruction

The primitive omphalomesenteric duct persists as a solid band between the umbilicus and a Meckel diverticulum of the small intestine (see Chapter 32). Intestinal obstruction may result from entrapment of a loop of intestine or volvulus around the band. Urgent surgery is required to prevent strangulation; at surgery, the obstruction is relieved, and the Meckel diverticulum is resected.

Peritoneal Adhesions

Adhesions within the peritoneal cavity occur most commonly following a surgical procedure but may also occur as the result of inflammatory and infectious conditions not requiring surgery or following radiotherapy. Adhesive obstruction is the result of entrapment of bowel loops and may lead to closed-loop obstruction and strangulation.

Postoperative adhesions occur to some degree in most patients following laparotomy, in particular for inflammatory conditions such as appendicitis, and in areas of serosal damage or intestinal ischemia. There is no reliable method for preventing adhesions. In the neonate, the most common associated conditions are gastroschisis and malrotation.[3] Obstruction may occur at any time after a laparotomy; in the report by Janik and colleagues, 80% of adhesive obstructions occurred within 2 years of the original operation,[4] and Wilkins and Spitz found that 75% occurred within 6 months and 90% within 12 months.[3]

The diagnosis must be suspected in any patient who presents with clinical and radiologic features of intestinal obstruction and has had previous abdominal surgery. Patients who have radiotherapy may present many years later; the possibility of recurrent tumor must also be borne in mind. Initial management is nonoperative, as discussed above, and in most patients, the obstruction will resolve within 24 to 48 hours. Because of the risk of strangulation, operation is advisable if there is no improvement within 6 to 12 hours. Urgent operation is indicated if there is unremitting abdominal pain, fever, tachycardia, abdominal tenderness, and guarding.

Hypertrophic Pyloric Stenosis

Hypertrophic pyloric stenosis (HPS) is the most common condition requiring surgery in the first 2 months of life. The incidence is approximately 2 to 3 per 1,000 live births, but there are wide geographic variations, and increasing[5,6] and decreasing[7–9] incidences over the past three decades have been reported. The underlying mechanism for HPS is not understood; in some infants, we have observed spasm of the pyloric muscle progressing to hypertrophy and evolution of the obstructive pyloric mass. Relaxation of the pyloric muscle appears to be dependent on inhibitory innervation through the nonadrenergic, noncholinergic neural system, mediators of which have been shown to be reduced or absent, notably certain neuropeptides and nitric oxide.[10] Other neural components, including the interstitial cells of Cajal,[11] neurotrophins, synapse formation, and neural supporting cells, have also been shown to be abnormal. The etiology of pyloric stenosis is not known, but a higher incidence in infants with a family history of HPS suggests a genetic predisposition.[12]

HPS is characterized by elongation and narrowing of the pyloric lumen; secondary gastritis and mucosal edema result in virtually complete obstruction. Typically, infants are well for the first 2 to 3 weeks of life. Nonbilious vomiting then begins intermittently after feeds, progressively increasing in frequency and volume, leading to dehydration, weight loss, and constipation. The vomitus may contain altered blood from secondary esophagitis or gastritis. Affected infants presenting late can be profoundly dehydrated with severe metabolic alkalosis. On examination, peristaltic waves may be seen traversing the epigastrium from left to right. Palpation of the pyloric tumor is diagnostic. Examination must be done with the stomach empty and the infant relaxed. This is achieved by aspirating the stomach through a nasogastric tube; the hungry infant is relaxed by allowing it to drink an electrolyte solution while the abdomen is palpated above and to the right of the umbilicus, the fingers probing under the liver. If the clinical findings are inconclusive, the diagnosis can be confirmed by ultrasonography (Figure 36-2) or contrast meal. HPS must be distinguished from gastroesophageal reflux, in which the vomiting is usually present from birth. Imaging studies and gastroscopy will clarify the diagnosis.

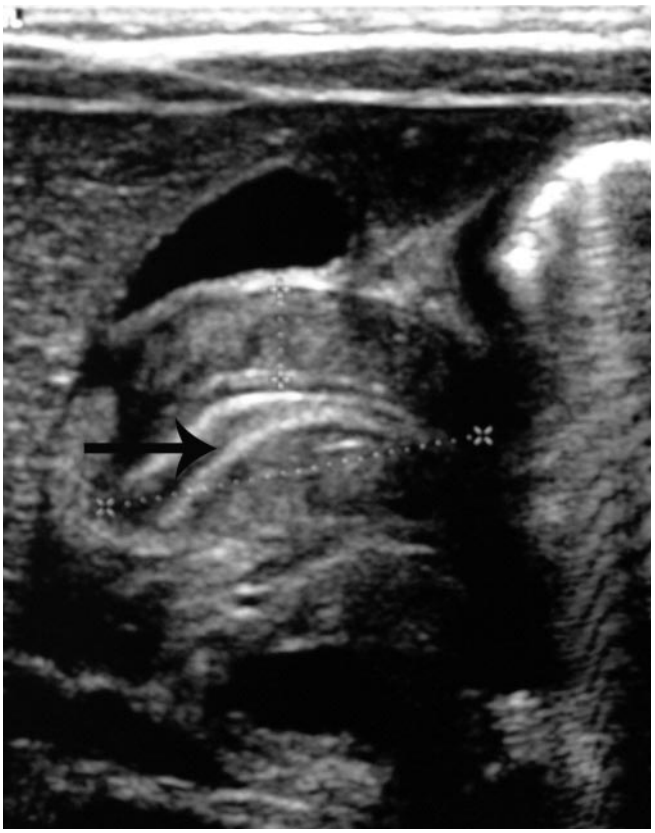

FIGURE 36-2 Idiopathic hypertrophic pyloric stenosis: sonogram showing the thickened pyloric muscle constricting the lumen (*arrow*).

Preoperative correction of fluid, electrolyte, and acid-base abnormalities, typically hypochloremic metabolic alkalosis, is essential. A nasogastric tube is passed to empty the stomach and for saline irrigations to alleviate the gastritis by removing residual feeds. Intravenous fluids are given as 0.45% saline with 10% dextrose and potassium chloride 20 mmol/L at an initial rate of approximately 5 to 6 mL/kg for 24 hours. Nasogastric aspirates are replaced with equal volumes of normal saline. Because of the risks of postoperative apnea and cardiac arrhythmia, surgery should not be undertaken until the serum electrolyte and blood gas levels have returned to normal.

Ramstedt pyloromyotomy, introduced in 1912, remains the treatment of choice. The pylorus is approached through a transverse right upper quadrant or a periumbilical incision.[13] A longitudinal incision along the pyloric tumor is deepened by blunt dissection to expose the mucosa. Laparoscopic pyloromyotomy is an established option.[14]

Postoperatively, oral feeds are given on demand, beginning with a small volume and increased progressively as tolerated. Most infants can be discharged 24 to 48 hours after operation. Postoperative complications include wound infection and dehiscence. Persistent vomiting after surgery is more common in younger infants and may reflect relative immaturity of the lower gastroesophageal sphincter[15]; rarely, it is due to unrecognized iatrogenic duodenal perforation or inadequate pyloromyotomy.

Meconium Ileus Equivalent (Distal Intestinal Obstruction Syndrome)

Patients with cystic fibrosis may develop acute intraluminal obstruction mimicking neonatal meconium ileus. This has been attributed to inadequate enzyme replacement therapy or dehydration. Oral Gastrografin may clear the obstruction; it is rare for surgery to be required.[16]

Milk Curd Inspissation

Obstruction by inspissated milk curd has been reported and is attributed to the use of concentrated formula preparations. If irrigation under radiologic control is unsuccessful, laparotomy is required to relieve the obstruction.[17]

Foreign Body Ingestion

Children frequently swallow coins, toys, and other objects, which usually impact in the esophagus. Retained esophageal foreign bodies require urgent removal, particularly button batteries and pins because these have the potential to cause esophageal ulceration or perforation, leading to mediastinal abscess or acquired tracheoesophageal fistula, and, rarely, aortoenteric fistula. Those that enter the stomach are likely to be passed per rectum but, occasionally, become impacted en route. The diagnosis is confirmed by plain radiography provided that the object is radiopaque. Commercially available metal detectors have been found to be as useful as radiographs for screening.[18] Patients who are asymptomatic are allowed a normal diet and are kept under observation as outpatients until the object has been retrieved. Special diets and laxatives are not necessary. If symptoms develop, or if the object remains in the stomach or becomes impacted in the intestine, it should be removed, particularly if it is a button battery, needle, or pin. Endoscopic removal from the stomach is often possible.

Intestinal Strictures

Pathologic narrowing of the intestine results in partial or complete obstruction. Local injury and impaired flow may result in fibrous strictures, for example, following blunt abdominal trauma or at the site of an intestinal anastomosis. Inflammatory strictures may complicate chronic diseases such as Crohn disease and intestinal tuberculosis.

Superior Mesenteric Artery Syndrome

In this rare condition, the third part of the duodenum is obstructed as it passes between the superior mesenteric artery anteriorly and the verterbral column posteriorly. Predisposing factors include rapid linear growth without weight gain, weight loss, scoliosis, spinal surgery, confinement to bed, and use of a body cast. The mechanism is not known, but absence of a cushion of retroperitoneal fat may be a factor.

The clinical features are nonspecific, with intermittent abdominal pain associated with anorexia, nausea, and bilious vomiting. Abdominal examination may reveal a succussion splash. A dilated stomach and proximal duodenum may be seen on plain abdominal radiography, and an upper intestinal contrast study will demonstrate partial obstruction in the third part of the duodenum (Figure 36-3). Management includes nasogastric drainage and intravenous fluids, depending on the severity of symptoms. Occasionally, intravenous feeding is advisable. With improved nutrition, symptoms may resolve. Occasionally, operative treatment is

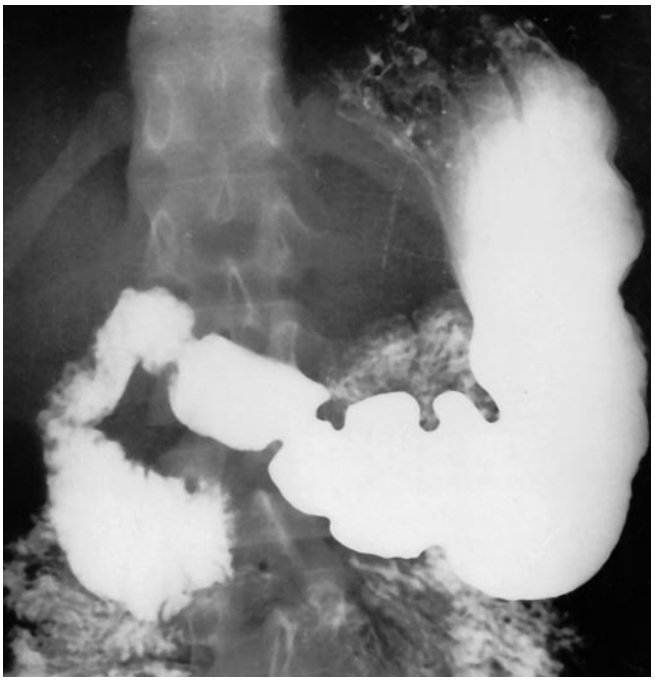

FIGURE 36-3 Superior mesenteric artery syndrome: contrast study showing partial obstruction in the third part of the duodenum by the superior mesenteric artery.

required to mobilize the ligament of Treitz and realign the duodenum; duodenojejunostomy has also been used.

Disordered Peristalsis

Paralytic Ileus. Paralytic ileus is a form of functional intestinal obstruction owing to transient loss of intestinal peristaltic activity. It is common to some degree after most abdominal operations. The causes include local factors, notably peritonitis, intestinal ischemia, surgical manipulation, retroperitoneal bleeding, or spinal surgery, and systemic factors, such as sepsis syndrome, hypokalemia, diabetic ketoacidosis, and uremia. The clinical picture resembles mechanical obstruction except for the absence of bowel sounds, an important diagnostic feature. On plain abdominal radiography, usually there is gas throughout the small and large intestine with no focal point of obstruction. Management is expectant, with nasogastric drainage and intravenous fluids.

Chronic Idiopathic Intestinal Pseudo-obstruction. Chronic idiopathic intestinal pseudo-obstruction is a rare cause of functional obstruction in children with ganglionic bowel. Acute episodes of intestinal obstruction may occur on a background of chronic peristaltic dysfunction. Treatment of these episodes is symptomatic, avoiding laparotomy if at all possible because this causes diagnostic problems for future episodes by introducing the possibility of adhesive obstruction.

CHILD WITH ACUTE ABDOMINAL PAIN

When evaluating a child with an acute abdomen, a thorough history and physical examination are essential to exclude nonsurgical and extra-abdominal conditions that may present with the clinical features of the surgical abdomen. These range from infective conditions such as acute tonsillitis and lower lobe pneumonia to vertebral discitis and metabolic conditions, including porphyria and lead poisoning.

The evaluation of the child with abdominal pain remains a challenge, and good interaction among the surgeon, child, and parents is essential. Successful evaluation requires patience and often deviates from the normal pattern of history taking and examination. General principles include addressing the child directly whenever possible and allowing time for him or her to answer questions; these should give the child several options without biasing the choice of answer: for example, "Is the pain getting better, worse, or staying the same?" "Is the pain bad all the time, or does it come and go?" As with all patients, showing an interest in the child as a person often helps build their confidence. No diagnostic information can be gleaned by attempting to examine the abdomen of a crying child who is being pinned to the examination couch. Often children pick up on the anxiety of the parents, and attempts should be made to calm their fears. In many cases, abdominal examination is best performed in an unorthodox manner; for example, the child on a parent's lap or standing up often feels safer and will relax, allowing relevant clinical signs to be detected. A useful strategy in the case of the uncooperative child is to defer examination until the child has been admitted; the fretful tearful child in the emergency room is often more cooperative when in the calm of a ward in a comfortable bed. Play specialists can also be invaluable when assessing children or if any invasive procedures need to be performed. Honesty with the child at all times is paramount; reassuring the child that a painful procedure will not hurt works only once, and all trust is then lost. This is not a minor consideration because doctor and needle phobia are real phenomena that may have lasting consequences on the future health of the individual.[19]

Some causes of abdominal pain in children are listed in Table 36-2. The clinical skills required to differentiate between them should not be underestimated. The choice of diagnostic methods will depend on the clinical picture. Ultrasonography, plain and contrast radiography studies, urine culture, serum amylase estimation, and upper and lower intestinal endoscopy all have a role.

The general principles of management for the child with acute peritonitis are similar to those for acute intestinal obstruction and include nasogastric drainage, intravenous fluids to correct hypovolemia and electrolyte and acid-base abnormalities, analgesia, and antibiotics.

Appendicitis

Appendicitis can be particularly difficult to diagnose in the very young and the neurologically impaired. Young children with appendicitis invariably present late, and perforation at the time of presentation is common in those under the age of 5 years. Abdominal signs can be subtle, and true peritonism may be absent. Abdominal distention is a common finding. Ultrasonography and/or computed tomography (CT) may provide useful diagnostic information in equivocal cases and save unnecessary surgery. Careful assessment for dehydration or shock is essential, and vigorous intravenous fluid resuscitation and intravenous antibiotics should be instituted when necessary, in addition to adequate intravenous opiate analgesia. The choice between an open or laparoscopic approach to appendicectomy in children remains controversial,

TABLE 36-2 SELECTED CAUSES OF ABDOMINAL PAIN IN CHILDHOOD

Appendicitis
Mesenteric adenitis
Intussusception
Midgut malrotation
Gastroenteritis
Constipation
Intestinal polyps
Intestinal ascariasis
Pancreatitis
Cholecystitis
Ovarian cyst
Ovarian torsion
Ovulatory/perimenstrual pain
Urinary tract infection

although a recent systematic review concluded that laparoscopic appendicectomy was "likely to be beneficial" when performed by an experienced laparoscopist.[20] Robust peripheral or central venous access inserted at the time of anesthesia is important to enable administration of postoperative intravenous fluids and antibiotics, particularly in cases of perforated appendicitis.

Mesenteric Adenitis

Inflammation of the mesenteric lymph nodes, particularly in the ileocecal region, may present with acute right iliac fossa pain mimicking appendicitis. A confident diagnosis of mesenteric adenitis can be made only when a definite cause can be identified, either abdominal or extra-abdominal, for example, acute tonsillitis. In the absence of a definitive diagnosis, acute appendicitis must be assumed to be the cause of the symptoms until positively excluded by direct inspection at laparotomy or laparoscopy or by reliable imaging. Tests such as the white blood cell count and C-reactive protein levels are not sufficiently specific but may provide useful correlates to clinical assessment.[21–23] Localized right iliac fossa contrast-enhanced CT has been shown to be highly specific and sensitive in adult patients admitted following surgical appraisal,[24] but its diagnostic value in children has not been rigorously evaluated.

Intussusception

With intussusception, a segment of bowel telescopes into the adjacent distal bowel, causing intestinal obstruction and impairing blood flow through the intussuscepted bowel segment. Although not confined to children, the peak incidence of intussusception is between 4 and 14 months, when most cases of intussusception are idiopathic, although enlarged gut-associated lymphoid tissue (Peyer patches) secondary to increased exposure to novel antigens during weaning may play a role as a lead point. In older children, a pathologic lead point such as a Meckel diverticulum or small bowel lymphoma may be found. Typically, infants will experience colicky abdominal pain often associated with bilious vomiting. The passage of blood and mucus per rectum is a later sign of intussusception. Most morbidity and mortality from intussusception arise from delays in diagnosis, and a high index of suspicion should be maintained. Between bouts of colic, infants are quiet but irritable, with evidence of hypovolemia. The characteristic sausage-shaped abdominal mass may be palpated between spasms of pain. Some children present atypically with lethargy but no colic; mild abdominal tenderness and mucoid rectal blood may be the clue to the cause. Hypovolemia is invariable, and vigorous fluid resuscitation with supplemental oxygen is often required. A plain abdominal radiograph will show nonspecific features of intestinal obstruction; occasionally, the outline of the intussuscepted bowel can be identified (see Figure 36-1). The diagnosis can be confirmed by ultrasonography (Figure 36-4) or, failing this, by contrast enema. Treatment is by either pneumostatic or hydrostatic reduction through a rectal Foley catheter under controlled pressure conditions by an experienced radiologist. Possible complications of this procedure include intestinal perforation and tension pneumoperitoneum. Children must be adequately resuscitated before the reduction and monitored by staff trained in advanced life support during the procedure. Enema reduction is contraindicated in children in refractive shock or with signs of peritonitis. In a minority of patients, reduction is unsuccessful, and open surgical reduction is then required. In advanced cases, the necrotic intussusception will need to be resected. Children over 2 years of age who are successfully treated nonoperatively must be investigated subsequently to exclude underlying pathology; this may include contrast radiography and colonoscopy.

Ileoileal intussusception is a rare cause of obstruction in the early postoperative period following abdominal surgery, probably the result of uncoordinated peristaltic activity. Diagnosis is difficult because the contrast enema will not show the obstructed ileum, and ultrasonography may be compromised by distended loops of bowel. Often the diagnosis is made at laparotomy for intestinal obstruction. Ileoileal intussusception can also occur in Henoch-Schönlein purpura and rarely may be the presenting sign. Such intussusceptions may spontaneously reduce, and if the child remains stable, with no signs of vascular compromise, a period of nonoperative observation may avoid the need for laparotomy.

Midgut Malrotation

Although volvulus complicating to midgut malrotation characteristically occurs in infancy (Chapter 32), duodenal malrotation or nonfixation of the cecum may allow intermittent volvulus to occur in later years. Most episodes are self-correcting and are not associated with intestinal ischemia, but acute strangulation remains a risk. Patients present with intermittent abdominal pain with or without

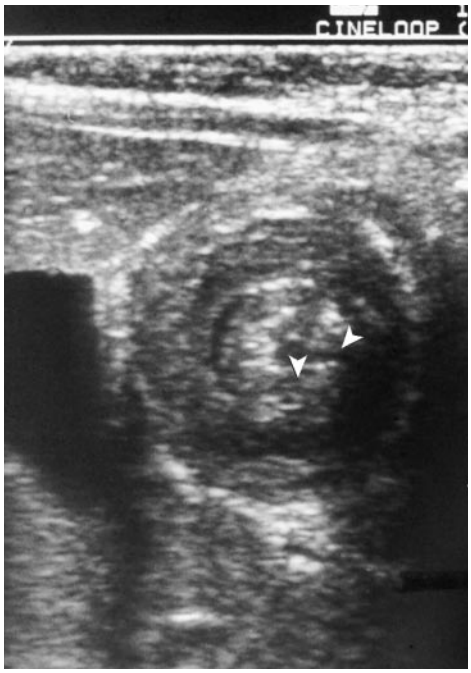

FIGURE 36-4 Intussusception: transverse sonogram showing the "doughnut sign." The arrowheads indicate the intussuscepted bowel.

vomiting and a history of chronic constipation. Upper and lower intestinal contrast studies, preferably while the patent is symptomatic, may reveal the diagnosis.

Gastroenteritis

Acute gastroenteritis may present with colicky abdominal pain. The presence of vomiting and diarrhea points to the diagnosis. Sudden cessation of stools and increasing pain raise the possibility of secondary intussusception.

Constipation

Childhood constipation, defined as the infrequent painful passage of stools, with or without soiling, is extremely common in the West as a consequence of both diet and parental and societal attitudes to toileting. It can be difficult to determine the pathologic basis of constipation in a child, and careful history taking is important. Although most cases are idiopathic, constipation may be a presenting feature of a wide range of disorders. Clinical examination should include evaluation for a pelvic mass, anorectal pathology, and neurologic deficit, including examination of the lumbar-sacral spine. When constipation first develops after the first few months of life, the incidence of Hirschsprung disease and related disorders is very low. Overinvestigation by invasive measures such as rectal biopsy may only increase parental anxiety, in addition to potentially increasing aversive behavior in the child. Typically, childhood constipation is characterized by avoidance of stooling, when the child will retain feces, which become increasingly firm and bulky, making defecation painful and provoking further retentive behavior. Fecal soiling owing to paradoxical overflow incontinence is common. Therapy should be centered on clear explanation of the nature of the problem and advice on diet, toileting, and stimulant and osmotic laxative use. Enemas are rarely required but can be of use where there is considerable fecal loading. Manual evacuation is occasionally useful in children who refuse enema treatments. The key to successful treatment of childhood constipation is a good relationship and clear communication among the clinician, the child, and the parents.

Parasitic Infestation: Ascariasis

The roundworm *Ascaris lumbricoides* is one of the most common human parasites. Infestation occurs as a result of ingesting larvae, which mature in the small intestine to reach 20 to 30 cm in length. Congregation of a large number of worms may obstruct the intestine, resulting in colicky abdominal pain mimicking intussusception (Figure 36-5). The diagnosis is suspected when there is a history of passing worms in the stool and more than one abdominal mass is palpated. The worm bolus may be visible on a plain abdominal radiograph (Figure 36-6). When in doubt, intussusception is excluded by sonogram or contrast enema. Management of *Ascaris* obstruction includes intravenous fluid replacement, with nasogastric drainage if there is significant vomiting. An intravenous antispasmodic (scopolamine butylbromide [Buscopan]) and an analgesic may be required for the colic. When the acute symptoms have abated, usually after 24 to 48 hours, an antihelminthic, for example, piperazine or mebendazole, is administered orally.[25] Dead worms may precipitate local intestinal inflammation, necrosis, and perforation, and antihelminthic therapy therefore should be avoided in the presence of acute bolus obstruction. Intestinal ascariasis may be complicated by intussusception, small bowel volvulus, or appendicitis.

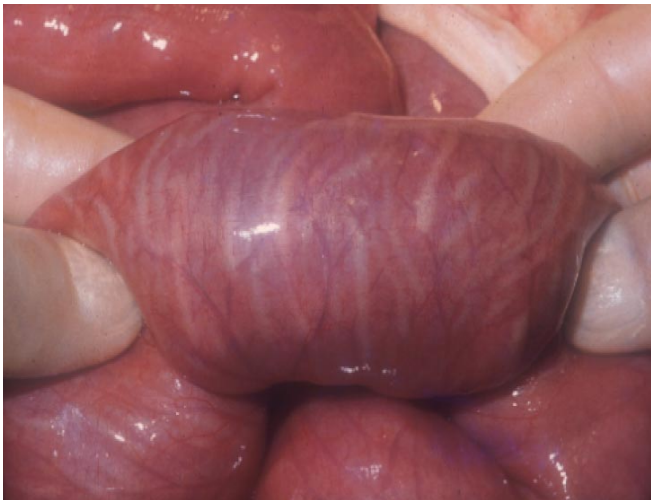

FIGURE 36-5 A bolus of *Ascaris lumbricoides* obstructing the small intestine.

Intestinal ascarids may enter the biliary tree, causing biliary colic and acute pancreatitis. Infestation of the intrahepatic ducts may lead to secondary abscess formation

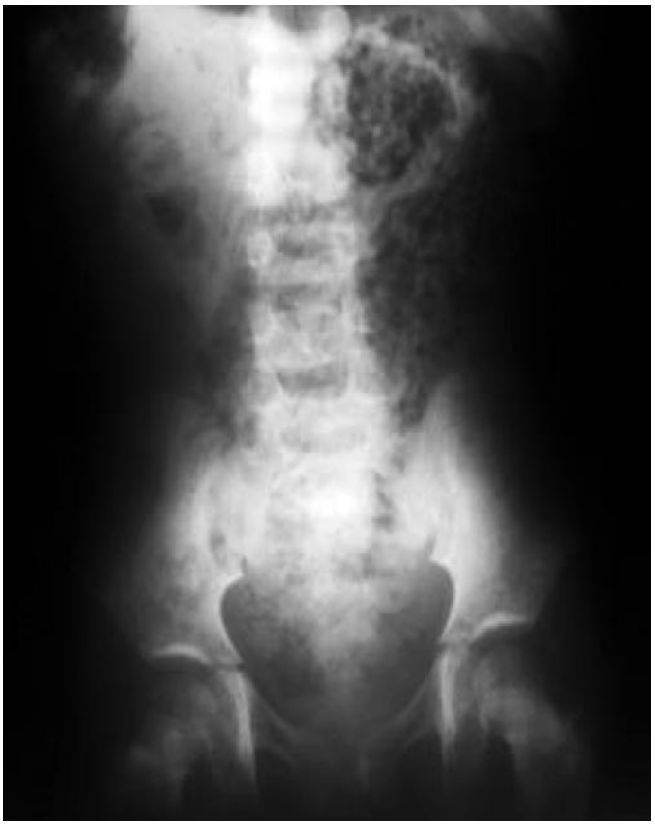

FIGURE 36-6 Plain abdominal radiograph of a child with *Ascaris* obstruction. Note the large mass of intraluminal worms.

(Figure 36-7).[26] Biliary ascariasis is initially treated with intravenous fluids, an antispasmodic, and analgesia, and, in most instances, the worm will evacuate the common duct. As soon as the symptoms have resolved, an oral antihelminthic is administered. If the biliary symptoms do not resolve within 24 to 48 hours, the worm may be removed endoscopically or by open operation. Intrahepatic worms are removed by direct exploration. Late complications include biliary and pancreatic duct strictures.

Intestinal Polyps

Intestinal polyps commonly present with colicky abdominal pain, which may be accompanied by the passage of blood in the stools. The "juvenile" polyp encountered in the rectum or colon and Peutz-Jeghers polyps are benign hamartomatous lesions; true adenomatous polyps may occur in isolation or as a manifestation of familial adenomatous polyposis. The initial investigation of choice is endoscopic biopsy or excision.

Pancreatitis

Pancreatitis is uncommon during childhood and may present as acute pancreatitis, as chronic relapsing pancreatitis, or in association with trauma. Acute pancreatitis may follow viral illness such as mumps, or in children with predisposing factors such as hyperlipidemia, cystic fibrosis, and polyarteritis nodosa. Gallstone pancreatitis is rare. Pancreatitis may complicate intestinal ascariasis. More usually, no predisposing factors can be identified. Chronic relapsing pancreatitis may lead to progressive irreversible changes in the pancreas; it is uncommon in childhood but should be suspected in the child with recurrent upper abdominal pain. There is a range of causes, including congenital pancreaticobiliary malunion.[27]

Diagnosis of acute pancreatitis is by detection of raised serum or urinary amylase levels (more than five times normal levels). Supportive treatment with intravenous fluids, analgesia, and antibiotics is often the only treatment required. Severe fulminant pancreatitis is fortunately rare and may follow severe systemic upset, such as burns or septicemia. Surgery in the form of necrosectomy is only rarely indicated and of no proven benefit in terms of outcome.

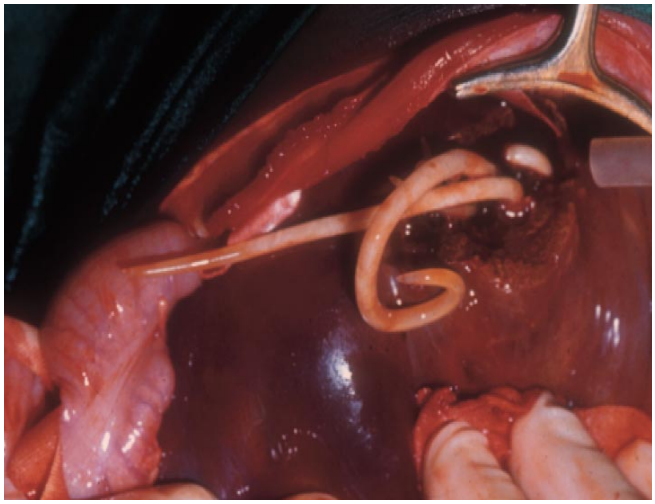

FIGURE 36-7 A nest of *Ascaris* worms being evacuated from the liver of a child with intestinal and biliary worms.

Cholecystitis

Gallstones are an uncommon cause of abdominal pain in childhood but should be considered in children with right upper quadrant pain and pyrexia. A family history of gallstones and a predisposing hemolytic condition such as sickle cell anemia or hereditary spherocytosis are important diagnostic clues. The diagnosis is also suggested by a positive Murphy sign (right upper quadrant tenderness on inspiration). Cholecystitis can be confirmed by abdominal ultrasonography, and treatment is by acute or interval cholecystectomy.

Gynecologic Causes of Acute Abdominal Pain

Adolescent girls may present with acute lower abdominal pain owing to a ruptured follicular cyst, hemorrhage into a cyst, ovarian torsion (particularly with a cyst larger than 5 cm in diameter), or premenstrual pain. Pelvic inflammatory disease may present with acute or chronic abdominal pain. When localized to the right iliac fossa, the clinical findings may mimic acute appendicitis. Ultrasonography has an important role in identifying or excluding ovarian and uterine abnormalities.[28] Urgent laparoscopy or laparotomy is indicated where acute appendicitis or ovarian torsion cannot be excluded.

Urinary Tract Infection

Urinary tract infections (UTIs) are common in children, affecting 1.5% of boys and 5% of girls by the age of 16 years.[29] UTI commonly occurs secondary to vesicoureteric reflux but may also complicate urinary tract obstruction or calculus disease, leading to suppurative pyelonephritis. Typical symptoms are pain in the lower abdomen or loin, pyrexia, and vomiting. The diagnosis is made by demonstrating bacteria on microscopy of a fresh urine specimen or pure culture of more than 10^7 bacteria/mL. The urine white cell count can be misleading because false-positives and -negatives can occur.

UTI associated with vesicoureteric reflux may lead to renal scarring and, ultimately, to hypertension or end-stage renal failure, and all children presenting with a UTI should therefore be screened with renal tract ultrasonography and technetium 99m dimercaptosuccinic acid scans regardless of age.[30] The diagnostic test for vesicoureteric reflux is a micturating cystourethrogram. Urinary tract obstruction and calculus disease are identified by ultrasonography, with specific imaging as indicated. Suppurative infection is a surgical emergency. Treatment includes oral or intravenous antibiotics and fluids and surgery as appropriate.

INFLAMMATORY CONDITIONS

Neonatal Necrotizing Enterocolitis

Necrotizing enterocolitis, a disease almost exclusively affecting premature infants, is characterized by multifocal progressive ischemic necrosis of the intestine. Predisposing factors include low birth weight, coexistent disease such as

congenital heart disease, umbilical vein catheterization, sepsis, or hypoglycemia. Necrotizing enterocolitis typically presents with the passage of blood per rectum and refusal to feed, and as the disease progresses, bilious vomiting, abdominal distention, and signs of generalized sepsis develop. Pneumatosis intestinalis and portal venous gas are the principal diagnostic radiologic features; in advanced cases, a pneumoperitoneum secondary to intestinal perforation may be seen. Most infants will respond to treatment consisting of withholding feeds, parenteral nutrition, and broad-spectrum intravenous antibiotics. Operation is indicated for complications, notably intestinal perforation or full-thickness necrosis with persistent sepsis. Percutaneous peritoneal drainage is a useful adjunct to resuscitating the low birth weight infant with perforation.[31] Subsequent surgical options will depend on the individual situation and include resection of nonviable bowel with stoma formation or primary anastomosis or stoma alone if there is extensive ischemia.

Hirschprung Enterocolitis

Hirschsprung disease may be complicated by enterocolitis, characterized by malaise, pyrexia, abdominal distention, constipation, or diarrhea. This potentially life-threatening complication can occur before or following surgery. The pathologic basis of Hirschsprung entercolitis is poorly understood and may be related to relative gut stasis, alterations of bacterial flora, and impaired mucosal or neuronal immunity. Current treatment is empiric, consisting of rectal washouts, antibiotics (vancomycin or metronidazole), probiotics,[32] and sodium cromoglycate.[33] Chemical (botulinum toxin[34] or topical glyceryl trinitrate)[35] or surgical internal sphincterotomy may be of benefit. Occasionally, it is necessary to perform an urgent colostomy.

Inflammatory Bowel Disease

Urgent operative intervention may be required for acute complications of ulcerative colitis (see Chapter 41.2, "Ulcerative Colitis") and Crohn disease (see Chapter 41.1, "Crohn Disease").

TRAUMA

Accidental injury is the most common cause of death in children over the age of 1 year. Most morbidity and mortality result from injuries to the head, but abdominal injuries account for approximately 7% of acute trauma admissions.[36]

Blunt Trauma

Falls are the most frequent cause of blunt injury, accounting for over 50% of acute trauma admissions. In Europe, motor vehicle accidents account for up to 15% of blunt injuries but are the major cause of death and disability. Motor vehicle–related trauma is also the most frequent cause of multiple injuries.

Patients with abdominal injury must be fully assessed according to Advanced Trauma Life Support guidelines.[37] The primary survey is followed by a thorough secondary survey to exclude associated injuries, particularly of the head. Surface injuries such as bruising from a seat belt or a bicycle handlebar are a clue to underlying organ injury. Management includes appropriate intravenous fluids and insertion of a nasogastric tube and urethral catheter if clinically indicated. Initial investigations include serum amylase estimation as a baseline for evolving pancreatitis and radiographs of the cervical spine, chest, and pelvis. In children, the abdomen is included with the pelvic radiograph and may reveal acute gastric distention requiring urgent decompression or pneumoperitoneum. The urine is examined for the presence of blood.

It is essential to remember that children have a considerable physiologic reserve in response to hypovolemia. Increasing heart rate, prolonged capillary return, and widening core-periphery temperature differences are early signs of significant blood loss, and changes in blood pressure and conscious state are late indicators of hypovolemic shock. Vascular access can be difficult in the child in hypovolemic shock, and an intraosseous needle may be invaluable.

Urgent laparotomy is indicated for patients who are hemodynamically unstable owing to ongoing intra-abdominal bleeding or who have evidence of peritonitis or pneumoperitoneum. For hemodynamically stable patients with clinical evidence of intra-abdominal injury, a CT scan with intravenous enhancement is the most sensitive modality for evaluating the solid intra-abdominal organs and retroperitoneum. CT is also indicated for patients with a head injury who are at high risk of abdominal injury but who cannot be evaluated clinically. Imaging is not reliable for identifying injuries to the intestine or bladder, and repeated clinical examination is essential to identify evolving peritonitis. Injuries to retroperitoneal organs, notably the duodenum and pancreas, are particularly difficult to recognize in the early phase after injury, and additional imaging may be helpful (Figures 36-8 and 36-9). Most injuries to the solid abdominal organs will heal without operative intervention. Indications for operation in stable

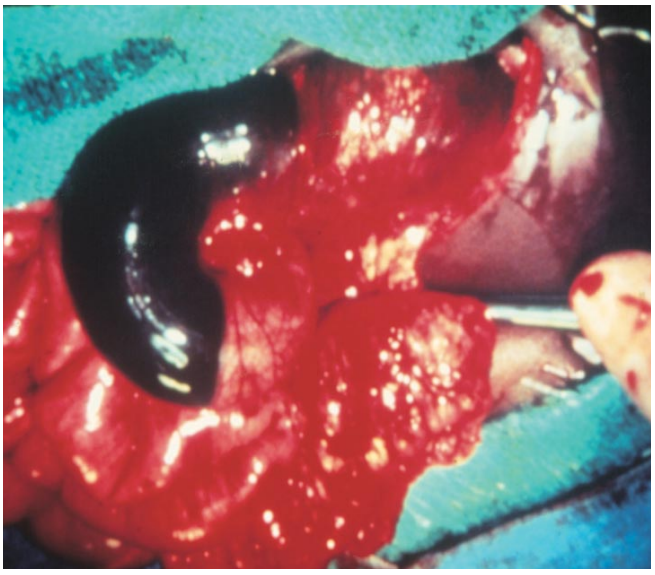

FIGURE 36-8 Operative appearance of a duodenal intramural hematoma caused by blunt abdominal trauma, in which the duodenum was crushed against the vertebral column.

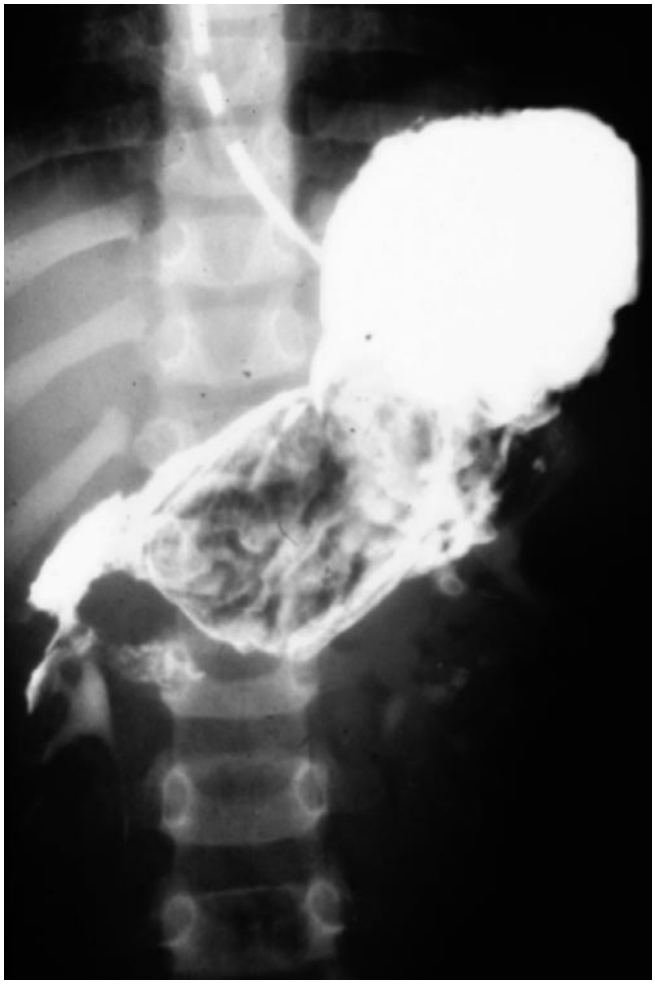

FIGURE 36-9 Contrast study in a child with traumatic duodenal hematoma showing partial obstruction.

patients include ongoing bleeding exceeding 40 mL/kg (50% of the circulatory volume), clinical evidence of peritonitis, and a rising serum amylase level indicating major pancreatic duct disruption.

Penetrating Injury

The incidence of penetrating injuries owing to stabbing or shooting varies regionally and nationally according to the availability of weapons. In European countries, penetrating injuries usually result from falling onto a sharp object such as a railing fence. In a patient with an entry wound between the level of the nipples and the symphysis pubis, the possibility of an intra-abdominal injury must be considered. Unlike blunt trauma, there is a high risk of multiple intestinal injuries. If there is suspicion that the peritoneum has been penetrated, the safest approach is a laparotomy to exclude organ injury. If laparoscopy is available, this may be used to examine the integrity of the peritoneum.

Nonaccidental (Intentional) Injury

The possibility of nonaccidental injury must be borne in mind at all times, in particular with injured children under the age of 1 year. Risk factors are a delay in seeking medical attention and inconsistencies in the explanation for the injury and the circumstances surrounding it. Careful examination is essential for bruising or other marks; these may be relevant to the current injury or may indicate previous injuries. Recognition of nonaccidental abdominal injury may be difficult because of a confusing history or subtle clinical findings, and an initial misdiagnosis may lead to delayed recognition of the intra-abdominal injuries.

A team approach is important, with early involvement of social services and other child protection experts. Thorough documentation of all injuries with photographic and physical evidence when appropriate is essential. In the case of young children with perineal injury, examination under anesthesia should be considered to minimize the trauma of examinations. Any such examination must be carried out in the presence of a child protection clinician or other expert.

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CHAPTER 37

APPENDICITIS

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Acute appendicitis, one of the most common surgical diseases in children, is also one of the most frequently misdiagnosed. The "Holy Grail" for appendicitis, that is, a simple and fail-safe test, is constantly being sought by diagnosticians but without success. More than 115 years after the description of the pathophysiology of this disease, it frequently continues to be inaccurately diagnosed and remains a continued source of considerable morbidity and occasional mortality.

Appendicitis is the most common cause of emergency abdominal operation in children. Over 250,000 cases of acute appendicitis occur each year in the United States, and almost one-third of these occur in children. Males develop appendicitis more commonly than females (incidence ratio 1.4 to1), and the peak age of appendicitis is between 10 and 14 years in boys and 15–19 years in girls. Roughly one-quarter of cases of acute appendicitis are perforated at the time of presentation. There remains a small but definite mortality associated with acute appendicitis, and this is usually related to delayed diagnosis or concomitant diseases. It is estimated that the lifetime risk of developing appendicitis is 8.6% for males and 6.7% for females.[1]

Accurate diagnosis of appendicitis may be very difficult. It is frequently underdiagnosed, which contributes in part to a high perforation rate. However, this disease is also frequently overdiagnosed. Most institutions will report a 10 to 20% incidence of normal, or "white," appendices having been removed when patients are explored for acute appendicitis. In fact, in most training programs, it is taught that if the incidence of normal appendices is less than 10%, the rate of perforation will be unacceptably high.

Reginald Fitz first described the clinical findings of acute appendicitis in 1886. Despite the many advances of modern medicine and improvements in diagnostic accuracy with tests such as ultrasonography (US) and computed tomography (CT), there is no single test to diagnose appendicitis short of pathologic examination. The diagnosis of appendicitis requires clinical acumen; the practitioner must make use of skills in obtaining a thorough history and a careful physical examination—which are more difficult to obtain in children—coupled with close observation of the tempo of the disease.

ANATOMY AND PATHOPHYSIOLOGY

The appendix, roughly the size and shape of one's fifth finger, is a diverticulum arising from the cecum. Its length and particularly its anatomic position can be quite variable, ranging from down in the pelvis to any place on the right side of the abdomen in patients with normal intestinal rotation. Patients in whom the appendix resides in a retrocecal location can present difficult diagnostic dilemmas because of the effect this may have on the presentation and location of signs and symptoms. In those who have abnormal intestinal rotation, the location may be highly variable, confusing the diagnosis even more (Figure 37-1).

Embryologically, the cecum is visible by the fifth gestational week as an enlargement of the hindgut, and the appendix begins to appear about the eighth week. Some villi are seen in the appendix during the fourth and fifth months, but these disappear prior to birth. Lymphatic nodules will be present by the seventh month. This lymph tissue continues to increase until puberty and then slowly recedes.[2] Despite much speculation based on comparative anatomy with other mammals, the function of the appendix remains unknown.

The pathophysiologic cause of acute appendicitis is thought to be obstruction of the lumen of the appendix either by fecal matter, such as a fecalith, or by swollen lymphoid tissue. This latter cause may explain why cases of appendicitis may follow soon after a viral illness. Multiplication of bacteria in the obstructed viscus leads to swelling and invasive infection of the wall of the appendix. This initially causes activation of stretch receptors in the wall of the intestine that is perceived in the tenth thoracic (T10) dermatome, the periumibilical region. As the infection proceeds, inflammatory fluid exudes from the organ. This fluid, which contains many inflammatory mediators, travels to the parietal peritoneum adjacent to the appendix, where it causes localized pain in the right lower quadrant owing to irritation of the sensitive nerves of the peritoneum. It is important to remember that the peritoneal pain is due to the irritating fluid, not necessarily to direct contact of the appendix with the peritoneal surface. This phenomenon may be helpful in diagnosing unusual cases of appendicitis in which the irritating fluid may travel some distance from the infected organ.

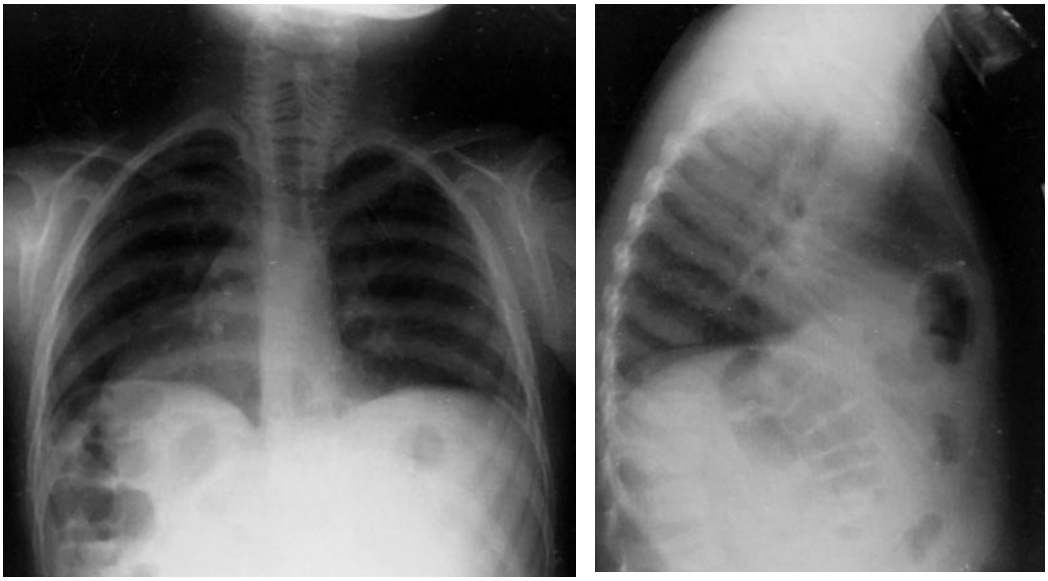

FIGURE 37-1 Posteroanterior and lateral chest radiograph from a young girl who presented with periumbilical pain that migrated to the epigastrium. She developed localized peritonitis in the epigastrium and bilious vomiting. At operation, she was found to have acute appendicitis contained within the diaphragmatic hernia of Morgagni. Note the gas-filled viscus above the diaphragm. Courtesy of R. C. Shamberger, MD.

If the inflammatory process proceeds unchecked, the appendix will usually "perforate" in about 36 hours. In children, it is estimated that 20% of cases of acute appendicitis perforate within 24 hours after the beginning of symptoms and up to 80% perforate within 48 hours. Perforation may be due to gangrene of the organ as a result of thrombosis from the invasive infection, or it may be due to direct erosion of the fecalith through the infected wall of the appendix. Alternatively, the swollen organ may begin to leak owing to high pressure from fluid and gas within the obstructed, infected lumen. If inflammatory fluid seeps throughout the abdomen, generalized peritonitis ensues. However, if the infection is confined to a local area by the body's natural defense mechanisms, such as the omentum walling off the infection, localized tenderness and a mass may be the presenting signs. When this infection is not drained in some fashion, the outcome will frequently be generalized shock and septicemia.

The bacterial flora present in acute appendicitis are those that inhabit the human colon, most of which are anaerobic. *Bacteroides fragilis*, *Escherichia coli*, *Enterococcus*, *Pseudomonas*, *Klebsiella*, and *Clostridium* species may all appear. Cultures of the peritoneum during simple appendicitis seldom yield organisms, but during the gangrenous and perforated stages of the disease, there may be a panoply of the organisms listed above.

Carcinoid tumors and parasites, particularly *Enterobius vermicularis*, or pinworm, and *Ascaris lumbricoides* may also rarely lead to obstruction of the lumen of the appendix and the development of acute appendicitis. These cases may or may not be accompanied by a history of gastrointestinal symptoms that preceded the development of the picture of appendicitis.

DIAGNOSIS

Accuracy in assessing the time of the onset of symptoms is critical in children because of the rapid tempo of the disease, that is, 36 hours to perforation from the start of the pain. The child's parents may be aware that the child awoke with pain in the night, or the child may not have eaten normally the evening before. Questioning patients and their parents about interest in the meals that immediately preceded the presentation may help pinpoint when their child first seemed unwell. Anorexia has been described as a reliable sign of appendicitis. Unfortunately, like many of the symptoms associated with appendicitis, it may or may not be present, and up to half of patients with appendicitis may say that they are hungry.

PAIN

The earliest symptom of appendicitis is usually periumbilical pain. After a few hours, the patients will frequently vomit, but the absence of vomiting does not exclude appendicitis. Many patients will progress to perforation without vomiting. Vomiting may become an important sign because it usually *follows* the periumbilical pain. In contrast, patients with gastroenteritis will vomit, but in these patients, the vomiting usually *precedes* abdominal pain. Vomiting in appendicitis is thought to be due to activation of the stretch receptors in the appendix itself; in operations done on awake, older patients under local anesthesia, traction on the bowel may induce vomiting despite complete somatic insensitivity.

After a few hours, the pain usually shifts to the right lower quadrant because of inflammatory fluid irritating the local peritoneum. This pain is stronger and overrides the

periumbilical discomfort. Thus, in the classic presentation, the pain of appendicitis starts around the umbilicus and migrates to the right lower quadrant. The pain from the inflammatory fluid is quite strong. Blood or urine in the peritoneum is less painful than inflammatory fluid or pus, whereas only bile causes consistently stronger pain than does inflammatory fluid.

McBurney first noted in 1889 that in the majority of cases, the point of maximal tenderness was localized to an area two-thirds of the way from the umbilicus to the anterior iliac spine. If the pain is due to fluid, why is this pain so localized rather than spread throughout the abdomen? The answer lies in the fact that the fluid is traveling by capillary action between the thin planes that exist among coils of intestine in the unopened abdomen, and this is commonly the point where the fluid is most concentrated. Further, the inflammatory fluid becomes gradually diluted as it moves away from the point of secretion. If the appendix lies in a retrocecal location, the peritoneal irritation may not occur, and the periumbilical pain may persist and dominate. This can go on for days and may persist even after perforation. In such cases, psoas or genitofemoral nerve irritation may be the dominant complaint, and attention is often focused on the hip or other musculoskeletal area. For this reason, retrocecal appendicitis may be very difficult to diagnose.[3]

Physical Signs

The first signs of tenderness usually appear after the pain has migrated to the right lower quadrant. This may first be mild tenderness on direct palpation. It is always wise to ask the child to localize the spot of maximal tenderness for you so that this area can be examined last (Figure 37-2). Because children are often apprehensive about examination, making the true nature of the tenderness difficult to assess, the examiner may find the stethoscope useful for "palpation" while appearing to be listening.

The most reliable diagnostic sign of acute appendicitis is localized tenderness in the right lower quadrant. As the peritoneal irritation progresses, guarding develops (voluntary stiffening of the rectus muscle), then spasm or involuntary guarding, and, finally, rebound tenderness. Stretch receptors in the peritoneum respond to the rate of stretch, not to the direction. Rebound tenderness is elicited by the examiner pressing down slowly, holding for a few seconds while the patient accommodates, and then removing the hand rapidly. If the patient suddenly winces, this is a very reliable sign of peritoneal irritation. The patient who claims the ride into the hospital caused pain with every bump in the road is describing rebound tenderness. So, too, is the patient who winces when he coughs or has pain as he jumps off the examining table. On the other hand, the patient who willingly hops up and down on one foot seldom will have appendicitis. However, we have seen patients with a gangrenous appendix walled off by omentum who performed even the hop test satisfactorily. Again, it is important for the diagnostician to remember that no single sign, symptom, or test is diagnostic of acute appendicitis aside from examining the appendix under the microscope.

The signs of peritonitis include voluntary and involuntary guarding, direct tenderness, and rebound tenderness. These signs are due only to irritation of the anterior abdominal wall. The abdomen, however, is a six-sided cavity. Each side of the cavity has physical signs unique to it. For example, retroperitoneal irritation may generate a psoas sign or an obturator sign owing to irritation of those muscles. We have seen a case of perforated appendicitis presenting with shoulder pain when the tip of the appendix perforated into the right subphrenic space. A pelvic mass or pelvic sidewall tenderness on rectal examination may be the manifestation of an inflamed or perforated appendix in the pelvis. Performing a rectal examination on a child frequently causes discomfort, making it difficult to discern true tenderness. Asking the patient to push down as if having a bowel movement and then to relax as the finger is gently inserted causes the least discomfort.

In postpubescent and especially in sexually active girls, it may be helpful to perform a pelvic examination. This should be done carefully and thoughtfully because it may be the first pelvic examination the patient has had. The patient may have localized tenderness or a mass with appendicitis. Cervical motion tenderness, however, is not usually present with appendicitis and is more suggestive of pelvic inflammatory disease.

The overall appearance of the child with abdominal pain is important in the diagnosis of appendicitis. Frequently, the child will simply "look sick"; signs and symptoms such as flushed cheeks, listlessness, low-grade fever, and unwillingness to move often accompany appendicitis even early in its course. In contrast, the child who is happy and talkative and willing to follow commands readily probably has some other cause of the abdominal pain. Fever is not a reliable sign of appendicitis. The temperature may be slightly elevated in acute appendicitis, usually by no more than one or two degrees in the child without perforation. Complicated appendicitis—perforated or gangrenous—may have high fever associated with it, however.

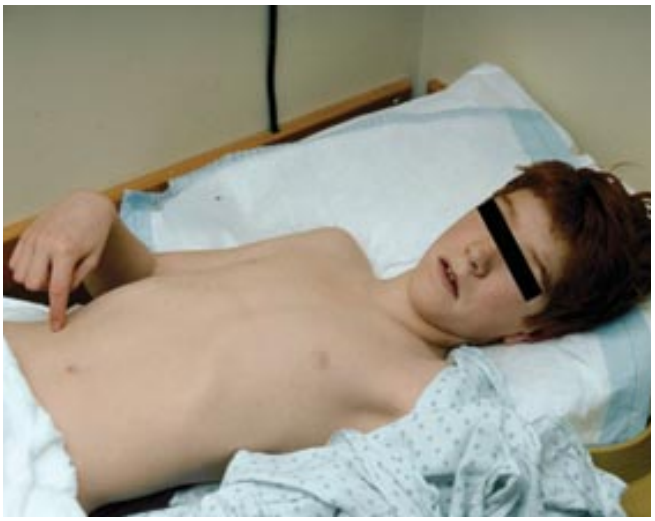

FIGURE 37-2 An 11-year-boy with appendicitis pointing to the area of maximal pain. Courtesy of Gary Williams, MD.

Laboratory Examination

Urinalysis is probably the only important laboratory test in the diagnosis of appendicitis, and it should be used to exclude urinary tract pathology. In a boy with symptoms that are not clearly appendicitis and a urinalysis with greater than 15 white blood cells (WBCs) per high-power field, urine should be sent for Gram stain and culture. If there is evidence of a urinary tract infection, the child may be treated with intravenous antibiotics for a few hours. However, this child should still be observed closely for response to therapy. A girl with greater than 30 WBCs per high-power field can be similarly treated, but only if the urine is obtained from a midstream specimen while the labia have been spread or by bladder catheterization. Urine obtained from a collecting bag applied to the patient may have many WBCs and squamous cells from the vagina and cannot be interpreted.

An elevated WBC count is the only laboratory test that has been shown to correlate with appendicitis.[4] Despite this, the value of the WBC count is limited. Most children who have been vomiting will have an elevated WBC count. Although there may be an increase in the polymorphonuclear leukocytes, the total WBC count does not usually exceed 20,000 cells/mm^3 in a patient with a nonperforated appendix. The WBC count is influenced by so many factors that it is not dependable in arriving at the diagnosis of appendicitis. It is not uncommon in a child without toxicity to see a WBC count below 5,000 cells/mm^3 when the appendix is not perforated. Such children may be recovering from a viral infection with leukopenia just prior to the onset of acute appendicitis. Indeed, such a viral episode may have led to swelling of the lymphoid tissue in the appendiceal wall and may have been the inciting factor in obstruction of the appendiceal lumen.

Experience has been reported using measurements of the C-reactive protein level. C-reactive protein is an acute-phase reactant, the concentration of which rises in whole blood within 12 hours of onset of an infection. It can be measured with a blood test. If patients have had symptoms for more than 12 hours and they have appendicitis, studies indicate that the C-reactive protein is elevated in over 85% of cases.[5] This test is not widely used because the result often takes time to obtain, but it may have benefit in excluding appendicitis in patients with symptoms of more than 1 day's duration[6]; however, this test is not specific, and any infectious process will have an elevated C-reactive protein level associated with it.

In an attempt to systematize the diagnosis of appendicitis, Alvarado used a combination of signs, symptoms, and laboratory values to develop an appendicitis score. In this score, eight variables were assessed: localized tenderness in the right lower quadrant, leukocytosis, migration of pain, WBC shift to the left, fever, nausea or vomiting, anorexia, and direct rebound tenderness. Each variable was assigned a score, and the scores were then added. In his scheme, Alvarado found excellent correlation with appendicitis in patients with higher scores.[7] Samuel applied a similar system based on signs and symptoms to children and found good statistical correlation with the presence of acute appendicitis.[8]

Radiologic Examination

Plain radiographs may be helpful if the diagnosis is in some doubt, but we do not routinely obtain films, except in infants. The most common finding on plain abdominal radiographs in the patient with appendicitis is curvature of the spine to the right. A dilated cecum containing an air-fluid level may be seen. A calcified fecalith can sometimes be seen if the films are well exposed and multiple films are obtained with the patient in different positions to portray the calcification unobscured by bony structures (Figure 37-3). Half of children with abdominal pain and a fecalith can be expected to have perforated appendicitis. When the appendix is perforated (especially in infants), there may be a paucity of gas in the right lower quadrant and an increase in the thickness of the lateral abdominal wall owing to soft tissue edema and evidence of free peritoneal fluid.[9] Use of routine plain radiographs is not recommended, however, because studies in adults have demonstrated no finding that correlated with appendicitis, and cost-benefit analysis showed that plain films have a very high cost-to-benefit ratio when used to diagnose this disease.[10]

In the confusing case, or if the diagnosis is in doubt, a chest radiograph may demonstrate a right lower lobe pneumonia that may be causing referred pain to the midabdomen.

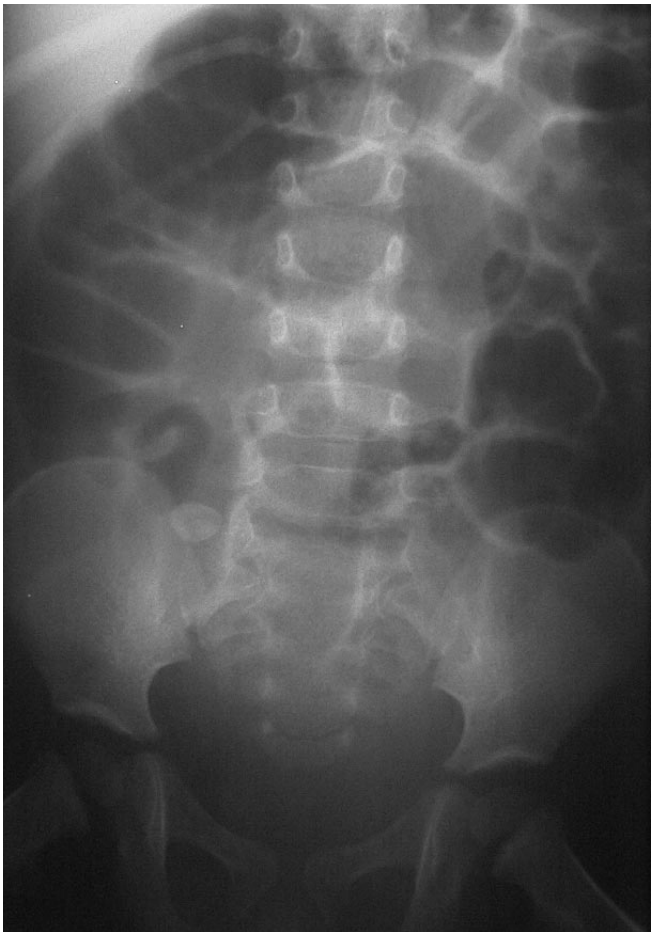

FIGURE 37-3 Plain radiograph of a teenage girl with appendicitis demonstrating a large, calcified appendicolith in the right lower quadrant. Appendicoliths are rarely this large and can be difficult to differentiate from surrounding bony structures.

A large amount of experience using US has been reported in the evaluation of abdominal pain in children, but the data provided by US are operator dependent and easily misinterpreted. The normal appendix is usually not visualized on US. However, a thickened or noncompressible appendix may be seen, as well as periappendiceal fluid, a fecalith, or even a periappendiceal abscess.[11] US should be reserved for the difficult cases because it delays surgery. However, in selected cases with an experienced ultrasonographer, this test may be of value, especially in females in whom adnexal pathology may confuse the issue. In fact, in cases in which pelvic pathology is high in the differential diagnosis, US may be the most valuable test because it is better for evaluating the adnexa than is CT. Data suggest that the use of US in evaluation of difficult cases of abdominal pain changed the treatment course and increased the level of certainty of the practitioner.[12]

The data on the utility of US are quite conflicting, however. The sensitivity and specificity of US examinations for appendicitis can be quite variable. More importantly, it is not clear that use of US affects the outcome in a population of children with appendicitis, that is, it may not lower the incidence of perforation or the cost of care.[13] This test must be factored into the overall clinical picture in deciding on operative intervention, and one must remember that a negative sonogram does not exclude appendicitis.

Use of CT in the evaluation of difficult cases of abdominal pain has also been reported extensively. The CT findings suggestive of appendicitis include appendiceal wall thickening, the presence of inflammatory changes in the periappendiceal fat, or the presence of an abscess or phlegmon (Figure 37-4). An appendicolith seen on a CT scan in a patient with right lower quadrant pain is also highly suggestive of appendicitis. The best results, meaning the highest specificity and accuracy, in children have come from using limited CT scanning with a thin-cut helical technique with rectal contrast. Using this technique, the sensitivity, specificity, and accuracy of the diagnosis were all reported to be 94%. This was much better than when the same group was examined with US.[14] Some of the US and CT signs of acute appendicitis are listed in Table 37-1. Because of the low false-negative rate with CT scanning, use of this test allows patients to be discharged home rather than admitted for observation, resulting in cost savings. However, we caution that 1 of 20 cases is still misdiagnosed, and careful follow-up of these patients is warranted, especially if they are discharged from care.[14] A follow-up telephone call the next day should reveal improvement of the patient; if this is not the case, the patient must be seen and re-evaluated.

Other radiologic tests, such as barium enema and intravenous pyelogram, are of little value these days because virtually all of the information that these might reveal, such as extrinsic compression of the cecum or ureteral obstruction, can be gleaned from CT scans. These tests should be reserved for selected chronic cases that present as diagnostic dilemmas.

Once again, however, it should be emphasized that obtaining a thorough history and performing a careful physical examination will accurately make the diagnosis of appendicitis in the majority of cases without radiographic testing. Use of any of the radiographic tests discussed above is not advocated unless the presentation is confusing or the diagnosis is in question.

Perforated Appendicitis

Immediately on perforation of the appendix, the child may have a period when he feels better owing to relief of the pressure that built up within the lumen of the appendix. Soon thereafter, the child will lie still, often with the right leg drawn up, and will become tachypneic. The vomiting pattern may change: although the child with early appendicitis may vomit once or twice or not at all, vomiting after perforation is more frequent, and the vomitus may contain small bowel contents from paralytic ileus. The child will be hot and dry, with temperatures of 101°F or higher. Signs of peritoneal irritation may be diffuse or localized, and a mass may be palpable. Peritoneal findings in the patient with perforation of a retrocecal appendix are more variable, and retroperitoneal signs such as the psoas or obturator signs may be more prevalent. Rectal examination may reveal lateralizing tenderness or a mass pushing on the rectum.

Many children with perforated appendicitis have what is described as "diarrhea," which leads to the erroneous diagnosis of gastroenteritis. Diarrhea accompanying perforated appendicitis is usually low-volume, irritative fluid from inflammation of the rectosigmoid. Peristalsis is often decreased. In contrast, gastroenteritis produces high-volume (profuse) diarrhea from the rectosigmoid, abetted by increased peristalsis.

The child who has been "sick for a week" may well have a large appendiceal abscess walled off from the peritoneal cavity. When the abscess begins to leak into the free abdominal cavity, the child shows signs of extreme toxicity, oliguria, mottling of the skin, evidence of gram-negative septicemia, and a falling platelet count. Radiographs may show signs of paralytic ileus or even partial small bowel obstruction. This is the type of patient most at risk for a disastrous outcome from appendicitis.

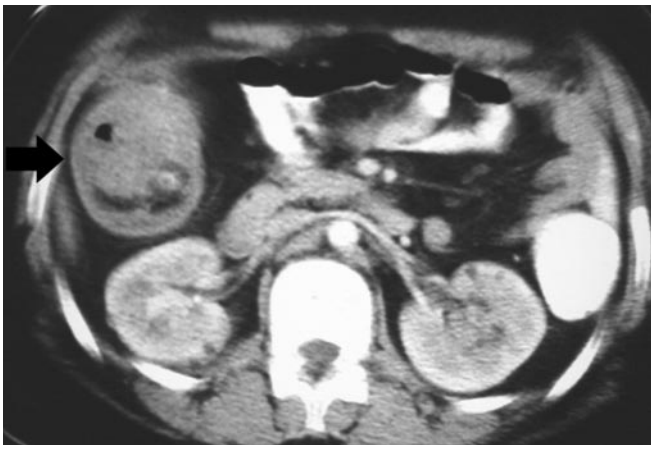

FIGURE 37-4 Computed tomographic scan showing a large periappendiceal abscess (*arrow*) containing gas. Note the contrast enhancement of the rim, which is typical with abscesses.

TABLE 37-1 ULTRASONOGRAPHY AND COMPUTED TOMOGRAPHY CRITERIA FOR APPENDICITIS

ULTRASONOGRAPHY	COMPUTED TOMOGRAPHY
Fluid-filled, noncompressible, distended tubular structure (≥ 6 mm)	Fluid-filled tubular structure measuring > 6 mm in maximum diameter
No peristalsis in appendix	Fat stranding, abscess, or phlegmon in adjacent tissue
With or without appendicolith	With or without appendicolith
Location: anterior to psoas or retrocecal	Focal cecal apical thickening
Pericecal inflammatory changes	

Adapted from Garcia-Pena BM et al [14]

Diagnostic Dilemmas

Appendicitis is a common disease with many uncommon presentations. We have seen appendicitis present as an incarcerated hernia, intermittent small bowel obstruction, and diverticulitis, just to name a few. *Appendicitis must be in the differential diagnosis of any child who presents with abdominal pain*. Children under the age of 2 years and obese patients, particularly perimenarchal girls, represent the most difficult diagnostic groups in our experience. Obesity interferes with the physical examination by making it difficult to elicit direct tenderness and guarding. Very young children are unable to give a history or tell where it hurts, and their parents often cannot pinpoint when the child began to feel ill. Although only 2% of children with appendicitis will be under the age of 2 years, over 70% of very young children with appendicitis will have perforated by the time of presentation.[15] Just as it was once said that if someone understood syphilis in all of its manifestations, he understood all of internal medicine, if one understands appendicitis in all of its presentations, one understands evaluation of the acute abdomen.

DIFFERENTIAL DIAGNOSIS

Gastroenteritis

This condition is the most common cause of abdominal pain in children presenting to emergency rooms. Retrocecal appendicitis can commonly be confused with gastroenteritis. Some of the signs that may help to distinguish appendicitis from gastroenteritis are listed in Table 37-2.[16] When there is any doubt of the diagnosis, the child should be observed closely. Gastroenteritis will usually improve gradually, whereas appendicitis will continue to get worse. It must also be remembered that the dehydrated child frequently appears quite ill and may improve dramatically simply as a result of rehydration. If improvement is seen after intravenous fluids are given, the child must still be closely examined for any signs of localized tenderness.

Of the bacterial enteritides, *Yersinia*, *Salmonella*, *Shigella*, or *Campylobacter* may present with abdominal pain. Usually, there is more than the expected amount of diarrhea, but right lower quadrant pain is common, and there may even be a shift of pain from the periumbilical region to the right lower quadrant. These patients often appear quite toxic, and leukocytosis is prominent. Cramps and high fever also are frequent, and there may be occult or frank blood in the stools, which is not usually present with appendicitis. The patient with appendicitis will rarely, if ever, thrash about in bed, but crampy, intermittent pain is frequent with gastroenteritis. If an operation is undertaken, the appendix is not inflamed. Culture of lymph nodes or stool may yield *Salmonella*. *Yersinia* may be diagnosed from stool cultures or serology. With *Campylobacter* infections, there are cramps, fever, watery diarrhea, and, frequently, blood per rectum. The diagnosis is made by culture of the organism from stool, and treatment is with oral erythromycin.

Constipation

This problem is a common cause of pain in some children, particularly in older children. The right lower quadrant pain may be intermittent, crampy, or steady, but it rarely progresses, and the pain may be perceived by the patient as quite severe. Usually, it is not possible to elicit a history of constipation. Patients will state that they have moved their bowels that day or the day before, but stool may be palpable on examination of the abdomen, or the colon may appear stool-filled on the KUB (kidney, ureter, bladder) radiograph. We see this problem most often in the heat of the summer months and in the dry winter months, when household heat is being used. At both times, chronic mild dehydration probably is a contributing factor. If there is little or no evidence of peritoneal irritation in the right lower quadrant and an abdominal film shows a colon filled with feces, it is safe to give a Fleet enema. If the symptoms disappear after a large bowel movement, the patient can be allowed to go home.

Urinary Tract Pathology

When urinary tract infection is present, the fever and leukocytosis may be increased out of proportion to the abdominal signs, in contrast to the usual signs of appendicitis. When pyelonephritis is present, there is usually flank pain and tenderness and high fever as opposed to right lower quadrant pain. If there is pyuria, the urine has been collected correctly, and the signs are not clearly consistent with appendicitis, then it is permissible to treat the patient for a few hours with intravenous antibiotics. However, if the patient does not improve rapidly, or if the diagnosis is still in doubt, we proceed with appendectomy. Crampy or intermittent severe pain in the right lower quadrant may be due to a right ureteral stone. Usually, there will be hematuria. If a stone is suspected, a CT scan without any type of contrast will be diagnostic. If this study is negative, however, and appendicitis is still in the differential diagnosis, the scan should then be repeated with gastrointestinal and oral or rectal contrast.

TABLE 37-2 DIFFERENTIAL DIAGNOSIS OF APPENDICITIS AND GASTROENTERITIS

SYMPTOM	GASTROENTERITIS	APPENDICITIS
Onset of periumbilical pain	Coexistent with or after vomiting	Before vomiting
Diarrhea	High volume, frequent	Mucus or low-volume irritative type of diarrhea, infrequent
Peristalsis	High frequency, low pitch	Low frequency or absent, high pitch if paralytic ileus
Rectal tenderness	Usually absent	Usually present
Rebound tenderness or referred rebound tenderness, such as "painful ride to hospital"	Usually absent	Often present, especially in cases of perforation

Adapted from Folkman MJ.[16]

Crohn Disease

Regional enteritis usually presents with a more protracted course than appendicitis. Often the child has had bowel symptoms for months or more, including crampy abdominal pain, diarrhea, and failure to thrive. There may be a family history of inflammatory bowel disease. Occasionally, however, the presentation will be similar to that of appendicitis, and the first discovery of the disease is during appendectomy. If this is the case at operation, the bowel, usually the terminal ileum, is thickened with mesenteric fat creeping over the bowel wall. Biopsy of an ileocolic mesenteric lymph node will often show granulomas. If the cecum is uninvolved and a secure closure of the appendiceal stump can be effected, we recommend removal of the appendix. If, on the other hand, the disease involves the region of the appendix, it is safest not to proceed with appendectomy for fear of development of an enterocutaneous fistula. Primary resection of the affected bowel should not be undertaken because early disease can frequently be treated medically, and bowel resection can be delayed or avoided.

Pelvic Inflammatory Disease

Pelvic inflammatory disease in girls over 12 years is not uncommon. The onset of abdominal pain is often preceded by the menstrual period. The pain usually begins in the lower quadrants rather than in the periumbilical area, as it does in appendicitis, and frequently accompanies the onset of menses. Pain with motion of the cervix is the hallmark of pelvic inflammatory disease, and there may be bilateral adnexal tenderness. Gram stain of the purulent cervical discharge may reveal gram-negative intracellular diplococci. The sedimentation rate is greater than 15 mm/h in the majority of cases, whereas in appendicitis, the sedimentation rate is almost always normal, that is, 1 to 10 mm/h. If the differential diagnosis is in doubt or if the signs persist after initiation of treatment, it is wise to do an appendectomy. We have operated on young adolescents with appendicitis who were originally thought to have gonorrhea because the cervical smear revealed gram-negative diplococci. Culture in these patients showed that these were only the saprophytic *Neisseria* that may be present normally in the vagina.

Ovarian Cyst

Pathology in the ovary can mimic appendicitis. The most common cause is rupture of an ovarian cyst. The pain is usually quite abrupt in onset and begins in the right lower quadrant. There is frequently tenderness. If the girl is menstruating, the pain is often midcycle, but ovarian cysts are frequent at menarche as well. US may be helpful to delineate free fluid or other cysts in the ovary, or a recently ruptured follicular cyst may be seen. If the diagnosis is in doubt, laparoscopy may help define the pathology, but this test does not rule out appendicitis unless the appendix is fully visualized. If diagnostic laparoscopy is undertaken and the appendix is found to be normal, we usually remove it in any case. US may also suggest torsion of an ovary, usually associated with a cyst. Pain and vomiting are common with this disease as well. Ovarian torsion should be treated operatively.

Pneumonia

Right lower lobe pneumonia may refer pain to the abdomen through the tenth and eleventh thoracic nerves. A chest film, the presence of abnormal respiratory signs (flaring, grunting, tachypnea), and increased toxicity should help to confirm the diagnosis. These patients often have high fever and a cough. We occasionally see a child in whom the right lower lobe infiltrate and the fever do not improve after 2 days of antibiotic treatment, and the underlying process turns out to be a localized rupture of a high retrocecal appendix with a collection of fluid beneath the right diaphragm.

Mesenteric Adenitis

This entity is due to viral infection or other inflammation of the lymph nodes clustered in the mesentery of the terminal ileum. This diagnosis tends to be a diagnosis of exclusion and should probably be made only at laparotomy or laparoscopy. US or CT may demonstrate enlarged mesenteric lymph nodes. The clinician must be careful in this case because enlarged mesenteric lymph nodes may coexist with acute appendicitis. If, at exploration, the appendix is found to be normal, Meckel diverticulum and adnexal pathology are ruled out, and enlarged lymph nodes are found, we may biopsy the node for culture and pathologic evaluation.

Typhlitis

Patients who are severely leukopenic as a result of disease or cancer chemotherapy may develop a syndrome of severe right lower quadrant pain and tenderness. This is thought to be related to invasive infection of the wall of the cecum. This usually occurs at the nadir of the leukocyte counts, and it can be confused with acute appendicitis. CT scan-

ning may reveal a thickened, irregular cecum and occasional pneumatosis coli. Operation in these patients has a prohibitive morbidity and mortality and is to be avoided if possible. Most patients will respond to bowel rest and high doses of intravenous broad-spectrum antibiotics.[17]

Cost of Diagnostic Error

The sequelae of perforation are so dangerous that if the diagnosis appears to be appendicitis, even if one is not absolutely certain, it is always preferable to remove the appendix before perforation and accept the occasional "error" of removing a normal appendix rather than waiting so long that perforation occurs. Despite improved accuracy in diagnosing this disease with modern technology, 5 to 10% of cases cannot be accurately diagnosed without an operation. Therefore, it is entirely acceptable practice for the surgeon to have occasional cases in which the appendix is found to be normal. The morbidity of a negative appendectomy should be quite small, and the alternative—a missed appendicitis leading to perforation—is quite high. If the diagnosis is doubtful, we prefer to admit the child for *repeated observation by the same physician*. Sending the child home to return the next day only ensures that a different physician who cannot accurately judge whether the signs have progressed will see him. The average length of hospital stay for acute appendicitis is 1 to 2 days, whereas the child with a perforated appendix will stay for as much as 10 days or more, incur much higher costs, and have greater potential for a complication or long-term sequelae.

TREATMENT

There are many possible ways to treat appendicitis, but, in general, appendectomy on the day of diagnosis is the treatment of choice. One exception to this rule is in the case of perforated appendicitis with a well-established abscess that can be drained. Even in a sick child who must be prepared with intravenous fluids, antibiotics, correction of electrolyte imbalances, and reduction of fever, we have always been able to perform the operation on the same day.

Operative Technique

For open appendectomy, a transverse right lower quadrant muscle-splitting incision is used, more lateral than in an adult because the rectus muscle is relatively wider in a child. It is virtually always possible to remove the appendix, even in the presence of an abscess or severe perforation with peritonitis. It is safest to mobilize the cecum so that the entire appendix lies above the abdominal wall. It may be necessary to incise the lateral cecal peritoneal attachments at the white line of Toldt to do so. The mesoappendix is divided so that the appendix is completely mobilized and free at its base. A purse-string suture is placed around the base of the appendix, and the appendix is crushed and ligated with plain catgut suture near its base. The stump of the appendix is inverted while the purse-string is tied. This will give a secure closure of the stump and prevent mucocele formation because the catgut will dissolve promptly inside the lumen of the cecum. The muscle layers are closed individually, and the skin is typically closed primarily. When removing a perforated appendix, it is important not to leave behind a fecalith that may have fallen out of a perforated appendix during the procedure. This will result in later formation of an abscess.

In cases of periappendiceal abscess, we use CT- or US-guided drainage of the abscess with delayed removal of the appendix, usually about 8 weeks later. This is safe and even preferable in cases in which there is a well-defined abscess cavity. Broad-spectrum antibiotics should be given, and the patient should respond promptly to this intervention with resolution of fever and return of bowel function. This method of treatment allows the child to defervesce quickly initially without becoming too ill and has the advantage of allowing later laparoscopic appendectomy with rapid recovery. In this treatment algorithm, the appendix should be later removed because pathologic abnormalities frequently exist, and if the appendix is not removed, these patients are at risk for recurrent bouts of appendicitis.[18]

On extremely rare occasions, the appendiceal stump is so gangrenous or the cecum so edematous that the stump cannot be inverted or closed safely (Figure 37-5). In this situation, it is wisest to do a limited ileocecal resection with an end-to-end two-layer ileo-right colic anastomosis. We try to position the anastomosis away from the abscess cavity and separate the two with omentum. Another option if the appendix cannot be inverted but the cecum is not thickened or inflamed is to resect a small portion of the cecal wall and perform a two-layer closure. In either option, it is critical that the tissue that is closed be soft, well vascularized, and without significant inflammation. Failure to close healthy tissue will result in a fecal fistula, and the patient will remain ill for a long period of time.

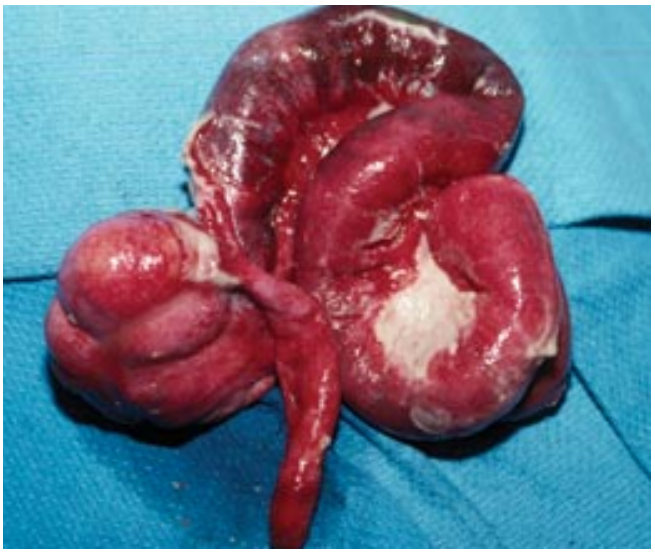

FIGURE 37-5 Gangrene of the terminal ileum secondary to perforated appendicitis with bowel obstruction. This patient was treated with ileocecectomy and primary reanastomosis. The anastomosis was placed in the right upper quadrant well away from the abscess cavity, and the patient did well.

Considerable experience has been gained in the use of laparoscopy for appendectomy. Many surgeons now prefer laparoscopy to the open technique for their patients, and the results are comparable to those with the open technique. Appendectomy through the laparoscope may offer the benefit of faster recovery to normal activity for older patients, but the hospital stay in uncomplicated cases is equal using both techniques. To date, operative costs associated with the laparoscopic approach still exceed those of open laparotomy, and these costs are not offset by a shorter length of hospital stay, as is usually the situation when laparoscopy is used for larger abdominal procedures. One situation in which laparoscopic appendectomy may offer an advantage is in the case of a patient—usually a teenage female—in whom there is a diagnostic dilemma, and laparoscopy offers a broader view of the abdomen and pelvis to search for other pathology.

Laparoscopic appendectomy can usually be accomplished using a three-trocar technique. Most surgeons divide the mesoappendix and the appendix with a linear stapling device, although a harmonic scalpel or another similar coagulating instrument may also be used for the mesentery. As opposed to an open technique in which the mesoappendix is typically divided first, with laparoscopy, it is often easier to divide the appendix before dividing the mesoappendix. It is very important to divide the appendix close to the cecum so that the entire viscus is removed. Cases of recurrent appendicitis have been reported when a length of appendiceal stump is left after laparoscopic appendectomy.[19]

Patients with nonperforated appendicitis receive a preoperative dose of intravenous antibiotic, usually a first- or second-generation cephalosporin, to cover skin flora and possibly some gram-negative organisms. This is continued for 24 hours if the operation reveals no evidence of perforation. Routine intraoperative cultures of the abdomen are of little value in such patients.[20]

Perforated Appendicitis

Optimal management of perforated appendicitis in the era of clinical outcome studies and managed care has been a source of some controversy. The management algorithm used at Boston's Children's Hospital since 1976 is outlined in Table 37-3.[21] This protocol has been consistently associated with an extremely low rate of postappendicitis complications.[22] Recently, this protocol was modified by substituting piperacillin and tazobactam (Zosyn, Lederle Laboratories, Carolina, Puerto Rico) instead of ampicillin, gentamicin, and clindamycin. This allows simplified drug dosing and avoids the potential complications associated with gentamicin. If these children are doing clinically well, they can frequently be discharged home to finish their 10-day intravenous antibiotic course. A recent prospective study of this clinical pathway again revealed an extremely low complication rate for the treatment of perforated appendicitis.[23] The duration of antibiotic therapy is also an area of debate among surgeons, but whatever the regimen used to treat perforated appendicitis, it should result in complication rates as low as possible, preferably no more than 5%.

The use of laparoscopy in patients with complicated appendicitis is also an area of considerable controversy among pediatric surgeons. Retrospective studies have shown a significantly higher incidence of postoperative intra-abdominal abscesses in children with perforated appendicitis who were treated laparoscopically when compared with children treated by the open technique.[24,25] Other surgeons, however, argue that they see no difference between the two techniques. Clearly, a large multicenter randomized trial of open versus laparoscopic appendectomy, while holding the other parameters such as antibiotic therapy constant, is needed to answer this question.

COMPLICATIONS

Infections

The incidence of infectious complications in appendicitis varies with the severity of the infection at the time of surgery. Usually, the wound infection rate with simple appendicitis is quite low. In fact, the wound infection rate associated with removal of a noninflamed appendix is often higher than with simple appendicitis. The incidence of wound infections with primary closure of the wound without drains in perforated appendicitis has been reported to be quite significant, over 10%.[26] In our experience, however, with use of a drain brought through the wound in perforated appendicitis, this rate can be lowered to about 1%. Primary wound closure with an absorbable suture is very much preferred by children because they

TABLE 37-3 PROTOCOL FOR MANAGEMENT OF PERFORATED APPENDICITIS

1. Fluid resuscitation; control fever and administration of intravenous antibiotics (ampicillin 100 mg/kg/24 h, gentamicin 5 mg/kg/24 h, and clindamycin 30 mg/kg/24 h on admission, or piperacillin/tazobactam 240 mg/kg/24 h of piperacillin component, up to 18 g/24 h).
2. Explore peritoneal cavity via right lower quadrant incision.
3. Perform appendectomy in all cases.
4. Perform limited peritoneal débridement.
5. Irrigate peritoneal cavity with cephalothin solution (4 g/L).
6. Place Penrose drains in pelvis and right pericolic space, which exit through the lateral margin of the wound.
7. Close the muscle layers, Scarpa fascia, and skin around the drains with absorbable sutures.
8. Encourage postoperative activity and position at will.
9. Continue parenteral antibiotics for 9 days, adjusting gentamicin dosage based on serum levels.
10. Remove drains slowly from the seventh to the ninth postoperative days. If the patient has been discharged for home antibiotics, he is usually seen sometime during this period in the clinic.
11. Discharge patient generally on the tenth postoperative day.

strongly dislike dressing changes, suture removal, or delayed wound closures. Removal of a small Penrose drain, on the other hand, is well tolerated.

Similarly, the incidence of abdominal and pelvic abscesses after perforated appendicitis is real—1.3% in our series[22]—and is one of the most frequent and significant complications seen. Many abdominal or pelvic abscesses subside spontaneously under antibiotic therapy and probably represent a phlegmon or cellulitis with agglutinated loops of bowel rather than a true abscess. The progress of these masses can be followed by repeat rectal examinations or CT scans. If the collection does not resolve or the child remains toxic, it is necessary to drain such an abscess. With use of US and CT, it is frequently possible to drain it percutaneously, leaving a small drain in the cavity. This can usually be done with sedation and local anesthesia.

A very small number of patients who have had very severe perforated appendicitis may develop persistent feculent drainage through their wound—a fecal fistula. Virtually all will resolve with bowel rest, antibiotics, and total parenteral nutrition. One must be sure, however, that there is no cause of obstruction distal to the fistula that is preventing its closure.

Intestinal Obstruction

Paralytic ileus may persist for 3 to 5 days following removal of a perforated appendix. Occasionally, this ileus is followed by a few days of normal intestinal function and then by mechanical obstruction with cramping pain. Most of these cases can be managed by nasogastric tube decompression until the inflammatory adhesions subside. Repeat laparotomy at this time is meddlesome and even dangerous unless there is evidence of strangulation or closed loop obstruction. In contrast, obstructions that occur more than 4 weeks postoperatively usually require prompt operation. This complication arises in 1 to 2% of patients with perforated appendicitis. Surgeons who are not experienced with children may be uncertain about the appropriate timing for re-exploration. We saw a child who had a re-exploration for intestinal obstruction 5 days after removal of a perforated appendix. No distinct point of obstruction was found at that time, but many inflammatory adhesions were present. After a number of postsurgical wound complications, the intestine began to function, but 1 month later, there was a new episode of intestinal obstruction. This was treated by a nasogastric tube, but after a few days, the child's condition deteriorated, and at operation, a loop of gangrenous bowel was found tethered by a single strong adhesive band. The initial reoperation was too early and the second too late.

Sterility

Pelvic abscess or pelvic inflammation associated with perforated appendicitis has been thought to be associated with an increased rate of infertility in female patients,[27] but the literature on this point remains quite controversial.[28] A recent historical cohort study from Sweden found no difference in the fertility rate of women who had perforated appendicitis as young girls when compared with normal controls.[29]

Antibiotic-Associated Colitis

A few patients may develop crampy diarrhea and fever after treatment for appendicitis. A stool smear should be sent to look for leukocytes and the stool checked for *Clostridium difficile*. If the titer is positive, these patients will respond to orally administered vancomycin or metronidazole.

SUMMARY

Appendicitis is most noteworthy for the difficulty it presents in diagnosis. Despite tremendous progress in medical diagnostic imaging and laboratory testing, the incidence of perforation and missed diagnosis has not changed significantly over the years. Success in diagnosing appendicitis still requires a thorough history and physical examination and a complete understanding of the tempo of the disease, as well as the anatomy and pathophysiology of the pain. Finally, the practitioner must be willing to follow the patient closely over time, with frequent, even hourly, reexamination to observe the progress of the symptoms. One must also recognize the difficulties inherent in diagnosing unusual cases of appendicitis, such as in very young children or in those cases in which the appendix is in a retrocecal location. In the end, successful management of this process can lead to the ultimate satisfaction in medicine, that is, timely and complete cure of a child with a potentially life-threatening illness.

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CHAPTER 38

INFECTIONS

1. *Bacterial Infections*

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Bacterial enteric infections exact a heavy toll on human populations, particularly among children. Despite the explosion of knowledge of the pathogenesis of enteric diseases experienced during the past decade, the number of diarrheal episodes and childhood deaths reported worldwide remains of apocalyptic dimensions.[1] The recent escalation of international terrorism is raising the risk of enteric pathogen epidemics occurring beyond the boundaries of natural endemic areas. However, bacterial genome sequencing and better understanding of the pathogenic mechanisms involved in the onset of diarrhea are finally leading to preventive interventions, such as enteric vaccines, which may have a significant impact on the magnitude of this human plague. This chapter reviews the major bacterial agents of infectious diarrhea (Table 38.1-1) and their interaction with the human host.

CHOLERA

Of all enteric pathogens, *Vibrio cholerae* is responsible for the most rapidly fatal diarrheal disease in humans.[2] Although cholera is rare in developed countries, it remains a major cause of diarrheal morbidity and mortality in many parts of the developing world.[3] However, with the occurrence of both natural (eg, earthquakes) and human-generated calamities (such as ethnic wars), the spreading of cholera infection in refugee camps, where sanitary conditions resemble those in cholera-endemic areas, represents a significant threat worldwide.

MICROBIOLOGY

Vibrio (from the Greek *comma*) *cholerae* are single, short-curved, gram-negative rods with a single long polar flagellum that confers to the microorganism the characteristic rapid linear motility that forms the basis for identification by an immobilization test.[4] Currently, 34 *Vibrio* species are recognized, a third of which are pathogenic in humans.[5] *V. cholerae* is divided into 139 serotypes on the basis of the O antigen of the cell surface polysaccharide. Work in the 1930s led to the concept that *V. cholerae* strains could be divided into two groups: those that agglutinated with antisera directed against antigens present on strains isolated from cholera patients (group O1) and other "nonagglutinating" or "noncholera" vibrios (non-O1), which were regarded primarily as nonpathogenic, environmental isolates.[5,6] Group O1 is further divided into two biotypes: classic and El Tor. The El Tor strains were first isolated in 1905 from returning Mecca pilgrims at the quarantine camp of El Tor in the Sinai Peninsula in Egypt.[7]

As more attention was paid to non-O1 vibrios, it became clear that they represented a heterogeneous group that includes 11 species, which have been associated with human illness.[8,9] Until 1993, only the O1 serotype was believed to be responsible for epidemics in humans, whereas the non-O1 group was considered responsible for sporadic cases of acute enteritis and extraintestinal infections.[10] However, a strain of *V. cholerae* non-O1 (O139 Bengal) associated with epidemic cholera appeared in southern and eastern India in October 1992 (see below).[11]

EPIDEMIOLOGY

V. cholerae O1 is transmitted by the fecal-oral route and is spread primarily through contaminated food and water. Since the original observation during the cholera epidemic in London in 1854,[12] water has been considered the main

TABLE 38.1-1 IDENTIFICATION OF BACTERIAL ENTERIC PATHOGENS IN SYMPTOMATIC PATIENTS FROM DEVELOPING AND INDUSTRIALIZED COUNTRIES (PERCENTAGE)

AGENT	INDUSTRIALIZED COUNTRIES (%)	DEVELOPING COUNTRIES (%)
Vibrio cholerae	< 1	0–3
Non-O1 *Vibrio* species	—	?
Salmonella	3–7	4–6
Shigella	1–3	5–9
Campylobacter	6–8	7–9
Yersinia	1–2	?
Escherichia coli	2–5	14–17
Clostridium difficile	?	?
Aeromonas, Plesiomonas, and *Edwardsiella*	0–2	4–5

vehicle for cholera transmission. During the outbreak in Peru, fecal contamination of public water was identified as responsible for the majority of cases.[13,14]

During the past two centuries, six pandemics spread throughout the world, starting in Asia and spreading through Europe and then to the Americas.[15] The current pandemic started in 1961 from an endemic focus in Indonesia and disseminated in southeast Asia and the Middle East, reaching Africa and Europe in 1970.[16–18] Finally, after almost a century, *V. cholerae* made its reappearance in South America in 1991, when the pandemic developed with explosive intensity in several coastal Peruvian cities.[19] Although the classic biotype of *V. cholerae* O1 caused the fifth and sixth pandemics, the seventh pandemic was caused by the El Tor biotype.

In October 1992, a new epidemic outbreak of cholera occurred in southern and eastern India[11] and spread 3 months later in Bangladesh.[20] For the first time, a non-O1 strain (named O139 Bengal) was identified as responsible for a cholera outbreak. This strain spread faster than the *V. cholerae* O1 El Tor biotype responsible for the seventh pandemic; it appeared in Thailand in April 1993[21] and as an isolated case imported from India in the United States in February 1993.[22] These observations raised the concern that the appearance of *V. cholerae* O139 may mark the beginning of the eighth cholera pandemic.[23]

Clinical Manifestations

The period of incubation of cholera ranges from a few hours to 5 days.[24–26] The vast majority of subjects infected with *V. cholerae* O1 remain asymptomatic or experience a mild disease indistinguishable from many other forms of infectious diarrhea, with a few episodes of watery stools, rare nausea or vomiting, and no significant dehydration.[27] In cholera gravis (Figure 38.1-1), the most severe form of the disease, profuse watery diarrhea and vomiting lead to massive fluid and electrolyte loss, which can occur at a rate of 1 L/h and can reach a total volume loss during illness of 100% of body weight.[28] Cholera stools are typically described as "rice water" owing to the presence of mucus in clear stools. Diarrhea is most severe during the first 48 hours of the disease, when dehydration can reach life-threatening levels, particularly in children. Diarrhea then slowly decreases, completely resolving after 4 to 6 days.[28]

Diagnosis, Treatment, and Prevention

The stools of patients affected by acute cholera contain a large number of vibrios that can be easily identified with a simple Gram stain of such stools. Direct placement of stool specimens on selective media is usually sufficient for the isolation of the microorganism. The recent spread of *V. cholerae* O139 Bengal has highlighted the need for specific tests to identify this new strain.[29]

Oral rehydration solutions are the cornerstone of cholera treatment and are typically the only necessary intervention for cholera patients. The introduction of this treatment has revolutionized the prognosis of cholera, reducing the mortality from over 50% to less than 1%.[13,30,31] If the dehydration is too severe (> 10%), the mental status of the patient is affected, or the presence of vomiting precludes the use of oral therapy, the use of intravenous solutions such as Ringer's lactate becomes the treatment of choice. The use of antibiotics for the treatment of cholera has limited indications. Antibiotics have been demonstrated to reduce the volume and duration of diarrhea by about half and to reduce the duration of *Vibrio* excretion to an average of 1 day.[32] Tetracycline (500 mg per dose four times/d) is the antibiotic most used; however, large outbreaks of tetracycline-resistant organisms have been reported.[33,34] Furazolidone (1.25 mg/kg four times/d), trimethoprim (TMP) (5 mg/kg two times/d) and sulfamethoxazole (SMX) (25 mg/kg two times/d), and erythromycin (10 mg/kg three times/d) have been suggested for children.[35]

Both killed whole-cell and live attenuated cholera vaccines have been proposed as a preventive intervention for cholera.[36] A large double-blind field trial of the killed vaccine showed 85% efficacy for a period of 4 to 6 months, dropping to 50% over 3 years of follow-up.[37] A locally produced killed vaccine in Vietnam provided 66% protection against El Tor cholera during an outbreak occurring 8 to 10 months after vaccination.[38] A genetically engineered attenuated cholera vaccine (CVD 103-HgR), obtained by deleting the active subunit of cholera toxin (see below) from a *V. cholerae* O1 classic biotype, was well tolerated when administered to volunteers. This vaccine elicited a high level of protection (82–100%) against homologous

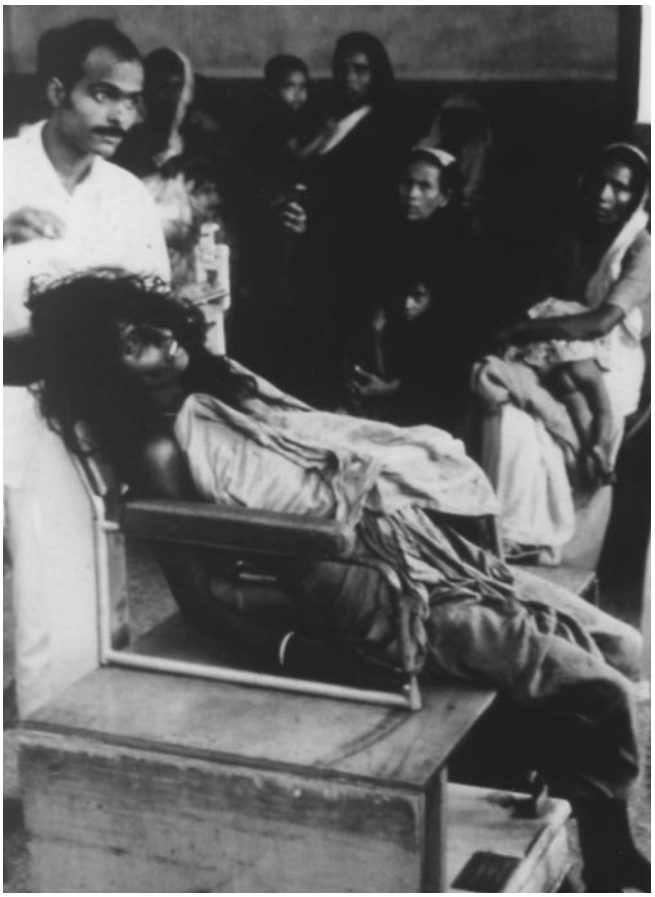

FIGURE 38.1-1 A case of cholera gravis in a young Bangladeshi woman.

challenge with a strain of the same biotype.[39] Protection across the biotype was also observed, albeit to a lesser extent,[40] lasting for at least 6 months after a single oral dose.[41] Live attenuated *V. cholerae* O139 vaccines have been developed, with promising preliminary results.[42,43]

Non-O1 Vibrio Species

As mentioned above, non-O1 *Vibrio* species (other than O139 Bengal) that infect the intestinal tract (*V. parahaemolyticus*, *V. fluvialis*, *V. mimicus*, *V. hollisae*, *V. furnissii*, and *V. vulnificus*) are responsible for sporadic cases of enteritis. These vibrios have been isolated from surface water in multiple sites in North America, Europe, Asia, and Australia, and it is likely that they are present in coastal and estuarine areas throughout the world.[44] Virtually all infections by non-O1 *V. cholerae* acquired in the United States are associated with the eating of raw or undercooked shellfish.[45] Seafood is also the main vehicle of infection for sporadic non-O1 disease outside the United States; however, the transmission can also occur through other routes, including water[46] and a variety of other foods.[47–49] Enteritis from non-O1 *V. cholerae* can range from mild illness to a profuse, watery diarrhea comparable to that seen in epidemic cholera. Diarrhea, abdominal cramps, and fever are the most common symptoms, with nausea and vomiting occurring less frequently.[45] Bloody diarrhea has been reported in 25% of cases.[45] As with *V. cholerae* O1, the mainstay of therapy for diarrheal disease is oral rehydration. In cases of septicemia (that typically occur in immunocompromised patients), supportive care and correction of shock are essential interventions associated with antibiotic treatment (tetracycline). In countries such as the United States, non-O1 infections can be prevented by not eating raw or undercooked seafood, particularly during the warm summer months.

SALMONELLA

For more than a century, *Salmonella* has fascinated physicians, microbiologists, epidemiologists, and geneticists by virtue of its diversity and success in nature. Nontyphi salmonellae are widely dispersed in animal hosts, including the intestinal tracts of both domestic and wild mammals, as well as reptiles, birds, and insects.[50] They are effective commensals and pathogens that cause a spectrum of diseases in humans and animals.

Microbiology

Salmonella is a genus of the family of Enterobacteriaceae. These microorganisms are gram-negative, motile bacilli that can be identified on selective media because they do not ferment lactose. Based on deoxyribonucleic acid (DNA) homology and host range, *Salmonella* isolates are currently classified into two species: *S. bongor*, which includes salmonellae that infect nonhuman organisms, and *S. enterica*, which is divided into six subspecies. Most human pathogens belong to *S. enterica*, subspecies *enterica*. On the basis of somatic O-oligosaccharide cell wall antigens and flagellar H-protein antigens, over 2,300 serovars have been identified. The most commonly reported human serovar in the United States is *S. enteritidis* (formally designated *S. enterica* subspecies *enterica*, serovar enteritidis), which recently surpassed *S. typhimurium*. *S. typhi* and *S. paratyphi* A, B, and C also belong to species *S. enterica*, subspecies enterica. For clarity, only the serovar names will be used in the discussion that follows.

Epidemiology

S. *typhi* and S. *paratyphi*. *S. typhi* and *S. paratyphi* colonize only humans; therefore, disease can be acquired only through close contact with a person who has had typhoid fever or is a chronic carrier. Often acquisition of the organism occurs through the ingestion of water or food contaminated with human excrement. Typhoid fever continues to represent a global health problem, with an estimated 12.5 million cases occurring per year (excluding China) and an annual incidence of 0.5% of the world population.[51] Certain subequatorial countries report high typhoid fever mortality rates (12–32%) despite antibiotic treatment.[51] In these areas, typhoid fever is often endemic and typically constitutes the most important enteric disease problem among school-age children. In the United States, substantial progress has been made in the eradication of *S. typhi*. The incidence of typhoid fever decreased from 1 case per 100,000 in 1955 to 0.2 case per 100,000 in 1966 and has remained fairly stable since then.[52] These changes were clearly related to better sanitation, particularly to food-handling practices and water treatment.

Nontyphoidal S*almonella*. In contrast to *S. typhi*, the incidence of cases of nontyphoidal *Salmonella* infections reported to the Centers for Disease Control and Prevention (CDC) increased between 1970 and 1987 from 12 to 20 per 100,000 population.[53] Because only an estimated 1 to 5% of cases are reported, it is likely that the true incidence is much higher. The incidence is greatest among children younger than 5 years of age (61.8 per 100,000), with a peak at under 1 year of age. Risk of infection and severity of disease are influenced by numerous host factors, including congenital and acquired immunodeficiency,[54,55] age younger than 3 months,[56] and impaired reticuloendothelial function, as is seen in patients with hemolytic anemia. Other risk factors include alterations in intestinal defenses such as achlorhydria, antacid therapy, and in situations in which there is rapid gastric emptying (neonates, postgastrectomy, and gastroenterostomy).[57,58] Ingestion of antibiotics to which the organism was resistant was the most important risk factor identified in an Illinois outbreak that was traced to milk, presumably because of a diminished competition of *Salmonella* growth by endogenous flora.[59] A wide range of domestic and wild animals, including poultry, swine, cattle, rodents, and reptiles, represents the typical reservoirs for nontyphoidal salmonellae.

S. enteriditis is the leading reported cause of foodborne disease outbreaks in the United States.[60] Intact and disinfected grade A eggs and egg-containing foods have been incriminated in over 80% of outbreaks with an identified vehicle. The potential role of cross-contamination is exem-

plified in several outbreaks in which the pulp of surface-contaminated raw fruits and vegetables became inoculated during slicing.[61] Person-to-person transmission, including vertical transmission from mother to child (resulting in neonatal hematochezia),[62] is occasionally seen. Pets (chicks, ducklings, reptiles, cats, and dogs) can also be a source of *Salmonella* infection.[63] Approximately 80% of *Salmonella* isolates reported in the United States appear to be unrelated to outbreaks. [60,61]

Clinical Manifestations

Enteritis. The incubation period ranges between 6 hours and 10 days (usually 6–48 hours).[64] The typical clinical manifestation of nontyphoidal *Salmonella* infection is an acute, self-limited enterocolitis sometimes accompanied by bacteremia. Diarrhea is usually watery but may contain blood, mucus, and fecal leukocytes. Associated headache, abdominal pain, and vomiting may occur. Fever is present in at least 70% of cases.[65] Most patients recover in about 1 week, but diarrhea occasionally becomes persistent.[65] *Salmonella* is usually detected in the stool for about 5 weeks, although approximately 5% of patients will excrete the organism for more than 1 year.[66] The reported incidence of *Salmonella* bacteremia is highest during the first year of life, with a peak during the first 3 months.[67] Estimates of the frequency of bacteremia in infants with *Salmonella* enterocolitis (generally derived from studies of small samples of children) range from 5 to 45%.[68] In the normal host, the bacteremia is transient and usually benign.

Extraintestinal Manifestations. Severe extraintestinal infections occasionally occur, mainly in young infants or in patients with impaired immunity. These infections manifest as life-threatening sepsis or focal infections at virtually any site in the body, particularly the meninges, bones, and lungs,[69] or in areas of localized tissue pathology or anatomic abnormality. *Salmonella* is the most common cause of osteomyelitis in patients with sickle cell anemia.[70] Meningitis is associated with high mortality and neurologic sequelae, even with prolonged antibiotic therapy,[71,72] and a high relapse rate, particularly in neonates.[73] Prolonged diarrhea, weight loss, persistent or recurrent bacteremia, and disseminated infection can develop in human immunodeficiency virus (HIV)-infected patients.[74]

Enteric Fever. Human typhoid and paratyphoid fever are severe systemic illnesses characterized by fever and intestinal symptoms. Case-fatality rates range from less than 1% in the United States to 10 to 30% in Africa and Asia.[67,70] The incubation period of *S. typhi* varies between 5 and 21 days (depending on the inoculum ingested) and may be followed by enterocolitis with diarrhea lasting several days; these symptoms typically resolve before the onset of fever. Constipation is present in 10 to 38% of patients.[75] Nonspecific symptoms such as chills, headache, cough, weakness, and muscle pain are frequent prodromes of typhoid fever. Neuropsychiatric manifestations, including psychosis and confusion (the so-called coma vigil), occur in 5 to 10% of patients with typhoid fever.[76] Approximately 30% of patients experience rose spots on the trunk.[77] Most symptoms resolve by the fourth week of infection without antimicrobial treatment in approximately 90% of patients who survive. Some patients improve initially only to develop high fever and increasing abdominal pain from inflammation of Peyer patches and intestinal microperforation, followed by secondary bacteremia with normal enteric flora.

Diagnosis, Treatment, and Prevention

Nontyphoidal Salmonellosis. The diagnosis of nontyphoidal salmonellosis does not represent a major challenge because the microorganism can easily be isolated from freshly passed stools or blood culture. Antimicrobial therapy is not indicated to treat asymptomatic carriage or uncomplicated nontyphoidal *Salmonella* infections in the normal host. There is considerable evidence that antibiotics neither speed resolution of clinical symptoms nor eliminate fecal excretion; conversely, treatment may prolong excretion or induce relapse.[78] These observations apply to both oral and parenteral antibiotics. Although efficacy is unproven, it is common clinical practice to administer antibiotics to patients with suspected or proven salmonellosis who are at high risk of complications. This includes infants younger than 3 months; patients with hemolytic anemia, malignancy, immunodeficiency, or chronic colitis; and patients who appear "ill" or "toxic," have documented bacteremia, or have an extraintestinal focus of infection. Increasing resistance to commonly used antibiotics is seen in the United States and elsewhere, so the choice of regimens should be guided by susceptibility data. Suggested therapies include trimethoprim-sulfamethoxazole (TMP-SMX), ampicillin (10–20% of isolates in the United States are resistant[78]), cefotaxime, ceftriaxone, or chloramphenicol. Parenteral antibiotics should be considered for infants younger than 3 months, for children at high risk for invasive infection if they have suspected or proven sepsis, and for those who appear "ill" or "toxic" or have a focal infection. Bacteremia is generally treated for 2 weeks, osteomyelitis for 4 to 6 weeks, and meningitis for 4 weeks.

Hygienic practices for preventing foodborne transmission are the most efficient prevention for nontyphoidal *Salmonella* infections because the vast majority of outbreaks and sporadic cases result from culinary practices that allow the organisms to survive and multiply in food. Parents should be instructed to avoid serving food containing raw or undercooked eggs and meat (especially poultry). Food should be thawed in the refrigerator, in the microwave, or under cold water but not at room temperature because surface bacteria begin to multiply when the outer layers warm. Eggs should be cooked until both the yolk and white are firm, and meats must reach an internal temperature of at least 74°C (165°F). Frequent hand washing is important. High-risk pets (especially chicks, ducklings, and reptiles) are not advisable for young children.

An extremely problematic situation is the management of an infected child who is attending day care. Excretion can go on for weeks and create a hardship to working parents if the

child must be excluded from day care. Although the decision to admit such a child must be made in concert with day care and public health officials, it is generally recommended that the infected children be excluded from day care if they are symptomatic or if adequate hygiene cannot be ensured. There is no vaccine to prevent nontyphoidal salmonellosis.

Enteric Fever. The definitive diagnosis of enteric fever requires the isolation of *S. typhi* or *S. paratyphi* from the patient. Cultures of blood, stool, urine, rose spots, bone marrow, and gastric and enteric secretions may all be useful in establishing the diagnosis. Chloramphenicol has been the treatment of choice since its introduction, given its low costs and high efficiency after oral administration. Treatment with chloramphenicol reduced typhoid fever mortality from approximately 20 to 1% and reduced the duration of the fever from 14 to 28 days to 3 to 5 days.[79]

The most effective attenuated vaccine for typhoid fever currently available, Ty21a, has proved to be free of adverse reactions in large-scale efficacy field trials involving almost 600,000 pediatric subjects.[80] When administered as a liquid suspension, Ty21a protected both young (82% vaccine efficacy) and older children (69% vaccine efficacy).[80] Currently, three new-generation attenuated vaccines, genetically engineered by deleting different pathogenic factors, are undergoing extensive phase II or III trials.

SHIGELLA

Shigella dysenteriae type 1 was first isolated by Kiyoshi Shiga during a severe dysentery epidemic in Japan in 1896, when more than 90,000 cases were described with a mortality rate approaching 30%.[81] Over the subsequent 50 years, the microbiology and epidemiology of *Shigella* species were clarified, and the mechanisms whereby the microorganism causes disease have been intensively investigated.

Microbiology

Shigellae are gram-negative, non–lactose-fermenting, nonmotile bacilli of the family Enterobacteriaceae. They are classified into four species: *S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei*, also designated groups A, B, C, and D, respectively. Groups A, B, and C contain multiple serotypes, whereas group D contains only a single serotype. The predominant serogroup of *Shigella* circulating in a community appears to be related to the level of development. *S. sonnei* is the main type found in industrialized countries, whereas *S. flexneri*, followed by *S. dysenteriae*, predominates in less developed countries.

Epidemiology

Humans are the only natural hosts for *Shigella*, and transmission is predominantly by fecal-oral contact. The low infectious inoculum (as few as 10 organisms)[82] renders *Shigella* highly contagious. Symptomatic persons with diarrhea are primarily responsible for transmission. Less commonly, transmission is related to contaminated food and water; however, the organism generally survives poorly in the environment. In certain settings where the disposal of human feces is inadequate, houseflies can serve as a mechanical vector for transmission.[83] According to a CDC report, isolation rates of *Shigella* (mostly *S. sonnei*) in the United States have gradually risen since the 1960s from 5.4 to more than 10 isolations per 100,000 population.[84] Endemic foci persist, primarily among indigent persons living in inner cities and in some Native American communities.[84] An elevated risk of shigellosis is also present in settings where hygiene is difficult to maintain, such as day-care centers,[85] in which attending children play an important role in disseminating shigellosis to others in the community.[86] In households with small children, transmission rates can exceed 50%.[87] Worldwide, the incidence of shigellosis is highest among children 1 to 4 years old, a trend also reflected in CDC surveillance data.[84] Nonetheless, *Shigella* infection is uncommon in the United States and accounts for fewer than 5% of episodes of diarrhea among children younger than 5 years of age.[88]

In developing countries, *Shigella* infections, most commonly caused by *S. flexneri*, are mainly endemics. In this setting, endemic shigellosis causes approximately 10% of all diarrheal episodes among children younger than 5 years of age.[89] The Institute of Medicine estimates that *Shigella* causes 250 million cases of diarrhea and 650,000 deaths each year worldwide, mostly in developing countries.[90] One serotype of *Shigella*, *S. dysenteriae* type 1, is capable of true pandemic transmission. Pandemics of Shiga dysentery have spread across Central America, Bangladesh, South Asia, and Central and East Africa during the past 30 years[91,92] and have been particularly problematic among refugee populations.[92] In the United States, *S. dysenteriae* infection is seen exclusively among travelers returning from abroad.

Clinical Manifestations

After an incubation period of 1 to 4 days, shigellosis usually begins with systemic symptoms, including fever, headache, malaise, anorexia, and occasional vomiting. Watery diarrhea typically precedes dysentery[93] and is often the sole clinical manifestation of mild infection.[94] Progression to frank dysentery may occur within hours to days, with frequent small stools containing blood and mucus accompanied by lower abdominal cramps and rectal tenesmus. Patients with severe infection may pass more than 20 dysenteric stools in 1 day. A variety of unusual extraintestinal manifestations may occur.[95] The microangiopathic hemolytic anemia that can complicate infection with organisms that produce Shiga toxin (see "Pathogenesis") manifests itself as hemolytic uremic syndrome (HUS) in children and as thrombotic thrombocytopenic purpura in adults.[96]

Most episodes of shigellosis in otherwise healthy individuals are self-limited and resolve within 5 to 7 days without sequelae. Acute, life-threatening complications are most often seen in malnourished infants and young children living in developing countries. In the United States, *Shigella* bacteremia has been reported among HIV-infected and other immunocompromised patients.[97]

Diagnosis, Treatment, and Prevention

Shigella are extremely fastidious to culture and readily die off if the stool sample is not well handled. The best way to isolate the organism is to (1) obtain stool (and not rectal

swab), (2) rapidly inoculate the specimens onto selective culture plates, preferably at the bedside, and (3) quickly incubate them at 37°C.

Many controlled clinical trials demonstrate that appropriate antibiotics decrease the duration of fever, diarrhea, intestinal protein loss, and pathogen excretion in shigellosis. Most patients in these studies were infected with either *S. flexneri* or *S. dysenteriae*. The advantages of treating *S. sonnei*, which is usually self-limited, are less clear. Susceptible strains can be treated with ampicillin (but not amoxicillin) or TMP-SMX. With the exception of severely ill patients, therapy can be administered orally. However, since the mid-1980s, strains of *S. dysenteriae*, *S. flexneri*, and *S. sonnei* that are resistant to one or both drugs have been identified with increasing frequency in Asia, Africa, and North America,[98] dictating a more cautious approach to empiric therapy. For infections acquired in the United States and for which susceptibility is unknown, TMP-SMX is given empirically for 5 days unless resistance is suspected or proved. Fewer than 5% of domestically acquired isolates are resistant to TMP-SMX, whereas about 10% are resistant to ampicillin.[98]

Interruption of transmission by individual hygienic behavior, such as hand washing, is an effective way to control and prevent endemic transmission. One intensely pursued strategy for constructing modern attenuated oral vaccine candidates involves the generation of defined deletions in virulence wild-type *Shigella* genes or genes that affect the ability to survive or proliferate in vivo. Several promising deletion mutants have entered clinical trials.

CAMPYLOBACTER

It is surprising that *Campylobacter* enteritis, the most common bacterial form of acute infective diarrheal disease in developed countries, was not recognized until the mid-1970s.[99] Why *Campylobacter* has been overlooked by microbiologists remains a matter for debate, but the too rigid methods of cultures and the failure to pick up ideas from the field of veterinary microbiology certainly played a role.

Microbiology

Organisms of the family Campylobacteriaceae are small, nonsporing, spiral-shaped gram-negative bacteria that exhibit rapid darting motility by means of a single flagellum at one or both ends. *Campylobacter* are largely microaerophilic, that is, they tolerate only low oxygen concentrations (5–10%). Molecular techniques have shown that *Campylobacter* (13 species pathogenic for humans), *Helicobacter*, *Arcobacter*, and *Wolinella* belong to a distinct phylogenetic group far removed from other gram-negative bacteria.[99] *C. jejuni* is by far the most common species isolated from patients with diarrhea in most areas (80–90 percent of infections), followed by *C. coli*.[100]

Epidemiology

Campylobacter enjoys a widespread reservoir in the intestines of both wild and domestic animals.[101] Case-control studies indicate that the vehicle for at least half of all endemic cases is poultry,[102] whereas common-source outbreaks are usually linked to consumption of unpasteurized milk[103] or contaminated water.[104] Up to 75% of raw poultry (but less than 5% of pork and beef) on sale in the United States is contaminated with *Campylobacter*.[105] As a result of its extensive animal reservoir, virtually all surface waters are contaminated with campylobacters, even in remote regions.[106]

Although they share many epidemiologic features, there are important differences between *Campylobacter* and *Salmonella*. *Salmonella* is more likely to infect animals in large-scale husbandry operations and has thus become an important problem in industrialized countries. In contrast, *Campylobacter* spp live naturally as commensals in a wide variety of animals and cause human infections globally.[107] *Campylobacter* does not multiply in food to high concentrations like *Salmonella* does; however, the inoculum required to cause infection is lower.[108] This may explain why, unlike *Salmonella*, *Campylobacter* rarely causes explosive foodborne outbreaks.

The annual incidence of *Campylobacter* infection in the United States is about 1%, making it the most frequently identified bacterial cause of diarrhea.[109] In industrialized countries with temperate climates, the peak incidence of infection occurs during the summer, and infections are more common in rural communities.[110] There is a bimodal age-specific incidence, with a principal climax during 0 to 5 years of age (highest, < 12 months) and a secondary rise among young adults 15 to 29 years of age.[111] In less developed countries, infection is hyperendemic, found in 8 to 45% of cases of diarrhea and in an equal number of asymptomatic controls during the first 5 years of life.[107]

Clinical Manifestations

After an incubation period of 3 to 6 days, *Campylobacter* enteritis begins abruptly with abdominal cramps and diarrhea.[111] Watery diarrhea often precedes the onset of blood-containing stools and is often the sole manifestation, especially among children from developing countries. However, abdominal pain may be so intense as to mimic appendicitis.[112] Diarrhea usually lasts 4 to 5 days, but in some patients, abdominal discomfort persists and brief relapses of diarrhea occur. The mean duration of fecal excretion is about 1 month in the normal host,[113] but carriage may be prolonged in patients with immunodeficiency. Neonates frequently experience milder illness, often with hematochezia in the absence of fever and diarrhea. However, severe or systemic illness may occur; *C. fetus* causes most cases of neonatal *Campylobacter* meningitis.[114]

Diagnosis, Treatment, and Prevention

A definitive diagnosis of *Campylobacter* infection can be made only by identifying the microorganism in a patient's stools. Properly taken rectal swabs are also satisfactory. The indication of antibiotic therapy for *Campylobacter* remains controversial. In some studies, early treatment shortened the course of diarrhea,[115] whereas in other studies, no clear clinical benefit was observed.[116] It is advisable to reserve antibiotics for patients with severe illness ongoing at presentation (either dysentery or suspected *Campylobacter*

infection on the basis of specific clinical or epidemiologic evidence) or if risk factors (pregnancy, systemic infection, immunosuppression) are present. Erythromycin remains the drug of choice for *Campylobacter* enteritis.[109]

Campylobacter vaccine development has proceeded cautiously because of concerns about postexposure arthritis or Guillain-Barré syndrome. The most developed approach is to orally administer killed *Campylobacter* cells. A monovalent, formalin-inactivated *C. jejuni* whole-cell vaccine with a mucosal adjuvant has entered human trial.[117]

YERSINIA

Like *Escherichia coli* and *Salmonella*, *Yersinia* is a heterogeneous species; however, only a few pathogenic serotypes commonly cause disease in humans.

Microbiology

The genus *Yersinia*, of the family Enterobacteriaceae, contains two important human enteropathogens: *Y. enterocolitica* and *Y. pseudotuberculosis*. *Y. enterocolitica* is divided into six biotypes and more than 50 O-antigen serotypes, whereas *Y. pseudotuberculosis* contains six serotypes with four subtypes. Several other *Yersinia* species, including *Y. bercovieri*, *Y. mollaretii*, *Y. intermedia*, and *Y. rodhey*, are widespread in the environment but are rarely human pathogens. These microorganisms are non–lactose-fermenting gram-negative aerobic and facultatively anaerobic bacilli that grow better at 25°C than at 37°C.

Epidemiology

Yersinia spp are distributed widely in the environment, with swine serving as the major reservoir for human pathogenic strains. Foodborne transmission is the suspected route for most infections, but the source is usually not identified.[118] The high infectious inoculum makes person-to-person transmission by fecal-oral spread an improbable event.[119] Most episodes of *Yersinia* enteritis occur in infants and young children.[120] *Yersinia*'s preference for cool temperatures makes this pathogen more common in regions in northern latitudes, such as in northern Europe, Scandinavia, Canada, the United States, and Japan, where it is responsible for 1 to 8% of sporadic diarrhea episodes.[121]

Clinical Manifestations

The incubation period is estimated to be 3 to 7 days. *Yersinia* enterocolitis occurs most often in children younger than 5 years of age and is characterized by watery diarrhea, usually with fever and abdominal pain.[122] The stools contain blood in 25 to 30% of patients. There may be vomiting, and approximately 20% of subjects exhibit pharyngitis that can be exudative and associated with cervical adenitis.[123] The organism can frequently be isolated from the pharyngeal exudate. Diarrhea typically lasts for 14 to 22 days, but fecal excretion may persist for 6 to 7 weeks or longer.[122] Abdominal complications may include appendicitis, diffuse ulceration of the intestine and colon, intestinal perforation, peritonitis, ileocecal intussusception, toxic megacolon, cholangitis, and mesenteric venous thrombosis.[121] The pseudoappendicitis syndrome occurs primarily in older patients and adults.[124] These patients typically present with fever and abdominal pain, with tenderness localized to the right lower quadrant, with or without diarrhea. Computed tomography may be helpful in distinguishing true appendicitis from *Yersinia* infection.[125] Case-fatality rates may reach 50%. Bacteremic spread may result in abscess formation and granulomatous lesions in the liver, spleen, lungs, kidneys, and bone and may also result in mycotic aneurysm, meningitis, and septic arthritis.[121] As with the other bacterial enteropathogens, *Y. enterocolitica* infection is associated with immunopathologic sequelae, including reactive arthritis, uveitis, Reiter syndrome, and erythema nodosum.[121]

Diagnosis, Treatment, and Prevention

Y. enterocolitica may be isolated from stool on commonly used selective media and appears as gram-negative colonies after 48 hours of growth at 25° to 28°C. Detection of the microorganism in stool by polymerase chain reaction (PCR) methodology may represent a valid future alternative.

Like *Campylobacter*, most uncomplicated cases of *Yersinia* gastroenteritis and pseudoappendicitis resolve without treatment. Therapy is reserved for patients with severe or extraintestinal infections and for immunocompromised individuals. Production of β-lactamases by *Y. enterocolitica* generally renders all but third-generation cephalosporins, aztreonam, and imipenem ineffective.[126] Broad-spectrum cephalosporins, often in combination with aminoglycosides, resulted in a good clinical outcome in 85% of cases of sepsis in one retrospective review.[126] The duration of therapy is generally 2 to 6 weeks, with an initial intravenous antibiotic followed by an oral agent to which the clinical isolate is sensitive. No enteric vaccines against *Y. enterocolitica* are currently available.

ESCHERICHIA COLI

An extremely heterogeneous group of microorganisms, *E. coli* encompasses almost all features of possible interactions between intestinal microflora and the host, ranging from a role of mere harmless presence to that of a highly pathogenic organism. In fact, the *E. coli* species is made up of many strains that profoundly differ from each other in terms of biologic characteristics and virulence properties.[127]

E. coli are gram-negative, lactose-fermenting motile bacilli of the family Enterobacteriaceae. Currently, 171 somatic (O) and 56 flagellar (H) antigens are recognized. Six distinct categories of *E. coli* are currently considered enteric pathogens (based on either outbreak data or volunteer studies) (see Table 38.1-1): enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC), diffusely adherent *E. coli* (DAEC), and enteroaggregative *E. coli* (EAggEC).

The diagnosis of diarrheagenic *E. coli* relies on isolation from stool and subsequent differentiation from commensal *E. coli* either by using genetic probes or by phenotypic assays. With the exception of *E. coli* O157:H7, assays for detection are not routinely available in clinical laboratories.

Enteropathogenic E. coli

This was the first group of *E. coli* species shown to be pathogens for humans and has been responsible for devastating outbreaks of nosocomial neonatal diarrhea and infant diarrhea in virtually every corner of the globe. Species of EPEC are distinguished from other *E. coli* species by their ability to induce a characteristic attaching and effacing lesion in the small intestinal enterocytes and by their inability to produce Shiga toxins.

Epidemiology. Between the 1940s and the 1960s, EPEC was associated with infant diarrhea in summertime and nursery outbreaks of diarrhea in the United States and other industrialized countries. Since then, it has become extremely uncommon in industrialized countries, although it is occasionally reported in child care settings.[128] However, EPEC persists as an important cause of infantile diarrhea in many developing countries.[129] In nursery outbreaks, transmission was thought to occur via the hands of caretakers and via fomites. In less developed countries, contaminated formula and weaning foods have been incriminated.

Clinical Manifestations. Volunteer studies and epidemiologic observations suggest that the infective dose for EPEC is high ($\approx 10^9$ colony-forming units).[130] EPEC causes a self-limited watery diarrhea with a short incubation period (6–48 hours). There may be associated fever, abdominal cramps, and vomiting. EPEC is a leading cause of persistent diarrhea (lasting 14 days or longer) in children in developing countries.[131]

Treatment and Prevention. Although few data exist to guide antibiotic therapy of EPEC diarrhea, administration of appropriate antibiotics seems to diminish morbidity and mortality. A 3-day course of oral, nonabsorbable antibiotics such as colistin or gentamicin (if available) has been shown to be effective.[132] Some clinicians also advocate the use of oral neomycin; however, this drug causes diarrhea in about 20% of people. In a placebo-controlled trial among Ethiopian infants with severe EPEC diarrhea, TMP-SMX and mecillinam resulted in significant clinical and bacteriologic cure rates by the third day compared with placebo.[133]

Strategies for the prevention of EPEC infection include efforts to improve social and economic conditions in developing countries, efforts to encourage breastfeeding, and prevention of nosocomial infections.

Enterotoxigenic E. coli

Species of ETEC are an important cause of diarrheal disease in humans and animals worldwide. The clinical importance of these microorgansims was first outlined in the 1970s by epidemiologic studies in India that identified them as a major cause of endemic diarrhea.[134] Their pathogenicity is related to the elaboration of one or more enterotoxins that are either heat stable (ST) or heat labile (LT) (see "Pathogenesis") without invading or damaging intestinal epithelial cells.

Epidemiology. Together with *Rotavirus*, ETEC is the leading cause of dehydrating diarrheal disease among weaning infants in the developing world. These children experience two to three episodes of ETEC diarrhea in each of the first 2 years of life. This represents over 25% of all diarrheal illness[135] and results in an estimated 700,000 deaths each year.[136] In industrialized countries, ETEC does not contribute to endemic disease[88] but is notorious for being the leading agent of traveler's diarrhea, accounting for about half of all episodes.[137] Transmission occurs by ingestion of contaminated food and water, with peaks during the warm, wet season.

Clinical Manifestations. Like EPEC, ETEC requires a relatively high inoculum[138] and has a short incubation period (14–30 hours). The cardinal symptom is watery diarrhea, sometimes with associated fever, abdominal cramps, and vomiting. In its most severe form, ETEC can cause cholera-like purging, even in adults. The illness is typically self-limited, lasting for less than 5 days, with few cases persisting beyond 3 weeks. Infection with ETEC has also been associated with short- and long-term adverse nutritional consequences in infants and children.

Treatment and Prevention. Most diarrheal illnesses owing to ETEC are self-limited and do not require specific antimicrobial therapy. Empiric therapy is reserved for those whose diarrhea is moderate to severe despite rehydration and supportive measures. Antibiotic regimens that have been efficacious in clinical trials, shortening the duration of illness by 1 to 2 days, include doxycycline, TMP-SMX, ciprofloxacin, quinolones, and furazolidone.[139] In the past, the drug of choice for children has been TMP-SMX; however, except in Central Mexico, a large proportion of ETEC is now resistant.[140] An alternative regimen for use in children is furazolidone.

Prevention of ETEC infection is based on avoiding contaminated vehicles. Although antibiotics are effective as prophylactic agents, their use is not recommended. Some experts advocate the use of bismuth subsalicylate to diminish the risk of traveler's diarrhea.[141] The development of vaccines against ETEC has received a great deal of attention because of the disease burden. Oral vaccines for ETEC are being developed by five different strategies, including killed whole cells, toxoids, purified fimbriae, living attenuated strains, and live carrier strains elaborating ETEC antigens. A killed whole-cell *V. cholerae* vaccine given with cholera toxin B provided 67% protection against LT-producing ETEC diarrhea for 3 months.[142] A formalin-inactivated whole-cell oral vaccine consisting of ETEC strains bearing colonization factor antigens in combination with cholera toxin B has entered field trial.[143]

Enteroinvasive E. coli

This group consists of invasive *E. coli* species that are genetically, biochemically, and clinically nearly identical to *Shigella*. This section serves only to highlight relevant characteristics that distinguish this pathogen.

Epidemiology. Species of EIEC are endemic in developing countries, where they exhibit similar epidemiology to *Shigella* and cause an estimated 1 to 5% of diarrheal episodes among patients visiting treatment centers.[144] The occurrence of EIEC in industrialized countries is limited to rare foodborne outbreaks.[145] From volunteer studies, it appears that the infectious inoculum is higher than that required to cause shigellosis.[146]

Clinical Manifestations, Treatment, and Prevention. Like *Shigella*, EIEC can produce dysentery, but watery diarrhea is more common.[147] The rare episodes for which treatment is desired are treated with antibiotics recommended for shigellosis. The same general preventive measures used for *Shigella* infections apply to EIEC-associated diarrhea.

Enterohemorrhagic E. coli

These *E. coli* species produce either one or both phage-encoded potent cytotoxins termed Shiga-like toxin (SLT) I (which is neutralized by antiserum to Shiga toxin produced by *S. dysenteriae* type I) or SLT II (which is not neutralized) and can cause diarrhea or HUS. *E. coli* O157:H7 is the prototypic (but not the exclusive) EHEC serotype because it is the predominant SLT-producing *E. coli*, the one most commonly associated with HUS in North America and the type most readily identified in stool specimens.[148]

Epidemiology. In 1982, a multistate outbreak of hemorrhagic colitis that was linked to the consumption of hamburgers at the same fast-food restaurant led to the identification of EHEC.[149] The causative organism was *E. coli* O157:H7, a serotype not previously recognized as a human pathogen. Soon after, Canadian investigators uncovered an association between O157:H7 and other SLT-producing strains of *E. coli* and HUS.[150] EHEC is now recognized as a global health problem; in 1996, an outbreak in Japan linked to eating radish sprouts affected over 6,000 persons.[151] One of the most severe EHEC outbreaks in the United States took place in New York State in 1999, with more than 1,000 ascertained cases, 2 HUS-related casualties, and 8 children on dialysis because of renal failure. Most of the infected individuals attended a fair whose underground water supply was contaminated by cow manure from a nearby cattle barn.

The predominant mode of transmission is ingestion of contaminated, undercooked ground beef. However, the spectrum of vehicles is widening to include raw fruits (including apple juice) and vegetables,[152,153] raw milk,[154] processed meats,[155] and drinking[156] or swimming[157] in contaminated water. The uncooked food vehicles are usually contaminated with manure from infected animals during growth or processing. Person-to-person transmission is the mode of spread in day-care outbreaks, for which secondary transmission rates of 22% have been reported.[158]

EHEC also causes sporadic diarrhea. Isolation from stools of unselected patients is low (< 1%), but isolation from stools of patients with bloody diarrhea may be as high as 20 to 30%.[159] A national laboratory-based study demonstrated that infection is more frequent in northern states and that it peaks from June through September.[159] The highest age-specific isolation rates are in patients 5 to 9 and 50 to 59 years of age. A population-based incidence rate based on stool samples submitted to a large health maintenance organization laboratory in the state of Washington was 8 per 100,000 person-years.[160]

Clinical Manifestations. Illness with EHEC follows 3 to 9 days after ingestion of as few as 100 organisms.[161] Crampy abdominal pain and nonbloody diarrhea are the first symptoms, sometimes associated with vomiting. By the second or third day of illness, diarrhea becomes bloody in ≈ 90% of cases, and abdominal pain worsens.[162] Bloody diarrhea lasts between 1 and 22 days (median 4 days). Unlike other infectious causes of bloody diarrhea, fever is usually absent or remains low grade. Younger children appear to excrete the organisms longer (median 3 weeks) than older children and adults.[163]

In outbreaks, approximately 25% of patients are hospitalized, 5 to 10% develop HUS, and 1% die.[164,165] Intestinal complications include rectal prolapse, appendicitis, intussusception, and pseudomembranous colitis.[148,166]

The most frightening complication of EHEC infection is HUS. It is usually diagnosed 2 to 14 days after the onset of diarrhea.[150] Risk factors include young and old age, bloody diarrhea, fever, an elevated leukocyte count, and treatment with antimotility agents.[166,167] Two-thirds of patients who develop HUS are no longer excreting the organism at presentation.[168]

Diagnosis. The most widely accepted indication for seeking a clinical diagnosis of *E. coli* O157:H7 infection is a patient with bloody diarrhea, in whom an accurate diagnosis may avoid unnecessary medical procedures because a surgical abdomen (such as appendicitis or intussusception) is suspected. A multicenter study found that when the presence of fecal blood was used as the sole criterion for culturing O157 strains, only 3% of stools would be cultured to detect 63% of infections.[159] Diagnosis may also be helpful in patients with HUS or with any type of diarrhea in a contact of a patient with HUS. *E. coli* O157:H7 is not detected by routine stool culture. A relatively inexpensive method exploits the inability of *E. coli* O157 to rapidly ferment sorbitol after 24 hours of incubation on sorbitol-MacConkey agar, in contrast to ~ 90% of commensal *E. coli*. The "sorbitol-negative" colonies can then be screened for the presence of O157 antigen, using commercially available antisera. These strains should be considered pathogenic pending the determination of the H type in a reference laboratory.

Treatment and Prevention. Although data are not available from prospective randomized double-blind trials, there is considerable evidence to suggest that patients who receive antibiotics to which the offending *E. coli* O157:H7 is sensitive have either the same or a poorer outcome when compared with untreated patients.[166,169,170] Therefore, antibiotic therapy is not recommended for EHEC infection. As mentioned above, antimotility agents have been

identified as a risk factor for the development of HUS and should be avoided.

Prevention of *E. coli* O157:H7 is a complex process. From a public health standpoint, control measures at the level of farms, slaughterhouses, and processing plants can decrease the risk of colonization of cattle and contamination of beef. Because these procedures are unlikely to achieve complete success, regulations governing proper processing and cooking of contaminated foods are also required. Advice to consumers should include recommending complete avoidance of raw foods of animal origin. Hamburger should be cooked until no pink remains and the juices are clear.

Because of the severity of the disease, there has been a recent focus on vaccine development for EHEC infection. Efforts have concentrated on three approaches: (1) parenteral toxoids and live oral carrier strains elaborating the B subunit of Shiga toxin[171]; (2) vaccines expressing the adhesin intimin, designed to prevent intestinal colonization[172]; and (3) a parenteral O157 polysaccharide protein conjugate.[173]

Diffusely Adhering E. coli

Until recently, DAEC was considered a nonpathogenic *E. coli* because early studies failed to find an association between this microorganism and diarrheal disease.[174–176] However, more recent studies have demonstrated such an association, particularly in children older than 2 years of age.

Epidemiology. A community-based, case-control study in southern Mexico revealed that DAEC was significantly associated with diarrhea in children less than 6 years of age.[177] Prospective cohort studies in Chile[178] and Bangladesh[179] also demonstrated a diarrheagenic role for DAEC that peaked in the 48- to 60-month age group.[178] This microorganism was more frequently isolated from cases of prolonged diarrhea,[179] and it showed a seasonal pattern similar to that of ETEC, occurring more frequently in the warm season.[178]

Clinical Manifestations, Diagnosis, and Treatment. The gastrointestinal symptoms that characterize DAEC infection are practically indistinguishable from those caused by ETEC, with self-limiting watery diarrhea rarely associated with vomiting and abdominal pain. The diagnosis is mainly based on DNA probe technique and on the pattern of adherence of the microorganism on Hep-2 cells. Given the technical challenge of both assays, their use is limited to epidemiologic surveys rather than the diagnosis of individuals.

Enteroaggregative E. coli

EAggEC are diarrheagenic *E. coli* defined by a characteristic aggregating pattern of adherence to Hep-2 cells and the intestinal mucosa (Figure 38.1-2). They have been particularly associated with cases of persistent diarrhea in the developing world. It has been hypothesized that the aggregating pattern of adherence may be a result of nonspecific, possibly hydrophobic, interaction; therefore, not all organisms meeting the definition of EAggEC may be pathogenic in humans. Moreover, because epidemiologic studies have not uniformly implicated EAggEC as pathogenic, some investigators have questioned the virulence of all EAggEC isolates. Volunteer studies performed to address both of these questions[180] confirmed that at least some EAggEC species are genuine human pathogens but that virulence is not uniform among isolates. More recently, EAggEC pathogenicity has also been proven in several outbreaks.

Epidemiology. From the earliest epidemiologic reports, EAggEC was most prominently associated with persistent cases of pediatric diarrhea (ie, lasting ≥ 14 days),[181] a condition that represents a disproportionate share of diarrheal mortality. On the Indian subcontinent, several independent studies have demonstrated the importance of EAggEC in pediatric diarrhea.[182] These studies include hospitalized patients with persistent diarrhea,[175] outpatients visiting health clinics,[182] and cases of sporadic diarrhea detected by household surveillance.[174] In Fortaleza, Brazil, Fang and colleagues demonstrated a consistent association between EAggEC and persistent diarrhea[183]; in this area, EAggEC accounts for more cases of persistent diarrhea than all other causes combined.[183] EAggEC have been implicated as a cause of sporadic diarrhea in other developing countries (including Mexico, Chile, Bangladesh, Congo, and Iran), as well as in industrialized countries such as Germany and England.[184] Besides being responsible for sporadic cases of diarrhea, EAggEC has also been associated with outbreaks in India,[185] Serbia,[186] Japan,[187] and England.[188]

Clinical Manifestations. The clinical features of EAggEC diarrhea are becoming increasingly well defined in outbreaks, sporadic cases, and the volunteer model. Typically, illness is manifested by a watery, mucoid, secretory diarrheal illness with low-grade fever and little or no vomiting.[174,189] However, in epidemiologic studies, grossly bloody stools have been reported in up to one-third of patients with EAggEC diarrhea.[190] This phenomenon may well be strain dependent. In volunteers infected with

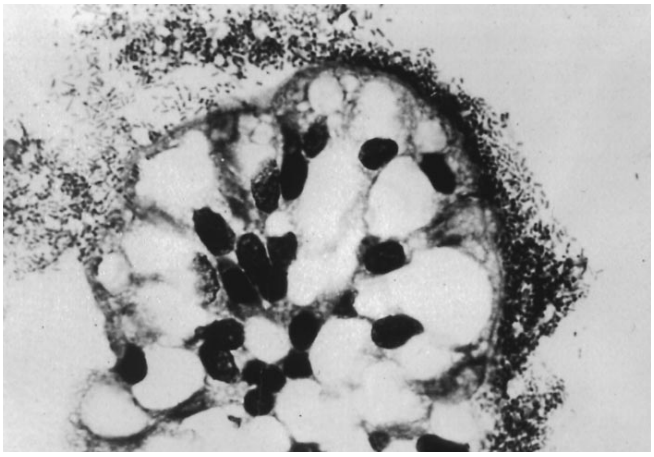

FIGURE 38.1-2 Histopathology of enteroaggregative *Escherichia coli* (EAggEC) infection in a gnotobiotic piglet ileum. The light photomicrograph shows the aggregate pattern of adherence to the intestinal mucosa that characterizes these microorganisms (hematoxylin and eosin; ×100 original magnification).

EAggEC strain 042, diarrhea was mucoid, of low volume, and, notably, without occult blood or fecal leukocytes; all patients remained afebrile. In such volunteers, the incubation period of the illness ranged from 8 to 18 hours.[180]

Perhaps even more significant than the association of EAggEC with diarrhea are the recent data from Brazil that link EAggEC with growth retardation in infants.[190] In this study, the isolation of EAggEC from the stools of infants was associated with a low z-score for height and/or weight, irrespective of the presence of diarrheal symptoms. Given the high prevalence of asymptomatic EAggEC excretion in many areas,[181,191] such an observation may imply that the contribution of EAggEC to the human disease burden is significantly greater than is currently appreciated.

Diagnosis and Treatment. Colonization of EAggEC is detected by the isolation of *E. coli* from the stools of patients and the demonstration of the aggregative pattern in the Hep-2 assay. Implication of EAggEC as the cause of the patient's disease must be done cautiously, given the high rate of asymptomatic colonization in many populations.[181,191] If no other organism is implicated in the patient's illness and EAggEC is isolated repeatedly, then EAggEC should be considered a potential cause of the patient's illness. A DNA fragment probe has proven highly specific in the detection of EAggEC strains. A PCR assay using primers derived from the aggregative probe sequence shows similar sensitivity and specificity.[192]

The optimal management of EAggEC infection has not been studied. Acute diarrhea is apparently self-limiting; however, more persistent cases may benefit from antibiotic and/or nutritional therapy. Given the high rate of antibiotic resistance among EAggEC,[193] susceptibility testing is recommended when available.

CLOSTRIDIUM DIFFICILE

Even though *Clostridium difficile* is now recognized as the single most common cause of bacterial diarrhea in hospitalized patients, its role as a pathogen had not been established as recently as the late 1970s. *C. difficile* has the ability to become established in the intestinal tract once the natural microflora have been modified by antibiotic therapy. The organism causes intestinal disease ranging from mild diarrhea to fatal pseudomembranous colitis (PMC). Although *C. difficile* is associated with almost all cases of PMC, only 25% of antibiotic-associated diarrheas are due to this pathogen.

Microbiology. *C. difficile* is a gram-positive anaerobe that forms spores, making this microorganism very difficult to remove from the hospital environment. Unlike some toxigenic clostridia, the production of spores is not associated with toxin production.

Epidemiology. *C. difficile* spreads from patient to patient[194] and tends to persist in the environment because of the formation of spores. The microorganism is not only present in the infected patient and soiled linens but can be isolated from bookshelves, curtains, and floors of rooms of infected patients, where it can persist for as long as 5 months.[194–196] The organism is spread primarily by health care workers; up to 60% of personnel attending patients infected with *C. difficile* in one study had the organism on their hands.[194] The isolation of *C. difficile* toxins from the feces of asymptomatic normal-term neonates and (in higher proportion) those admitted into neonatal intensive care units[197] further supports the concept of the nosocomial spreading of the infection.

Several outbreaks of *C. difficile* infection have been reported in the United States and throughout the world, and the incidence continues to rise. Whether this increase represents a true increment or an increased awareness of the disease is not clear at this stage.

Clinical Manifestations. Infections with *C. difficile* range in severity from asymptomatic forms to clinical syndromes, such as severe diarrhea, PMC, and toxic megacolon, and can even lead to death.[198] The onset of symptomatic forms usually begins several days after starting antibiotic therapy up to 2 months following cessation of treatment. Diarrhea and abdominal cramps are usually the first symptoms, followed by the development of fever and chills in severe cases.

Diagnosis and Treatment. Mild forms of colitis, with bloody stools and mucus, particularly if they are preceded by antibiotic treatment, should be considered suspicious for *C. difficile* infection. Clinical microbiologists face an array of methods and commercial tests when considering which procedure to use for the detection of *C. difficile* and its toxins. Culturing of the organism, latex agglutination, tissue culture assay, and enzyme-linked immunosorbent assay are all used as aids for the diagnosis of *C. difficile* infection.

In many instances, *C. difficile* disease is self-limiting, and the patient may respond simply to the withdrawal of the offending antibiotic. In more severe forms, particularly if complicated by PMC, antibiotic treatment with either oral vancomycin[199] (5–10 mg/kg, maximum 500 mg, given every 6 hours for 7 days) or metronidazole[200] (5–10/per kg, maximum 500 mg, given every 8 hours for 7 days) is recommended. Despite pharmacologic treatment, the rate of relapse is significant (up to 40–50% of cases). In these complicated patients, the use of probiotics, particularly *Lactobacillus* GG[201] and *Saccharomyces boulardii*,[202] has been associated with a significant eradication of *C. difficile* and a substantial decrease in the recurrence of the infection.

AEROMONAS, PLESIOMONAS, AND EDWARDSIELLA

This group includes microorganisms of which the existence has been known for a long time; however, only recently have they been associated with human diseases.

AEROMONAS

Aeromonads are gram-negative facultative anaerobic, motile bacilli. Although their association with enteritis is still con-

troversial, experimental, clinical, and epidemiologic data continue to support the evidence that at least certain strains are involved in diarrheal diseases.[203] The highest attack rate appears to be in young children, particularly those under 3 years of age.[204] *Aeromonas* infections occur more frequently during the warm months, with an isolation rate that varies from as little as 0.7% to peaks of 50%.[205] Despite these data, a number of troubling aspects regarding the association between *Aeromonas* and diarrhea remain unresolved. In contrast to other waterborne and foodborne pathogens, no clearly defined outbreaks of diarrheal illnesses associated with the pathogen have ever been reported, even though the microorganism is often isolated from water, food, and other environmental sources.

The intestinal diseases caused by *Aeromonas* cover the same spectrum of clinical manifestations secondary to other classic enteric pathogens. *Aeromonas* spp have been associated with several distinctive clinical syndromes, including watery diarrhea, dysentery, and prolonged or chronic diarrhea. Acute secretory diarrhea is the most commonly reported, with as many as 20 bowel movements a day. Abdominal pain, fever, nausea, and vomiting are common associated symptoms.[206,207] Although the infection is usually self-limited (< 7 days in duration), dehydration or persistent diarrhea may occur in one-third of the cases. The most common *Aeromonas* species isolated in these cases is *A. caviae*. Some children with this infection experience abdominal complications secondary to their diarrheal episodes, including failure to thrive, gram-negative sepsis, and HUS.[207]

The mainstay of therapy in *Aeromonas*-associated enteritis, as in any diarrheal disease, is rehydration, via either the oral or the intravenous route. The illness is usually self-limited, and previously healthy subjects who experience this form of enteritis who are not treated with antibiotics appear to do well, with rapid resolution of the symptoms and clearance of the microorganism from the stools. TMP-SMX is considered the drug of choice for the chronic forms that seem to benefit from antibiotic treatment.[208]

Plesiomonas

Plesiomonas, originally assigned to the family Vibrionaceae but recently reassigned to Enterobacteriaceae,[209] are gram-negative, facultative anaerobic, motile, primarily freshwater organisms, with isolation rates increasing during the warm months. Fish and shellfish, especially if associated with mud or sediment, frequently harbor plesiomonads.[210] However, the microorganism can also be isolated from the feces of asymptomatic animals, including cats and dogs.[211] Although less frequently encountered in the United States, *Plesiomonas shigelloides* is commonly isolated in other areas, particularly in Bangladesh, where this organism represents the fourth leading cause of bacterial enteritis.[212] Typical symptoms of *P. shigelloides* infection include secretory or a colitis/proctitis type of diarrhea (one-third of patients experience frank bloody diarrhea), abdominal pain, nausea, vomiting, and fever. Fatal outcomes of severe intestinal infections without apparent dissemination by *Plesiomonas* have also been described.[213] Quinolones and TMP-SMX are the best oral agents for the treatment of uncomplicated infections whose course seems to be shortened by antibiotic treatment.[214]

Edwardsiella

The genus *Edwardsiella* is composed of bacteria that are gram-negative, facultative anaerobic rods. *E. tarda*, the only species in this genus consistently associated with both intestinal and extraintestinal human illness, has been isolated from the feces of persons suffering from diarrheal diseases and from fish, freshwater ecosystems, and animals that inhabit these locales, such as reptiles and amphibia. Enteritis associated with *E. tarda* exists either as a benign secretory diarrhea or as a more invasive process resembling dysentery or enterocolitis. The most common symptoms include low-grade fever, vomiting, and watery stools.[215] Symptoms may be more severe (resembling PMC or invasive enterocolitis) and include cramping, abdominal pain, nausea, tenesmus, and up to 20 bowel movements per day. Occasionally, disseminated *E. tarda* infections (septicemia, hepatic abscess) can occur in subjects with liver dysfunction or iron overload conditions.[216] Ampicillin, TMP-SMX, and ciprofloxacin are all reasonable choices for the treatment of *E. tarda* infections.

PATHOGENESIS

The distinguishing characteristics of bacteria (small size, concise deployment of genetic information, and the ability to survive in highly varied circumstances) contribute to their acclaimed virtuosic ability to adapt and learn fast in order to survive. To be a successful enteric pathogen, a bacterium must be a good colonizer, must compete for nutrients, and must be able to interact with the target eukaryotic cell to induce secretion of water and electrolytes. Because the basic metabolism of enteric pathogens and commensals is the same, it follows that pathogens must possess highly specialized attributes that enable them to activate one of the eukaryotic intracellular pathways leading to intestinal secretion.[217] This cross-communication between enteric bacteria and the intestinal host is typically activated by the elaboration of enterotoxins (Table 38.1-2) that subvert host-cell signal transduction pathways, leading to the secretion of water and electrolytes and thus to diarrhea.

Toxins that Activate Enterocyte Signal Pathways

Intestinal cells operate through three main intracellular signal transduction pathways to regulate ion transport vectorially: (1) cyclic adenosine monophosphate (cAMP), (2) cyclic guanosine monophosphate (cGMP), and (3) calcium-dependent pathways (Figure 38.1-3). A fourth pathway involving cytoskeleton rearrangement has also been described (see Figure 38.1-3).

Cyclic Adenosine Monophosphate. Cholera toxin elaborated by *V. cholerae* represents the archetype of the family of cAMP-mediated toxins and is certainly the most extensively investigated. Cholera toxin is a protein with a relative molecular mass (M_r) of 84 kD, made up of five B subunits with

TABLE 38.1-2 BACTERIA-DERIVED ENTERIC TOXINS

TOXINS THAT ACTIVATE ENTEROCYTE SIGNAL PATHWAYS
Cyclic AMP
Cholera toxin
Heat-labile *Escherichia coli* enterotoxin (LT)
Salmonella enterotoxin
Pseudomonas aeruginosa enterotoxin
Shigella dysenteriae enterotoxin
Cyclic GMP
Heat-stable *Escherichia coli* enterotoxin (ST)
Yersinia enterocolitica (STI, STII)
Yersinia bercovieri enterotoxin
Klebsiella pneumoniae enterotoxin
Heat-stable *Vibrio cholerae* non-O1 enterotoxin
Enteroaggregative *Escherichia coli* heat-stable enterotoxin (EAST 1)
Ca^{2+}
Clostridium difficile enterotoxin
Ciguatera enterotoxin
Helicobacter pylori vacuolating toxin
Vibrio parahaemolyticus thermostable direct hemolysin (TDH)
PORE-FORMING TOXINS
Clostridium perfrigens enterotoxin (CPE)
Staphylococcus aureus α-toxin
Vibrio cholerae cytolysin (CTC)
TOXINS BLOCKING PROTEIN SYNTHESIS
Shigella dysenteriae Shiga toxin
EHEC Shiga-like toxin 1 (SLT1) and 2 (SLT2)
TOXINS INDUCING PROTEIN SYNTHESIS
Staphylococcus aureus enterotoxin A
Enteroaggregative *Escherichia coli* (EAggEC) toxin
TOXINS AFFECTING THE ENTEROCYTE CYTOSKELETON
Clostridium difficile toxin A and B
Clostridium sordelli toxin
Clostridium botulinum C2 and C3 toxins
Escherichia coli cytotoxic necrotizing factor 1 (CNF1)
Campylobacter jejuni cytolethal distending toxin
Vibrio cholerae zonula occludens toxin (ZOT)
EAggEC plasmid-encoded protein (Pet)
Bacteroides fragilis toxin (BFT)
Vibrio parahaemolyticus thermostable direct hemolysin (TDH)

AMP = adenosine monophosphate; GMP = guanosine monophosphate.

M_r of 10.5 kD each and one A subunit with M_r of 27.2 kD. The A subunit is proteolytically cleaved to yield two polypeptide chains, a 195-residue A_1 peptide of 21.8 kD and a 45-residue A_2 peptide of 5.4 kD.[218] As with other toxins in this group, the functions of the two subunits are specific: the B subunit serves to bind the holotoxin to the eukaryotic cell receptor, and the A subunit possesses a specific enzymatic function that acts intracellularly. The single A subunit is presumably located on the axis of the pentameric B subunit ring, with the fragment A_2 extending some distance into the central hole.[219–221] The cholera toxin receptor on the surface of the enterocyte is a ganglioside GM_1 that is ubiquitous in the body, being present on such diverse cell types as ovarian and neural cells as well as intestinal cells.[219] The neuraminidase produced by *V. cholerae* can increase the number of receptors by acting on higher-order gangliosides to convert them to GM_1 gangliosides.[222] Reduction of the disulfide bond between A_1 and A_2 peptides on the external surface of the membrane is necessary for penetration of the A_1 peptide into the cell. The fate of the A_2 peptide is not known, but there is little evidence that it actually enters the cell. Once within the cell, the A_1 peptide activates adenylate cyclase at the basolateral membrane, where the enzyme is localized in intestinal epithelial cells. The A_1 peptide is thought to migrate to the basolateral membrane through the cytosol, although there is no convincing evidence that this actually occurs. An alternative model proposes that generation of the A_1 peptide and activation of adenylate cyclase are functionally linked to toxin endocytosis. The A_1 peptide acts as an enzyme to adenosine diphosphate ribosylate, the α subunit of G stimulatory (G_s) at an arginine residue. Once activated, the α subunit of G_s dissociates from the membrane-bound subunit of G_s, leaving it free to transverse the cell and attach to the catalytic subunit of adenylate cyclase in the basolateral membrane.[223] The adenylate cyclase so activated induces the formation of cAMP, which then activates the catalytic unit of cAMP-dependent protein kinase (protein kinase A). Finally, the phosphorylation of membrane proteins is responsible for the transepithelial ion transport changes induced by cholera toxin. These changes consist of the inhibition of the linked sodium and chloride absorptive process in the villous cells and the stimulation of electrogenic chloride secretion in the crypt cells.[224,225] The nature of the target protein(s) phosphorylated by protein kinase A remains uncertain. One attractive candidate is the cystic fibrosis transmembrane conductance regulator, which is a chloride channel[226] and which has multiple potential substrate sequences for protein kinase A. Unlike healthy intestinal tissue, tissues obtained from patients with cystic fibrosis do not respond to either cAMP- or Ca-mediated secretagogues.[227] Heterozygotes presumably have only half of the normal number of chloride channels responsive to kinase. After infection with *V. cholerae*, the cystic fibrosis heterozygote may have less intestinal chloride secretion and, therefore, less diarrhea, suggesting a selective advantage over "normal" homozygotes in surviving cholera.

ETEC elaborate an LT (see Table 38.1-2) that closely resembles cholera toxin in structure and biochemical mode of action. Unlike cholera toxin, LT can also bind to GM_2 and asialo-GM_1 gangliosides in addition to GM_1 ganglioside.[228] Toxin binding is followed by activation of the adenylate cyclase–cAMP system, resulting in water and electrolyte secretion into the lumen of the intestine, with a mechanism similar to that of cholera toxin.[229] However, whereas LT induces a mild diarrhea known as "traveler's diarrhea," cholera toxin is responsible for the severe, sometimes fatal, clinical condition typical of cholera. Rodighiero and colleagues have reported that the differential toxicity of cholera toxin and LT is related to a 10–amino acid segment within the A_2 fragment of cholera toxin that confers a higher stability to the cholera toxin holotoxin during uptake and transport into intestinal epithelia.[230]

In addition to its invasiveness, *S. typhimurium* elaborates an enterotoxin, whose role in inducing diarrhea remains controversial (see Table 38.1-2).[231] Cell-free lysates of *Salmonella* can cause intestinal secretion and activate intestinal epithelial cell adenylate cyclase independently of any change in inflammation.[231] How the *Salmonella* toxin activates adenylate cyclase has not been determined.

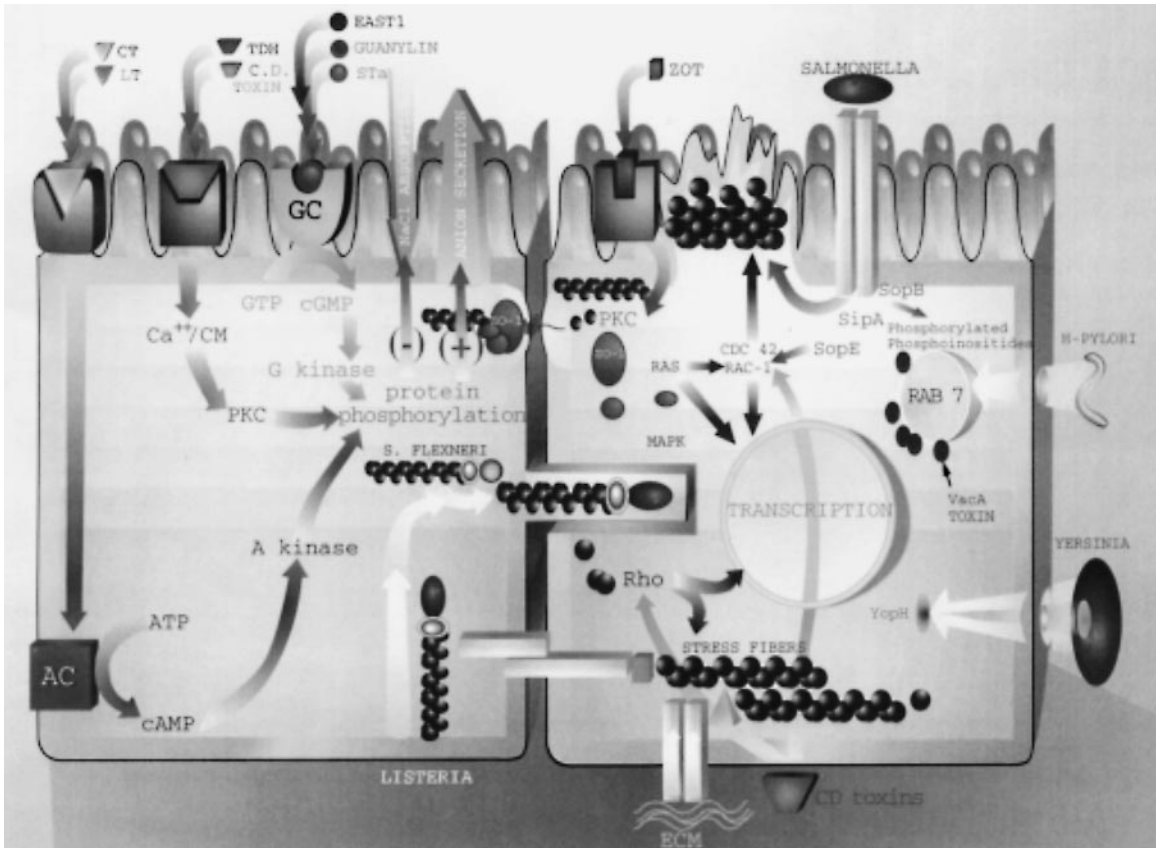

FIGURE 38.1-3 Enterocyte intracellular signaling leading to intestinal secretion. Four main pathways seem to be involved in the intestinal secretion of water and electrolytes: cyclic adenosine monophosphate (cAMP), cyclic guanosine monophosphate (cGMP), calcium (Ca), and cytoskeleton. These pathways are activated by several enteric pathogens, either directly or through the elaboration of enterotoxic products. AC = adenylate cyclase; C.D. = *Clostridium difficile*; CM = calmodulin; CT = cholera toxin; EAST1 = enteroaggregative *Escherichia coli* heat-stable toxin 1; ECM = extracellular matrix; EGF-r = epidermal growth factor receptor; GC = guanylate cyclase; LT = heat-labile enterotoxin; PKC = protein kinase C; ST_a = heat-stable toxin $_a$; TDH = thermostable direct hemolysin; ZOT = zonula occludens toxin.

Cyclic Guanosine Monophosphate. Besides LT, ETEC elaborates a family of ST enterotoxins. STIp is a small peptide that stimulates guanylate cyclase (GC), causing an increased intracellular concentration of cGMP, which evokes chloride secretion and diarrhea.[217] The STIp is a typical extracellular toxin, consisting of 18–amino acid residues synthesized as a precursor protein. The precursor translocates across the inner bacterial membrane, using the general export pathway consisting of the Sec proteins.[233] The ST_a epithelial surface receptor is distinct from the cholera toxin and LT receptor and coincides with the GC activity.[234,235] Ileal villus epithelial cells have approximately twice as many receptors as crypt cells for the enterotoxin.[236] GC exists in two major forms: soluble and particulate. These are distinct proteins encoded by separate genes. Soluble GC is a dimeric cytosolic protein that is activated by nitric oxide.[237] Particulate GC is a family of brush border membrane glycoproteins that are activated by only two classes of substances, atrial natriuretic peptides and ST_a. In the intestine, approximately 80% of total GC is particulate.[238,239] So far, three different members of the particulate GC family have been cloned.[238] GC-A and -B are atrial natriuretic peptide receptor cyclases, whereas GC-C is the specific receptor for ST_a. All three members of the GC family are proteins that span the cell membrane and contain an extracellular domain, a transmembrane domain, an intracytoplasmic domain made up of a protein kinase–like enzyme, and a catalytic domain. These proteins show minimal similarities in their extracellular domain, whereas there is a higher degree of similarity of their intracellular domains. This finding suggests that the extracellular domain represents the ligand-binding domain.[238,239] In addition to LT and ST exotoxins, ETEC also contains a lipopolysaccharide endotoxin. When orally administered to mice, lipopolysaccharide markedly increased the expression of the inducible nitric oxide synthase II and its effector enzyme-soluble GC in colonic cells.[240] This creates the pathophysiologic autocrine pathway, producing increased levels of cGMP leading to hypersecretion and diarrhea.[240] Another heat-stable enterotoxin, EAST 1, which is genetically and structurally distinct from ST, was discovered in EAggEC[241] and subsequently found in other *E. coli* belonging to several distinct diarrheagenic categories.[242] A case-control study demonstrated that 19% of children with diarrhea harbored EAST 1–positive *E. coli* in their stools compared with 3.5% of healthy individuals,[243] confirming the pathogenic role of EAST 1 in diarrheal diseases in children. The last described member of the ST fam-

ily was reported by Sulakvelidze and coworkers.[244] This toxin, elaborated by *Y. bercovieri*, elicited a secretory response in both in vitro and in vivo animal models; these results were genetically and immunologically distinct from the response to *Y. enterocolitica* STI and STII.[244]

Calcium. Several toxins, including ciguatera toxin,[245] *C. difficile* toxin,[246] *Cryptosporidium* toxin,[247] and the *Helicobacter pylori* vacuolating toxin,[248] seem to act through Ca. However, the involvement of Ca in the secretory effect of these toxins has been only indirectly demonstrated. A more definitive proof of Ca-mediated secretory effect was provided by Raimondi and colleagues.[249] Using direct intracellular ($[Ca]_i$) measurement, they demonstrated that the enterotoxic effect of the thermostable direct hemolysin elaborated by *V. parahaemolyticus* is mediated by Ca.[249] This toxin seems to interact with a polysialoganglioside GT1b surface receptor, whose physiologic function remains to be established.[249]

Pore-Forming Toxins. *Clostridium perfrigens* is a common agent of foodborne intoxication, the symptoms of which are caused by the elaboration of *C. perfrigens* enterotoxin.[250] This enterotoxin is a very hydrophobic protein that is released by bacterial lysis and subsequently binds to a brush border receptor of the host enterocyte.[250] Following binding, *C. perfrigens* enterotoxin associates with a 70 kD membrane protein, with subsequent formation of pores through which water, ions, nucleotides, and amino acids leak. *Staphylococcus aureus* α toxin also forms pores; however, its mechanism of action involves the formation of oligomers containing only toxin molecules.[250] According to Zitzer and coworkers, the *V. cholerae* cytolysin represents a novel prototype of pore-forming toxin.[251] The authors have demonstrated that the oligomerization of *V. cholerae* cytolysin yields a pentameric pore and has a dual specificity for both cholesterol and ceramides present in the mammalian brush border membrane of enterocytes.[251]

Toxins Blocking Protein Synthesis. Shiga toxin elaborated by *S. dysenteriae* represents the archetype of this family of toxins. SLTI and II are related toxins elaborated by EHEC. Shiga toxin and SLTs share the AB5 structure typical of cholera toxin and LT; however, they act through a different mechanism of action. The A_1 subunit of Shiga toxin and SLTs binds and inactivates the 60S subunit of the host cell ribosome and, consequently, completely interrupts cell protein synthesis.[252] To induce this inhibitory effect, the toxins must interact with a glycolipid surface receptor (Gb3 receptor), whose expression in different endothelial districts varies.[253] In fact, whereas endothelial cells of large blood vessels such as the umbilical and saphenous veins produce minimal amounts of Gb3,[253] human renal[253] and intestinal[254] microvascular endothelial cells constitutively express maximal quantities of the receptor. These results provide a rationale for the targeting of the glomeruli in HUS and the endothelial cells of the colon in hemorrhagic colitis. Recent epidemiologic data suggest that the elaboration of SLTs may not be sufficient in itself to induce disease in humans. By applying a multivariate logistic regression analysis, Boerlin and coworkers showed a significant association between the presence of genes for intimin (a protein involved in the intimate attachment of EHEC to the host intestinal cell) and SLT2 and isolates from cases of hemorrhagic colitis and HUS.[255] Further analysis revealed an interaction between the intimin gene and the *SLT2* gene, thus supporting the hypothesis of the synergism between the adhesin intimin and SLT2.[255]

Toxins Inducing Protein Synthesis. Up-regulation of protein synthesis, particularly of proinflammatory mediators, is one of the most recently described mechanisms through which bacterial toxins induce diarrhea. Nielsen and coworkers have demonstrated that staphylococcal enterotoxin A induces tyrosine phosphorylation of several host intracellular proteins, down-regulation of the T-cell receptor, and production of interferon-γ, a key cytokine in the pathogenesis of intestinal inflammatory and secretory processes.[256,257] Transcriptional up-regulation of proinflammatory cytokines seems also to be involved in the pathogenesis of EAggEC-associated diarrhea. It has been reported that EAggEC produces a cell-free factor that up-regulates interleukin-8 (IL-8) messenger ribonucleic acid in CaCo2 cells.[258] This up-regulation correlates with the clinical observation that increased lactoferrin (as a marker of inflammation) and IL-8 can be found in the stools of children in Brazil with EAggEC infections.[258]

Toxins Affecting the Enterocyte Actin Cytoskeleton. A growing number of toxins have been reported to act by affecting the host-cell cytoskeleton. *C. difficile* has emerged as the most important pathogen causing the syndrome of antibiotic-associated colitis.[259] The virulence of this pathogen depends on its elaboration of two related toxins, TxA and TxB. These toxins are among the largest monomeric toxins described, with molecular weights of 308,000 for TxA and 270,000 for TxB. Despite the fact that TxA has traditionally been referred to as an enterotoxin and TxB as a cytotoxin,[259] they both exert a cytotoxic effect in vitro. Both TxA and TxB are glucosyltransferases and use uridine diphosphate (UDP) glucose as a substrate to inactivate, by monoglucosylation, members of the Rho family of small guanosine triphosphatases (GTPases) at Thr,[37] an amino acid residue located within the putative effector domain of the Rho proteins.[260] Rho GTPases regulate a variety of cytoskeleton-dependent cellular functions, such as cell adhesion and motility, growth factor–mediated signaling, cellular transformation, and induction of apoptosis.[261] The dramatic effects of TxA and TxB on tissues and cells, including cytoskeletal depolymerization, increased intestinal permeability and diarrhea, cellular retraction and rounding, disruption of cell adhesion and chemotaxis, and activation of apoptosis,[262] are therefore all related to the TxA- and TxB-dependent inactivation of the Rho proteins. *Clostridium sordelli* toxin also functions as a UDP glucosyltransferase and inactivates Ras, Rap, and Rac.[259] *Clostridium botulinum* C2 and C3 toxins exert their enterotoxic effect by inactivating actin and Rho, respectively.[259]

Besides the inactivation of Rho proteins, their activation is also associated with increased intestinal permeability and diarrhea. Cytotoxic necrotizing factor 1 (CNF1), a protein of ≈ 115 kD produced by pathogenic *E. coli* strains,[263] activates Rho GTPases by deamination of Gln[62,264] and consequently induces polymerization of F actin. When tested on CaCo2 monolayers, CNF1 reduced the monolayer resistance by 40% after 4 hours of incubation,[265] suggesting that not only depolymerization but also polymerization of actin and subsequent reorganization of the actin cytoskeleton alter the barrier function of intestinal tight junctions.

A similar mechanism was previously described for zonula occludens toxin, a toxin elaborated by *V. cholerae*.[266,267] Zonula occludens toxin is a single polypeptide chain of 44.8 kD, encoded by the bacteriophage CTXϕ present in toxigenic strains of *V. cholerae*.[268] The mechanism of action of zonula occludens toxin involves the rearrangement of the epithelial cell cytoskeleton owing to protein kinase C α-dependent polymerization of actin filaments strategically located to modulate intercellular tight junctions.[269] The plasmid-encoded protein (Pet) elaborated by EAggEC is a member of the autotransporter class of secreted proteins that induces contraction of the cytoskeleton and loss of the actin stress fibers when tested on either Hep-2 cells or HT29 C-cell monolayers.[270] Both the cytopathic and enterotoxic effects of Pet seem related to the serine protease activity of the toxin that elicits cytoskeletal changes without compromising cell viability.[270]

Enterotoxigenic *Bacteroides fragilis* elaborates a 20 kD zinc-dependent metalloprotease toxin (*B. fragilis* enterotoxin) that alters tight junctions and intestinal permeability.[271] This enterotoxin specifically cleaves the extracellular domain of the zonula adherens protein E-cadherin. Its protease activity appears to be specific for E-cadherin because no proteolytic activity was detected for other cytoskeleton-associated proteins, including occludin, β_1 integrin, zonula occludens (ZO)1, or α- and β-catenins.[271]

Beside its Ca-mediated enterotoxic effect, mentioned above, the *V. parahaemolyticus* enterotoxin thermostable direct hemolysin also induces a significant (though reversible) decreased rate of progression through the cell cycle and morphologic changes related to the organization of the microtubular network, which appears to be the preferential cytoskeletal element involved in the cellular response to the toxin.[272]

ENTERIC BACTERIA GENOMIC REVOLUTION

Our knowledge of the complexity of the prokaryotic kingdom, including enteric pathogens, has been a dynamic process of learning that progressed hand in hand with the advent of cornerstone technologies. The microscope has been the first instrument that allowed us to appreciate the variety of microorganisms initially classified based on their appearance. Over time, disciplines such as cell and molecular biology and biochemistry contributed to new discoveries in microbiology. However, the full appreciation of the extent of genetic complexity and diversity among prokaryotic organisms could not be estimated until the advent of high-throughput genome sequencing and genome annotation.[273] So far, the genomes of more than 100 bacterial species have been sequenced, including enterobacteriaceae such as *V. cholerae*, EHEC, and *S. enterica*. For several of these species, the genome sequences of both pathogenic and nonpathogenic strains have been determined, thereby launching the field of comparative bacterial genomics. This information is of crucial importance in assisting us to identify pathogenic traits and, therefore, to develop alternative strategies for the treatment of enteric bacteria-associated infections.

V. cholerae represents the typical exemplification of the genomic revolution and its possible outcomes.[274] One of the most intriguing and least understood features of *V. cholerae* is its annual epidemic profile in the Bengal region of Bangladesh and India. In this region, nearly all cases occur in synchronized, massive outbreaks toward the end of the monsoon season. Between epidemics, the microorganism resides in a stable environmental reservoir, suggesting that changes in rainfall and sunlight dictate its shift from aquatic habitat host to human pathogen. The functional annotation of *V. cholerae* genomic sequence[275] shed some light on this extraordinary adaptability to two extremely different lifestyles. The *V. cholerae* genome consists of two circular chromosomes of ~ 3 million and ~ 1 million bp, respectively, that together encode almost 4,000 putative genes. The vast majority of recognizable genes for essential cell functions and pathogenicity are located on the large chromosome, whereas the small chromosome contains many genes that appear to have origins other than *V. cholerae*. This two-chromosome configuration of *V. cholerae* seems to confer to the microorganism its plasticity to adapt to climate changes and to different lifestyles. One of the most accredited hypotheses predicts that the large chromosome genes are in charge for the microorganism's adaptation to the human intestine, whereas the small chromosome genes are essential for environmental survival.[274] If this hypothesis proves correct, it will be theoretically possible to develop strategies that will prevent *V. cholerae* to switch from environmental to human host and, consequently, its survival in the human intestine.

ENTERIC BACTERIA AND BIOTERRORISM

Recent discoveries involving bacterial genomes and virulence machinery have promoted strategies to reduce the morbidity and mortality of microbial infections. Recent progress has also been made, which has resulted in effective interventions for reducing environmental risks and preventing human-to-human transmission of infectious agents. These approaches have had a huge positive impact on childhood mortality. Both the events of September 11, 2001, and the subsequent highly publicized use of the US Postal Service to spread anthrax infection have drastically changed our sense of confidence toward the prevention and treatment of infectious diseases. It has become clear that present disease control strategies are ineffective and possibly obsolete in dealing with bioterrorism. The standard goals of public health organizations—confinement of specific pathogens to endemic areas, eradication of

microorganisms, and vaccination campaigns to eliminate specific pathogens—are now undergoing a process of reprioritization in light of the new realities that we face with bioterrorism and biologic warfare. Despite the fact that hundreds of potential agents could be used in biologic warfare or bioterrorism, attention has been focused on microorganisms, including anthrax and smallpox, which have the potential for aerosol dissemination.[276] It is surprising, however, that little attention has been paid so far to enteric pathogens listed in the category B agents by the CDC. Despite the fact that these microorganisms typically cause moderate morbidity and low mortality, they pose a major risk because they are easy to produce and disseminate, they do not require complex production and stocking facilities, and they can be handled by nonprofessional personnel.[276] The threats to food and water safety represented by these agents make them an obvious choice for bioterrorism initiatives executed by terrorist groups whose level of scientific expertise may be relatively primitive. Unfortunately, deliberate contamination of food with enteric pathogens has already been perpetrated.[277] With free trade, centralized production, and wide distribution of products worldwide, the biosecurity of commercial food products is becoming a challenging task because deliberate contamination of foodstuff could present either as a slow, diffuse, and initially unremarkable increase in sporadic cases or as an explosive epidemic suddenly producing many illnesses. Among the potential enteric pathogens to be used as bioweapons, *Salmonella* serotypes need to be considered at high risk for their high rate of infectivity and their ability to survive in the environment. The bioterrorism potential of *S. typhi* was recognized in the 1970s by the World Health Organization, which assessed a potential attack on municipal water supplies with the organism.[277] *Shigella* spp, which are frequent worldwide, have a low infectious dose and cause dysentery, with severe complications and death rates of up to 20%. *S. dysenteriae* is rare in the United States, but most clinical laboratories have reference strains. *E. coli* O157:H7 causes bloody diarrhea and abdominal cramps. It is the most common cause of HUS in children, has a low infectious dose, and, therefore, is highly transmissible, and reference strains are kept by clinical laboratories.[277] The mortality rate of *V. cholerae* secondary to dehydration is limited when appropriate rehydrating therapy is enforced. However, widespread disease could overwhelm unprepared medical care facilities, and cases of severe untreated cholera have mortality rates that can reach 50%. Both cultures and purified cholera toxin are available commercially for research purposes.[277]

CONCLUSION

Despite the tremendous increase in knowledge of bacterial pathogenesis experienced during the past decade, intestinal infections remain a major cause of disease and death because they are responsible for 6 to 60 billion cases of diarrhea every year and claim the life of a child every 15 seconds. Widespread travel to developing countries and the recent events of bioterrorism have brought diseases transmitted by contaminated food and water to immunologically naive populations. These changes, along with an increased number of immunocompromised individuals and the pandemic of HIV infection, have elevated bacteria-associated diarrheal diseases to a worldwide threat. However, the widespread use of oral rehydration solutions has revolutionized the way in which this plague has being fought, and more positive results are anticipated from the development of safe enteric vaccines and a better preparedness to face possible bioterrorist attacks exploiting enteric pathogens as bioweapons.

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2. *Food- and* Waterborne Infections

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Food- and waterborne illnesses typically conjure up the image of an individual who develops an infection following exposure to food. However, the definition of foodborne illness is broad and encompasses exposure to toxins, carcinogens, metals, prions, and other factors, in addition to the classic infective pathogens. Because reviewing each of these agents in detail is beyond the scope of this chapter, our focus is on food- and waterborne infections. An extensive list of foodborne pathogens is shown in Table 38.2-1; many of these are discussed individually in other chapters. This chapter discusses the current epidemiology of foodborne illness and provides an overview of the various toxins and organisms considered to be the more important foodborne agents. The clinical symptoms, treatment, and long-term consequences of various foodborne infections are also reviewed.

Everyone reading this text will likely have had a personal experience with foodborne infection. In its classic form, this illness consists of acute gastrointestinal upset with nausea, vomiting, diarrhea, and abdominal cramps. Typically, symptoms resolve spontaneously without the need for significant medical intervention and without long-term consequence. However, on occasion, foodborne infection causes severe illness or death. Unfortunately, in the early stages of illness, differentiating between a patient with an inconsequential infection and a patient who may develop life-threatening sequelae can be difficult. Some systemic consequences of infection occur several days or weeks after the initial exposure. Examples include hemolytic uremic syndrome (HUS) secondary to Shiga toxin–producing *Escherichia coli* (STEC), the development of Guillain-Barré syndrome (GBS) after *Campylobacter* infection, and the association of a number of enteric bacterial pathogens with reactive arthritis and postinfectious irritable bowel syndrome. We have much to learn about the pathogenic mechanisms of the common foodborne microbes and the illnesses they cause, but we are gradually elucidating the processes whereby pathogens such as *Listeria*, *Salmonella*, and *E. coli* subvert the host.

CURRENT EPIDEMIOLOGY OF FOOD- AND WATERBORNE ILLNESSES

The true burden of foodborne illnesses in the United States and other parts of the world is largely unknown, yet the number of suspected deaths worldwide from food- or water-related pathogen exposure is staggering. Several million children die each year worldwide from acute diarrheal disease and resulting dehydration, the majority of which is likely due to contaminated food or water. In the United States, until recently, we have had very few data on the numbers and outcomes of foodborne infection. The development of the Foodborne Diseases Active Surveillance Network (FoodNet) by the Centers for Disease Control and Prevention (CDC) has provided, for the first time, the opportunity to determine the epidemiology of foodborne disease in the US population. FoodNet is the main foodborne disease component of the CDC's Emerging Infections Program and is a collaborative venture with Emerging Infections Program sites, the US Department of Agriculture (USDA), and the US Food and Drug Administration (FDA). FoodNet performs population-based active surveillance for confirmed cases of *Campylobacter*, *E. coli* O157:H7, *Listeria*, *Salmonella*, *Shigella*, *Vibrio*, *Yersinia*, and HUS, as well as *Cryptosporidium* and *Cyclospora* infections. Currently, surveillance occurs within a defined population of 37.4 million Americans using information from clinical microbiology laboratories in nine states. FoodNet monitors only confirmed cases of diarrheal infection, missing cases that never present to medical attention. However, through additional surveys, FoodNet has the capacity to determine the frequency of diarrhea and the number of physician visits within the study population. Using FoodNet and other data, a recent publication from the CDC provides our current best estimate of the true burden of foodborne infections in the United States.[1]

Mead and his colleagues from the CDC estimate that there are 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths annually owing to foodborne infections.[1] This means that, on average, somewhere between 1 in 3 and 1 in 4 Americans will have a foodborne infection each year. Although these data provide an excellent estimate of disease prevalence in the United States, they also illustrate some major gaps in our knowledge of foodborne infections. For example, in the context of sporadic infections, whether a particular case is due to consumption of contaminated food or water or is acquired through person-to-person spread is difficult to ascertain. Also, in 62 million cases, or 82% of the estimated 76 million infections each year, no specific pathogen is identified. Disease attributable to unidentified agents results in 265,000 hospitalizations and 3,200 deaths.

Our ignorance as to the cause of more than 80% of the estimated foodborne illness is a daunting problem. However, many new agents have been discovered and linked to foodborne disease in the last 20 years. Table 38.2-2 offers a list of some recently described food- and waterborne pathogens. Some are newly discovered, such as *E. coli* O157 and *Vibrio cholerae* O139. Others are agents previously rec-

TABLE 38.2-1 BACTERIAL, VIRAL, PROTOZOAL, WORM, AND TOXIC AGENTS THAT ARE ASSOCIATED WITH FOOD- AND WATERBORNE ILLNESS IN HUMANS

BACTERIAL PATHOGENS
Bacteria causing disease primarily mediated by a preformed toxin
Clostridum botulinum
Staphylococcus aureus
Bacillus cereus
Bacteria causing disease by production of toxins within the intestine
Vibrio species
Clostridum perfringens
Shiga toxin–producing *Escherichia coli*
Enterotoxigenic *E. coli*
Bacteria causing disease primarily by invading the intestinal epithelial cells
Salmonella spp
Camplylobacter spp
Yersinia spp
Listeria monocytogenes
Shigella spp
Enteroinvasive *E. coli*
Other bacterial causes of foodborne illness
Aeromonas spp
Plesiomonas shigelloides
Enteropathogenic *E. coli*
Enteroaggregative *E. coli*
Enterobacter sakazakii
VIRAL PATHOGENS
Hepatitis A virus
Hepatitis E virus
Rotavirus
Noroviruses
Enteric adenovirus
Coronaviruses
Toroviruses
Reoviruses
Saporo-like viruses
Astroviruses
Parvoviruses
Picobirnaviruses
PROTOZOAL PATHOGENS
Toxoplasma gondii
Cryptosporidium parvum
Giardia lamblia
Entamoeba histolytica
Cyclospora cayetanensis
Microsporidium (*Enterocytozon bieneusi, Septata intestinalis*)
Isospora belli
Dientamoeba fragilis
Blastocystis hominis
CESTODES AND WORMS
Taenia saginata
Taenia solium
Diphyllobothrium latum
Hymenolepis nana
Ascariasis
Trichuriasis
Trichinella spiralis
NATURAL TOXINS
Ciguatera
Scrombroid
Shellfish poisoning (neurotoxic, diarrheic, and toxic-encephalopathic)
Tetrodotoxin
Mushroom toxins
Aflatoxins

TABLE 38.2-2 FOODBORNE PATHOGENS DESCRIBED SINCE 1977

Campylobacter jejuni
Campylobacter fetus ssp fetus
Cryptosporidium parvum
Cyclospora cayentanesis
Shiga toxin–producing *Escherichia coli* (eg, O157:H7, O111:H8)
Listeria monocytogenes
Norwalk-like viruses
Salmonella enteritidis
Salmonella typhimurim DT104
Spongiform encephalopathy prions
Vibrio cholerae O139
Vibrio vulnificus
Vibrio parahaemolyticus
Yersinia enterocolitica

ognized but regarded as rare or nonpathogenic organisms. For example, *Campylobacter jejuni* was once thought to be an unusual cause of bacteremia but is now one of the most frequent bacterial causes of enteritis in the United States.

In 2002, the most recent year for which the FoodNet data have been fully tabulated, the CDC confirmed 16,580 infections from the FoodNet sites.[2] Incidence varied dramatically among the FoodNet sites. For example, *Campylobacter* affected 6.7 per 100,000 people in Maryland and 31.7 per 100,000 in California. *Salmonella* infections varied from 9.5 per 100,000 in Oregon to 22.5 per 100,000 in Georgia. Although the explanation for these geographic differences is unknown, they seem to suggest true regional variation of foodborne pathogens. Another trend observed in recent FoodNet data was the preponderance of cases in the young and elderly. Children under the age of 1 year had a much higher incidence of *Salmonella* (134.1 per 100,000) and *Campylobacter* (33.5 per 100,000) than did adults. Across all age groups, clinical outcomes differed by pathogen. Although the total number of *Listeria monocytogenes* and *E. coli* O157:H7 infections was considerably lower than some of the other pathogens, they were associated with much higher hospitalization rates and death rates than any of the other bacterial pathogens monitored (Table 38.2-3). Table 38.2-3 reflects the lack of correlation between the propensity for an organism to cause disease and its propensity to result in the death of the patient.

Since FoodNet began to operate in 1996, the accumulated data have also revealed a seasonal trend, with a spike in infection with the three major pathogens (*Salmonella*, *C. jejuni*, and *E. coli* O157:H7) during the summer months (Figure 38.2-1). The summer predominance of bacterial foodborne infections is likely multifactorial. Clearly, warmer weather allows for a more rapid bacterial growth on food that is not refrigerated. Consumer habits also change in the warmer months, with more picnics and barbecues, contributing to abuse of food. One compelling study suggests that the summer spike of bacterial pathogens goes right back to the farm. Hancock and colleagues examined *E. coli* O157 shedding in 14 cattle herds at 1-month intervals for up to 13 months. The overall prevalence was 1.0% (113 of 10,832 fecal samples), and for all herds, the highest prevalence occurred in the summer months.[3]

TABLE 38.2-3 DEATH RATES OF THE COMMON FOODBORNE BACTERIA

ORGANISM	NUMBERS OF DEATHS/INFECTIONS	PERCENT DEATHS PER INFECTION
Listeria	22/105	20.9
E. coli O157:H7	7/626	1.1
Salmonella	13/4,330	0.3
Shigella	2/2,355	0.08
Campylobacter	4/4,713	0.08
Vibrio	1/54	1.85

Adapted from FoodNet, Centers for Disease Control and Prevention/US Department of Agriculture/Food and Drug Administration.[2]

The FoodNet surveillance effectively monitors trends in the rates of infection over time. In the last several years, the rates of *Campylobacter* and *Yersinia* have declined, whereas the incidence of *Salmonella* has been stable. A change in the frequency of infection with different *Salmonella* species has occurred, however, with a decline in *S. typhimurium* and a rise in less common species such as *S. newport*. Infection with *E. coli* O157:H7, *Shigella* and HUS cases have not declined in the survey period, which may be due, in part, to increased recognition. This time period coincides with the implementation of several control measures designed to limit the occurrence of bacterial food contamination, including in 1997 the USDA's Pathogen Reduction/Hazard Analysis and Critical Care Point (HAACP) system to regulate meat and poultry slaughter and processing plants. Similarly, the FDA has emphasized food safety education and introduced guidelines on better agricultural practices, refrigeration, and product labeling. The current challenge is to assess whether the improved surveillance system, through capturing more cases, masks the decline in infection rates resulting from the regulatory measures or whether there really is little change in the levels of some of these pathogens.

Although FoodNet produces excellent data on the epidemiology of foodborne illness overall, it has several important areas of weakness. It does not survey for many of the common foodborne pathogens, including viruses, which are thought to cause the majority of foodborne illness. Similarly, it does not address the cause of illness in patients who do not have a stool sample sent for analysis: those who either do not seek medical care or who do seek care but do not have a stool sample analyzed. In an adjunctive study reported by the CDC, 11% of 10,000 residents interviewed through random telephone consultations reported an episode of diarrhea during the previous month.[4] This translates to 1.4 episodes of diarrhea per person per year, which, if multiplied roughly by the population of the United States, represents approximately 375 million diarrheal cases per year. In this study, merely 8% of those with a diarrheal episode sought medical care, and of those, only 20% reported submitting a stool sample for culture. Thus, our best data on the causes of acute gastrointestinal disturbance from FoodNet surveillance are based on cultures of less than 2% of diarrheal episodes. Nonetheless, despite the current limitations of our evaluation of foodborne illness, the endeavors of federal authorities, including the CDC, FDA, and USDA, have been critical to improving our knowledge of disease frequency, pathogen epidemiology, and the establishment of control systems to limit food contamination. The knowledge gained from FoodNet surveillance allows for targeted efforts to improve food safety and education.

SPECIFIC FOOD- AND WATERBORNE MICROBES

As noted previously, the diversity of foodborne pathogens listed in Table 38.2-1 is too extensive to be described completely in the scope of this chapter. In the following sections, many of these microbial agents are discussed briefly, with a focus on typical modes of transmission, the foods they frequently contaminate, and the specific serious consequences that may ensue from infection. Although foodborne agents cause disease by a wide variety of mechanisms, often the mode of infection falls into one of the following three categories: (1) ingestion of preformed toxins produced by bacteria in food prior to consumption; (2) infection with pathogens present in food that, following ingestion, produce toxins in the gastrointestinal tract; and (3) infection with organisms with various virulence factors that permit the microbes to be invasive, cause local damage, or create physiologic perturbations that result in clinical disease.

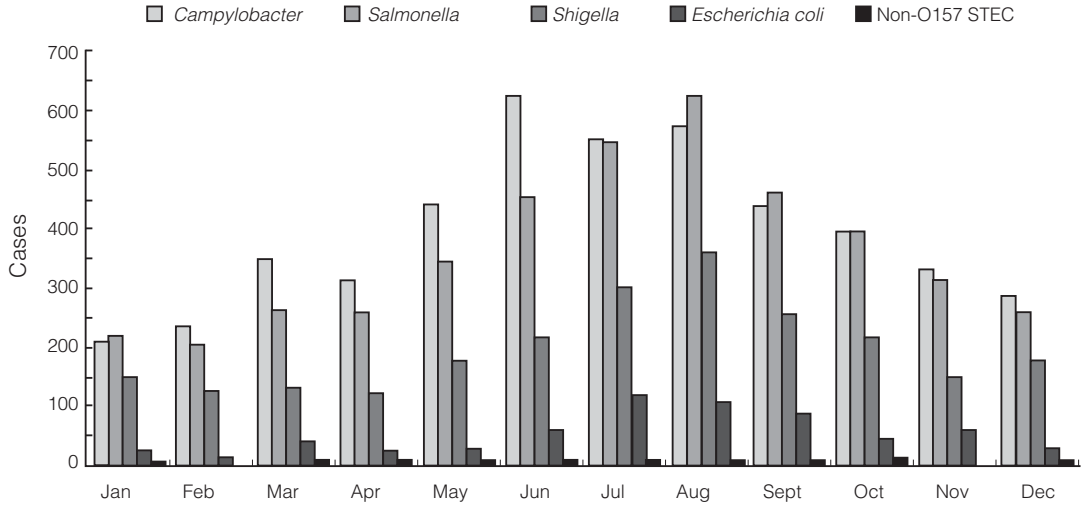

FIGURE 38.2-1 Cases of foodborne disease caused by specific pathogens, by month, FoodNet sites, 2000. Adapted from FoodNet, Centers for Disease Control and Prevention/US Department of Agriculture/Food and Drug Administration.[2] STEC = Shiga toxin–producing *Escherichia coli*.

FOODBORNE ILLNESS OWING TO PREFORMED TOXINS

Of the three mechanisms, the preformed toxin is the most consistently transmitted via food. As each toxigenic organism requires a specific environment to stimulate toxin production, each has a predilection for certain types of food. As a result, different types of foods confer different risks for toxin ingestion. The major toxins of the common foodborne pathogens are discussed in detail in the next sections and were reviewed by Sears and Kaper in 1996.[5]

Clostridium botulinum Toxins

C. botulinum produces one of the best known preformed toxins.[6] The organism is a gram-positive, spore-forming, toxin-producing, obligate anaerobe. Its natural habitat is soil; therefore, its spores frequently contaminate fresh fruits and vegetables. Commercial food sources have been occasionally incriminated, but the majority of outbreaks have been traced to home-produced canned foods, especially vegetables, fruits, fish, and condiments.[7,8] To prevent botulism, the *Clostridium* spores must be destroyed by heating food to a temperature of 120°C for 30 minutes, usually with the aid of a pressure cooker. In an anaerobic environment, any surviving spores will germinate and produce their deadly toxins.

There are seven antigenically distinct types of botulinum toxin, each of which is designated by a letter, A to G. Types A, B, E, F, and G are associated with human disease. Once ingested, the toxin is absorbed through the proximal small intestine and spreads via the bloodstream to the peripheral cholinergic nerve synapses, where it irreversibly blocks acetylcholine release. A flaccid paralysis results, with cranial nerves affected first, followed by respiratory muscle paralysis and death if left untreated.[9] Despite being a foodborne illness, the only common gastrointestinal manifestation is severe constipation. Symptoms tend to occur 18 to 36 hours after toxin ingestion but may occur as quickly as a few hours or as late as 8 days.

The diagnosis of botulism is clinical, and treatment should be initiated prior to confirmation with laboratory data because the traditional mouse bioassay for toxin detection requires approximately 4 days for final results. Samples such as food, vomitus, serum, gastrointestinal washings, and feces are all reasonable specimens to test. Newer polymerase chain reaction (PCR)- and enzyme immunoassay–based detection methods are being explored. Early in the course of the disease, treatment may include emetics or gastric lavage to remove unabsorbed toxin. A trivalent (A, B, E) horse serum antitoxin decreases the progression and duration of paralysis, but it does not reverse existing paralysis.[10] Pentavalent and heptavalent antitoxins are also being investigated. Intravenous human botulism immunoglobulin may also be beneficial.[11] Botulism carries a significant mortality rate, of up to 25%, with type A toxin. Of those who survive the acute phase of illness, most recover completely.

Staphylococcus aureus Toxin

A second well-known group of preformed toxins is those produced by *S. aureus*.[12] *S. aureus* produces a variety of enterotoxins, defined by their antigenicity as staphylococcal enterotoxin (SE)A through E. In recent years, SEG through SEO have been described, but the original strains are responsible for 95% of staphylococcal food poisoning outbreaks.[13] On rare occasions, other staphylococcal species, including coagulase-negative staphylococci, have been found to produce similar enterotoxins. The toxins are small proteins with similar tertiary structures and biologic activity, including superantigen properties.[14] Ingestion of as little as 100 to 200 ng of toxin is considered sufficient to cause disease in humans. Compared with botulinum toxin, staphylococcal toxins are not inactivated by heating or boiling, nor are they susceptible to pH extremes, proteases, or radiation. As a result, once formed in food, these toxins are almost impossible to remove.

The mechanism through which the toxin acts is not fully understood but is suspected to be via stimulation of the autonomic nervous system and gut inflammation. Because the toxin is not absorbed systemically, protective immunity is not induced following exposure. Typically, patients become symptomatic within 6 hours of ingestion, with nausea (73–90%), vomiting (82%), and abdominal cramps (64–74%). Diarrhea occurs in a large proportion of patients (41–88%), but fever is rare.[15–17] Treatment of affected individuals is supportive, and symptoms usually abate within 8 hours. There is no need to treat with antibiotics directed toward *S. aureus*.

Food is most often contaminated with *S. aureus* through the fingers or nose of a food handler. The toxin is produced when contaminated food is stored at room temperature for any length of time, thereby allowing the organism to grow and produce toxin. A number of different foods have been associated with staphylococcal food poisoning, including egg products, meat products, poultry, tuna, mayonnaise, and particularly dairy-based products such as cream-filled desserts and cakes. This disease is more frequently associated with food from the home or a service establishment rather than commercially prepared food. It has also occasionally occurred in large outbreaks with thousands of affected individuals. For example, in 2003, over 13,000 individuals in Japan became ill from a contaminated powdered milk source.[18]

Bacillus cereus Toxin

A third example of preformed toxins is that of *B. cereus*, a gram-positive, spore-forming aerobe that causes two distinct clinical syndromes: a short-incubation period emetic syndrome and a longer-incubation period diarrheal syndrome.[19] The diarrheal phase is caused by at least three enterotoxins, which are not preformed but are produced by the organism during the vegetative growth phase in the small intestine. The emetic toxin, recently named cereulide, is thought to be an enzymatically synthesized peptide produced as the organism grows in food, especially starchy foods such as rice and pasta.[20] Like staphylococcal toxin, cereulide is resistant to heat, pH variation, and proteolysis and is therefore rarely destroyed during food preparation. Its exact mechanism remains unknown, but it has been shown to stimulate the vagus afferent by binding

to the 5-hydroxytryptamine$_3$ receptor.[21] The emetic syndrome presents much like *S. aureus*–related foodborne disease, occurring 1 to 6 hours after exposure and causing nausea and vomiting. Fever is not characteristic of the illness, and full recovery usually occurs, although there has been at least one report of acute hepatic necrosis associated with exposure to emetic toxin.[22] *B. cereus* is ubiquitous in the environment, present in both soil and water, and easily spread to most raw foods; even 10 to 40% of humans are colonized with this bacterium.[23] Diagnosis can be made by finding the organism in the food or vomitus of the patient or through detection of the emetic toxin through bioassays or the enterotoxins by commercial immunoassays.

Natural Toxins

Derived from various types of food, a number of naturally occurring toxins may cause human foodborne illness. Many are associated with consumption of seafood contaminated by algae. Others are due to fungal contamination of food or inherent to certain fruits and vegetables.

Scombroid

Scombroid poisoning typically occurs after the ingestion of spoiled, dark-fleshed fish, especially tuna and mackerel.[24,25] The clinical symptoms of poisoning, including flushing, headache, palpitations, dizziness, nausea, vomiting, and diarrhea, are attributable to excess levels of histamine present in temperature-abused fish.[26] Histamine is produced by bacterial metabolism of the amino acid histidine in fish muscle. Bacterial replication and histamine production occur when fish is not frozen promptly after being caught or is stored at room temperature for several hours. A second mediator, *cis*-urocanic acid, may augment the response to histamine from spoiled fish through mast cell degranulation.[27] Symptoms of intoxication begin within minutes to several hours following ingestion. Most resolve fully within hours, but, occasionally, bronchospasm or circulatory collapse may occur. The diagnosis is clinical, and treatment consists of antihistamines. Elevated histamine levels in the contaminated fish or the patient's serum may be diagnostic, but few laboratories, other than regulatory laboratories, are equipped to undertake this analysis.

Ciguatera

Ciguatera poisoning is due to the ingestion of neurotoxins from tropical and subtropical marine fin fish, including mackerel, groupers, barracudas, snappers, amberjack, and triggerfish.[28] It affects 50,000 individuals yearly, mainly in the Caribbean and South Pacific islands.[29] The toxin is produced in reef algae, the dinoflagellates (eg, *Gambierdiscus toxicus*). It spreads through the food chain via consumption of smaller organisms and fish by larger predators, accumulating at dangerous levels in the flesh of large fish. Two groups of compounds are implicated in ciguatera fish poisoning: the lipid-soluble ciguatoxins, which activate nerve synapse sodium channels, and water-soluble maitotoxin, which induces neurotransmitter release by binding to calcium channels.[30] In humans, these toxins cause gastrointestinal symptoms 3 to 6 hours after ingestion, including nausea, vomiting, and watery diarrhea. Neurologic symptoms follow, with weakness, heat-cold temperature reversal, vertigo, ataxia, paresthesias, and dysathesias of the perioral region, palms, and soles.[31] Death and serious cardiovascular complications are uncommon. Most symptoms resolve within a week, but neurologic symptoms can persist for months. Mannitol therapy was thought to improve symptoms but did not show a significant benefit in a recent randomized controlled trial.[32] The diagnosis of ciguatera is clinical; however, the toxin can be detected in fish using a mouse bioassay or newer enzyme immunoassays.

Shellfish Poisoning

Five main types of shellfish poisoning have been described: paralytic, neurotoxic, diarrheic, amnestic, and azaspiracid.[33,34] Like ciguatera, illness is due to toxins generated by algae, usually dinoflagellates, which accumulate in the shellfish. The paralytic variant of shellfish poisoning is due to saxitoxin, an agent that blocks neuronal sodium channels and prevents propagation of the action potential. Clinically, this results in a rapid-onset, life-threatening paralysis. Brevitoxin, the agent responsible for neurotoxic shellfish poisoning, also binds sodium channels but does not cause paralysis; instead, it produces a clinical syndrome similar to but less severe than ciguatera. Symptoms of nausea, vomiting, and paresthesias occur within hours of exposure and resolve completely within 3 days. Diarrheic shellfish poisoning causes gastrointestinal disturbance with nausea, vomiting, and diarrhea. The toxin acts by increasing protein phosphorylation.[30]

Amnestic shellfish poisoning, also known as toxic-encephalopathic poisoning, causes outbreaks of disease in association with consumption of mussels.[35] Manifestations include nausea, vomiting, diarrhea, severe headache, and, occasionally, memory loss. The toxin domoic acid is a glutamate receptor agonist that causes excitatory cell death. In 1995, a new shellfish poison was discovered in Ireland, where several individuals became sick with nausea, vomiting, diarrhea, and abdominal pain after eating mussels. The illness resembled diarrheal shellfish poisoning, but chemical analysis revealed little diarrheic shellfish poisoning toxin. A new class of compound, the azaspiracids, was isolated. These are derived from a phytoplankton previously thought to be benign.[36]

Diagnosis of human illness due to shellfish toxins is clinical based on symptom profile and prompt onset of symptoms after shellfish consumption. The exception to this is amnestic poisoning, which may not cause symptoms until 24 to 48 hours after exposure. The toxins can be detected using either mouse bioassays or by high-performance liquid chromatography (HPLC), but this is done primarily for research purposes or in monitoring. Owing to the serious consequence of shellfish poisoning, large-scale surveillance systems for contamination of shellfish populations have been implemented.

Tetrodotoxin

Tetrodotoxin is present in certain organs of the puffer fish and if ingested can cause rapid paralysis and death. Symp-

toms may occur in as little as 20 minutes or after several hours. The illness progresses from gastrointestinal disturbance to almost total paralysis, cardiac arrhythmias, and death within 4 to 6 hours after ingestion of the toxin. The diagnosis is clinical and based on history of exposure. Mouse bioassays and HPLC have been used to detect tetrodotoxin in food.

Aflatoxins

Aflatoxins are produced by certain strains of fungi (eg, *Aspergillus flavus* and *Aspergillus parasiticus*) that grow in various types of food. Most human exposure occurs through mold-contaminated corn or nuts, especially tree nuts (Brazil nuts, pecans, pistachio nuts, and walnuts), peanuts, and other oilseeds. Because mycotoxins can be produced prior to or after harvest, eliminating them from food is nearly impossible.[37] Aflatoxin B1 is the most common and toxic, but there are several types of toxins (B2, G1, and G2). They are potent mutagens and carcinogens, with B1 causing deoxyribonucleic acid (DNA) damage in the *P53* tumor suppressor gene.[38] Exposure to the aflatoxin predisposes the patient to hepatocellular carcinoma, especially in conjunction with chronic hepatitis B infection.[39] With a high ingested dose of aflatoxin, a condition known as aflatoxicosis may occur, characterized by fever, jaundice, abdominal pain, and vomiting. Aflatoxin exposure is common in Asia and parts of Africa but uncommon in the United States. The diagnosis is clinical, but assays exist to detect the toxins in food. Serum and urine markers also have been developed to quantify exposure.

Other Natural Toxins

A number of other naturally occurring foodborne toxins have been reported. Grayanotoxin derives from honey produced from rhododendrons and causes self-limiting nausea, vomiting, and weakness. The Jamaican akee fruit contains hypoglycin A, resulting in vomiting and hypoglycemia. The bitter cucumber contains curcurbitacin E, which causes cramps and diarrhea 1 to 2 hours after ingestion. Hydrogen cyanide may be present in lima beans or cassava root and can lead to death within minutes. Castor beans can contain a hemagglutinin that may cause nausea and vomiting. Red kidney beans also produce a hemagglutinin known as phytohemagglutinin. Illness occurs after eating raw or undercooked red kidney beans and consists of severe nausea, vomiting, and diarrhea. The toxin is heat sensitive but requires a high temperature to be inactivated; as a result, a number of cases have been linked to beans cooked in slow cookers in which the temperature does not get high enough. Many other agents, such as toxins, chemicals, and heavy metals, may also cause foodborne illness but are not discussed further here.

FOODBORNE MICROBES THAT PRODUCE TOXINS FOLLOWING INGESTION

Vibrio

Currently, there are over 40 *Vibrio* species, a group of gram-negative marine organisms, most of which are not human pathogens. The most common and severe human illness is caused by *V. cholerae* O1, the species responsible for seven cholera pandemics. The previous six were caused by the "classic" biotype, and the seventh pandemic, which began in 1961, was caused by the "El Tor" biotype.[40,41] Because *Vibrio* species inhabit aquatic ecosystems, sporadic infection and pandemics are typically due to contaminated water. In the United States, cholera is mainly acquired through consumption of Gulf Coast seafood or through foreign travel. A clean water supply is critical to cholera prevention because the organism is resistant to washing, refrigeration, and freezing of a wide variety of seafood and fresh produce.[42] Simple water filtration for particulates greater than 20 μ reduced cholera infection by 48% in a Bangladesh study.[43]

Because stomach acidity does kill many of the organisms, more than 10^6 *V. cholerae* are usually required for infection; those with decreased gastric acidity are infected with lower doses. The incubation period is usually 1 to 3 days but may be as short as a few hours or as long as 5 days. Infection causes voluminous watery diarrhea. Hypotension and shock may result within the first 12 hours of infection. The primary virulence factor is the cholera toxin, which targets an intestinal G protein, producing cyclic adenosine monophosphate (cAMP). The increase in cAMP produces watery diarrhea by inhibiting intestinal sodium absorption and increasing chloride and bicarbonate secretion.[44] The toxin is transmitted to the organism via a bacteriophage. Indeed, in recent years, a new pathogen, *V. cholerae* O139, evolved in the Indian subcontinent. Non-O1 strains were not previously associated with human epidemics, but this pathogen appears to have acquired the cholera toxin and other virulence factors through horizontal transmission and bacteriophage infection.[45,46] Recent large epidemics of this organism in Africa and South East Asia may represent the beginnings of an eighth cholera pandemic.

Vibrio parahaemolyticus also inhabits marine environments and is acquired principally through the ingestion of shellfish. This *Vibrio* has been a major foodborne pathogen in Japan but is less common in the United States. Infection is characterized by diarrhea, abdominal cramps, nausea, and vomiting, with fever and chills present in about 25% of cases. Dysentery occurs in a minority of patients, more often in children than in adults.[47] Occasionally, wound infections and septicemia occur. Symptoms may appear in as little as 4 hours but are typically present 12 to 24 hours after exposure. Disease is attributed to a 23 kD protein called thermostable direct hemolysin.[5] Recently, studies have also located type III secretion system (TTSS) genes in the bacterial genome. TTSS is a virulence factor causing inflammatory diarrhea in other invasive bacteria such as *Shigella*, *Salmonella*, and enteropathogenic *E. coli*; significant enteric inflammation has been documented in *V. parahaemolyticus* infection.[48,49] The enteritis is usually self-limiting. Patients require fluids, and antibiotics may be useful if intestinal symptoms persist.

Vibrio vulnificus is another free-living estuarine organism that is frequently isolated from shellfish, most often acquired through raw oyster or clam consumption. It is the most

common life-threatening *Vibrio* infection in the United States. Individuals with diabetes, immunosuppressive disorders, and liver disease, including hemochromatosis and alcoholic liver disease, are especially susceptible to infection.[50,51] In these groups, the case-fatality ratio may exceed 50%. Infection presents with fevers, chills, nausea, vomiting, and diarrhea. Hypotension and sepsis ensue. Large hemorrhagic bullae erupt and progress to necrotic ulcers. *V. vulnificus* is an encapsulated organism and is therefore resistant to the bactericidal activity of normal human serum. The organisms are sensitive to the amount of transferrin-bound iron in the host, which may explain the increased susceptibility in patients with hemochromatosis. Definitive diagnosis may be made from blood, stool, or wound cultures. Owing to the severity of infection, antibiotics should be initiated promptly. *V. vulnificus* is susceptible to many antimicrobials, including tetracycline, ciprofloxacin, trimethoprim-sulfamethoxazole, ampicillin, and chloramphenicol.

Clostridia

Clostridium perfringens is an anaerobic, spore-forming, gram-positive rod associated with two main types of foodborne disease. The species has been divided into five distinct types, A to E. Type A causes the majority of human infections and is usually linked to the consumption of meat or poultry (typically high-protein foods) that have been stored between 15° and 60°C for more than 2 hours.[52,53] At this temperature, clostridial spores germinate and begin vegetative growth. At an infective dose of 10^5 vegetative cells, ingested clostridial spores transiently colonize portions of the intestine and produce enterotoxin. Ingestion of preformed toxin or nongerminated spores will not usually result in disease. The enterotoxin (CPE) is a heat-labile 35 kD protein encoded by the *cpe* gene. *C. perfringens* types A, C, and D all carry this gene, but for unclear reasons only type A is frequently associated with foodborne disease. CPE functions by a complex mechanism, inserting itself into the host cell membrane and altering membrane permeability.[5] Clinically, diarrhea and severe abdominal cramps develop 6 to 14 hours after exposure; vomiting and fever are less common. Diagnosis is complicated by the presence of *C. perfringens* in the bowel microflora of many asymptomatic individuals.[54] However, a number of tests are able to detect the enterotoxins in stool, including enzyme immunoassays or latex agglutination.[55]

C. perfringens type C causes a distinct foodborne illness, mainly in developing countries. It causes a necrotizing enterocolitis seen in the context of malnutrition. The type C strains produce enterotoxin and types "α" and "β" toxins. The β toxin appears to be responsible for the cell necrosis associated with infection. As the β toxin is inactivated by intestinal proteases, illness occurs in patients in whom these enzymes are inadequate (eg, in malnutrition) or in the presence of trypsin inhibitors found in undercooked pork or sweet potatoes.

Escherichia coli

The two main *E. coli* species associated with foodborne illness are STEC and enterotoxigenic *E. coli* (ETEC). The former are relative newcomers to the scene of foodborne pathogens. The first STEC to be associated with disease in humans was *E. coli* O157:H7 following two outbreaks of hemorrhagic colitis in 1982. Since then, at least 60 different types of STEC have been associated with clinical disease and have become recognized as the most common cause of HUS. Studies suggest that approximately 1% of samples submitted to clinical microbiology laboratories in the United States contain STEC, of which two-thirds are O157:H7 and the remainder non-O157.[56] These bacteria colonize the intestinal tracts of many mammalian species, particularly ruminants (cattle, sheep, and goats). Most human illness is due to the ingestion of contaminated bovine products, but an increasing number of reports associate infection with fecally contaminated fresh produce (lettuce, alfalfa sprouts, apple cider) and water. The main virulence factors of STEC are bacteriophage-encoded Shiga toxins (Stx). The two main types are the Stx1 and Stx2, but there are at least five subtypes of Stx2 (2c, 2d, 2e, and 2f). The infectious dose of STEC is very low, in the region of 10 to 100 organisms. Following ingestion, the bacteria colonize portions of the lower intestinal tract and produce toxin. Stx crosses the intestinal epithelial cell barrier and damages distant target sites, especially the kidney and brain, by a direct effect on endothelial cells in the microvasculature. These distant effects are responsible for HUS. Symptoms typically develop 2 to 4 days after ingestion but may occur in as little as 1 day or as long as 8 days. Nonbloody or bloody diarrhea is the primary acute manifestation.

Treatment of STEC and its major complications is currently largely supportive. Controversy exists as to the role of antibiotics, with concern that treatment of pediatric patients with certain antimicrobials (eg, fluoroquinolones and trimethoprim-sulfamethoxazole) may actually increase the likelihood of serious complications such as HUS.[57] Several recent reviews relating to foodborne *E. coli* infections have been written, and the reader is referred to them for more details.[58–60]

ETEC infection is a common cause of disease in developing countries and is frequently associated with traveler's diarrhea. Like many other *E. coli* strains, ETEC are transmitted through contaminated water and food. They have caused a number of large outbreaks in the United States; however, their importance in sporadic disease is not known. Incubation periods range from 12 hours to 2 days, and typical symptoms are abdominal discomfort and watery, nonbloody diarrhea without fever. ETEC have two significant virulence characteristics: the ability to colonize the intestine and the capacity to produce enterotoxins. A variety of colonization factor antigens and two different types of toxins, known as heat-stable and heat-labile toxins, have been found in ETEC. The heat-stable group consists of small peptides that affect intracellular concentrations of cyclic guanosine monophosphate. The heat-labile toxins are structurally and functionally much like cholera toxin. Oral rehydration is the mainstay of treatment and is often lifesaving. Antibiotic therapy is not routinely required.

FOODBORNE INFECTIONS THAT CAUSE DISEASE BY MECHANISMS OTHER THAN TOXIN PRODUCTION

An enormous diversity of microbes fall into this category, including bacterial, viral, and protozoal organisms. Many of these agents are discussed in great detail in other chapters and are addressed only briefly here.

SALMONELLA

Salmonella species are one of the most common causes of foodborne illness in humans. They can be divided into two broad categories: those that cause typhoid and those that do not. The typhoidal *Salmonella*, such as *S. typhi* and *S. paratyphi*, colonize humans and are acquired through the consumption of food or water contaminated with human fecal material. The much larger group of nontyphoidal *Salmonella* is found in the intestines of other mammals and therefore is transmitted through food or water that has been contaminated with fecal material from a wide variety of animals and poultry. More than 2,300 species of *Salmonella* are differentiated by their somatic (O) and flagellar (H) antigens. Many isolates have been named after the towns in which they were first discovered or by the individuals who first discovered them.

In the United States, most typhoid is the result of food contamination by an asymptomatic chronic carrier or from foreign travel. Typhoid fever continues to be a global health problem but is less common in the United States; only 60 outbreaks occurred between 1960 and 1999.[61] In contrast, the number of cases of nontyphoidal *Salmonella* increased steadily over the last four decades. *Salmonella enteritidis* infection owing to contamination of hen eggs is a particular problem, with an estimated contamination rate of 1 in 10,000 eggs. The bacteria penetrate intact eggs lying in fecal material or infect them transovarially before the shell is formed.[62] Other common sources of nontyphoidal salmonellosis are milk, foods prepared with raw eggs, meat, poultry, and fresh produce. To reduce contamination, the agricultural industry has implemented many safeguards. For example, Promsopone and colleagues used spray vaccination of young chicks with a combination of avian-specific probiotic and *S. typhimurium*–specific antibodies and found a significant reduction in the cecal and colonic concentration and fecal shedding of *S. typhimurium*.[63] Another study using a commercial preparation known as PREEMPT showed a similar reduction of *S. gallinarum*, with reduced mortality in the vaccinated chicks.[64]

The infectious dose of *S. typhi* is thought to be around 10^5 organisms. Typhoid infection is characterized by high fevers, abdominal discomfort, and a rose-colored macular rash. The infective dose of nontyphoidal *Salmonella* may vary from less than 100 to 10^6 depending on the host, food vehicle, and type of *Salmonella*. These species tend to cause bloody or nonbloody diarrhea, fever, nausea, vomiting, and abdominal discomfort. In all types of *Salmonella*, the most critical virulence determinant is their ability to cross the intestinal epithelium and cause invasive disease. The most concerning problem regarding *Salmonella* is the emergence of multidrug-resistant strains. For example, *S. typhimurium* phage type DT104 is resistant to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline. In Europe, quinolone-resistant strains of *Salmonella* have been detected. Some of these issues are reviewed by Poppe and colleagues.[65]

CAMPYLOBACTER

Campylobacter, which was not recognized as a foodborne disease until the mid-1970s, is now one of the most common bacterial foodborne infections diagnosed in the United States.[66–68] *Campylobacter* are gram-negative, spiral, microaerophilic organisms. The two species *C. jejuni* and *C. coli* are responsible for the vast majority of human disease, with *C. jejuni* causing 90% of infections and *C. coli* nearly 10%. *C. fetus*, *C. upsaliensis*, *C. hyointestinalis*, and *C. lari* have occasionally been associated with enteritis. In human studies, infectious doses as low as 100 organisms may result in disease, and one drop of chicken juice may contain 500 infectious organisms.[69] *Campylobacter* species are more frequently associated with sporadic disease than outbreaks, and person-to-person spread does not appear to be common. *C. jejuni* and *C. coli* are intestinal commensals in many animals and birds, including domestic pets. The main vehicle for human infection is poultry, but other raw meats, milk, and water have also been implicated. Surface water can be contaminated with *Campylobacter*, and waterborne outbreaks have been reported.[70]

The pathogenicity of *Campylobacter* depends on its motility; in vitro, nonmotile strains are not capable of invading intestinal epithelial cells. Typical infection causes diffuse colonic inflammation with marked inflammatory cell infiltration of the lamina propria, which may be mistaken for inflammatory bowel disease.[71] Symptoms usually occur within 2 to 3 days after exposure but may occur as quickly as 10 hours or as late as 7 days.[72] High fevers, headache, and myalgias may precede the onset of nausea, vomiting, and diarrhea. The diarrhea may be loose and watery or grossly bloody. Abdominal cramps and pain may predominate. Interestingly, the disease is sometimes biphasic, with an apparent settling of symptoms after 4 to 5 days followed by a recrudescence. Local complications resulting from direct spread of the organisms from the intestinal tract include cholecystitis, hepatitis, acute appendicitis, pancreatitis, and focal extraintestinal infections. The case-fatality rate is low, approximately 0.5 per 1,000 infections. However, long-term complications may occur, including reactive arthritis, uveitis, HUS, and, most importantly, GBS. GBS affects 1 to 2 persons per 100,000 in the United States each year, or less than 1 person per 1,000 infected.[73] The extensive axonal injury and potentially irreversible neurologic damage following infection are likely the result of molecular mimicry of *C. jejuni* antigens and myelin proteins or peripheral nerve glycolipids.[71,74] GBS typically develops 1 to 3 weeks after infection.

Diagnosis of *Campylobacter* is confirmed by stool culture. PCR and enzyme immunoassays are now available and may become useful for species-specific antigen detection. As with *Salmonella*, a growing number of *Campylobacter* are developing antimicrobial resistance.[75]

Yersinia

Of the three members of the genus *Yersinia*, *Y. enterocolitica* and *Y. pseudotuberculosis* are considered to be foodborne pathogens, whereas *Y. pestis* is typically not.[76,77] Overall, *Yersinia* cause less foodborne illness than *Salmonella* or *Campylobacter*, and the majority of isolates in food, environmental samples, and human stool are nonpathogenic species. One of the challenges, therefore, has been how to determine the pathogenicity of an isolated organism.[78] *Y. enterocolitica* is divided into biogroups, with more than 50 "O" antigens used to designate strains. Most human disease is associated with serotypes O3, O5, O8, or O9. *Y. enterocolitica* is an invasive organism. All pathogenic strains carry a plasmid pYV, coding for the virulence proteins *Yersinia* outer proteins and adhesin A, which block phagocytosis, opsonization, and complement activation; and *Yersinia* enterotoxin, invasin, and attachment-invasion proteins, which mediate invasion and serum resistance.[79] A variety of tests can be used to determine if a strain is pathogenic, including PCR and DNA hybridization, Congo red absorption, salicin fermentation, and esculin hydrolis.

Y. enterocolitica infection results in a mesenteric lymphadenitis, enteritis, and diarrhea. Most infections are self-limited, but symptoms can be prolonged, lasting several weeks or longer. Complications such as ulceration and intestinal perforation may occur. The classic long-term complication following yersiniosis is the development of reactive arthritis, occurring most commonly in patients who are HLA-B27 positive. Although antibiotic therapy is not routinely required, many antimicrobials are effective; ceftriaxone or fluoroquinolones are recommended for serious infection. *Yersinia* infection is most frequently associated with raw or undercooked pork consumption.[80,81] Swine are the major reservoir of these organisms, although they have been found in sheep, dogs, cats, and cattle. Milk is a frequently reported source, and because *Y. enterocolitica* can survive and indeed multiply in milk at 4°C, small numbers of organisms can become a significant health threat, even if the milk is refrigerated.

Infection with *Y. pseudotuberculosis* has also been associated with consumption of contaminated water or unpasteurized milk. Six serotypes and four subtypes of *Y. pseudotuberculosis* have been described. The clinical picture is similar to that of *Y. enterocolitica*.

Listeria

Listeria monocytogenes is one of the most concerning of foodborne pathogens because of the high mortality rate associated with infection.[82,83] Of the 1,800 cases per year estimated to occur in the United States, there are over 400 deaths, giving a case-fatality rate of over 20%. Of the seven *Listeria* species, only *L. monocytogenes* is a significant human pathogen. It is common in the environment, present in soil, in water, on plants, and in the intestinal tracts of many animals. Thirty-seven different types of mammals, at least 17 species of birds, and between 1 and 10% of humans are carriers of *Listeria*. Although the organism is readily killed by heat and cooking, the fact that it is ubiquitous makes recontamination a real risk. The organism is able to grow and multiply at refrigerator temperatures, so even minor contamination of a product may result in high levels of bacteria after storage. The infectious dose is not known. The more critical determinant of *Listeria* infection is likely individual susceptibility, with the elderly, pregnant women, the immunocompromised, and newborns having higher rates of infection and higher mortality rates.[84–86]

Foods incriminated in *Listeria* infection include milk, cheese, raw vegetables, undercooked meat, and even ready-to-eat foods such as hot dogs.[87] Human infection occurs sporadically and in outbreaks. Infected individuals suffer a mild, transient enteritis 2 to 3 days after contaminated food is consumed. Most immunocompetent adults have no further symptoms. Susceptible individuals may suffer, after a period of days, fevers and mylagias, septicemia, meningitis, or encephalitis. Pregnant women have a 12-fold increased risk of infection, and transplacental transmission may cause spontaneous abortion, premature birth, neonatal sepsis, and meningitis.[88] Once the diagnosis is established, *L. monocytogenes* is readily treated by penicillins or aminoglycosides.

L. monocytogenes has also been associated with a febrile enteritis and linked with a variety of food items. Generally, such episodes are self-limiting and do not lead to the listeriosis. It is unclear how frequent *L. monocytogenes* causes enteritis because it is not an organism that is routinely looked for in this context.[89,90]

Shigella

Shigellae are unusual in that they are not present in fecal material from animals such as poultry, beef, and pork and are therefore not transmitted in the same manner as *Salmonella*, *Campylobacter*, or *E. coli*. Instead, these bacteria are highly host adapted, infecting only humans and some nonhuman primates. Transmission occurs when a food product is contaminated by human fecal material. There are four different species of shigellae (*S. dysenteriae*, *S. flexneri*, *S. sonnei*, and *S. boydii*), and all cause human disease. In the United States and other developed countries, most infection is due to *S. sonnei*, although *S. flexneri* is also common. One of the most striking features of shigellosis is the very small inoculum of organisms required to cause disease: as few as 10 to 100 of the most virulent genus, *S. dysenteriae*, are sufficient to cause dysentery in healthy adult volunteers. This low infectious dose permits person-to-person spread, with approximately 20% of persons in a household becoming infected when an index case is identified in a family.[91] Given that these organisms are not typically present in food other than through human contamination—either directly during food preparation or indirectly from contamination with human fecal material—all shigellosis could be considered to be due to person-to-person spread.

A variety of foods have been implicated in the spread of *Shigella*, including salads (potato, tuna, shrimp, macaroni, and chicken), raw vegetables, dairy products, poultry, and common-source water supplies. In 1998, eight restaurant-associated outbreaks of shigellosis revealed contamination of parsley imported from a common farm in Baja, Califor-

nia.[92] At a wake in Australia, 13 of 32 people developed shigellosis: *S. sonnei* was found in 4 people and *S. dysenteriae* type 2 in 1 person. Ham was considered to be the vehicle, and the person who handled the ham had recovered earlier that week from a diarrheal illness of unknown type.[93] Twenty-four individuals in southeastern Texas were infected with *Shigella* after eating oysters. All consumed oysters in the previous 5 days from different restaurants, but all of the implicated restaurants obtained oysters from the same supplier. Indeed, all of the contaminated oysters were traced to a single boat. One of the crew was found to be an asymptomatic carrier of *S. sonnei* similar to the strain that infected the 24 people. Subsequent investigation found that the toilets on the boat were 5-gallon pails that were tipped overboard in the oyster harvesting area after use.[94]

Shigella often cause bloody diarrhea. Some species carry the Shiga toxin and may cause a HUS similar to *E. coli* O157:H7. Treatment with antibiotics shortens the duration of fever, diarrhea, and bacteremia and reduces the risk of lethal complications. It also shortens the duration of pathogen excretion in stool, thereby limiting the spread of infection. A recent concern, however, is the increasing antibiotic resistance of *Shigella* species. Antibiotic resistance occurs quickly in *Shigella*, attributed to horizontal transfer of resistance genes on integrons.[95] Multidrug-resistant isolates have been discovered in several developing countries.[96]

Other Bacterial Agents that May Be Foodborne and Waterborne

Enteroinvasive *E. coli* (EIEC) is not frequently recognized as a foodborne pathogen, but infection has been linked to water and other foods, such as cheese.[97] EIEC causes morbidity and mortality in young children in developed countries but is a more important menace in developing countries owing to poor hygiene and sanitation. A number of prominent serogroups found to be EIEC have been described, including O28, O112, O124, O136, O143, O144, O147, and O164. Clinically, EIEC produces disease similar to shigellosis, with a watery diarrhea or dysentery. Like shigellae, they invade colonocytes and cause an intense inflammatory response by means of several virulence factors encoded on a 120 to 140 MD plasmid.[98] EIEC do not, however, produce Shiga toxins. EIEC should be considered in those subjects with dysentery and substantial fecal leukocytes, in whom other invasive organisms have been ruled out.

Enteropathogenic *E. coli* (EPEC), like *Shigella* species, is transmitted mainly by the fecal-oral route from one infected individual to another.[59] EPEC has no known animal reservoir and is transmitted via food and water once contaminated by an infected person. EPEC is a major cause of infantile diarrhea worldwide but affects mostly the developing world.[98] The organisms have caused major outbreaks in various developed countries, but their role in sporadic disease is unknown because we lack routine diagnostic testing for these bacteria. Clinically, EPEC infection presents with a watery, nonbloody diarrhea. Low-grade fever and vomiting are common. In the developing world, mortality rates may be high, especially among infants.

Enteroaggregative *E. coli* (EAEC) get their name from the way in which they adhere to epithelial cells in culture, in a "stacked brick" pattern.[59] These bacteria have been associated with an acute or persistent diarrhea among immunocompromised patients and in developing countries. Currently, there is no known animal reservoir for EAEC, and fecal-oral spread from one person to another is considered to be the usual route of transmission. As with EPEC, contamination of food and water from infected individuals is probably important. In human immunodeficiency virus (HIV) patients with persistent EAEC-associated diarrhea, antibiotic treatment has resulted in clearing of the organisms and improvement in symptoms,[99,100] suggesting that these bacteria are true pathogens, but they may be more opportunistic than other foodborne bacteria.

Aeromonads are gram-negative, facultatively anaerobic, motile, oxidase-positive bacilli that have been associated with foodborne illness.[101,102] They are present in soil, freshwater, and sewage and can contaminate fresh produce, meat, and dairy products. The infection rate tends to peak during the summer months. Of the various species, *Aeromonas hydrophila*, *A. caviea*, *A. veronii*, and *A. jandaei* are most frequently associated with acute enteritis and foodborne infections. All typically cause persistent watery diarrhea. Patients often have abdominal pain, and dysenteric-like symptoms can occur, but fecal leukocytes and red cells are usually absent from stool. Nausea, vomiting, and fever may occur in up to 50% of patients.[102] Stool cultures may be unreliable in making the diagnosis because asymptomatic carriage of *Aeromonas* has been reported. Immunologic tests can confirm recent infection. However, because infection is usually self-limiting and full recovery occurs in most healthy individuals without antimicrobial therapy, making the diagnosis is often of academic interest only. The exception may be the patient with persistent diarrhea in whom no other cause has been identified.

Plesiomonas shigelloides was placed in its own genus in 1962.[102] It is primarily a freshwater organism, and, like *Aeromonas*, the isolation rates increase in warmer months. Plesiomonads are gram-negative, motile, and facultative anaerobes. Initially, these species were regarded as nonpathogenic bacteria, but studies in Asia have demonstrated an association between the consumption of contaminated food and diarrheal illness owing to *Plesiomonas*. One review suggests that this organism may cause traveler's diarrhea in Japan and that its frequency is increasing.[103] Symptoms occur 24 to 48 hours after exposure and are thought to be linked to the production of an enterotoxin. Infection is associated with abdominal cramping and occasionally with bloody stools. Nausea, vomiting, and fever are less common. Fluid resuscitation is important in treating *Plesiomonas* infection, but antimicrobials are seldom required. If antibiotics are required, most strains are sensitive to quinolones and trimethoprim-sulfamethoxazole.

Protozoal Foodborne Pathogens

A number of protozoa have been associated with consumption of contaminated food and water. *Cryptosporid-*

ium parvum is an apicomplexan protozoan parasite that causes diarrhea in both immunocompetent and immunocompromised individuals.[104,105] Its pathogenic potential in immunocompromised patients first became evident during the early acquired immune deficiency syndrome (AIDS) epidemic. Its ability to affect healthy individuals was confirmed in 1993, when more than 400,000 people in Milwaukee developed cryptosporidiosis as a result of contaminated municipal drinking water.[106] This infection is endemic in developing countries and is a common cause of persistent diarrhea in young children. Cryptosporidia are typically waterborne, but foodborne and person-to-person spread have occurred. The primary reservoirs are bovine and human. Symptoms tend to occur 5 days after ingestion of the oocysts. Once ingested, the oocysts release four sporozoites, which then attach to and invade intestinal epithelial cells, especially in the jejunum and ileum. As a result, infection may be missed by diagnostic evaluation such as endoscopy. The diagnosis is made by a modified acid-fast or Kinyoun stain for oocysts in the stool or using commercially available immunofluorescence assays.[107]

Typically, cryptosporidiosis causes watery diarrhea, abdominal cramping, nausea, and vomiting. Fever is infrequent. In the immunocompetent, infection is self-limiting, and recovery is the rule after a week or two. Immunocompromised hosts do not clear the infection, and malabsorption may become a significant and life-threatening problem. Unfortunately, there is no known treatment for *C. parvum* infection, and current methods of water purification are ineffective for removal of the organism from the public water supply.

Giardia lamblia is probably the most common enteric protozoan worldwide.[108] Although it may not cause dramatic enteric disease and has few systemic complications, giardiasis can lead to profound malabsorption and misery. Only *G. lamblia* is known to infect humans. Like other enteric protozoa, it is transmitted via the fecal-oral route and is most commonly spread through contaminated water. Disease is caused by ingestion of cysts, which excyst in the proximal small intestine and release trophozoites. The trophozoites divide by binary fission and attach intimately to the intestinal epithelium via a ventral disk. The infectious dose is as low as 10 to 100 organisms. Clinical symptoms vary greatly; infection may be asymptomatic or, at the other extreme, may result in substantial abdominal discomfort, chronic diarrhea, protein-losing enteropathy, and intestinal malabsorption. *G. lamblia* can be diagnosed by fecal microscopy looking for either cysts or trophozoites. Currently, many laboratories use commercially available kits using either fluorescence microscopy with specific antibodies or enzyme immunoassays. Metronidazole is the drug of choice for treatment.

Entamoeba histolytica is the second leading cause of parasitic death in the world, with more than 40,000 deaths annually.[109] It is spread through fecal contamination of food and water or by person-to-person contact. Amebic cysts are the infectious agent. They may survive for weeks in an appropriate environment. Following ingestion, they pass unharmed through the stomach, travel to the small intestine, and excyst to form trophozoites. The trophozoites then colonize the large bowel and either multiply or encyst, depending on local conditions. The trophozoites invade the colonic epithelium, resulting in ulceration of the mucosa and amebic dysentery. They may also spread hematogenously to the portal circulation, causing parenchymal liver damage and amebic abscesses. The onset of symptoms in amebic dysentery may be gradual, initially presenting with mucoid stools and constitutional symptoms before progressing to bloody stools, abdominal pain, and fever. Amebic abscesses may develop months to years after exposure.

There are two types of *Entamoeba*: *E. histolytica*, which is pathogenic, and *E. dispar*, which is a commensal. Microscopic examination of the stool has been the standard technique used to diagnose amebic dysentery, but this technique cannot distinguish between the two species. In the patient with classic symptoms of amebic dysentery, this distinction may not be important. However, enzyme-linked immunosorbent assay and stool PCR techniques are now commercially available and allow specific identification of *E. histolytica*. Once the diagnosis is made, in the United States, metronidazole is the only drug available for treatment. In fulminant amebic colitis, luminal agents such as paromomycin or iodoquinol should be used to eliminate bowel colonization. Amebic abscesses can be eliminated with antiparasitic agents alone; percutaneous drainage should be reserved for those who do not improve with metronizadole therapy or those with large left-lobe abscesses.[109]

Cyclospora cayetanensis is a recently described apicomplexan parasite that has been found in food. Most recently, it has caused a number of outbreaks in North America; in 1996 to 1999 associated with consumption of imported raspberries. *Cyclospora* has also been associated with other fresh produce, undercooked meat and poultry, and contaminated drinking and swimming water. In immunocompetent patients, *Cyclospora* infection results in a self-limiting diarrhea with nausea, vomiting, and abdominal pain. In immunocompromised patients, there can be a chronic cycle of diarrhea with anorexia, malaise, nausea, and abdominal discomfort followed by transient remissions. Both *Cyclospora* and *Cryptosporidium* infections have been linked to a number of postinfectious complications, including GBS, reactive arthritis, and acalculous cholecystitis.[110] Infection is diagnosed through detection of oocysts in stool by direct stool microscopy and oocyst autofluorescence. The infection can be treated successfully with trimethoprim-sulfamethoxazole.

A number of other protozoa have been associated with food- and waterborne infections in humans. *Microsporidium* causes watery diarrhea and malabsorption in the immunocompromised host. Various microsporidia species, including *Enterocytozoon bieneusi* and *Septata intestinalis*, cause human disease. The apicomplexan protozoan *Isospora belli* also causes diarrhea in the immunocompromised host. Sarcocystosis is a rare zoonotic infection that, on occasion, causes a necrotizing enteritis in humans. Although *Dientamoeba fragilis* was originally thought to be a commensal, there are increasing data to indicate that it is pathogenic, causing abdominal pain, nausea, diarrhea, and anorexia. *Balantidium coli* is the only ciliate known to par-

asitize humans. Most infections are asymptomatic, but dysentery can occur. *Blastocystis hominis* is a strict anaerobic protozoan that infects both immunocompetent and immunocompromised hosts and results in a variety of gastrointestinal symptoms, including diarrhea, abdominal pain, nausea, vomiting, anorexia, and malaise.

Cestodes and Worms

A variety of cestodes and worms may be transmitted in food.[111–114] The beef tapeworm *Taenia saginata* is highly endemic in parts of South America, Africa, and Asia. These worms may live as long as 20 years and grow up to 25 meters in length. Humans are their definitive host, whereas cattle represent an intermediate host. In the cow, hexacanth embryos emerge from eggs and pass by blood or lymph to muscle, subcutaneous tissue, or viscera. When humans consume raw or undercooked beef, they are infected with the living larval forms, and the life cycle is completed. In the human intestine, the worms are remarkably quiescent, typically causing nausea or a sense of fullness. However, vomiting and diarrhea may occur. Worms can be eradicated with praziquantel or albendazole. The diagnosis is made by detecting the proglottids in stool.

The pork tapeworm *Taenia solium* is distributed worldwide and is acquired by ingesting pork containing the infectious cysts cysticerci. The adult worm in humans sheds proglottids, which are then eaten by pigs. In the pig, the hexacanth embryos emerge, penetrate the intestinal wall, and migrate to the muscle and other tissues. With consumption of raw or undercooked pork, humans acquire the larval forms. These larval forms, unlike those of *T. saginata*, are capable of migrating to the human nervous system. In the brain, the larvae encyst, become calcified, and may cause seizures. However, in a recent study of a hyperendemic region of Mexico, 9% of the population had calcified lesions on computed tomographic scan and were asymptomatic.[115] *T. solium* is smaller than *T. saginata*, and gastrointestinal symptoms are even less remarkable. Infected individuals often make the diagnosis by noticing proglottids in their stool. The diagnosis is confirmed by identifying the proglottids, and treatment consists of either praziquantel or albendazole.

Diphyllobothrium latum is a fish tapeworm, most commonly found in Northern Europe and Scandinavia. Eating raw fish is the primary mode of infection, and, as a result, the incidence in the United States has increased with the rise in sushi consumption.[116] The worm's life cycle is complex: the eggs are passed in human feces, hatch in freshwater, and are eaten by copepods, which are freshwater crustaceans. In the copepod, they develop into larval forms and are eaten by fish. The procecoids then invade the stomach wall of the fish and come to reside in the fish muscle. Humans become infected by eating a fish that harbors a viable plerocercoid larva. Infection is usually asymptomatic, but diarrhea and fatigue may occur. Because the worm absorbs free vitamin B_{12} in the intestine, the host may develop vitamin B_{12} deficiency and neurologic symptoms. Diagnosis is made by identifying the ova or proglottids in stool. Treatment with praziquantel or niclosamide is effective.

Ascaris lumbricoides is the most common intestinal helminth worldwide, with an estimated 1.5 billion cases globally.[117] Humans are the only host for *Ascaris* and become infected by fecally contaminated food or water. Humans ingest the mature ova, which develop into larvae in the small intestine. The larvae enter the circulation and lodge in the pulmonary alveoli, where they mature. Pulmonary infestation may cause pneumonitis and allergic manifestations. Finally, the larvae migrate up the bronchial tree and are swallowed. Although most of the life cycle is completed in humans, soil is necessary for egg development and acts as a reservoir and a contaminant. Ascariasis contributes to childhood malnutrition, growth, and cognitive delay and is an important cause of intestinal and biliary tract obstruction. Its diagnosis is made by finding adult worms, larvae, or eggs in the stool. Treatment consists of mebendazole or pyrantel pamoate.

Trichuris trichiura, commonly known as whipworm, is found in the same parts of the world as *Ascaris*. Humans are the definitive hosts. Eggs are passed in human feces and mature in warm, moist soil to become infective. They contaminate food and are ingested by a new host. The worms remain within the intestine, and their carriage may be asymptomatic or may result in chronic diarrhea. With a heavy worm burden, malnutrition or dysentery may develop. The diagnosis is made by finding adult worms or eggs in stool. Treatment with mebendazole is usually curative.

Trichinella spiralis is a nematode that infects humans following the ingestion of the first-stage larvae and its nurse cell in striated skeletal muscle tissue, typically in pork. The larvae are released from the meat in the stomach and pass to the small intestine, where they infect epithelial cells. They develop into adult worms and are shed in the stool. They may also penetrate into lymph or blood vessels, travel to the skeletal muscles, and form the nurse cell. The principal mode of transmission to humans is through the consumption of undercooked meat; outbreak sources have included pork, horse, and bear meat.[118] The major clinical features of infection relate to the cellular destruction caused by parasitic penetration of cardiac or nervous tissue. Gastrointestinal symptoms are common, including diarrhea and vomiting. The diagnosis is dependent on the histologic identification of cells containing larvae within infected muscle tissue. Serologic tests are also of value. Trichinella may be treated with thiabendazole.

Viral Foodborne Infections

According to a recent CDC report, viruses account for many more cases of foodborne infection than bacterial causes.[1] Viral syndromes range from simple enteritis to life-threatening hepatitis. Viruses contaminate both food and water, but they do not reproduce in these media, nor do they produce toxins. Several viruses, such as the noroviruses, cause large outbreaks, whereas others are associated only with sporadic disease. Noroviruses (genus *Norovirus*, family Caliciviridae) are a group of related, single-stranded RNA, nonenveloped viruses that cause acute enteritis in humans. *Norovirus* was recently approved as the official genus name for the group of viruses provision-

ally described as "Norwalk-like viruses." The difficulty in diagnosing viral illness has precluded the acquisition of large amounts of epidemiologic data. However, the advent of rapid tests such as enzyme immunoassays is beginning to change this and will eventually lead to a better understanding of the epidemiology and disease burden caused by the various foodborne viral pathogens.[119]

Noroviruses or the Norwalk-Like Viruses

Noroviruses, or Norwalk-like viruses, are the principal cause of epidemic, nonbacterial enteritis in the United States. Mead and colleagues estimate that these viruses cause 23 million infections, 50,000 hospitalizations, and 300 deaths annually.[1] Norwalk virus was first described after a large outbreak in 1972. It is a small, round, structured virus of the Caliciviridae family. Noroviruses have been associated with many large outbreaks in cruise ships, nursing homes, banquet halls, and other institutional settings. The primary source of infection is feces-contaminated drinking water, but the virus may also be spread through food that has been stored or washed in contaminated water or handled by an infected food service worker. Noroviruses are highly contagious, with fewer than 100 viral particles sufficient to cause disease, and are resistant to freezing, heating, pH extremes, and disinfection.[120] Symptoms tend to occur 48 hours after exposure and consist of vomiting and diarrhea. The diarrhea is watery, without red cells, leukocytes, or mucus. The disease is usually self-limiting, resolving in 1 to 3 days without long-term sequelae. Diagnosis can be made using transmission electron microscopy to find Norovirus particles in stool, vomitus, or food. Serologic testing, enzyme immunoassays, and PCR techniques also establish the diagnosis. The only treatment required is to prevent dehydration. Hand washing will prevent spread of the infection.

A number of other viruses have also been associated with outbreaks of acute enteritis and are suspected to be spread through the fecal-oral route. The list of potential foodborne viruses includes rotavirus, enteric adenovirus, saporo-like viruses, coronaviruses, toroviruses, reoviruses, and the smaller-sized viruses such as caliciviruses, astroviruses, parvoviruses, and picobirnaviruses.[121] All cause a similar acute illness with a self-limiting, noninflammatory, watery diarrhea.

Hepatitis A Virus

Hepatitis A is an RNA virus, belonging to the family Picornaviridae, with a worldwide distribution. It is spread via the fecal-oral route through contaminated food and water and person-to-person spread. In sporadic infections, up to 50 to 75% of susceptible household contacts of the affected individual are infected with hepatitis A.[122] Large outbreaks have been traced to contaminated water, shellfish, milk, potato salad, and fresh fruits. Symptoms develop 30 days after exposure on average, with a range of 15 to 50 days. The lengthy incubation period complicates tracing the source of infection. During the incubation period and the first week of acute illness, hepatitis A virus can usually be detected in stool. Therefore, there is a prolonged phase when an individual is asymptomatic but may transmit the disease to others—a significant concern in relation to food workers and foodborne transmission. An inactivated viral vaccine exists and is recommended for travelers to endemic regions but is not routinely recommended for food handlers. In endemic countries, childhood infection and immunity are almost universal; childhood disease tends to be asymptomatic. In the United States, disease typically occurs after foreign travel to an endemic region. It may present with fever, jaundice, fatigue, abdominal pain, nausea, and diarrhea. Diagnosis of the acute infection may be established serologically, and treatment is supportive. An immunoglobulin may also be used for pre- or postexposure prophylaxis.

Hepatitis E Virus

Hepatitis E virus was first described in 1978 after an epidemic affecting 52,000 individuals occurred in Kashmir, causing 1,650 cases of fulminant hepatic failure and 1,560 deaths.[122] It is a small RNA virus from the Caliciviridae family that is usually transmitted through contaminated drinking water. Foodborne spread has not yet been documented. Hepatitis E is endemic to India, Southeast and Central Asia, parts of Africa, and Mexico. It has an incubation period of 2 to 9 weeks, although most people develop symptoms around 40 days postexposure. Clinically, the disease is similar to hepatitis A, with constitutional symptoms followed by jaundice. Most patients recover, but mortality rates of up to 3% have been reported, with pregnant women at higher risk. The diagnosis is made serologically. Hepatitis E vaccines remain experimental.[123]

Bovine Spongiform Encephalopathy and Variant Creutzfeldt-Jakob Disease

Bovine spongiform encephalopathy or "mad cow disease" was first diagnosed in the United Kingdom in 1986. It is a member of the transmissible spongiform encephalopathies, a group of infectious neurologic diseases affecting a variety of species. Transmissible spongiform encephalopathies were recognized in the eighteenth century, when the condition scrapie was described in sheep,[124] but they did not become a public health or food safety concern until the mid-1990s, when a link was established between mad cow disease and a fatal, degenerative neurologic disease in humans known as variant Creutzfeldt-Jakob. Human infection occurs through beef consumption, with symptoms developing 10 years or more after exposure. The disease is transmitted only through nervous system tissue, included with or contaminating other meats during the slaughtering process. The disease is due to a novel infectious agent, the prion, a protein encoded on the host genome and produced as a normal cellular constituent that is altered and becomes infectious through the disease process.[125,126] In variant Creutzfeldt-Jakob, the bovine neurologic proteins are suspected to induce structural changes in the human protein PrP^c using an unknown cofactor. The epidemic of variant Creutzfeldt-Jakob is likely to have originated in changes in the rendering process, in which cattle were fed sheep and cattle remnants in a concentrated form.[127] In the United Kingdom, nearly 4.5 million asymptomatic cattle were slaughtered to prevent

disease spread. There has been one recent case in Canada, and one infected cow in the United States. A number of cattle are routinely tested for bovine spongiform encephalopathy to ensure early detection.

LONG-TERM CONSEQUENCES OF FOODBORNE INFECTIONS

As previously mentioned, most foodborne illness is characterized by rapid onset and resolution of disease. Even if the intervening hours and days result in profound illness or critical dehydration, most infected individuals recover fully. In recent years, however, we have learned that some food- and waterborne pathogens have more sinister long-term consequences. For example, osteomyelitis may result from *Salmonella* infection. Reactive arthropathy follows infection with a number of enteric bacteria, including *Salmonella*, *Shigella*, and *Yersinia*. Two of the most significant long-term consequences include *Campylobacter*-associated GBS and Shiga toxin–producing *E. coli*–associated renal failure.

In recent years, a clear association between *C. jejuni* infection and GBS has emerged.[74,128] GBS is an autoimmune disorder characterized by flaccid paralysis. The pathogenesis of injury is molecular mimicry, in which the immunologic response to the core oligosaccharides of *Campylobacter* lipopolysaccharide cross-reacts with a variety of neuronal glycosphingolipids.[129] Up to 20% of individuals affected by GBS require mechanical ventilation, and another 20% will have permanent neurologic deficits. The overall risk of developing GBS following *Campylobacter* infection is considered to be approximately 1 in 1,000.[74] The percentage of cases of GBS linked to prior infection with *Campylobacter* is estimated to be 30 to 40%.[74] At least 11 *Campylobacter* serotypes have been associated with GBS, but serotype O:19 is thought to be the most common association. The interval between infection and the development of GBS may be as short as 1 week or as long as 6 weeks. Those with a rapid onset of GBS are suspected to have had prior exposure to the critical *Campylobacter* serotypes and are therefore primed for a rapid immune response.

Another well-described example of a long-term consequence following infection with a food- and waterborne pathogen is the HUS resulting from Shiga toxin–producing *E. coli* infection. Shiga toxin crosses the intestinal epithelial cell monolayer and damages distant target sites, especially the kidney and brain, by direct and immune-mediated effects on endothelial cells in the microvasculature. Permanent renal failure may result. In the United States, 1.5% of patients will require a renal transplant following HUS.[130] In the United Kingdom and South Africa, the rate of renal transplant post-HUS is less than 5%,[131,132] whereas in Argentina, it may be as high as 20%.[133] In up to 20% of patients with HUS, the pancreas is also damaged, causing some patients to develop permanent diabetes mellitus. The majority of patients with HUS have some neurologic involvement, ranging from minor symptoms such as irritability or lethargy to major neurologic dysfunction such as seizures, coma, or stroke.[130] Of the one-third of patients who suffer major neurologic illness, a small proportion sustain permanent neurologic damage. One of the most thorough reviews of post-HUS sequelae was published by Siegler and colleagues,[134] who investigated over 100 patients with postdiarrheal HUS to evaluate outcomes. Overall, 11% had a poor outcome: death, stroke, or chronic renal failure. Approximately 50% had evidence of permanent renal damage in the form of hypertension, reduced glomerular filtration rate, or proteinuria.

SUMMARY

Over time, many new microbes and other infectious agents are discovered and associated with food- and waterborne illness. Despite this growing list of pathogens, data from the CDC suggest that over 80% of such illnesses are due to organisms that are yet to be identified.[1] We constantly have to be aware of new foodborne pathogens coming onto the horizon. One recent example is *Enterobacter sakazakii*, which has been associated with meningitis and sepsis in young infants. Several epidemiologic studies linked this infection with the presence of the organism in powdered infant formula.[135,136]

The majority of available epidemiologic data in the United States from FoodNet and other sources are based on less than 5% of enteritis cases, and many laboratories do not routinely screen for the pathogens, such as enteric viruses, that are probably causing much of the disease burden. In recent years, following a number of highly publicized outbreaks involving a variety of enteric pathogens, food and water safety has become a major public health concern. The food industry has made substantial efforts to improve the safety of food processing, and we are beginning to see the benefits of this with lower levels of bacterial pathogens in poultry and a decline in the incidence of infection with certain bacterial pathogens. Continued monitoring is necessary, both to document trends in the prevalence of pathogens and incidence of infections and to determine the outcomes of foodborne illness. We presume that the outcome is good for the vast majority of foodborne disease, but we do not know this for a fact. Clearly, the outcome is occasionally poor, with sequelae such as HUS, GBS, or reactive arthropathy following infection with enteric pathogens.

One of the primary goals in food safety is prevention through consumer education, conveying information and encouraging behaviors that reduce the risk of infection. High-risk individuals must be made aware that they have a high risk for infection. This includes the young and elderly, the immunocompromised, pregnant women, and possibly even those taking medications that markedly reduce gastric acidity. Simply paying attention to personal hygiene, hand washing, food handling, proper cooking, and food preparation and storage can go a long way in reducing the burden of foodborne illness.

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3. Viral Infections

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Although viruses had long been suspected as pathogens in acute enteritis, the first report of reovirus-like particles in epithelial cells in small bowel biopsies from children with acute nonspecific diarrhea was made only 30 years ago.[1] That original discovery led to a cascade of clinical and laboratory studies that have established rotavirus as the leading cause of dehydrating acute pediatric diarrhea, which is responsible for 30 to 50% of episodes of acute enteritis in children in developed countries. Now 40 to 70% of cases can be attributed to one of several pathogens, compared with only 10 to 20% in the 1970s. It is now known that astroviruses account for up to 15% of cases of acute pediatric diarrhea.[2–4] Specific types of adenoviruses account for 3 to 5% of cases.[5,6] Noroviruses, such as Norwalk and Snow Mountain agents, are also emerging as significant pediatric pathogens. Norwalk agent, one such small round virus known since 1969 to cause epidemic vomiting, can cause outbreaks of vomiting and diarrhea in children at school camps,[7] as well as nosocomial infections in children's hospital wards.[8] Noroviruses are now thought to be second in frequency to rotaviruses as causative agents of acute enteritis in young children,[4] and these infections can result in children having moderately severe or severe episodes of enteritis.[9]

None of the currently recognized intestinal viruses are "new" viruses, but they are newly discovered viruses. This is illustrated by the examination of stool samples from children with acute infectious diarrhea that were stored in 1943 when Light and Hodes were searching for the elusive etiology. In 1975, after rotavirus had been discovered, Hodes tested these samples again and found rotavirus in several of them.[10] Other "novel" viruses have been described in association with acute diarrhea, but cause and effect have yet to be established for many of them. Some of these candidates are still named after the locality in which they were discovered (Breda virus) or for their appearance on electron microscopy (coronavirus) (Table 38.3-1).

Viruses also account for up to 40% of cases of severe infectious diarrhea in children in developing countries. Rotavirus is one of the single most important pathogens because of its frequency and because it is overrepresented in more severe dehydrating disease.[11] Rotavirus alone is estimated to cause 600,000 deaths per year in the developing world. Development of a rotavirus vaccine has been an objective of the World Health Organization for many years.

Enteric viruses spread mainly by contact with feces (the fecal-oral route) or person-to-person contact. The epidemiology of rotavirus closely resembles that of childhood viruses that are spread by the respiratory route.[12] There is some evidence that transmission via contaminated water supplies may be important in developing countries.[13] Excretion after infection may be more prolonged than previously thought. Polymerase chain reaction (PCR) techniques have shown excretion of rotavirus ribonucleic acid (RNA) for up to 57 days after infection.[14] If this is indicative of viable virus, there are considerable implications for the management of cross-infection in day-care centers and schools.

Discovery of these various enteric viruses was the result of the application of new technology, especially electron microscopy, to diarrheal disease. Most enteric viruses have proven difficult to grow in the laboratory; hence, they eluded recognition by traditional tissue culture techniques. Electron microscopic identification of rotavirus has been replaced by less cumbersome enzyme immunoassay (EIA) antigen detection tests, which can be undertaken on large batches of stools. EIAs have been developed for group A rotaviruses, enteric adenoviruses, and astrovirus. More recently, PCR techniques have been emerging as important diagnostic methodology.

There is a large overlap between human and veterinary medicine in this field. Knowledge of animal enteritis viruses has often preceded their discovery in humans. Animal studies have provided much of the understanding of current pathologic and pathophysiologic sequelae of viral enteritis.

PATHOPHYSIOLOGY OF VIRAL ENTERITIS

Viruses that cause diarrhea in humans and animals generally show strong tropism for epithelial cells of the small intestine. The traditional view is that these agents cause disease by selectively destroying large numbers of mature, absorptive enterocytes via lysis, and/ or apoptosis leading to inadequate absorption of fluid, electrolytes, and luminal nutrients (Figure 38.3-1). In contrast to invasive bacterial pathogens, the host inflammatory response in viral enteritis is relatively mild and is not thought to contribute much to the diarrhea. Elevations of cyclic nucleotides, such as cyclic adenosine monophosphate and cyclic guanosine monophosphate, seen with some of the toxin-producing bacterial pathogens that stimulate chloride secretion through the cystic fibrosis transmembrane regulator (CFTR) channel, are not observed during viral enteritis.

New concepts of rotaviral pathogenesis have been evoked by the demonstration that a nonstructural rotavirus protein may function as an enterotoxin.[15] Other proposed mechanisms include neuronally mediated intestinal secre-

TABLE 38.3-1 VIRAL AGENTS OF GASTROENTERITIS

AGENT	VIROLOGY	CLINICAL/EPIDEMIOLOGY	DIAGNOSTIC TESTS
Group A rotavirus	80 nm, segmented dsRNA, grow in tissue culture	Major cause of infantile dehydrating diarrhea Peak incidence in winter in temporate climate	EIA (commercial) EM PAGE RT-PCR
Astrovirus	34 nm, ssRNA, 8 human serotypes	Infant diarrhea, outbreaks in elderly, immunocompromised Peak incidence in winter in temporate climate	EIA EM RT-PCR
Calicivirus including noroviruses and sapoviruses	28 nm, ssRNA, never adapted to tissue culture	Common cause of outbreaks among adults and children, also infantile diarrhea Peak incidence in winter in temporate climate	EM RT-PCR EIA for noroviruses
Enteric adenovirus	80 nm, dsDNA, only serotypes 40 and 41 definitely associated with diarrhea	Prolonged diarrhea in infants and young children. Year-round prevalence	EIA EM
Picobirnavirus	Segmented dsRNA	? Diarrhea in immunocompromised	EM PAGE
CMV	Enveloped dsDNA	Enterocolitis in immunocompromized	Culture Serology PCR Histology
Groups B and C rotavirus	80 nm, segmented dsRNA, do not grow in tissue culture	Mostly animal pathogens with occasional human outbreaks in adults and children	EM PCR PAGE
Toroviruses	ssRNA enveloped 100–150 nm pleomorphic	? Infantile gastroenteritis	EM Experimental EIA

CMV = cytomegalovirus; dsDNA = double-stranded deoxyribonucleic acid; dsRNA = double-stranded ribonucleic acid; EIA = enzyme immunoassay; EM = electron microscopy; PAGE = polyacrylamide gel electrophoresis; PCR = polymerase chain reaction; RT-PCR = reverse transcriptase polymerase chain reaction; ssRNA = single-stranded ribonucleic acid.

tion,[16] loss of tight junctions with paracellular flux of water and electrolytes (Dr. Robert Shaw, personal communication, 1999), and villus tip ischemia.[17]

These comments refer to classic agents of viral enteritis such as rotavirus, astrovirus, calicivirus, and enteric adenoviruses. Viruses such as cytomegalovirus (CMV), Epstein-Barr virus (EBV), and human immunodeficiency virus (HIV) can be associated with diarrhea that is probably due to other mechanisms, such as local or systemic inflammatory cytokines.

EFFECTS OF UNDERNUTRITION

The impact of viral enteritis has an added dimension if the child is malnourished. Although susceptibility to infection may not be greatly different, animal studies suggest that the severity of infection is greater,[18] and there is evidence from both laboratory studies[19,20] and clinical studies in malnourished children[21] that recovery is delayed.

A detailed study of the effects of chronic protein-calorie malnutrition on small intestinal repair after acute viral enteritis was reported by Butzner and colleagues in 1985.[22] Malnourished and normally fed germ-free piglets were infected with a coronavirus called transmissible enteritis virus. In control piglets, structural changes present in the intestine at 40 hours had virtually recovered by 4 days, but changes persisted through 15 days in malnourished animals. Recovery of mucosal enzymes and glucose-stimulated sodium absorption was also delayed in malnourished piglets, suggesting that malnutrition delays intestinal repair after viral injury. This observation reinforces the need for early and effective nutritional rehabilitation during episodes of diarrhea.

Studies of rotavirus in malnourished piglets have shown that the small intestinal inflammatory response is elevated, and diarrhea persists in malnourished animals.[19] Such prolonged disease in malnourished animals is associated with local mediators or markers of intestinal inflammation. The identification of specific rotavirus-induced alterations that are responsive to malnutrition may allow determination of how macronutrients contribute to host responses to viral infection and viral clearance.

Vitamin A and zinc deficiencies are associated with diarrheal diseases in developing countries. In mouse studies, vitamin A deficiency resulted in much more severe intestinal pathology, as well as impaired antibody- and cell-mediated responses to rotavirus infection.[23,24] Zinc supplements have shown some efficacy in the treatment of acute watery diarrhea in the developing world.[25]

BREASTFEEDING

Breastfeeding reduces the incidence of diarrheal diseases in infants and reduces mortality in children hospitalized with diarrhea in developing countries.[26] It also reduces diarrheal disease in children who contract rotavirus as a nosocomial infection. There is some information about the influence of breastfeeding on rotaviral diarrhea. A prospective study in Finland showed that if breastfeeding

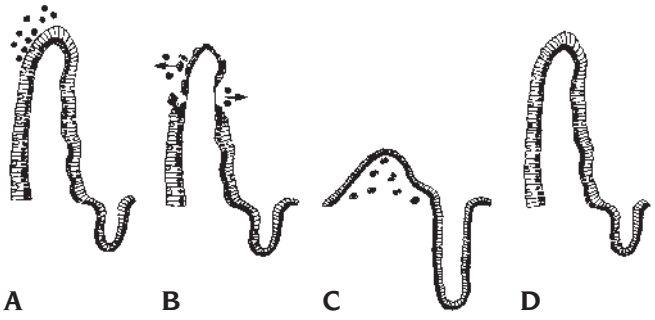

FIGURE 38.3-1 Traditional view of pathogenesis of viral diarrhea. *A*, Mature enterocytes selectively infected. *B*, Virus multiplies in enterocytes that are damaged and shed by 24 hours. *C*, Crypts hypertrophy by 42 hours, repopulating the villi with immature enterocytes. Villi are variably stunted; mononuclear cells increase in lamina propria. Disaccharidases, glucose absorption, glucose-stimulated sodium absorption at the brush border, and sodium-potasium adenosine triphosphatase at the basolateral membrane are diminished. Thymidine kinase is increased. Cyclic adenosine monophosphate and cyclic guanosine monophosphate are not increased. *D*, Structure and function return to normal in 7 to 14 days.

ceased before 6 months of age, the incidence of rotavirus diarrhea between 7 and 12 months of age increased but was the same thereafter.[27] A study of risk factors associated with rotavirus in England found that breastfeeding is protective.[28] There is also some evidence of increased risk of rotavirus diarrhea in children who continue to be breastfed after a certain age, suggesting that infection is delayed but not prevented.[29]

An interesting animal study suggested that persistent asymptomatic excretion of rotavirus by cows may protect their calves from infection, perhaps by reinforcing the immune stimulus to the cow, thus encouraging secretion of antirotavirus antibodies in the milk.[30] Thus, breast milk may be an attenuator of viral diarrheal disease, as well as providing nutritional advantages to affected infants.

IMMUNODEFICIENCY

Virus infections of the intestinal tract are common in patients with immunodeficiency. One child with severe combined immunodeficiency chronically excreted five different viruses.[31] Prolonged excretion of rotavirus may occur in such children. One group demonstrated changes in the strain of virus, suggesting that reinfection, rather than prolonged infection, was the problem.[32] Enteric viral infection may be seen after bone marrow transplant[33] and as a complication of acquired immune deficiency virus infection.[34] CMV is frequently associated with diarrhea in HIV-infected individuals, but the detection of other enteric viral infections varies among studies, and their role in inducing disease remains controversial. rotavirus appears to be more invasive after liver transplant.[35] Passive treatment with oral gammaglobulin has been used in immunodeficiency[36] and as prophylaxis in premature newborn infants.[37]

ENTERIC VIRUSES THAT ARE NOT PRIMARY GUT PATHOGENS

Most viruses that traverse or even replicate within the gut cause no discernable enteric disease. Such viruses can be found in many stool samples from patients with and without symptoms of enteritis. Enteroviruses such as echovirus, hepatitis A virus, coxsackievirus, and poliovirus are excellent examples of viruses that enter the host through the gut, often via M cells overlying Peyer's patches, prior to primary replication in intestinal lymphoid tissue. Other commonly identified stool viruses that do not usually cause diarrhea include nonenteric adenoviruses, reoviruses, and bacterial phages.

VIRAL GUT PATHOGENS

ROTAVIRUSES

Epidemiology. These viruses are the single most important cause of diarrhea requiring admission to hospital during the first 6 to 24 months of life, although most infections are asymptomatic or associated with mild symptoms. Infection is common worldwide from birth to old age.[11] Every child in the world becomes infected with rotavirus, and approximately 2% of infected children are hospitalized for these infections in the United States. The attack rates for *Rotavirus* diarrhea in children aged 6 to 24 months are 0.3 to 0.8 episode per child per year in both developing and developed countries,[38] resulting in at least 600,000 deaths annually in developing countries. The frequency and severity of rotavirus infection provide clear and compelling data for the need for an effective vaccine.[39]

Rotaviruses have been identified in stools from 10 to 40% of children admitted to hospital with acute diarrhea in developing countries and in 35 to 50% in developed countries. Severe infection occurs at a younger age in children in developing countries, where the majority of children admitted to hospital are 6 to 12 months old[38] compared with 12 to 18 months for children in developed countries.[40] All children can be expected to come in contact with rotavirus by 5 years of age, and most will experience asymptomatic boosts in mucosal immunity several times each year. Rotavirus is endemic in the community, and repeated contacts maintain a high level of protection against symptomatic disease after primary infection. In temperate climates, severe rotavirus infections peak during the winter months, but some cases are seen throughout the summer. In tropical areas, seasonal variations are less pronounced.

Neonatal rotavirus infection is often asymptomatic in healthy full-term infants; presumably, these infections are modified by passive immunity from placental and breast milk antibodies. Clinical observations that unique strains of rotavirus may be responsible for endemic nursery infection have been supported by RNA/RNA hybridization experiments using nursery strains and strains from children with acute enteritis.[41]

Infection in children after 3 years of age is not usually severe, but it can necessitate admission to hospital.

Rotaviruses have been identified in 16% of children aged over 5 years admitted for enteritis in Australia[40] and in 5% of adults admitted to hospital in Thailand for severe diarrheal disease.

Some studies from developing countries have reported similar rates of rotavirus isolation from both controls and diarrhea patients. The balance among an ingested dose of virus, mucosal immunity, breastfeeding, and sensitivity of detection methods probably influences these observations.

Outbreaks of diarrhea caused by "atypical" rotaviruses have been widespread in China, where they have affected adults, children, and newborn infants.[42] These atypical rotaviruses lack the group A–specific common antigen, but they are morphologically identical to conventional human strains. They account for 1 to 3% of cases in Finland, Mexico, and the United States.[39] Overall, they account for less than 1% of all severe rotavirus disease worldwide, but if they become more common, current vaccine development strategies may need to be reconsidered. Other recent outbreaks in adults have been caused by rotavirus strains that are not the common circulating strains, suggesting that natural immunity to common strains does not always provide adequate heterotypic protection.[43]

Virology. Rotaviruses are classified as a genus within the family Reoviridae. The prefix "rota-" refers to the wheel-like appearance of particles in feces seen by negative-contrast electron microscopy (Figure 38.3-2). Complete particles, about 70 nm in diameter, exhibit a triple-shelled capsid (Figure 38.3-3). Incomplete double-shelled particles are common but not infectious.

There are four major groups (A, B, C, and D) of rotaviruses as determined by antigens on the middle layer of the viral capsid. Group A rotaviruses are responsible for the great majority of human infections. Epitopes on the outer capsid layer (VP7 and VP4) of group A rotaviruses determine the G (glycoprotein) and P (protease-sensitive) serotypes. G1, G2, G3, and G4 are the most common infecting serotypes in humans, against which current vaccines are directed. In recent years, a global increase in G9 strains has been observed.[44,45] Serotypes can be identified by EIA or by PCR methods. Rotavirus strains can be further subdivided by RNA gel electrophoresis to follow epidemiologic patterns in a given locality. Multiple electropherotypes and serotypes exist simultaneously in most communities, but, usually, one or two are dominant in any 1 year in children admitted to hospital.

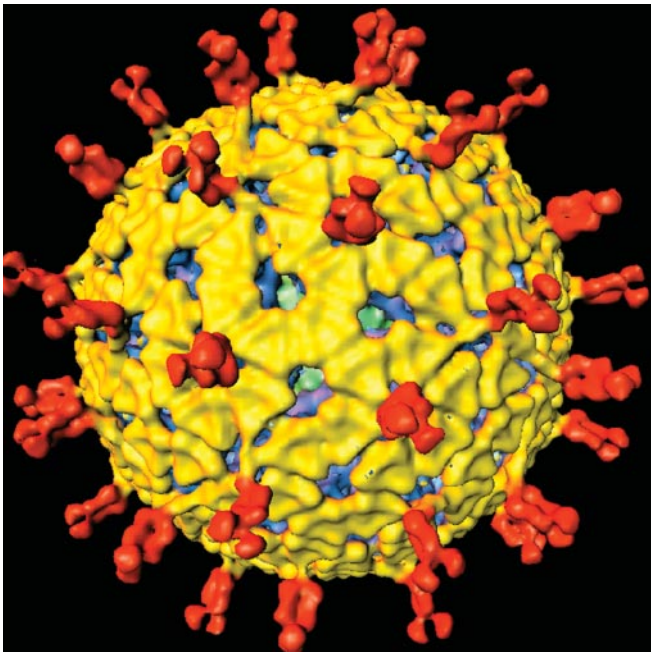

FIGURE 38.3-3 Three-dimensional structure of rotavirus as determined by cryoelectron microscopy and image processing. Note the red spike-like projections (VP4), which mediate cell attachment, the yellow surface consisting of the glycoprotein, VP7, which is the determinant of the G serotype. Small amounts of the inner capsid layers (blue and green) can be seen through channels that penetrate the virion core (×500,000 original magnification) (see CD-ROM for color image). Courtesy of B. V. V. Prasad, Baylor College of Medicine.

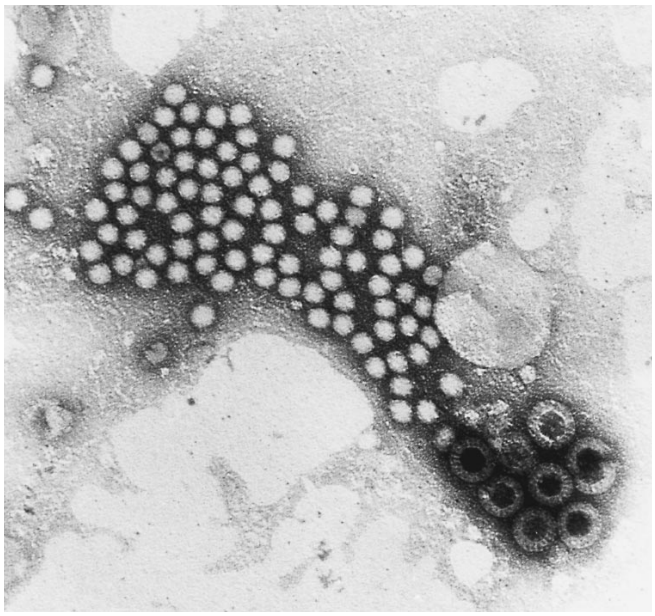

FIGURE 38.3-2 Electron microscopy of feces from an infant with acute diarrhea. The larger (70 nm diameter) particles are rotaviruses that have lost their electron-dense core. A group of smaller unidentified virus particles is seen here in association with rotavirus (×17,000 original magnification.)

Pathophysiology. The traditional view of rotavirus pathophysiology is that diarrhea is a direct result of rotavirus tissue tropism. Rotavirus infects only differentiated villus enterocytes in the small intestine. Virions are ingested, activated by trypsin in the small intestine, and infect the villus enterocytes, leading to their destruction and the release of thousands of progeny, which are locally activated by trypsin to infect more enterocytes. The epithelium is rapidly repopulated with less differentiated enterocytes from the crypts, which lack both digestive enzymes such as lactase and mechanisms for active sodium and water absorption such as sodium-potassium adenosine triphosphatase (ATPase) (Figure 38.3-4). This results in diarrhea

by two mechanisms. Undigested and therefore unabsorbable carbohydrates such as lactose lead to an osmotic diarrhea, whereas the loss of active absorption of water in the face of intact or hypertrophied crypt secretion of chloride and water leads to a low-grade secretory diarrhea. According to this model, symptoms resolve when the new enterocytes have differentiated, which may require 7 days or more in the setting of a severe primary infection.

Recently, Ball and colleagues have proposed that a rotavirus nonstructural protein, NSP4, functions as a novel viral enterotoxin. They have reported that this protein (or a derivative peptide) is capable of inducing diarrhea in susceptible suckling mice when administered by either intraluminal or intraperitoneal routes.[15] NSP4 is reported to cause chloride secretion and thus diarrhea by increasing intracellular calcium when it is either expressed intracellularly or applied externally to cells.[46] Further evidence for the role of NSP4 in the pathophysiology of *Rotavirus* diarrhea is the fact that antibodies against NSP4 can ameliorate rotavirus diarrhea in suckling mice. It has been suggested that changes in NSP4 sequence correlate with rotavirus virulence in porcine rotavirus strains.[47] Of note, NSP4 appears to exert its chloride secretory effects through a previously unrecognized, age-dependent anion channel that is distinct from the CFTR.[48] The ability of NSP4 to induce diarrhea in sucking mice has been reported for several rotavirus strains, including group C and avian rotaviruses, suggesting that the structural elements of this protein are important in enterotoxin function.[49,50] A few studies have questioned the role of NSP4 as an enterotoxin. One group was unable to replicate the secretory diarrhea seen with administration of the NSP4 peptide.[51] Furthermore, although one study found a correlation of virulence with NSP4 sequence of human rotavirus strains, different studies have failed to find correlation of virulence with NSP4 sequence in other human and mouse rotavirus strains.[51–53] This lack of correlation between NSP4 sequences may have been due to mutations in other virulence genes. The role of NSP4 as an enterotoxin in pathogenesis of rotavirus diarrhea remains an area of active investigation.

Another recent hypothesis is that modifications in intracellular tight junctions between enterocytes during rotavirus infection lead to an enhanced paracellular flux of ions and small macromolecules. Morphologic studies show alterations in components of the tight junctions during *Rotavirus* infection. Ussing chamber experiments demonstrate enhanced transepithelial flux of 458 Da and 4 kDa but not 70 kDa markers in rotavirus-infected murine jejunal epithelium.[54] In another study, rotavirus NSP4 increased paracellular fluxes in vitro.[55]

Other possible mechanisms of diarrhea during intestinal viral infection include microischemia of villi,[17] impaired polar transport of sucrase-isomaltase and other apical proteins to the correct membrane surface,[56] cytokine genera-

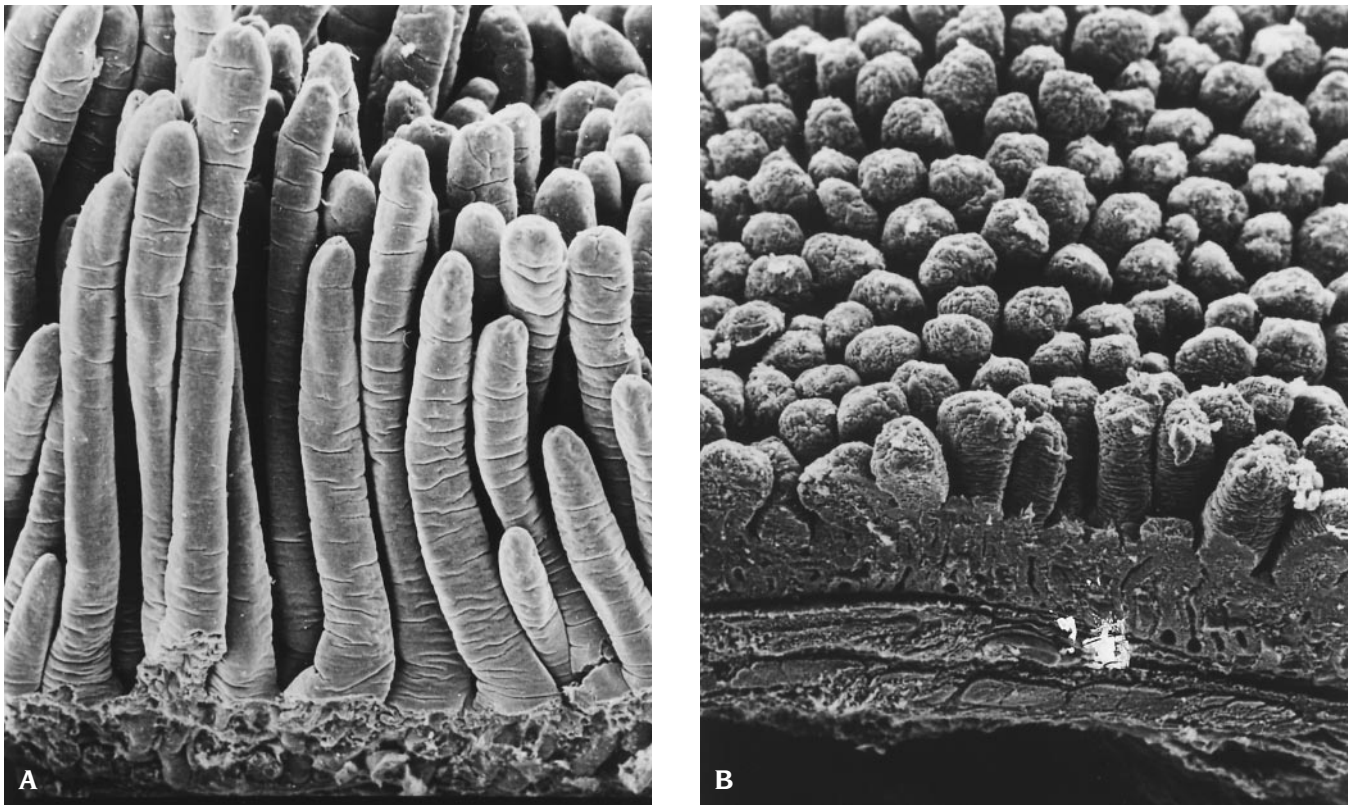

FIGURE 38.3-4 Scanning electron microscopic appearance of normal and rotavirus-infected calf jejunum. *A*, Jejunum from a normal, conventionally reared calf showing tall, fingerlike villi. *B*, rotavirus-damaged intestine from a moribound calf. rotavirus antigen was detected by immunoperoxidase staining of paraffin sections from adjacent tissue. Most of the epithelial cells on the surface of the stunted villi contained antigen. Note epithelial damage, decreased villous height, and increased depth of crypts (uranyl acetate and lead stain; ×60 original magnification.) Courtesy of Dr. G. A. Hall, Institute for Animal Health, Compton, UK.

tion by the epithelium or underlying mononuclear cells,[57] and neuronally mediated intestinal hypersecretion.[16] It is noteworthy that none of these proposed pathophysiologic mechanisms are mutually exclusive, and the etiology of rotavirus diarrhea may well be multifactorial. Discernment of the relative contribution to disease of these mechanisms may be important in devising treatment strategies.

Clinical Features. In severe cases, after an incubation period of 2 to 7 days, there is an abrupt onset of vomiting and fever.[58] Profuse watery diarrhea soon follows, leading to dehydration, acidosis, and electrolyte imbalance. In contrast to bacterial enterocolitis, the stool fluid does not contain blood, white cells, or mucus. Abdominal cramps are less frequent, but irritability and lethargy are often present. Respiratory symptoms have been reported in 20 to 40% of patients, but in several studies, an equally high incidence has been found in controls. The temperature falls to normal quickly. Usually, vomiting settles within 24 to 48 hours and diarrhea in 2 to 7 days.

Acute complications include hypernatremia or hyponatremia when water and electrolyte losses are discrepant. Febrile convulsions can occur as with any other cause of sudden high fever. Reye syndrome, encephalitis, rectal bleeding, afebrile seizures, and intussusception have been described in association with rotavirus infection, but the evidence linking them as cause and effect is tenuous. Case reports of PCR detection of the rotavirus genome in cerebrospinal fluid are noteworthy, but their significance is not clear.[59] Raised aminoaspartate transferase levels are common in severe disease.[60] Depressed mucosal lactase activity is frequent,[61] but persistent lactose malabsorption is not common because disaccharidase activities return to normal within a few days.

Treatment. Initial management is directed at correcting dehydration, acidosis, and electrolyte imbalance. The assessment of the degree of dehydration and treatment with oral or intravenous fluids are considered elsewhere (see Chapter 75.1, "Fluid and Dietary Therapy of Diarrhea"). Early resumption of normal feeding is encouraged, especially in undernourished children, with particular emphasis on breastfeeding. Breastfeeding can almost always be continued, even in children with lactose malabsorption. However, in very small infants, lactose malabsorption can be a serious problem, requiring lactose-free feeding for days or even weeks. These cases, although rare, should not be overlooked in the appropriate enthusiasm for continued breastfeeding.

For the breastfed infant with severe diarrhea, a combination of oral rehydration solution and increasing volumes of breast milk can be offered. In older children, early introduction of a balanced diet should be encouraged, capitalizing on the fact that temporary mucosal damage leaves a much larger reserve of maltase and isomaltase than lactase. Small amounts of fruit juices can be given, but large volumes of sucrose-containing fluids should be avoided in the acute phase.

Drugs are contraindicated. Antibiotics have no place in viral diarrhea. Antiperistaltic agents may lead to pooling of fluid, which is effectively removed from the circulation and yet not revealed to the observer. Complications include ileus and respiratory depression.[62] Antiemetics should be avoided. Vomiting is usually self-limiting, and dystonic reactions to these drugs may occur. Probiotics, such as *Lactobacillus* GG, have been reported to decrease the duration of diarrhea in relatively mild disease[63] but were not of benefit in severe disease.[64]

Prevention. Improved sanitation and hygiene are unlikely to radically alter the incidence of rotavirus diarrhea in developing countries. Such measures have proved unsuccessful in North America and Europe, where the attack rates are similar to those in less developed parts of the world. In pediatric wards, hand washing and isolation procedures may limit nosocomial outbreaks.

Breastfeeding reduces the incidence of diarrheal diseases overall in the first year of life.[65] This is especially true for nonviral pathogens but is not consistent for rotavirus infection.[66] The period immediately after weaning is associated with higher risk of diarrhea in general[67] and rotavirus diarrhea in particular.[29] Breastfeeding may thus delay rotavirus infection rather than prevent it. However, if infants are older when they contract the illness, they are likely to tolerate it better, and the many other benefits of breastfeeding will not be lost.

Passive prophylaxis has been tried in special situations. Human gammaglobulin, given by mouth to newborn premature infants, delays onset of viral excretion and decreases the severity of rotavirus disease.[37] Additions of bovine milk rotavirus antibody to formula given to infants protected them against symptomatic infection[68] and have been reported to hasten recovery.[69]

Vaccine Development. Because of the high morbidity and mortality of rotavirus infection in pediatric populations throughout the world, vaccine development has been a major priority. The initial vaccine prototypes have been based on animal rotavirus strains that are not virulent in humans. This approach has been termed "Jennerian" after Edward Jenner, who used cowpox to immunize against smallpox in the late eighteenth century.[70] Both simian and bovine strains were employed in early trials, which were promising but failed to provide sufficient protection from rotavirus disease. A second generation of vaccines included reassortant viruses, which contained the RNA segment that encodes the viral glycoprotein for each of the four epidemiologically significant human rotavirus G types inserted in a background of the genome of an avirulent simian rotavirus.[71] A tetravalent rotavirus vaccine, Rotashield, based on such simian/human reassortant viruses, was shown in major trials to provide 80 to 100% protection against dehydrating rotavirus diarrhea.[72] In 1998, the Advisory Committee on Immunization Practices endorsed the vaccine and the US Food and Drug Administration granted a license to Wyeth-Ayerst Pharmaceuticals (Philadelphia, PA) to produce it. Subsequently, the American Academy of Pediatrics included, for the first time in its recommended childhood immunization schedule, three doses of oral rotavirus vaccine to be given at 2, 4, and

6 months of age. Nine months later, after the administration of approximately 1.5 million doses of Rotashield, the Centers for Disease Control and Prevention (CDC) reported 15 cases of intussusception in infants who had received the vaccine.[73] Eleven of the intussusceptions occurred within 1 week of the first vaccine dose. Therefore, the CDC recommended suspending further vaccination while further investigation was carried out.[74] Subsequent case-control and case-series investigations confirmed the temporal association, and the manufacturer removed the vaccine from the market.[75] Despite widespread vaccination in some states, no significant increase in intussusception was detected in ecologic studies.[76] The most recent consensus of the increased attributable risk for intussusception for infants receiving Rotashield vaccine was on the order of 1 in 10,000.[72] As shown in Table 38.3-2, immunization of the entire cohort of US infants would have resulted in a net reduction of approximately 50,000 rotavirus hospitalizations and 15 to 30 deaths in young children in the United States.

Other rotavirus live attenuated vaccine candidates are currently under development, including a multivalent bovine-human reassortant vaccine that is similar in concept to Rotashield and an attenuated human rotavirus. Eventually, other strategies may be employed, such as noninfectious virus-like particles, which can be produced using recombinant technology, parenteral inactivated virus vaccines, or DNA vaccines.

Astrovirus

Epidemiology. Astroviruses were first observed in 1975 by Madley and Cosgrove using negative-stain electron microscopy to examine stools obtained from children with acute enteritis.[77] Astroviruses were distinguished by their size (28–34 nm) and morphology containing five- or six-pointed stars. Because the only method of detection was electron microscopy and because the star-like morphology was variably observed, the true significance of this agent of enteritis was grossly underestimated until the development of EIAs and reverse transcriptase (RT)-PCR assays. Astroviruses are now known to be an important cause of infantile enteritis, second or third only to rotavirus in several studies.[78–81]

Astrovirus infection accounts for 7 to 15% of infantile diarrhea in a variety of settings. Most symptomatic infections occur in infants less than 1 year of age, with a peak incidence in winter months in temperate climates. Transmission appears to be fecal-oral. Astrovirus is an important agent of diarrhea in the developing world,[78–80, 82] in nosocomial outbreaks,[81,83] and in day-care–related diarrhea.[3] Asymptomatic infection appears to be common, particularly in day-care and hospital settings. Such asymptomatic infections are important in maintaining and amplifying outbreaks. Most children have developed antibody against astrovirus by age 5 years. Astrovirus has also been reported as an important cause of diarrhea in immunocompromised hosts such as acquired immune deficiency syndrome (AIDS)[81,84] and bone marrow transplant patients.[85] Less common serotypes of astrovirus have been reported as responsible for outbreaks of enteritis among immunocompetent military personnel[86] and nursing home residents.[87,88] Astroviruses also infect a variety of animals. To date, it appears that strains are species specific, so that animal viruses are not commonly transmitted to humans.

Virology. Astroviruses have a unique genome organization, resulting in these viruses being assigned to their own viral family, Astroviridae. Particles consist of small (28–34 nm) nonenveloped capsids that consist of two to three proteins of 20 to 34 kD mass. The genome is positive-sense, single-stranded RNA containing three open reading frames that encode a viral protease, a 90 kD capsid precursor protein, and an RNA polymerase. Infected cells contain considerable amounts of subgenomic RNA that encodes the capsid precursor. Mammalian astroviruses grow efficiently only in tissue culture in the presence of exogenous trypsin. Eight human serotypes have been identified, with serotype 1 being the most common. It is not known whether infection with one serotype confers protection against subsequent infection with other serotypes.

Pathogenesis. Knowledge of pathogenesis in humans is limited to a single observation by electron microscopy of astrovirus particles in the epithelium of a child with enteritis. Studies in lambs have shown infection of villus tip epithelial cells, with subsequent shortening of the villi, and crypt hypertrophy.[89] Studies in calves with bovine astrovirus have shown preferential infection of M cells overlying Peyers patches.[90] In calves, astrovirus appeared to be pathogenic only as a coinfection with either Breda virus or bovine rotavirus.

Clinical Features. Astrovirus infection is associated with a moderate enteritis syndrome that is usually milder than primary rotavirus disease. The incubation period is between 1

TABLE 38.3-2 PROJECTED RESULTS OF UNIVERSAL USE OF ROTASHIELD IN THE UNITED STATES

EVENTS	UNIVERSAL ROTASHIELD	NO ROTAVIRUS VACCINE
Hospitalization: rotavirus dehydration	2,750	55,000
Hospitalization: intussusception (excess)	320	0
Death: (secondary to rotavirus dehydration)	2	20–40
Death: intussusception* (excess)	4	0

Adapted from Kapikian AZ[72] and *Annals of Pharmotherapy*.[73]
Assume birth cohort of 4 million, 95% efficacy of vaccine against very severe disease, excess intussusception at 1 in 12,000 vaccinees.

and 4 days, probably depending on the size of the inoculum. The illness is characterized initially by low-grade fever and vomiting, followed by 3 to 4 days of watery diarrhea without blood or white blood cells. Immunocompromised hosts may experience more severe and prolonged illness. Similarly, coinfection (which is common) with rotavirus or other enteric pathogens may lead to particularly severe symptoms. Adult volunteers have very mild or no symptoms after inoculation with astrovirus.[91] Adults almost uniformly possess serum antibodies against astrovirus and astrovirus-specific T helper cells in their small intestine.[92]

Astrovirus infection can be diagnosed by electron microscopy, EIA, or RT-PCR assay. A commercial monoclonal antibody–based diagnostic EIA kit is available in Europe but is not currently approved for use in the United States.

Treatment and Prevention. As with other viral enteritides, there is no specific treatment for astrovirus. Attention should be directed toward maintaining hydration and nutritional status. A single case report describes successful administration of intravenous immunoglobulin to an immunocompromised adult with severe astrovirus disease.[93] There are currently no active vaccine development programs.

Human Caliciviruses (Noroviruses and Sapoviruses)

Norwalk virus was observed by electron microscopy in stools from a severe outbreak of enteritis in Norwalk, Ohio, in 1972.[94] This was the first direct confirmation of a viral etiology for human enteritis. Subsequent volunteer studies proved its pathogenicity. Other morphologically similar viruses were observed and named for their outbreak sites (Montgomery County, Hawaii, Snow Mountain, Sapporo, Toronto), and these viruses were often called "Norwalk-like viruses" or small round structured viruses.

Recently, these human viruses, which cause enteritis, have been reclassified as members of a distinct genus called *Norovirus* in the Caliciviridae family. None of these agents has been adapted to tissue culture, but the genomes of several, incuding the prototypic Norwalk virus, have been completely sequenced and characterized.[95]

Other human caliciviruses are classified in a separate genus called *Sapovirus*. These viruses exhibit typical calicivirus structure when observed by electron microscopy, cause disease primarily in young children, and have a slightly different genome organization when compared with the noroviruses.

Epidemiology. Knowledge of the full extent of norovirus epidemiology remains limited because the only available diagnostic tests until quite recently were electron microscopy and immunoelectron microscopy. These methods are not sufficiently sensitive to detect the low number of virus particles found in most diarrhea samples. Norovirus enteritis is best known for explosive outbreaks of disease that affect both children and adults. Such outbreaks frequently occur in closed or semiclosed settings, such as schools, camps, and cruise ships. The CDC has shown that more than 95% of such outbreaks, which are not caused by conventional bacterial or parasitic pathogens, are caused by noroviruses.[96] Transmission in these outbreaks has been linked to a variety of foodstuffs, including shellfish, cake icing, and lettuce (washed with contaminated water). Outbreaks are distinguished from those caused by preformed toxins by the appearance of secondary cases in household contacts and the slightly longer incubation period (12–24 hours versus 2–6 hours for toxins). Vomitus has been shown to contain infectious virus and can rapidly amplify outbreaks. Noroviruses are also common nosocomial pathogens in children.[97] In addition, noroviruses are emerging as important causes of endemic childhood enteritis. Like rotavirus, there appears to be an increase in the incidence of norovirus disease in winter months in temperate climates. Sapoviruses cause disease in young children but are generally less severe than norovirus- and rotavirus-induced disease.[9]

Virology. Caliciviruses are small, 28 to 34 nm, nonenveloped, positive-sense RNA viruses. Human caliciviruses are classified into several distinct genogroups within each genus and viruses in each genogroup that are genetically and serologically distinct. Despite this, there are some common epitopes among viruses in genogroups 1 and 2 of the noroviruses, and an EIA based on cross-reactive monoclonal antibodies has been developed and is commercially available in Europe.[98] The RNA genome contains three open reading frames that encode a polyprotein that makes a nucleotide triphosphatase, viral proteinase, RNA polymerase, the capsid protein, and a small basic protein that is found in virions. The capsid consists of 180 molecules of a single major protein and a few molecules of a minor protein. When the capsid protein is expressed in a recombinant baculovirus system, it spontaneously assembles into virus-like particles, which have been used to create diagnostic reagents and candidate vaccines (Figure 38.3-5). The most common means of diagnosis of infections is by RT-PCR using primers, but this is not straightforward because several sets of primers or probes must be used to confirm infection. The EIA for noroviruses currently available in Europe is being evaluated for use in the United States, and other EIAs are being developed.

Pathophysiology. Human volunteer studies demonstrate villus shortening and crypt hypertrophy in the proximal duodenum associated with villus tip vacuolization and infiltration of the lamina propria with inflammatory cells.[99] Intestinal sucrase, alkaline phosphatase, and trehalase are diminished with demonstrable mild carbohydrate intolerance.[100] Mild steatorrhea is also noted. Gastric and colonic mucosa are completely normal. Delayed gastric emptying has been formally demonstrated in symptomatic volunteers and may account for the pronounced nausea and vomiting associated with this infection.[101] Animal studies show similar histologic features. Several recent studies have suggested that specific blood group determinants may confer susceptibility or resistance to specific noroviruses.[102,103]

Clinical Features. The most striking characteristics of calicivirus infections are the rapid onset of symptoms, rapid spread of disease through groups, predominance of vomiting as a symptom, and the high attack rate across all age groups. Generally, the illness is mild and self-limited to 12 to 24 hours after a 1- to 2-day incubation. Attack rates are usually about 50% of exposed populations.[104] Asymptomatic infections are common. Recent studies have shown that virus is excreted for much longer times than previously recognized.[105,106] This asymptomatic shedding of virus likely contributes to virus transmission. Infantile norovirus enteritis is clinically similar to rotavirus enteritis, although it results in less severe dehydration.

Treatment and Prevention. No specific treatment is available. There is evidence that protective short-term immunity to the same serotype of calicivirus develops after infection. However, this immune protection wanes fairly rapidly. Because of the economic and military liability associated with large outbreaks, there is considerable interest in finding methods of prevention. A virus-like particle vaccine is being evaluated.

Enteric Adenovirus

Adenoviruses are ubiquitous human pathogens and cause a variety of syndromes, ranging from respiratory infections to hepatitis. Only 2 of more than 50 serotypes, types 40 and 41, are clearly associated with human enteritis, although a variety of serotypes may be found in stool samples. Adenovirus enteritis tends to last longer (up to 2 weeks) than disease caused by other enteritis viruses.

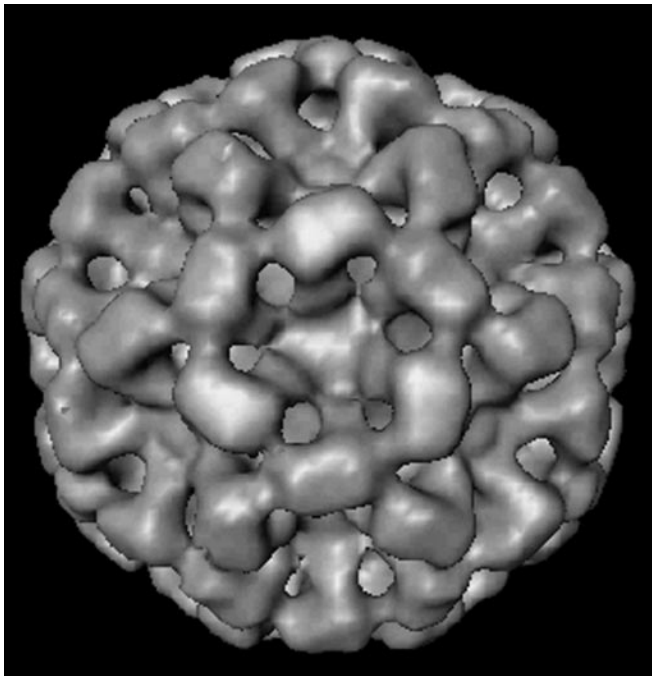

FIGURE 38.3-5 Three-dimensional structure of recombinant Norwalk virus–like particles generated by expression of the capsid protein in insect cells. Images were prepared by cryoelectron microscopy, followed by image processing (uranyl acetate and lead stain; ×1,000,000 original magnification). Courtesy of B. V. V. Prasad, Baylor College of Medicine.

Epidemiology. Like all of the agents of viral enteritis, enteric adenoviruses have a worldwide distribution. Most symptomatic infections occur in children less than 2 years of age. Unlike rotavirus and astrovirus, there does not seem to be a winter peak in the incidence of adenovirus enteritis. In longitudinal studies, there is considerable year-to-year variation in the extent of adenovirus diarrheal disease in a given geographic area.[107] Adenovirus infections generally account for 3 to 5% of acute pediatric enteritis. Like the other agents of viral enteritis, enteric adenoviruses commonly cause asymptomatic infections, especially in day-care centers.[108] Seroconversion studies suggest that enteric adenovirus infection is not as common as rotavirus infection during early childhood. Fecal shedding of adenovirus of various serotypes is common in AIDS patients but is not clearly associated with disease manifestations.[109–112] Despite the fact that up to 10^{11} virions/g stool have been reported in patients with enteric adenovirus enteritis, there are fewer reports of explosive diarrhea outbreaks from enteric adenovirus than from rotavirus, calicivirus, or astrovirus.[112]

Virology. Adenoviruses are 80 nm nonenveloped particles containing a double-stranded DNA genome. Enteric adenoviruses are more fastidious in tissue culture than the other adenovirus serotypes but can be grown on selected cell lines, such as CaCo-2 cells. Diagnosis can be made by commercially available EIAs.[113]

Pathophysiology. Adenoviruses replicate in host nuclei, and intranuclear inclusions are observed in enterocyte nuclei in patients with diarrhea. There are no systematic studies of the pathophysiology of these infections in humans or animals.

Treatment and Prevention. There is no known specific treatment, although the use of ribavirin in immunocompromised patients has been reported.[114] No major efforts are under way to develop a vaccine.

Other Related Pathogens and Infections

Cytomegalovirus. In the modern era of both acquired and iatrogenic immunodeficiency states, CMV has emerged with increasing frequency as an enteric pathogen. Several clinical syndromes have been described, including a protein-losing gastropathy,[115] deep ulcers, which may occur anywhere in the gastrointestinal tract, and an enterocolitis endoscopically similar to Crohn disease. Most cases occur in immunocompromised or very young patients.[116,117] Diagnosis is made by finding characteristic nuclear inclusions in mucosal biopsies or by culture of such material. Yield is much higher from the center of ulcer craters. Treatment consists of restoration of immunologic function, if possible, and the administration of ganciclovir. Selected cases may also benefit from CMV immunoglobulin.

Epstein-Barr Virus. In immunosuppressed, solid organ transplant patients, infection with EBV may trigger an immunoproliferative syndrome, which may present with fever and diarrhea. Endoscopic evaluation shows nodular or ulcerated lesions in the bowel that may contain many cells with EBV genome demonstrable by in situ hybridization. Treatment consists mainly of withdrawal of immunosuppression and the use of ganciclovir.

Small Round Viruses. Small round viruses are morphologically characterized particles observed by electron microscopy in diarrheal stools. Almost all of the previously described small round viruses and "minireoviruses" are now known to be caliciviruses and astroviruses. Parvoviruses and picobirnaviruses also share this morphology. It is likely that some small round viruses represent uncharacterized, novel viral agents of diarrhea or bacterial phages.

Aichi Virus. An example of a newly described small round enteritis virus is the Aichi virus. This virus was isolated in cultured cells from individuals who had enteritis associated with eating oysters.[118] Aichi virus has since been detected in Pakistani children with acute enteritis children and Japanese travelers from Southeast Asia.[119] The virus contains a single-stranded RNA genome and is a new member of the Picornaviridae family.[120] RT-PCR assays have been developed to detect the viral genome.[121]

Picobirnavirus. These are small (35 nm) double-stranded RNA viruses with a two-segment genome. They have been associated with animal diarrhea and have been found on occasion in humans with HIV infection.[122,123] Diagnosis can be made by visualizing two distinct bands of genomic RNA on electrophorectic gels of RNA extracted from stool samples.

Parvovirus. This is a small round virus containing single-stranded DNA that causes severe diarrhea in young animals. Human diarrheal disease has not been established.

Torovirus. Large (100–150 nm) pleomorphic, enveloped, single-stranded RNA viruses have been associated with diarrhea in livestock. Several reports suggest that they may be pathogenic in young children.[124–126]

Coronavirus. Large enveloped single-stranded RNA viruses related to toroviruses, coronaviruses cause severe enteritis in several species of animals and are common respiratory pathogens in humans. The pleomorphic particles have been observed in stools from infants with enteritis and from patients with tropical sprue, but their true role in human disease remains uncertain.

Measles. In the developing world, severe measles infection is often accompanied by severe diarrhea. The pathophysiology of measles enteritis is not well understood, although viral-mediated intestinal inflammation has been described.[127] The incidence of measles-associated diarrhea has been greatly reduced with use of the measles vaccine.

Human Immunodeficiency Virus. Even in the absence of identifiable pathogens, AIDS patients frequently develop diarrhea that is likely related to HIV infection of the intestinal mucosa. Pathology in such cases shows apoptosis and disproportionate CD4+ T-cell depletion as well as demonstrable viral nucleic acid in tissue mononuclear cells.[128] The diarrhea usually responds to appropriate multiagent antiretroviral therapy.[112,128,129]

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4. *Parasitic and Fungal Infections*

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Parasitic infections of the gastrointestinal (GI) tract occur in all geographic regions of the world and produce a substantial morbidity in children. Recent evidence confirms that there is increased mortality in children with some parasitic infections such as that due to *Cryptosporidium parvum*, especially when infection is associated with undernutrition and other comorbidities. Prevalence is highest in the economically deprived regions of the world, notably in the tropics. Infants and young children are particularly susceptible to *Giardia lamblia*, *C. parvum*, *Ascaris lumbricoides*, and *Trichuris trichiura*. In addition to producing GI symptoms, these parasites may impair growth and development.

There has been controversy regarding the clinical relevance of many of these common intestinal parasitic infections, which often appear to coexist with their hosts without causing significant clinical problems. However, recent studies confirm the importance of many of these infections, particularly in immunocompromised children with severe undernutrition or human immunodeficiency virus (HIV) infection.

Diagnostic tests for many GI parasites continue to be limited, usually relying on microscopic techniques and a skilled observer. Similarly, the development of new drugs for the control of these infections has been slower than that for other infectious diseases because drug development programs tend to focus on the needs of the more profitable industrialized world.

These common infections can have a major impact on child health and, therefore, need to be considered as one of the objectives of any diarrhea control and nutritional intervention program. Several GI parasites (*G. lamblia*, *C. parvum*) have become common in industrialized parts of the world, partly owing to increased foreign travel and immigration.

PARASITES OF THE STOMACH: ANISAKIS ANISAKIS

Anisakis is a nematode parasite that is transmitted to humans by the ingestion of uncooked fish. It is found most commonly in Japan, Holland, Scandinavia, and the Pacific coast of South America.[1–3]

Following the ingestion of contaminated food, there is usually an acute upper GI illness with epigastric pain, nausea, and vomiting. Symptoms are produced through the direct attachment of larvae to the gastric mucosa, where they cause ulceration and, occasionally, perforation.

Larvae attaching to the gastric mucosa can be identified at endoscopy and can be removed by biopsy or by grasping forceps. It is suggested that endoscopy should be performed early in suspected anisakiasis because this is the mainstay of therapy.

PARASITES OF THE SMALL INTESTINE

A variety of protozoa and helminths may infect the small intestine (Table 38.4-1); some can simultaneously colonize the large intestine as well, notably *Crypotosporidium* species and *Strongyloides stercoralis*, the latter as part of the hyperinfection syndrome.

PROTOZOA

Giardia lamblia. This flagellate protozoan exists as a motile trophozoite and as a cyst, the latter being the infective form of the parasite. The trophozoite has a smooth

TABLE 38.4-1 PARASITES OF THE SMALL INTESTINE

	ESTIMATED GLOBAL IMPORTANCE*		
PARASITE	INFANCY	CHILDHOOD	ADOLESCENCE
PROTOZOA			
Giardia intestinalis sp†	±	+++	+
Cryptosporidium sp†	±	+++	+
Microsporidium sp†	±	+	+
Isospora belli†	±	++	+
Sarcocystis sp†	–	+	+
Cyclospora cayetanensis†	–	++	+
NEMATODES			
Strongyloides stercoralis†	±	+++	+
Capillaria philippinensis†	–	++	+
Trichinella spiralis†	–	+	+
Trichostrongylus orientalis†	–	+	+
Ascaris lumbricoides	±	+++	++
Ankylostoma duodenale	±	+++	++
Necator americanus	±	+++	++
CESTODES			
Taenia saginata	–	++	++
Taenia solium	–	++	++
Hymenolepis nana	–	+	+
TREMATODES			
Fasciolopsis buski	–	+	+
Heterophyes heterophyes	–	+	+
Metagonimus yokogawai	–	+	+

*These are rough approximations that attempt to take into account marked geographic variations.
†Parasites that can cause diarrhea and malabsorption.

dorsal surface and a convex ventral surface occupied by the ventral disk (Figure 38.4-1). This disk consists of contractile proteins that are thought to mediate the attachment of the parasite to the intestinal epithelium. The surface membrane of *Giardia* contains a lectin that is activated by trypsin and is thought to participate in the attachment to intestinal epithelial cells.

The chemotaxonomy of *G. lamblia* has been reported using a variety of techniques, including antigen, isoenzyme, and deoxyribonucleic acid (DNA) analyses.[4–6] These approaches have confirmed that *G. lamblia* isolates differ from one another, although the sensitivity of the techniques varies. Molecular genetic approaches show that *G. lamblia* isolated from humans can be divided into two major genotypes, types I and II.[7] Some animal isolates have been shown to possess the same genotypes, thereby providing evidence that giardiasis can be a zoonosis.

Epidemiology. *Giardia* is found in most countries of the world, its prevalence being highest in the developing world, where rates can approach 30%,[4,8] particularly in young children. Age-specific prevalence rates increase throughout infancy and childhood but approach adult levels only during adolescence.[4] *G. lamblia* is transmitted by food, water, and direct person-to-person contact. There is now compelling evidence to suggest that giardiasis is a zoonotic infection.[8,9]

The precise mechanisms by which *Giardia* causes diarrhea and malabsorption have not been determined.[5,9] Jejunal morphology may be normal, although partial atrophy and even total villous atrophy are reported.[10,11] The presence of a mucosal inflammatory response, with an early increase in intraepithelial lymphocytes,[12,13] suggests that mucosal damage may be immunologically mediated. Nude mice with T-cell deficiency fail to develop significant alterations in villous architecture during experimental infection, supporting the view that activated T cells are responsible for the mucosal abnormality.[9] Steatorrhea, however, can occur in the absence of significant histopathologic abnormality, suggesting that other factors, such as bacterial overgrowth, bile salt uptake by the parasite, and inhibition of pancreatic lipase, may be additional pathogenic mechanisms.[5,9] Evidence indicates that different *Giardia* isolates vary in virulence in experimental models of infection.[14] This relates specifically to the ability of different isolates to affect water and electrolyte absorption and to alter the expression of disaccharidases in the microvillous membrane.

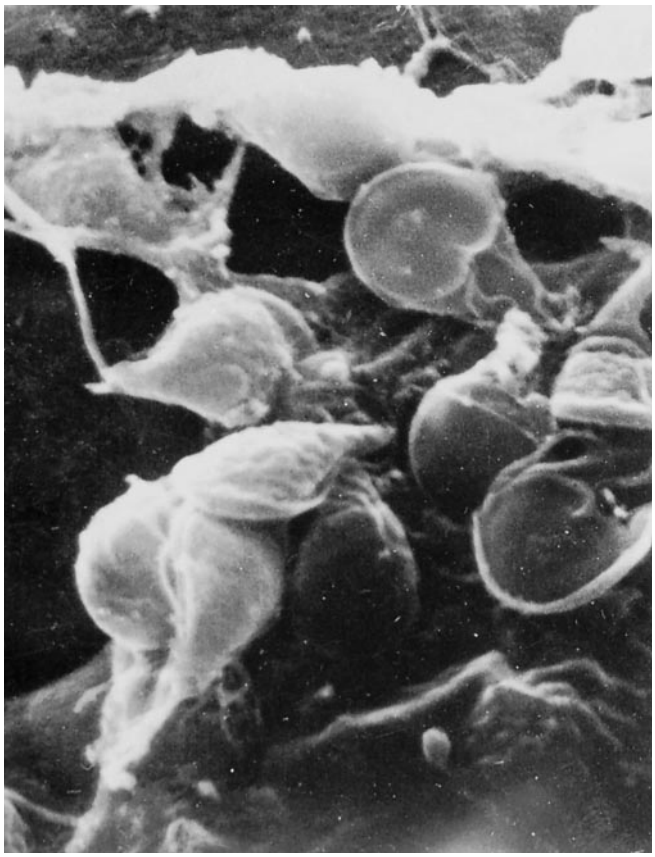

FIGURE 38.4-1 Scanning electron micrograph of *Giardia lamblia* trophozoites in mucus on human jejunal mucosa.

Clinical Aspects. Adults and older children commonly harbor *Giardia* without symptoms, but infection early in life is usually symptomatic. Acute infection often begins with watery diarrhea that persists and is associated with anorexia and abdominal distention. Untreated chronic diarrhea with steatorrhea ensues, and growth may be impaired.[15] Chronic giardiasis is associated with immunoglobulin (Ig) deficiency,[16] which may be accompanied by diffuse nodular lymphoid hyperplasia involving the small and sometimes the large intestine.[17]

Diagnosis. Identification of *Giardia* forms by microscopy of feces, duodenal fluid, or mucosal biopsy specimen remains the "gold standard" for diagnosis.[9] However, even after examination of multiple stool specimens, only 80% of positive individuals will be detected. The measurement of specific anti-*Giardia* IgG antibody has not been helpful, but there is an early IgM response in acute giardiasis that can distinguish current from past infection. Sensitive and specific IgG-based immunoassays have been developed, and some of these assays are now marketed commercially.[18,19] Sensitivity and specificity are reported to be between 87% and 100%.[20,21] However, further studies are awaited to determine if their use can be recommended in the routine diagnostic laboratory setting.[22,23]

Treatment. The drugs of choice are nitroimidazole derivatives, namely metronidazole (30 mg/kg as a single dose on 3 consecutive days) or tinidazole (30 mg/kg as a single dose).[8] Alternatives include mepacrine (2 mg/kg 3 times daily for 7 days) and furazolidone (1.25 mg/kg 4 times daily for 7 days). Recent trials indicate that the antihelminthic drug albendazole is also effective in giardiasis (400 mg once daily for 5 to 7 days). Adverse effects with nitroimidazole derivatives include anorexia, nausea, vomiting, and peripheral neuropathy. In addition to GI side effects, mepacrine causes yellowing of the skin, sclerae, and blood dyscrasias.

***Cryptosporidium* Species.** This coccidian takes up an intracellular but extracytoplasmic location in host intestinal epithelial cells. Although recognized by veterinarians as an important cause of diarrhea in animals, the first human infection with *Cryptosporidium* was discovered in a

3-year-old immunocompetent child in 1976.[24] Subsequently, the majority of reported cases have occurred in immunocompromised individuals, particularly those with HIV infection.[25] The parasite can be found in both the small bowel and large intestine and reproduces both sexually and asexually. Its ability to complete its life cycle within the human host is probably a major factor in persistent infection.[26,27]

Epidemiology. *C. parvum* is found throughout the developed and developing world, with prevalence rates of 10% or more in the latter. As with *Giardia*, asymptomatic carriage is well recognized. Infection is spread by water and by direct person-to-person transmission.[27]

The mechanism by which this parasite produces acute watery diarrhea is unknown, although various morphologic abnormalities have been described, including disruption of the microvillous membrane (Figure 38.4-2) and a spectrum of morphologic abnormalities from partial to subtotal villous atrophy.[28] Although specific virulence factors have not been identified, the organism does possess an *N*-acetylgalactose–binding lectin thought to be involved in the mediation of adherence to the epithelial cells. In addition, a parasite phospholipase has been identified that appears to have a role in parasite invasion in in vitro models.[29] Studies in experimental models indicate that impairment of water and sodium absorption is mediated, at least in part, by local production of prostaglandins in the intestinal mucosa. Intestinal perfusion studies in HIV-infected humans with cryptosporidiosis, however, fail to demonstrate any abnormality of water and electrolyte absorption in the jejunum.

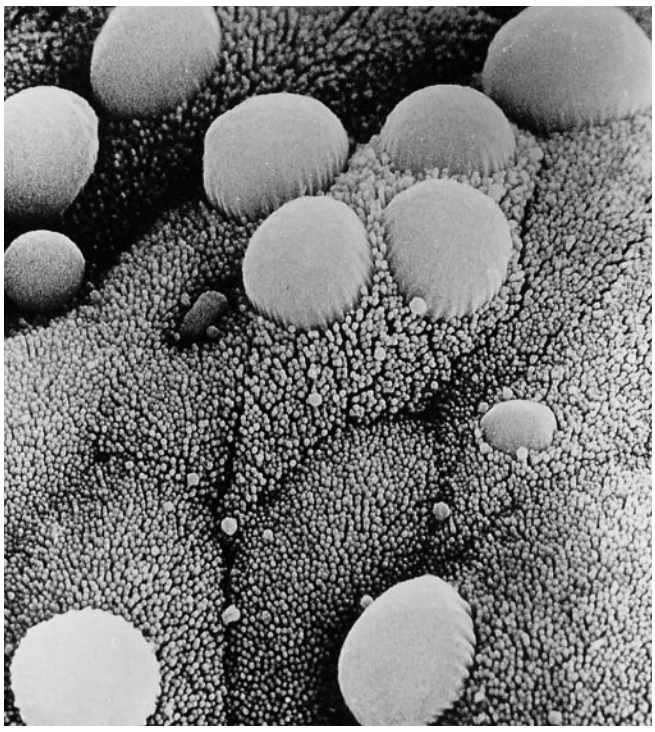

FIGURE 38.4-2 Scanning electron micrograph of *Cryptosporidium* species. Courtesy of Patricia Bland and David Burden, ARFC Institute for Animal Disease Research, Compton.

Molecular genetic typing techniques clearly identify two different strains of *C. parvum*: one that appears to infect animals (particularly cattle) exclusively and another that can infect both humans and animals. The ability to detect differences between animal and human isolates has been helpful in understanding the sources of human waterborne epidemics originating from domestic water supplies.

Clinical Features. Acute infection in an immunocompetent individual usually has an incubation period of 1 to 7 days, followed by fever, abdominal discomfort, nausea, vomiting, and high-volume watery diarrhea. The illness may resolve within 2 days or continue for 2 to 3 weeks.[26] Dehydration occurs in children, and the illness tends to be more severe in the malnourished. Work from West Africa indicates that cryptosporidiosis is an important risk factor for poor survival in infants and young children and is associated with a 3.5-fold increased risk of death. Isaacs and colleagues found that 7 of 213 children (3.2%) with chronic diarrhea in the United Kingdom had cryptosporidiosis.[30] Two had symptoms for more than 4 months, with failure to thrive. Asymptomatic carriage is reported in children, particularly those in the developing world. Symptoms tend to be more severe and prolonged in the immunocompromised host and may contribute to the terminal illness of patients with acquired immune deficiency syndrome (AIDS).[25]

Diagnosis. Oocysts can be detected in feces, duodenal fluid, and, occasionally, sputum with a variety of stains, including trichrome, modified acid fast, and auramine.[31] Cyst concentration techniques may be required. Diagnostic precision can be increased by exposing cyst preparations to fluorescent-labeled monoclonal antibody directed toward cyst antigens. Serum antibody responses occur in cryptosporidiosis, but serology has not found a place in routine laboratory diagnosis.[32]

Treatment. Macrolide antibiotics such as erythromycin, spiramycin, and clindamycin fail to eradicate the parasite but reduce parasite numbers and, transiently, stool volume.[33] Paromomycin and diclazuril also reduce parasite load, but neither is able to achieve eradication. A broad-spectrum antihelminthic drug, nitazoxanide, appears to have activity against *C. parvum* in vitro, and preliminary clinical studies suggest that it may be able to eradicate the parasite.[34] Further controlled studies are required before this drug can be widely recommended.

In patients with HIV infection, azidothymidine is reported to reduce stool volume and eradicate the parasite.[35] Recent experience with highly active antiretroviral therapy (HAART) has had a major impact on the prevalence and severity of opportunistic infections such that cryptosporidiosis is now rarely seen in HIV-infected patients treated with this regimen. Although HAART may not completely eradicate infection, it reduces parasite numbers to such low levels that they no longer produce clinically relevant disease. However, infection may recur if HAART is discontinued and CD4 counts decrease. Hyperimmune bovine colostrum induced a remission in a 3-year-old child with hypogammaglobulinemia and cryptosporidiosis but has not found a place in routine therapy.[36] Treatment is otherwise

supportive, with oral glucose electrolyte solutions for dehydration. In the past, before HAART, immunocompromised individuals required parenteral fluids and total parenteral nutrition. Antidiarrheal medications, such as opiates (morphine) and opioids (loperamide), or the somatostatin analogue octreotide also may prove useful in those patients who are intolerant or who fail to respond to HAART.

Microsporidia Species. In many respects, species of *Microsporidia* resemble *Cryptosporidium*, both in life cycle and in clinical features. Two parasites, *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* (formerly *Septata intestinalis*) are now known to infect humans. The clinical importance of these organisms is recognized in immunocompromised patients, particularly those with AIDS-related diarrhea.[37–40]

Epidemiology. Microsporidia species constitute their own phylum within the protozoa and are obligate intracellular spore-forming organisms with a wide range of hosts. Infection is acquired via the spore, which, following ingestion, extrudes a polar tube through which the sporoplasm is passed into the enterocyte. The organism then multiplies within the infected cell by binary fission, taking up an intracytoplasmic location surrounded by a simple membrane (parasitophorous vacuole). In industrialized countries, *Microsporidium* species appear to be emerging as the dominant coccidial parasite in AIDS and have recently been described in African patients.[40]

The pathogenesis of infection is poorly understood, largely because of the difficulties of establishing in vitro cultures of these organisms and the limited availability of animal models representative of intestinal disease. In humans, infection appears to be confined to the small intestine and is generally associated with varying degrees of villous atrophy.[39]

Clinical Features. Clinically, infection with *E. bieneusi* resembles cryptosporidiosis, with chronic watery diarrhea, anorexia, nausea, and abdominal pain. This organism has also been associated with sclerosing cholangitis in patients with HIV infection. *E. intestinalis* also causes diarrhea, but dissemination can also occur, particularly into the kidneys, with shedding of spores in the urine. *E. intestinalis* can be distinguished from *E. bieneusi* by its development within a septate parasitophorous vacuole.

Diagnosis. The diagnosis of these infections is made by detection of spores in stool with a chromotrope stain or the fluorescent stain calcafluor. Experienced parasitologists are able to distinguish these different *Microsporidium* species using light microscopy, although this differentiation is more reliably done by electron microscopy of small intestinal biopsy specimens. Giemsa-stained small bowel biopsy specimens may also reveal the parasite.

Treatment. Albendazole is effective against microsporidia, although this usually results in suppression rather than eradication of infection. Albendazole inhibits microtubule formation and, thus, reduces cell division and, possibly, polar tubule action. The usual dose is 400 mg twice daily for 4 weeks. There is evidence that *E. bieneusi* responds much less satisfactorily than *E. intestinalis*. There is preliminary evidence suggesting that the antihelminthic drug nitazoxanide also may have activity against microsporidia.

Isospora belli and Sarcocystis Species. These intracellular coccidian parasites are rare in immunocompetent individuals but have been recognized in increasing numbers of patients with HIV infection and AIDS. Oocysts are ingested in contaminated food, namely, undercooked beef and pork. Like *Cryptosporidium* species, these parasites produce varying degrees of partial villous atrophy and an associated inflammatory response in the mucosa, consisting of lymphocytes, plasma cells, and eosinophils.[41,42]

Although these parasites are carried without symptoms, children can develop profuse watery diarrhea and go on to a chronic malabsorptive state with steatorrhea and weight loss. Diagnosis is by the detection of oocysts in feces, duodenal fluid, or jejunal mucosal biopsy specimens.[42] Nitroimidazole derivatives, furazolidone, and trimethoprim-sulfamethoxazole are effective, but recurrence of infection is common. Treatment may need to be continued for many weeks.

Cyclospora cayetanensis. This is another relatively newly recognized member of the intracellular intestinal protozoa. The organism was first detected in travelers returning from Nepal with persistent diarrhea but has subsequently been isolated in parts of the developing world and North America; in the latter, the infection has been found in immunocompromised individuals.[43,44] The infection is seasonal, with peak prevalence during the periods of high rainfall, strongly suggesting that it is waterborne. Diarrhea is usually prolonged, lasting approximately 7 weeks if untreated. The organism has been identified within enterocytes and is associated with varying degrees of villous atrophy.[45]

Oocysts can be identified in feces by light microscopy, and the addition of potassium dichromate induces the oocysts to sporulate. Intracellular parasites are identified in small intestinal biopsy specimens by electron microscopy. The parasite is probably underdiagnosed, and its precise role as a cause of acute and chronic diarrhea worldwide needs to be established by further epidemiologic studies. The parasite can be eradicated with cotrimoxazole given in conventional doses for 7 days. This therapy eradicates more than 90% of infections; the remainder can be cured by continuing therapy for a further 3 to 5 days.[46]

Nematodes

Many helminths appear to be able to coexist with their human hosts without causing marked disturbance of intestinal function. Small intestinal nematodes may be considered as two groups: those that cause diarrhea and those that do not (see Table 38.4-1).

Strongyloides stercoralis. The adult worms of this parasite live predominantly in the duodenum and jejunum, although, occasionally, there is extensive involvement of the whole gut. The life cycle is summarized in Figure 38.4-3. *S. stercoralis* is found in the tropics and subtropics and also in eastern Europe, Italy, Australia, and the south-

ern United States. An important group of individuals still carrying this parasite are ex-servicemen who served in Southeast Asia, particularly those who were forced to work on the Thai-Burma railroad.[47,48]

Adult worms invade the intestinal mucosa (Figure 38.4-4) and produce an inflammatory response involving mononuclear cells and eosinophils. In addition, there may be varying degrees of partial villous atrophy.

Clinical Features. Penetration of the skin by filariform larvae often produces a local reaction, followed 1 week later by respiratory symptoms, including coughing, wheezing, and transient pulmonary infiltrates as adolescent worms migrate through the airways. Diarrhea follows some 2 weeks later as the parasite colonizes the small intestine.

Abdominal symptoms may pass unnoticed, but children can develop a sprue-like syndrome. Occasionally, severe infection is associated with protein-losing enteropathy[49,50] and intestinal obstruction.[51]

Autoinfection can occur in the same individual by invasion of either the colon or the perianal area, which results in a form of cutaneous larva migrans known as larva currens. The serious and often fatal hyperinfection syndrome may occur in immunocompromised individuals.

Diagnosis. Larvae or adult females can be detected in feces, duodenal fluid, sputum, or jejunal biopsy specimens. Multiple stool specimens may need to be examined to find larvae. Thus, a negative stool examination does not exclude infection.[48] Serology is usually positive in up to 80% of patients.

Treatment. Ivermectin as a single oral dose (200 µg/kg) repeated after 1 week or 200 µg/kg daily for 3 days is an effective intervention. Albendazole and thiabendazole are also effective, but the latter, in particular, is frequently accompanied by unwanted side effects, including nausea, anorexia, vomiting, and diarrhea.

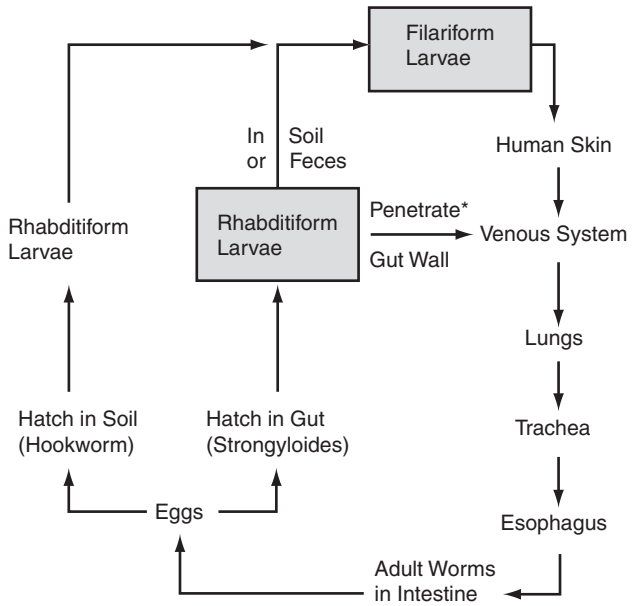

FIGURE 38.4-3 Life cycle of *Strongyloides stercoralis* and hookworm.

Capillaria philippinensis. Infection with this important parasite of Southeast Asia, particularly the Philippines and Thailand, results in severe diarrhea and malabsorption.

Infection generally follows the ingestion of raw fish. After an incubation period of 1 to 2 months, nonspecific abdominal symptoms may be noted, followed by severe, watery diarrhea. In some cases, this progresses to intestinal malabsorption with profound weight loss. If untreated, mortality approaches 10%.[52]

Parasite forms (ova, larvae, adult worms) are detected in stool or intestinal biopsy specimens. Infection is successfully eradicated by mebendazole and thiabendazole, provided that treatment is continued for 3 to 4 weeks.[52] Albendazole is an effective alternative therapy.

Trichinella spiralis. *Trichinella spiralis* occurs worldwide in communities that eat pork.[53] Unlike other nematodes, *T. spiralis* requires two hosts to complete its life cycle (Figure 38.4-5). Initially, diarrhea and abdominal pain predominate, usually occurring several days after eating contaminated pork. After 1 to 2 weeks, infected individuals experience an acute febrile illness associated with periorbital edema, an erythematous rash, and severe muscular pains, which may last for up to 6 weeks. Associated complications include pneumonitis, myocarditis, and encephalitis.[53,54]

Diagnosis can be confirmed by demonstrating larvae in skeletal muscle biopsy specimens. Often there is eosinophilia and elevation of serum creatinine phosphokinase and serum glutamicoxaloacetic transaminase. Treatment is with mebendazole or thiabendazole for 10 days, but in patients with disseminated infection, concurrent corticosteroid therapy is recommended to minimize allergic reactions.

Trichostrongylus orientalis. Found predominantly in the Far East, this small roundworm infects those who ingest contaminated food or drink. Diarrhea occurs, but usually infection is asymptomatic. Ova can be detected in duodenal fluid or feces, and treatment is with a single dose of levamisole (2.5 mg/kg). Thiabendazole is less effective.

Ascaris lumbricoides. *Ascaris*, one of the most common parasitic infections of humans, is the largest human intesti-

FIGURE 38.4-4 Scanning electron micrograph of *Strongyloides* adult worms invading intestinal mucosa. Courtesy of Tim McHugh.

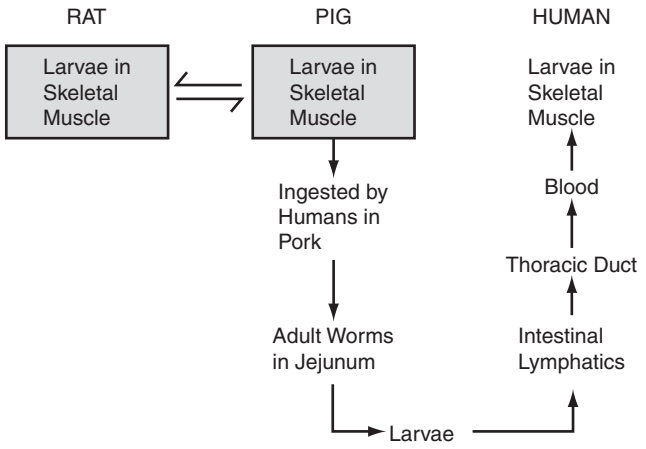

FIGURE 38.4-5 Life cycle of *Trichinella spiralis*.

nal nematode. It is found worldwide, but it is most evident in the developing world, where its prevalence may exceed 90% in very deprived communities.[55] The life cycle is summarized in Figure 38.4-6.

***Clinical* Features.** Most individuals with *Ascaris* infection are symptom free, but during the pulmonary phase, migrating larvae may produce coughing with sputum, wheezing, fever, and eosinophilia. Heavy infections, particularly in children, may cause anorexia and abdominal cramps. Some evidence suggests that the parasite also may impair growth and development. Large worm burdens can produce intestinal obstruction, particularly in children. Worms may migrate into a variety of locations, including the pancreatic and biliary system, causing duct obstruction with jaundice and pancreatitis, obstruction of the appendix, appendicitis, volvulus, intussusception, intestinal perforation, and peritonitis.[55]

Diagnosis and Treatment. Ova and adult worms can be detected in feces and larvae in sputum or gastric washings. Albendazole (200 to 400 mg as a single dose), mebendazole (100 mg twice daily for 1 day), and levamisole (2.5 mg/kg as a single dose) are the drugs of choice. Other drugs include piperazine citrate and pyrantel pamoate. Intestinal obstruction from nematode infestation may require operative treatment, but antihelminthic drugs combined with intestinal suction and intravenous fluids are often tried first. Bile and pancreatic duct obstructions can be relieved endoscopically.

***Ankylostoma duodenale* and *Necator americanus*.** *A. duodenale* (Old World hookworm) is found in Africa, Asia, Australia, and parts of southern Europe, whereas *N. americanus* predominates in Central and South America, together with some locations in Southeast Asia, the Pacific, and Nigeria. Morphologically, the parasites are similar, with identical life cycles (see Figure 38.4-3). Adult worms attach firmly to the small intestinal mucosa by a buccal capsule consisting of tooth-like or plate-like cutting organs. It is estimated that more than 500 million persons in the world are infected with these parasites.

***Clinical* Features.** Filariform larvae penetrate the skin, where a local inflammatory reaction may develop (ground itch). Pulmonary symptoms are less dramatic than those of *Ascaris* infections, but upper abdominal discomfort, mild diarrhea, and associated eosinophilia usually ensue. The dominant clinical response to infection is iron deficiency anemia, which is proportional to the worm load and the amount of iron taken in the diet. Occasionally, heavy infection can result in protein-losing enteropathy and hypoproteinemia.

Diagnosis and Treatment. Ova and rhabditiform larvae can be detected in stool and duodenal fluid. Mebendazole (100 mg twice daily for 3 days) and albendazole (200 mg daily for 3 days) are effective against both species of the hookworm, but other drugs, including pyrantel pamoate and levamisole, are also active. Oral iron supplements should be given to treat iron deficiency.

Cestodes (Tapeworms). Four tapeworms are common human pathogens: *Taenia saginata, Taenia solium, Diphyllobothrium latum,* and *Hymenolepis nana*. These flatworms are similar structurally, and their heads have suckers; *T. solium* has additional hooks. The head is joined by a short, slender neck to several segments called proglottids, which form the body of the worm. Nutrients are absorbed directly through the cuticle because these worms do not possess an intestinal tract. Tapeworms are hermaphrodites, with cross-fertilization occurring between proglottids. Adult worms reside in the intestinal tract, whereas larvae exist in tissues, particularly muscle; infection is transmitted to humans by the eating of infected tissues (Figure 38.4-7).

T. saginata (*Beef Tapeworm*). Most patients infected with this tapeworm are symptom free, although mild vague abdominal discomfort and occasional diarrhea may occur. Occasionally, adult worms obstruct the appendix or pancreatic duct, causing appendicitis and pancreatitis, respectively. Generally, infection is apparent to the host only when proglottids are identified in the feces. Praziquantel (10 mg/kg as a single dose) is the treatment of choice, although niclosamide (2 g as a single chewed dose) is also effective. Infection can be avoided by thorough examination of beef for encysted larvae known as cysticerci,

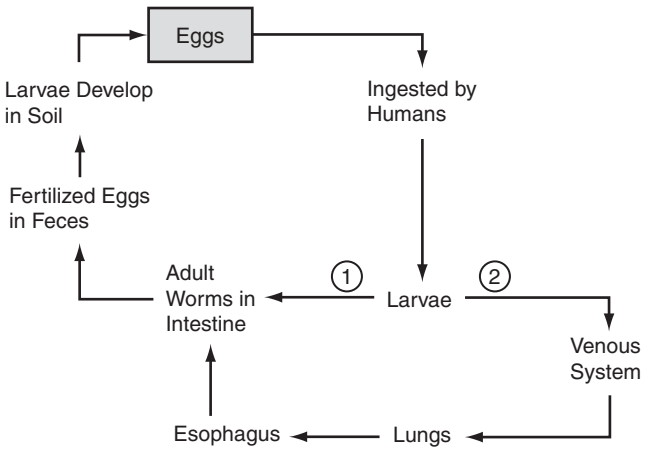

FIGURE 38.4-6 Life cycle of (1) *Trichuris trichiura* and *Enterobius vermicularis* and (2) *Ascaris lumbricoides*. The shaded area indicates the infective form of parasites.

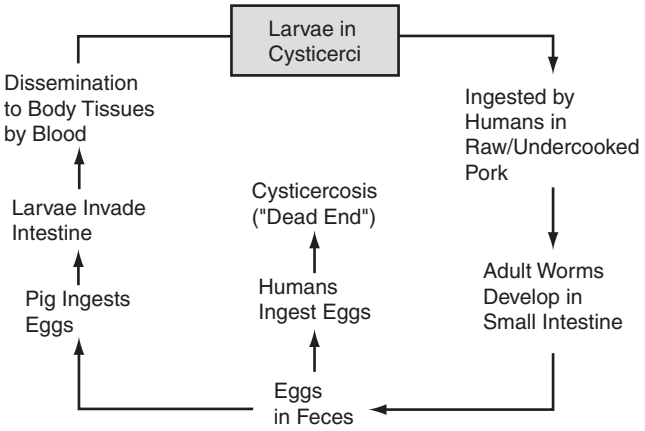

FIGURE 38.4-7 Life cycle of *Taenia solium* (pork tapeworm).

although freezing at –10°C for 5 days or cooking at 57°C for several minutes destroys them.

T. solium (*Pork Tapeworm*). The clinical features and treatment of the adult worm infection are similar to those of *T. saginata* infection. However, a serious complication occurs when infection with *T. solium* larvae results in their dissemination to many sites, including skeletal muscle, brain, subcutaneous tissue, the eye, and myocardium, a condition known as cysticercosis. The cysts remain alive for many years but eventually produce a local inflammatory reaction and calcify. Cerebral involvement presents as epilepsy, as a space-occupying lesion, or as focal neurologic deficits. Ocular involvement produces retinitis, uveitis, conjunctivitis, or choroidal atrophy. Diagnosis can be established by biopsy of skin nodules, although calcified cysticerci usually can be detected radiographically. Encouraging results in the treatment of cysticercosis have been obtained with the use of praziquantel (10 mg/kg). Surgery or photocoagulation may be required for retinal lesions.

D. latum (*Fish Tapeworm*). This tapeworm is found mainly in Scandinavia, the Baltic countries, Japan, and the Swiss lakes region. Infection in humans occurs by the ingestion of raw or undercooked fish containing the infective plerocercoid form of the parasite. Infection is usually asymptomatic, although there may be abdominal discomfort, vomiting, and weight loss. Occasionally, intestinal obstruction can occur. *D. latum* cleaves the vitamin B_{12}–intrinsic factor complex and consumes more than 80% of dietary vitamin B_{12}. Nevertheless, megaloblastic anemia owing to vitamin B_{12} deficiency is relatively uncommon, although it is well recognized in Finland. Diagnosis and treatment are as for other tapeworms.

H. nana (*Dwarf Tapeworm*). This worm infects children more frequently than adults but has other natural hosts in rats and mice. Infection generally produces no symptoms, although very heavy infection may result in diarrhea and abdominal pain. Treatment is with praziquantel or niclosamide.

Trematodes. A large number of flukes infect the biliary and intestinal tracts of humans, producing a broad spectrum of disease. *Fasciolopsis buski*, the largest human fluke (up to 7 cm in length), is found most often in the Far East.[56] It attaches to the proximal small intestine, causing ulceration, bleeding, and abscesses. Although asymptomatic infection occurs, heavy parasite burdens in undernourished children result in intermittent diarrhea, abdominal pain, and protein-losing enteropathy with hypoalbuminemia, edema, and ascites. Progressive weight loss and even ileus may develop. Although hexylresorcinol and tetrachloroethylene were used previously, praziquantel is now the treatment of choice.

Heterophyes heterophyes and *Metagonimus yokogawai* are very small flukes found in the Far East. Natural hosts include dogs, cats, foxes, humans, and other fish-eating mammals. Infection may be asymptomatic, but heavy infections produce intermittent diarrhea and abdominal discomfort. Ova can be detected in feces, and treatment is the same as for *F. buski*.

PARASITES OF THE COLON AND RECTUM

PROTOZOA

Entamoeba histolytica and a ciliate, *Balantidium coli*, are the important protozoal pathogens of the large intestine (Table 38.4-2). However, it is now clear that *C.* parvum can infect the entire small and large intestine; indeed, the first case of human infection was diagnosed by rectal biopsy. *G. lamblia* is predominantly a pathogen of the small intestine, but isolated reports in both humans and animals purport that this parasite occasionally causes colitis.

E. *histolytica*. This organism is found worldwide, but its prevalence is highest in developing countries. Up to 500 million individuals carry the parasite, with an annual mortality of approximately 75,000.[57] Amebiasis is relatively uncommon in infancy and childhood,[58] but when infection occurs, morbidity and mortality tend to be high.

The parasite exists in two forms, the motile trophozoite and the cyst. In the infective form of *E. histolytica*, the cyst can exist for long periods outside the human host.[59] Cysts are spread in food and water and also by person-to-person contact.

Virulence varies among strains of *E. histolytica*, which can be distinguished by isoenzyme electrophoresis and DNA analysis.[60] This finding may explain some of the clinical diversity of *E. histolytica* infection, which varies from asymptomatic carriage to severe invasive disease. It is now clear that there is a phenotypically and genotypically distinct nonpathogenic ameba that morphologically resembles *E. histolytica* but does not produce invasive disease. This organism is now known as *Entamoeba dispar*. These two species can be distinguished by using specific monoclonal antibodies or discriminatory DNA probes, which confirm that many individuals with asymptomatic carriage, in fact, have the nonpathogenic form, *E. dispar*.

The capacity of *E. histolytica* to kill colonic epithelial cells on contact appears to depend on a number of factors, including a surface lectin that mediates adherence to epithelial cells together with a range of cytotoxic proteins, including proteolytic and hydrolytic enzymes and probably a pore-forming protein known as amebapore.[61]

Clinical Features. The term *amebiasis* covers a wide range of clinical syndromes, from asymptomatic infection to amebic colitis and extraintestinal amebiasis, usually hepatic abscess. Most infected individuals throughout the world (80–90%) are asymptomatic carriers.[57] Acute amebic dysentery produces symptoms similar to those of bacterial dysentery; in its most severe form, it may progress to colonic dilatation and severe toxemia. This progression is particularly likely to occur in pregnancy, in the puerperium, and in malnourished infants and young children.[62]

Chronic amebic colitis begins more insidiously, with cyclic remissions during which bowel function may return to normal or even be reduced to constipation. The pattern may mimic nonspecific inflammatory bowel disease such as Crohn disease.[63] Constitutional upset is mild to moderate, although complications such as colonic stricture and fibrotic masses of granulation tissue (known as an ameboma) may occur.[64]

An amebic liver abscess can develop within days, months, or even years after the onset of amebic colitis, but in up to 50% of cases, there is no clear antecedent history of colonic involvement.[65] Nonspecific symptoms, such as low-grade fever and weight loss, may begin insidiously, or there may be an acute fulminant illness with localized hepatic pain or evidence of direct extension into pleural or pericardial cavities. Distant spread to lung, brain, and kidney occurs when the abscess ruptures into a hepatic vein.

Diagnosis. Demonstration of *E. histolytica* trophozoites and cysts in feces remains the mainstay of diagnosis. Examination of a fresh saline wet mount should reveal motile trophozoites containing ingested red blood cells. Nonpathogenic intestinal amebae are not erythrophagic. Trophozoites may be seen in rectal mucosal biopsy specimens or in slough from rectal ulcers. It is unusual to see trophozoites in pus from a liver abscess. Serology for IgG antibodies is positive in 70 to 80% of patients with amebic colitis and approaches 100% in those with amebic liver abscess.[66] The treatment of amebiasis in children is summarized in Table 38.4-3.

TABLE 38.4-2 PARASITES OF THE COLON AND RECTUM

	ESTIMATED GLOBAL IMPORTANCE*		
PARASITE	INFANCY	CHILDHOOD	ADOLESCENCE
PROTOZOA			
Entamoeba histolytica†	±	++	++
Balantidium coli†	–	++	+
Cryptosporidium sp†	±	+++	++
Trypanosoma cruzi	±	++	++
NEMATODES			
Trichuris trichiura	±	+++	++
Enterobius vermicularis	±	+++	++
Oesophagostomum sp	–	+	+
Angiostrongylus costaricensis	–	+	+
TREMATODES			
Schistosoma sp†	–	+++	++

*These are rough approximations that attempt to take into account marked geographic variations.
†Parasites that can cause diarrhea and malabsorption.

B. *coli*. This protozoan is the only ciliate that produces clinically significant infection in humans; it is restricted largely to communities that live in close proximity to pigs, the preferred host.[67–69] It occurs mainly in Papua New Guinea, the Philippines, and Central and South America. The cyst is the infective form, although the trophozoite can survive outside the human host for a week or more in moist conditions. Clinically, *B. coli* infection closely resembles amebiasis, and diagnosis is made by identification of the large motile trophozoite in feces. Cysts are seen relatively rarely. Tetracycline (500 mg 4 times daily for 10 days) is the usual treatment, but the parasite is also sensitive to ampicillin, metronidazole, and paromomycin.

***Trypanosoma cruzi* (South American Trypanosomiasis or Chagas Disease).** *T. cruzi*, a protozoan hemoflagellate, does not directly affect the GI tract. The primary infection generally occurs in early childhood. The parasite is transmitted through the bite of a blood-sucking vector insect of the family Reduviidae. During the blood meal, the infective form of the parasite is deposited with the insect's feces on the skin and rubbed into the bite wound or a mucous membrane susceptible to invasion, such as the conjunctiva. The vast majority of initial illnesses pass unnoticed, but in some cases, there is marked fever, lymphadenopathy, and hepatosplenomegaly. In severe infection, there may be signs and symptoms of acute myocarditis. Death can occur as a result of this acute illness, but the individual may recover within a few weeks or months.

The development of the "megasyndromes" occurs many years later, with more than 90% of symptomatic patients being 20 or more years of age. In Brazil, achalasia and megaesophagus occur in approximately 6% of seropositive patients, whereas megacolon appears to be less common, affecting only 1%. In Argentina and Chile, however, megacolon is more common than megaesophagus.

A medical approach to the management of these conditions is usually attempted with either nifurtimox (10 mg/kg in three divided doses for at least 90 days) or benznidazole (5 to 10 mg/kg in two divided doses for 60 days).

NEMATODES

The most common nematodes to infect the colon and rectum are *T. trichiura* (whipworm) and *Enterobius vermicularis* (threadworm). In the hyperinfection syndrome, *S. stercoralis* also colonizes the large intestine, producing ulceration and inflammatory changes after invasion and autoinfection.

T. *trichiura*. This parasite is found worldwide, with a high prevalence in the developing world. In some particularly deprived communities, prevalence may be as high as 90%.[70,71] Infection is transmitted by ingestion of ova that have matured outside the host for several weeks. Colonization involves the distal ileum and cecum, although the entire colon may be involved.

TABLE 38.4-3 TREATMENT OF AMEBIASIS IN CHILDREN

INTESTINAL AMEBIASIS
Metronidazole
50 mg/kg daily for 10 d, followed by diloxanide furoate, 20 mg/kg daily for 10 d
AMEBIC LIVER ABSCESS
Metronidazole
50 mg/kg daily for 10 d
ASYMPTOMATIC CYST PASSER
Diloxanide furoate
20 mg/kg daily for 10 d + metronidazole

Clinical Features. Light infections are often asymptomatic, but when larger numbers of parasites are present (greater than 20,000 ova per gram of feces), diarrhea with blood and mucus is characteristic.[72,73] Other symptoms include abdominal pain, anorexia, weight loss, tenesmus, and rectal prolapse. Evidence suggests that within endemic areas, some children are more susceptible than others to whipworm infection. There is now persuasive evidence indicating that chronic heavy infection is an important contributor to the impairment of growth and development in young children.[71,74]

Diagnosis and Treatment. Typical barrel-shaped eggs can be detected in feces, and adult worms can endoscopically be seen attached to the colonic mucosa, often with the presence of ulceration and inflammatory changes. Albendazole (400 mg) or mebendazole as a single dose is now the treatment of choice, although mebendazole should not be used in children under the age of 2 years. Multiple courses may be necessary to clear infection. Ivermectin (200 µg/kg) is also highly effective.

Enterobius vermicularis. This parasite is found worldwide, although it is more prevalent in temperate and cold climates. Children are most often infected, but infection can spread rapidly among family members, those in residential institutions, and any group living in overcrowded circumstances. Infection is spread by direct transmission of ova from person to person or indirectly, on clothing or housedust. The life cycle is summarized in Figure 38.4-6.

Anal pruritus is usually the only symptom, occurring mainly at night, when adult females lay their eggs in the perianal region. Symptoms of appendicitis may result from worms entering the lumen of the appendix. Occasionally, adult worms migrate through the intestinal wall and are found in the genital tract, peritoneum, omentum, lung, urinary tract, liver, spleen, or kidney.[75–77.]

Diagnosis. Ova can be detected in the perianal region by applying clear adhesive tape to the perianal skin and examining this microscopically. Ova can also be found under fingernails. Adult worms may be observed directly emerging from the anal canal or on the perineal skin.

Treatment. Albendazole is the drug of choice (in a single oral dose of 10 to 14 mg/kg), although mebendazole, pyrantel pamoate, and piperazine are also effective. It is wise to treat the entire family and to consider a second course of treatment 2 to 4 weeks later to eradicate worms that may have matured since the first treatment.

***Oesophagostomum* Species.** This organism infects mainly ruminants, primates and pigs, but occasionally causes human infection. The worm often penetrates the intestinal wall, resulting in multiple nodules along the intestine, some of which develop into paracolic abscesses requiring surgical drainage.

Angiostrongylus costaricensis. Infection with this nematode was first described in Costa Rican children presenting with severe pain in the right iliac fossa, fever, and anorexia.[78] An inflammatory mass involving the cecum, appendix, and terminal ileum is characteristic.[78] In its acute form, the syndrome can be confused with appendicitis. The inflammatory reaction is the result of intramural eggs that have been discharged from adult worms living in terminal mesenteric arterials. Surgical resection may be required.

TREMATODES

***Schistosoma* Species.** Schistosomiasis is one of the most important parasitic infections worldwide, with a high morbidity and mortality. More than 200 million individuals are infected with this parasite.[79] Five species are known to cause intestinal disease in humans: *Schistosoma mansoni* (Africa, Central and South America, the Caribbean, and the Middle East), *Schistosoma japonicum* (Japan, the Philippines, southeast China, and Taiwan), *Schistosoma haematobium* (Africa), *Schistosoma mekongi* (Southeast Asia), and *Schistosoma intercalatum* (Zaire and Gabon). Human infection is totally dependent on the intermediate host, the freshwater snail. The life cycle is summarized in Figure 38.4-8.

Clinical Features. Invasion of the skin by cercariae produces a local inflammatory response known as "swimmer's itch." Within a week, there may be a generalized allergic response, with fever, urticaria, myalgia, general malaise, and associated eosinophilia. The acute phase of *S. japonicum* infection is known as Katayama fever. Hepatosplenomegaly also occurs in the early stages; in children, it is more marked in those with heavy parasite loads.[79] Intestinal symptoms of diarrhea, blood, and mucus can occur immediately but may be delayed for months or even years. Extensive colonic ulceration and polyp formation are characteristic of *S. mansoni* infections.[80] In severe cases, there may be marked iron and protein loss. Stricture formation and intestinal obstruction are characteristic, as are localized granulomatous masses within the gut wall, known as bilharziomas. *S. japonicum* can involve both small and large intestines, and infection may be more severe than that from *S. mansoni*. *S. japonicum* colitis, like extensive long-standing ulcerative colitis, has premalignant potential.[81] *S. haematobium* produces rectal inflammation and bladder involvement. The inflammatory changes in the intestine in schistosomiasis are due entirely to an intense T lymphocyte–related immune response to eggs deposited in the intestinal wall.

Diagnosis and Treatment. Characteristic ova of each species can be detected in feces or in intestinal biopsy

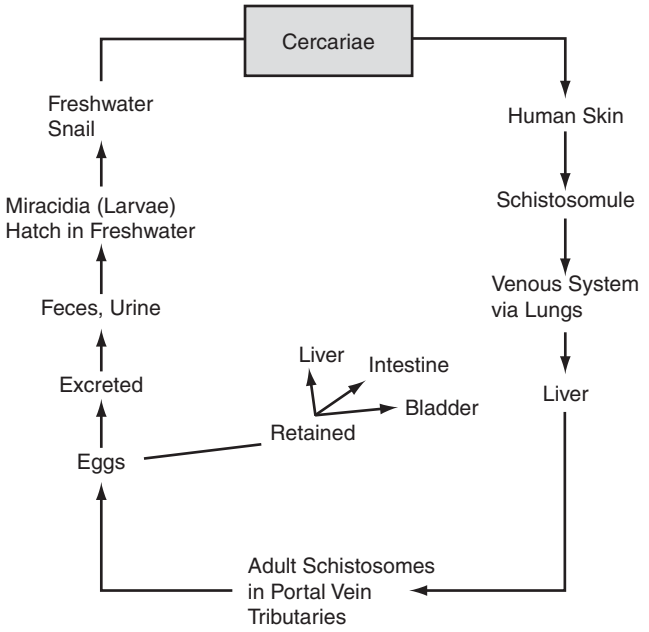

FIGURE 38.4-8 Life cycle of *Schistosoma* species.

specimens. Specific antibodies can be detected by immune assay in more than 95% of patients during the first few weeks of infection.

Praziquantel given as a single dose (40 mg/kg for *S. mansoni*; 60 mg/kg in divided doses for *S. haematobium*) is probably the drug of choice. Oxamniquine is also effective.[82,83]

FUNGAL INFECTONS

A well child with intact host defense mechanisms is generally not considered to be susceptible to fungal infections of the digestive tract. As immunosuppressive therapies become more aggressive and myelotoxic regimens more effective, opportunities increase for fungi to invade and establish themselves in humans. Patients disabled from chronic malnutrition and those exposed to intense antimicrobial infection are also susceptible to these organisms. HIV infection and AIDS have produced a group of chronically immunosuppressed patients susceptible to a wide range of organisms, including fungi. The digestive tract is not a preferred site of infection in cases of disseminated fungal infection, but certain species are capable of infecting the esophagus, the stomach, or the intestine.

A consequence of modern treatment and recently evolving patterns of disease, these opportunistic infections have been recognized only in recent years. No doubt, additional GI fungal infections of significance will emerge and will be recognized in the years to come.

CANDIDIASIS

Candida species are oval cells (4 to 6 μm in size) that reproduce by budding. There are at least 80 species, of which 8, including *Candida albicans*, are of GI significance. In disseminated candidiasis, there is widespread involvement of several organs. The major risk factor leading to this serious problem is neutropenia. The liver may be involved in addition to the heart, brain, kidney, lung, spleen, and eye.[84]

Esophagitis caused by *Candida* species is seen in immunosuppressed children and in those with hematologic malignancy. In such cases, oral thrush may be seen in as few as 20% of cases.[85] Treatment with nystatin usually is effective. Ketoconazole and fluconazole also can be used, and in severe disease (particularly when oral medication cannot be taken), intravenous amphotericin B is employed.

Candida is isolated from up to 15% of gastric ulcers, but no pathogenetic role for the organism in ulcer disease has been established.[86] *Candida* peritonitis, with infection localized usually to the peritoneum, is seen after bowel surgery and in patients undergoing chronic ambulatory peritoneal dialysis. *Candida* infection has been associated with acute watery diarrhea in newborn infants, although its causative role has not been definitely established.[87] *C. albicans* can invade the small bowel and large intestine in terminally ill patients.

ASPERGILLOSIS

The molds of the genus *Aspergillus* reproduce by means of spores that germinate, resulting in hyphae, the form in which they are associated with disease. Most cases of invasive *Aspergillus* infection are seen in severely immunocompromised patients. In about 20% of such cases of invasive infection, the small and large intestines are involved, in addition to the esophagus and stomach.[88] Amphotericin B is the treatment of choice.

ZYGOMYCOSIS

Zygomycetes are ubiquitous agents found in organic debris, on fruit, and in soil. They grow rapidly on any carbohydrate substrate. The terms "mucormycosis" and "phycomatosis" have been used in the past for these infections. These agents can infect the subcutaneous and submucosal tissues in an immunocompetent host, but in the debilitated host, they can cause acute fulminant invasive infection.[89] Intestinal zygomycosis is encountered in severely malnourished children and sometimes as a complication of severe chronic intestinal disease, such as amebic colitis.[90] On occasion, the infection can occur without apparent predisposition.

COCCIDIOIDOMYCOSIS

Coccidioides is a dimorphic fungus endemic in the southwest of the United States. Arthroconidia arising from mycelial growth causes infection when inhaled. Coccidioidomycosis is usually a pulmonary infection; spores can escape the chest during primary infection and with dissemination; on rare occasions, the terminal ileum and colon are involved.[91]

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5. Bacterial Overgrowth

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Bacteria do not inhabit the upper small intestine and stomach in significant numbers, whereas in the colon, concentrations of 100 billion organisms/mL are the norm. Colonic microflora will proliferate in the small intestine, however, whenever intrinsic cleansing mechanisms are interrupted. Classically, colonic flora proliferate in the small intestine in areas of stasis. The clinical syndrome that results has been given a variety of names: stagnant loop, blind loop, contaminated small bowel, small bowel stasis, and small bowel bacterial overgrowth (SBBO) syndrome. In this chapter, I use the latter term, small bowel bacterial overgrowth. The characteristic features of SBBO are (1) abnormal colonization of the upper small intestine by organisms that characteristically reside in the colon, (2) steatorrhea, and (3) anemia.

NORMAL INTESTINE

At birth, the intestine is sterile, but soon after parturition, orally ingested organisms begin to colonize the gut.[1] Commensal bacterial populations are not uniform in either number or type (Table 38.5-1). In the upper small intestine, aerobic bacteria typical of the oral cavity predominate. Their numbers do not exceed 10^6 organisms/mL. In the colon, strict and facultative anaerobes, adapted to growth within the fecal mass, where bacterial metabolism quickly deprives the environment of oxygen, are most common. The total number of colonic bacteria/mL is at least 1 million times greater than that of the upper small intestine. Colonic species are listed in some detail in Table 38.5-1, but the list is, nevertheless, incomplete. There are at least 60 different bacterial species.[2] Many are present in trace numbers, but under specific circumstances, some of them, such as *Clostridium difficile*, proliferate and cause disease, such as antibiotic-associated colitis. The number of bacteria in the distal small intestine is greater than that of the proximal small bowel. Near the cecum, there may be 10^9 organisms/mL, and the composition of the microflora is similar to that of the colon.

TABLE 38.5-1 COMMENSAL ENTERIC FLORA OF THE NORMAL INTESTINAL TRACT

PROXIMAL SMALL INTESTINE
$< 10^6$ organisms/mL
Aerobic, oral flora dominate
Streptococcus, Lactobacillus, Neisseria
DISTAL SMALL INTESTINE
$> 10^9$ organisms/mL
Greater numbers of anaerobic and facultative anaerobic bacteria
Bacteroides, *Escherichia coli*, *Bifidobacterium*
COLON
$10^{9–10}$ organisms/mL
Anaerobic and facultative anaerobic bacteria
Bacteroides, *Escherichia coli*, *Bifidobacterium*, *Clostridium*

PRESERVATION OF THE NORMAL ENVIRONMENT

The relative sterility of the small intestine depends on a number of factors (Figure 38.5-1) that act to reduce bacterial load and prevent colonization. They may be conveniently divided into nonimmune and immune categories.

NONIMMUNE ANTIBACTERIAL FACTORS

Gastric acidity acts as an initial line of defense against ingested bacteria. Gastric juice with a pH < 4.0 is bactericidal for most organisms, although not immediately. In one experiment, bacteria instilled into the normal lumen of the stomach were killed within 15 minutes, but when instilled into the achlorhydric stomach, they remained viable for at least an hour.[3] Chronic inhibition of gastric acid increases

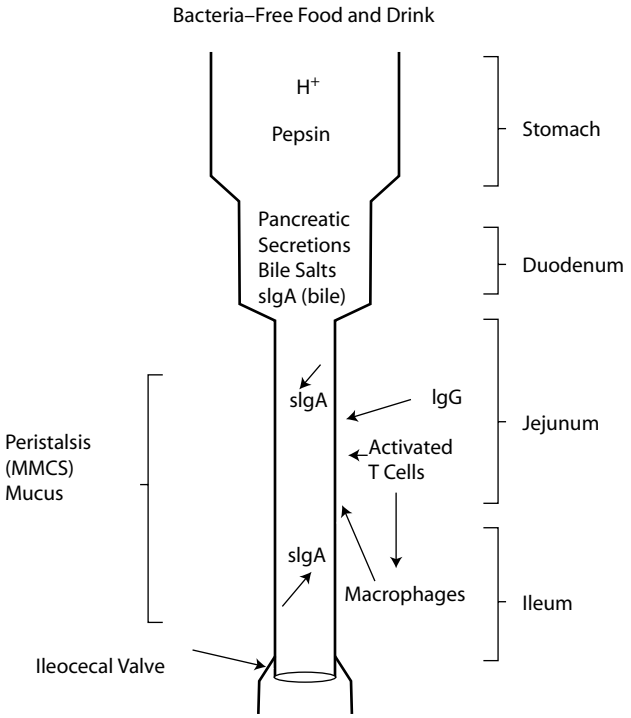

FIGURE 38.5-1 Prevention of small bowel bacterial overgrowth in health.

the number of gastric bacteria substantially.[4] The number of bacteria presented to the duodenum, therefore, depends on the number of organisms ingested and the length of their exposure to low pH.

Two concerns have been raised about low gastric acid and increased bacterial counts induced by the chronic use of drugs that prevent gastric acid secretion. First, *N*-nitroso compounds are associated with an increased incidence of gastric cancer. Second, when histamine$_2$ blockers were used in an intensive care unit to prevent stress ulcers, there was a twofold increased incidence of gram-negative bacteria-induced pneumonia in ventilated patients compared with those taking sucralfate, an agent that does not alter gastric pH or bacterial numbers.[5] No studies have been performed on children to determine the effects of acid suppression on SBBO. However, several studies in the elderly show that both omeprazole and ranitidine cause high duodenal bacterial counts and induce intestinal symptoms,[6,7] but this was not found in a third study.[8]

Peristaltic propagation of luminal contents in a steady distal flow toward the colon is of major importance in reducing the growth of bacteria in the proximal intestine. Interdigestive migratory motor complexes (MMCs) are especially important in this role. Bacterial populations rise within hours when the complexes are ablated pharmacologically.[9]

Enzymatic digestion is probably of significance as well because small numbers of bacteria might be expected to undergo normal digestive degradation. Pancreatic juice has antibacterial activity,[10] possibly because of its proteolytic and lipolytic enzymes, although other factors, such as competitive binding and specific antibacterial activities, cannot be ruled out. Bile acids and digestive secretions probably help to limit growth as well. Colonization requires access to preferential growth sites in stagnant niches, such as the lumina of intestinal glands and membrane sites to which bacteria can bind. Just as importantly, it also requires a relatively large number of available bacteria to exploit these niches at any given instance. Intestinal secretions from the stomach, intestine, and pancreas are, therefore, a deterrent to growth owing to their ability to dilute the bacterial mass.

Mucus is an example of an epithelial secretion with special properties that enable it to trap bacteria in an intraluminal location while moving organisms distally in bolus fashion. Mucus is composed of a variety of proteins and salts anchored within a viscous gel formed by a single carbohydrate-rich protein. About 80% of the mucus glycoprotein by weight is carbohydrate, and the protein itself is organized as a long thread of disulfide-bonded units, often amounting to a molecular weight of $5 - 10 \times 10^7$ D. These huge threads bind bacteria through specific lectin-like reactions with carbohydrate,[11] by hydrophobic interactions,[12] and probably also through simple trapping. The carbohydrate serves as a nutrient for some bacteria, thus attracting them to a mobile colonization site that is continuously replaced. Huge numbers of organisms (Figure 38.5-2) are found in the mucus coats of the colon[13] and stagnant loops,[14] whereas bacteria are relatively scarce on the underlying mucosa and glands, suggesting that mucus limits access of bacteria to the intestinal surface even in stagnant circumstances. This may explain why cellular damage is often minor in stagnant portions of the bowel even when bacterial counts are high.[15]

The ileocecal valve prevents retrograde colonization of the distal small bowel to a significant extent.[16] As noted in Table 38.5-1, the concentration gradient across the valve is not large, on the order of 100 times. In the absence of the valve, however, free reflux of right-sided liquid colonic content occurs, and the total load of colonic bacteria exposed to the distal small intestine increases greatly.

Bacterial load also conditions intestinal colonization. Coprophagic animals, for example, harbor higher numbers of bacteria in the proximal small intestine than do humans, although colonic bacterial populations are equivalent. Poor environmental sanitation, particularly in warm climates, encourages ingestion of excessive numbers of bacteria and may increase the small intestinal bacterial count in this manner.

Immune Antibacterial Factors

Antibodies to indigenous intestinal bacteria develop early in life[17] and probably play an important role in controlling membrane colonization and mucosal penetration by bacteria and bacterial products. Antibodies to bacterial pilus proteins have been shown to inhibit binding to specific attachment sites on intestinal membranes.[18] Combined immunodeficiency states, notably acquired immunodeficiency syndrome (AIDS) and combined B- and T-cell immunodeficiency, predispose the upper intestine to opportunistic infestation by a variety of parasites. Surprisingly, studies of the prevalence of SBBO in patients with human immunodeficiency virus (HIV) infection yield conflicting results. For example, no relationship was found between gastric pH, diarrhea, and small bowel bacterial colonization in HIV-infected patients.[19] Loss of the capacity to secrete immunoglobulins into the intestinal lumen generally produces less dramatic effects, but upper intestinal colonization by specific parasites, such as *Giardia lamblia*, occurs frequently in hypogammaglobulinemia and secretory immunoglobulin A (sIgA) deficiency. Specific IgG and IgA antibodies hasten the elimination of intestinal parasites such as *Giardia* and nematodes,[20] possibly by activating macrophages. The role that the immune system plays, if any, in modulating growth of intraluminal commensal bacteria in the intestine is less clear. There is evidence that agammaglobulinemic and hypogammaglobulinemic patients develop small intestinal bacterial overgrowth,[21–23] but it has been argued that other complications arising in these conditions, such as achlorhydria, are critically important. Dolby and others, for example, compared three groups of patients: one with pernicious anemia, another with hypogammaglobulinemia and achlorhydria, and a third with hypogammaglobulinemia and normal gastric acidity.[23] Bacterial overgrowth was found in the first two groups but not in the third. Although an impressive amount of sIgA enters the intestinal lumen in bile, direct transmucosal secretion is also important. Upper intestinal blind loops, produced experimentally in rats, produce and

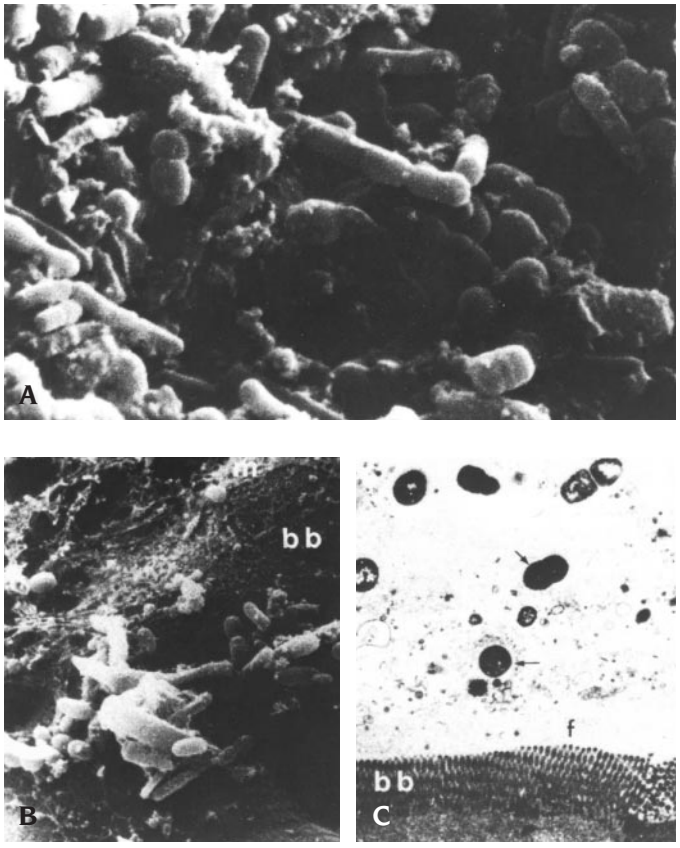

FIGURE 38.5-2 Association of bacteria with the mucous coat in small bowel bacterial overgrowth. *A,* Scanning electron micrograph of the unwashed small intestinal surface showing bacterial rods and chains embedded in multiple layers within the surface mucous coat. *B,* After gentle washing with saline, the brush border surface (bb) is exposed under strands of mucus. Bacteria remain bound to the mucous strands and not to the brush border. *C,* Transmission electron microscopy of an enterocyte with a layer corresponding to surface filaments (f) on the surface of the brush border (bb). Bacteria (*arrows*) are confined to the mucus outside the filamentous layer.

secrete sIgA with specificity for the colonic-type bacteria.[24] Antibodies secreted by the mucosa may help to prevent mucosal attachment by luminal bacteria. Secretory IgA may also enhance binding of certain bacteria to mucus.[25]

FACTORS PREDISPOSING TO THE DEVELOPMENT OF SBBO

A large number of specific clinical entities are reported to cause SBBO. Seemingly unrelated illnesses, however, can be grouped into four categories, depending on the mechanism by which the SBBO is produced. As summarized in Table 38.5-2, these include (1) anatomic abnormalities, (2) disorders of intestinal motility, (3) lesions that increase the number of bacteria presented to the upper small intestine, and (4) deficiencies of host defense.

Anatomic lesions include diverticula (Figure 38.5-3), duplications, and mucosal strictures.[26] These lesions each interrupt normal gut motility and thereby provide a site of relative stasis for bacterial colonization and replication. Surgical procedures such as side-to-side anastomoses,[27] jejunoileal bypass,[28] and neoreservoirs[29] (Koch pouch, ileoanal anastomosis) may create areas of poorly drained bowel and SBBO. Disruption of normal intestinal motor activity causes intestinal stasis by interfering with peristaltic clearing function. Short-term disruption, such as occurs with abdominal surgery, is rarely a problem because although SBBO develops, it rapidly clears with return of motor function. Severe clinical symptoms develop when motility is adversely affected on a more long-term basis. Idiopathic intestinal pseudo-obstruction syndrome is a frequent cause of symptomatic SBBO in children. Secondary causes of a disrupted motility pattern, such as progressive systemic scleroderma and diabetes mellitus with associated autonomic neuropathy, are more frequent considerations in adults. In the elderly, otherwise isolated absence of the MMC is associated with SBBO.[30] The MMC normally has an important "housekeeper" function, helping to keep the proximal small intestine relatively sterile.[31,32] Vantrappen and colleagues showed that 5 of 18 patients with clinically documented SBBO had an absent or disordered MMC pattern.[32] These results have been confirmed and extended.[33] In addition, several studies show that SBBO is associated with delayed gastric emptying.[33,34] Because the activity of the MMC is not fully developed in the immature gut,[35] the premature infant may be at particular risk. Motor abnormalities have subsequently been shown to occur in experimentally produced SBBO,[36] indicating that bacterial overgrowth can cause a motor abnormality independently and thus exacerbate the tendency to stasis.

An increased bacterial load that overwhelms the normal host defenses also can result in SBBO. Coloenteric fistulae (Crohn disease, surgical misadventures), loss of the ileocecal valve (postresection, eg, in Crohn disease or necrotizing enterocolitis), and loss of normal gastric acid output (autoimmune gastritis, malnutrition) may all permit entry into the small intestine of abnormally large numbers of bacteria and initiate SBBO even without appreciable early stasis. Poor sanitation, particularly in the absence of a clean

TABLE 38.5-2 FACTORS PREDISPOSING TO THE DEVELOPMENT OF SMALL BOWEL BACTERIAL OVERGROWTH

ANATOMIC ABNORMALITIES
Diverticula, duplication
Stricture, stenosis, web
Blind loop
MOTILITY DISORDERS
Pseudo-obstruction
Absence of migratory motor complexes
Autonomic neuropathy (eg, diabetes mellitus)
Collagen vascular diseases (eg, scleroderma)
EXCESSIVE BACTERIAL LOAD
Achlorhydria
Fistula
Loss of ileocecal valve
ABNORMAL HOST DEFENSES
Immunodeficiency
Malnutrition
Prematurity

water supply, may also lead to the habitual ingestion of such a large oral load of bacteria that gastric acidity is overwhelmed. Abnormalities of host defense, such as, for example, the achlorhydria associated with hypogammaglobulinemia, are frequently associated with SBBO. Protein-calorie undernutrition is associated with SBBO in children[37–39] and causes loss of gastric acidity,[40,41] decreased mucin production,[42] and impaired cellular and humoral immune function.[43] End-stage renal failure, with creatinine above 6 mg/dL, also has been associated with SBBO.[44] SBBO is reported in patients following liver transplant.[45] Patients with chronic pancreatitis may have an increased incidence of SBBO owing to dysmotility or because of the antibacterial properties of pancreatic juices.[46]

In many clinical settings, etiologic risk factors for SBBO overlap. For example, in underdeveloped countries, it is difficult to separate the effects of an increased bacterial load owing to poor hygenic conditions from the effects of coexisting protein-calorie undernutrition. In experimental animals, using the self-filling blind-loop model of SBBO, malnutrition hastens the onset of deficiencies in mucosal hydrolase activities following intestinal stasis.[47] However, in the rat model, protein-calorie deprivation without surgical intervention to induce intestinal stasis does not depress specific activities of brush border disaccharidases. Infants with short-bowel syndrome frequently have associated SBBO that complicates their clinical course and medical management. In this setting, SBBO is multifactorial because it can be related to intestinal dysmotility, loss of the ileocecal valve, prior abdominal surgery, and associated malnutrition of the host. In some cases of postinfectious enteropathy, symptoms of chronic diarrhea appear to be related to associated SBBO.[48–51] Although the etiology of bacterial overgrowth in this setting is not clearly established, changes in gut motility, altered host defenses, and coexisting malnutrition each may be contributing factors. Inadequate preparation of food may also be a factor in some countries. For example, the red kidney bean contains a lectin, phytohemagglutinin, which is readily destroyed by heating but which can produce bacterial overgrowth in experimental animals when raw beans are fed to them.[52]

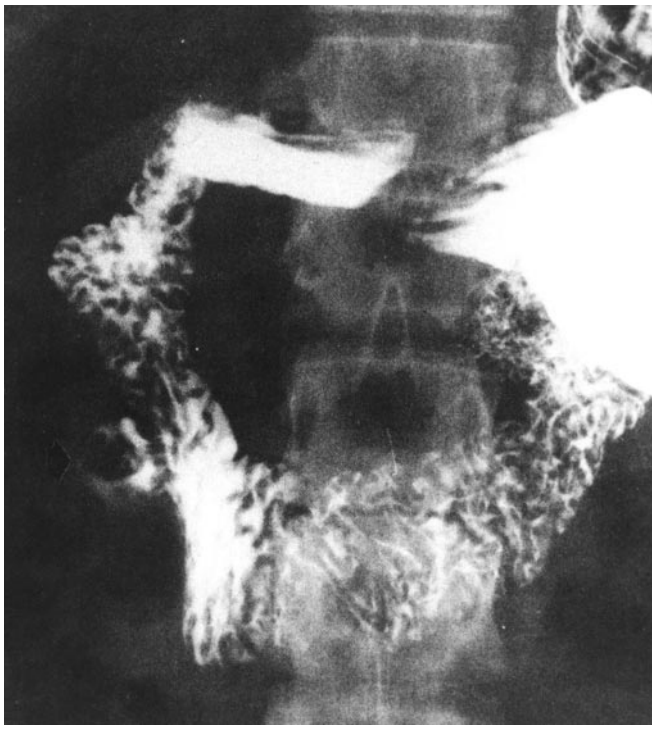

FIGURE 38.5-3 Barium meal with follow-through in a 13-year-old female demonstrates a diverticulum of the duodenum. Courtesy of Dr. David Stringer, Department of Radiology, The Hospital for Sick Children, Toronto.

PATHOPHYSIOLOGY

Excessive numbers of intraluminal bacteria alter intraluminal secretions and produce metabolic products, enzymes, and toxins that damage the mucosa and are absorbed. As a consequence, they produce intraluminal, mucosal, and systemic effects (Table 38.5-3) that greatly alter the performance of their human host.

INTRALUMINAL EFFECTS

Bacteria are metabolically active organisms, and it is not surprising, therefore, that their nutritional demands conflict with those of the host when their numbers increase in a metabolically active area of the intestine. Pathologic effects are maximal when overgrowth involves the proximal small intestine. Intraluminal anaerobic bacteria, particularly fecal strains, possess enzymes that deconjugate bile salts and convert their component cholic and chenodeoxycholic acids to the secondary bile acids deoxycholate and lithocholate.[53] The net result is to lower the concentration of bile salts in the duodenum and jejunum below the critical micellar concentration (CMC).[54,55] Above the CMC, much of the triglyceride and cholesterol in the lumen is present in mixed micelles containing hydrolyzed lipid products (fatty acids and mono- and diglycerides) and bile salts. Below the CMC, large liquid crystalline and insoluble emulsoid forms predominate. Because pancreatic lipase is

TABLE 38.5-3 INTRALUMINAL BACTERIA: EFFECTS ON THE HOST

INTRALUMINAL EFFECTS	MUCOSAL EFFECTS	SYSTEMIC EFFECTS
Bile salt deconjugation	Disaccharidase loss	Absorption of bacterial toxins, antigens
11α-Hydroxylation	Enterocyte damage	Hepatic inflammation
Bile salt depletion		
Lipid malabsorption	Inflammation	Immune complex formation
Vitamin B_{12} malabsorption	Protein loss	Cutaneous vasculitis
Fermentation—short-chain fatty acids	Bleeding	Polyarthritis
Release of proteases, toxins		

water soluble and must operate at a lipid–water interface, the great reduction in lipid surface area has disastrous consequences for fat digestion, and malabsorption of triglyceride, fat-soluble vitamins, and other lipid molecules results. Patchy histologic abnormalities, impaired uptake of lipolytic products, and slow chylomicron transport also may contribute to fat malabsorption.[56]

Intraluminal bacteria, particularly *Bacteroides* species and coliforms, also use vitamin B_{12} and thus directly compete for dietary vitamin B_{12}, preventing its absorption. When radioactive vitamin B_{12} is administered to animals or patients with SBBO, most of the radioactivity subsequently recovered from the small bowel contents is bound to enteric microorganisms.[57,58] Once bound, the vitamin is unavailable to the host unless the bacteria die. Luminal bacteria also produce inactive cobamides that are not available to the affected host.[59] Vitamin B_{12} malabsorption not correctable by exogenous intrinsic factor may be the most consistent feature of clinically significant SBBO.[54]

Mucosal Effects

An enteropathy is less dramatically expressed but in aggregate is equally deleterious. Although bacteria that accumulate in SBBO do not produce classic enterotoxins,[60] they do produce enzymes[61,62] and metabolic products[63,64] that are potentially capable of injuring the mucosa. In experimental blind loops, anaerobic bacteria elaborate proteases with elastase-like properties that remove or destroy glycoprotein enzymes on the brush border surface.[61,62] As a result, mucosal disaccharidase activities are reduced.[65] Monosaccharide transport may also be impaired, reflecting damage to the microvillus plasma membrane and the toxic effects of deconjugated bile salts.[63] Impaired transport of sodium and chloride has also been demonstrated.[66] In the self-filling blind loop, bacterial overgrowth produces a relatively mild morphologic lesion. Both villus height and crypt depth are increased.[67,68] Approximately 10 to 20% of the columnar cells in the upper half of the villi are swollen and vesiculated. Apical membrane microvilli of some, but not all, enterocytes are blunted, swollen, and budded. Damaged mitochondria and endoplasmic reticulum can be found. These experimental findings are in keeping with morphologic reports in humans,[69] which suggest that bacterial overgrowth causes a patchy mucosal lesion with segments of subtotal villus atrophy and a marked subepithelial inflammatory response. In infants, particularly, there is a well-established association between carbohydrate intolerance and small intestinal bacterial overgrowth.[47,70] Protein loss in the intestine may be sufficiently profound in both experimental animals[71] and humans[71,72] to cause hypoproteinemia. Chronic intestinal blood loss also has been documented as a cause of anemia.[73]

Batt and colleagues described German shepherd dogs that spontaneously develop SBBO.[74] These dogs have chronic diarrhea, weight loss, vitamin B_{12} deficiency, increased folate, and a relative serum IgA deficiency.[75] This model of SBBO differs from that in rats and humans because aerobic bacteria play a more important role in dogs, and total bacterial numbers are generally lower. This causes different types of brush border injury because dogs with aerobic bacterial overgrowth have normal disaccharidase and aminopeptidase N activities. Ten of 17 dogs had aerobic bacterial overgrowth that induced increased γ-glutamyl transferase and decreased alkaline phosphatase.[76] Other than the relative IgA deficiency, the etiology of this spontaneously occurring SBBO is unknown. Riordan and colleagues examined the difference between overgrowth of colonic-type and oropharyngeal-type organisms and found no difference in the morphology of villus height and crypt depth.[77]

Systemic Effects

Bacterial products and antigens are absorbed through the damaged mucosa, causing systemic effects. SBBO increases intestinal permeability in humans[78] and in rats causes enhanced absorption of the bacterial polymer peptidoglycan.[79]

Abnormalities in both hepatic function and liver architecture develop in the rat self-filling blind-loop model of bacterial overgrowth.[80–82] Immune responses are probably responsible for complaints of arthritis and dermatitis. Circulating immune complexes containing IgG, IgA, IgM, and complement are detected during episodes of arthritis associated with SBBO caused by intestinal bypass surgery.[83,84] IgM, IgA, and C3 depositions in the reticular dermis have been demonstrated in association with necrosis of the upper dermis and adjacent vasculitis.[85,86]

Following surgical creation of jejunal self-filling blind loops, SBBO in susceptible rat strains induces hepatobiliary injury.[80] Female Lewis and male and female Wistar and Sprague-Dawley rats develop liver injury by 4 to 9, 12, and 14 weeks, respectively, but Fischer and Buffalo rats do not develop hepatobiliary injury even after 16 weeks. Total anaerobic bacteria within the loops are similar (approximately $10^{8–10}$ organisms/mL), and loop sizes are similar in each rat strain. Metronidazole and tetracycline prevent the lesions in the livers of susceptible strains.[81] Histopathology

demonstrates inflammatory infiltration in the portal tracts, with bile duct proliferation and destruction, as well as fibrosis with some inflammation of the parenchyma.[80] Cholangiograms demonstrate widened extrahepatic bile ducts that are thickened and intrahepatic bile ducts that are dilated, tortuous, and irregular.[82] Taken together, these histologic and cholangiographic features resemble primary sclerosing cholangitis in humans. Further studies are required to determine the differences and similarities between SBBO-induced hepatic injury and primary sclerosing cholangitis. It should be noted that SBBO causes translocation of viable bacteria to mesenteric lymph nodes in the rat model, but its significance is unknown because translocation occurs in all rat strains and in the presence of metronidazole, tetracycline, and mutanolysin, which prevent hepatic injury.[80,81] Intravenous doses of lipopolysaccharide and SBBO (which increases serum lipopolysaccharide levels) worsened liver disease in rats with bile duct ligation.[87] The precise relevance of these animal models to humans remains speculative because in one study with eight adults with anaerobic colonic-type SBBO, only one patient had elevated γ-glutamyl transferase and alkaline phosphatase.[88]

Recently, Kaufman and colleagues showed that in patients with short-bowel syndrome, SBBO causes longer total parenteral nutrition dependency and correlates with small intestinal inflammation.[89] They noted that antibiotic therapy was not always successful, resulting in the use of other therapies, including saline enemas, polyethylene glycol (Golytely) infusions, and probiotics.[90]

IMMUNOLOGIC EFFECTS

Rats with self-filling blind loops produce high levels of luminal sIgA specifically targeted against colonic bacteria.[24] Luminal IgA2 and IgM are increased in humans, but IgG1–4 is not.[91] C4 is also not influenced by SBBO.[92] Interleukin-6 is elevated in the lumen of patients with SBBO, but tumor necrosis factor (TNF)-α and interferon-γ are not.[93] Interestingly, increased luminal levels of antigliadin IgA antibodies are observed in patients with SBBO.[94] Serum IgG3 levels are decreased in humans with SBBO but not other serum immunoglobulins and interleukin-2 receptor levels.[93] SBBO may reduce IgG3 in humans via an interaction with mucosa-related immunoregulatory mechanisms, a type of "tolerance."[95] In a rat model of monoarticular arthritis, the acute creation of SBBO reactivates arthritis that may be mediated by release of cytokines from the blind loop.[96] Interestingly, one study found that adults with rheumatoid arthritis have an increased incidence of SBBO.[97] In German shepherd dogs with SBBO, duodenal biopsies show increased TNF-α and transforming growth factor-β messenger ribonucleic acid expression that decreases after antibiotic treatment.[98] A single-dose of oral cholera vaccine administered to children results in less seroconversion when the children have SBBO.[99] Studies are, therefore, now showing that SBBO affects the local intestinal immune responses, which can have significant distant effects.

CLINICAL FEATURES OF SBBO

Clinical symptoms (Table 38.5-4) occur in approximately one-third of all patients.[56,100] In those who are symptomatic, clinical effects range from mild inconvenience to complaints that are both chronic and disabling. In general, overgrowth of bacteria in the proximal small bowel results in greater disability than overgrowth in the distal small intestine. SBBO may occur at any age, but relative deficiencies in mucosal defenses in the immature host may place the very young at increased risk.

Diarrhea is a common presenting symptom. Stools may be foul and greasy owing to steatorrhea or watery and explosive owing to maldigestion of dietary carbohydrates. In children, growth failure may be an important additional presenting clinical feature. Arrested weight velocity is usually the first feature to be noticed, but later a delay in height velocity and resultant short stature can appear. Nutritional debilitation is multifactorial. Appetite is frequently diminished. Diets may be restricted in an attempt to decrease stool frequency. Maldigestion of fat, dietary carbohydrate,[63] and protein[72] and increased intestinal losses of endogenous proteins[71] also contribute.

Clinical evidence of vitamin deficiency is rarely seen. Usually, vitamin B_{12} deficiency is prevented in the pediatric population by adequate body stores of cobalamin. Iron deficiency anemia can occur, however, secondary to enteric iron losses.[73] Osteomalacia, rickets, and pellagra-like symptoms each have been reported as a consequence of vitamin D malabsorption[101] and water-soluble B-vitamin[102] deficiency. Because luminal bacteria produce vitamin K and folic acid and these become available to the host, absorption may be enhanced. Serum folic acid levels may be elevated.[103]

Davidson and colleagues indicate that SBBO may be a cause of abdominal pain in young children owing to sec-

TABLE 38.5-4 CLINICAL FEATURES OF SMALL BOWEL BACTERIAL OVERGROWTH

CLASSIC
Chronic diarrhea
Steatorrhea
Anemia
SYSTEMIC
Arthritis
Tenosynovitis
Vesiculopapular rash
Erythema nodosum
Raynaud's phenomenon
Nephritis
Hepatitis
Hepatic steatosis
OTHERS
Weight loss
Short stature
Abdominal pain
Protein-losing enteropathy
Hypoalbuminemia
Osteomalacia
Night blindness
Ataxia

ondary carbohydrate intolerance.[104] De Boissieu and coworkers confirmed this observation in children under age 2 years and produced a dramatic improvement in diarrhea and abdominal pain using metronidazole and colistin.[105] Pimental and colleagues reported that 78% of 202 adults with irritable bowel syndrome had SBBO, and that eradication, using antibiotic therapy, reduced clinical symptoms.[106] Others have commented that, by definition, irritable bowel syndrome is not associated with underlying intestinal pathology, so these patients may not truly have irritable bowel syndrome.[107,108]

Recurrent episodes of arthralgia, nondeforming polyarthritis, tenosynovitis, and cutaneous vasculitis (the arthritis-dermatitis syndrome) occur commonly following intestinal bypass operations but also have been reported in other forms of SBBO.[85,109] Skin lesions are typically vesiculopustular or erythema nodosum–like. Paresthesias and Raynaud phenomenon are common. Renal damage[110] and hepatic steatosis[111] have also been reported following jejunoileal bypass surgery and could conceivably appear in other forms of SBBO. SBBO may affect drug metabolism. The half-life of antipyrine is increased by 78% in rats with SBBO.[112] Oral digoxin bioavailability is decreased owing to bacterial degradation.[113] A case of pneumoperitoneum and ascites was reported secondary to SBBO.[114]

LIVER DISEASE AND SBBO

Many studies have reported that liver disease is associated with SBBO in humans. Dame Sheila Sherlock first described this relationship in alcoholics.[115] Subsequent studies confirmed this finding and extended it to other causes of liver disease, including viral hepatitis.[116,117] These studies show that the incidence of SBBO increases with the severity of liver disease and may exceed 50% in patients with Childs-Pugh Class C liver disease.[116,117] Data suggest that the incidence of spontaneous bacterial peritonitis (SBP) increases with SBBO, but both SBP and SBBO are more frequent as liver disease worsens.[118] Runyon and colleagues used a rat model of cirrhosis to show that increased bacterial translocation is associated with SBBO, with or without SBP.[119] They postulated that the first step toward SBP is SBBO, which causes translocation of bacteria, and then local factors (such as ascites and ascitic complement levels) ultimately determine whether SBP will occur.[119] Chang and colleagues showed that risk factors for SBP in humans were lower serum C_3 and C_4 concentrations, lower ascitic total protein, and SBBO.[120] The incidence of SBBO in patients with SBP was 68% compared with a frequency of only 17% SBBO in cirrhotics without SBP.[120] However, two other groups, although confirming that adult cirrhotics had a higher incidence of SBBO than noncirrhotics, could not correlate SBBO with SBP.[121,122] In another study of adults with cirrhosis, a 6-month treatment trial with cisapride (n = 12) or antibiotics (neomycin and norfloxacin; n = 12) enhanced small bowel motility, diminished SBBO, and was associated with slightly improved liver function.[123] Finally, as interest in nonalcoholic steatohepatitis increases,[124] Wigg and colleagues showed that patients with nonalcoholic steatohepatitis have a 50% prevalence of SBBO compared with only 22% of controls.[125] No study of SBBO in the setting of liver diseases in children has yet been published.

DIAGNOSIS OF SBBO

A well-directed medical history often provides important clues to indicate that a more detailed investigation related to the possibility of underlying bacterial contamination of the small intestine is warranted. A history of previous abdominal surgery should always be sought because SBBO owing to either alterations of intestinal motility or the creation of anatomic regions of intestinal stasis can occur as a long-term adverse complication of intestinal surgery. Specific surgical procedures that affect normal nonimmunologic host defenses may also predispose the patient to SBBO. For example, interruption of the vagus nerve inhibits output of gastric acid and thereby can result in an increase in the load of viable organisms that enter into the proximal small intestine. Similarly, signs and symptoms suggestive of systemic disease—in particular, collagen vascular diseases—should be explored in a detailed history. Long-standing diabetes mellitus can result in alterations in intestinal motility owing to an autonomic neuropathy. Although not uncommon in adults, SBBO owing to autonomic dysfunction is very unusual in children with diabetes mellitus. This age-related difference may simply relate to the duration of the underlying chronic disease.

Table 38.5-5 summarizes alternatives that are useful in establishing the diagnosis of clinically significant bacterial overgrowth. Not all patients with overgrowth of bacteria in the small bowel have clinical symptoms. Therefore, an initial evaluation should be directed toward first determining if there is evidence of malabsorption of either fat or cobalamin (vitamin B_{12}). Serum levels of cobalamin are not, in general, a useful screening test for SBBO in children because body stores of the vitamin are sufficient to maintain normal circulating levels for at least 5 years after the onset of cobalamin malabsorption in the gut. Elevated levels of folic acid may be useful as a screening test.

In the appropriate clinical setting, abnormal values of quantitative fecal fat excretion, Schilling test with intrinsic factor, and serum folate indicate the need for more detailed investigations to establish a diagnosis of bacterial overgrowth. Although some physicians would proceed directly to an empiric course of antibiotic therapy, it should be emphasized that clinical symptoms and abnormalities in screening laboratory tests are not specific to this diagnostic consideration.

A barium meal with follow-through should be performed to document the presence of intestinal strictures, diverticula, and delayed intestinal transit. Abnormalities in the radiologic study should prompt more extensive, directed investigations. A normal barium meal study does not exclude the presence of clinically significant bacterial overgrowth in the small intestine. A biopsy of the duodenum should also be obtained because the presenting symptoms of gluten-sensitive enteropathy in children can mimic

TABLE 38.5-5 DIAGNOSTIC TESTS FOR SMALL BOWEL BACTERIAL OVERGROWTH

SCREENING
Sudan stain for neutral fat
72-Hour fecal fat
Schilling test with intrinsic factor
Folic acid, vitamin B_{12}
Barium meal with follow-through
DIAGNOSTIC
Invasive
Duodenal aspiration for culture (aerobic, anaerobic bacteria and exclude known pathogens)
Deconjugated bile salts
Short-chain fatty acids
Noninvasive
Indicanuria
Serum bile acids
Breath tests

SBBO. Patchy enteropathic changes with increased numbers of inflammatory cells are typical of SBBO.

Specific Diagnostic Tests

Quantitative culture of increased numbers of anaerobic bacteria in luminal fluid obtained from the proximal small intestine establishes the diagnosis of SBBO. The presence of more than 10^6 colony-forming units/mL of bacteria that are not typical residents of the oral cavity is an abnormal finding. Documentation of coliforms and anaerobic colonic-type bacteria is important because these bacterial species normally do not reside in the mouth or stomach and do not colonize the upper human small intestine. However, the culture of duodenal fluid as a diagnostic technique is not without its problems. Many hospitals do not have bacteriology laboratories with the ability to routinely culture fastidious strict anaerobes. Fortunately, the presence of more than 10^6 colony-forming units of facultative anaerobic bacteria, such as *Escherichia coli* strains, is relatively good evidence of associated colonization by strict anaerobic bacteria. Therefore, quantitative duodenal cultures should be performed even if one lacks anaerobic culture facilities. When culture of duodenal aspirates was compared with that of gastric aspirates and duodenal biopsies in 75 adults, duodenal aspirates proved to be significantly more sensitive.[126]

Neither duodenal aspiration nor quantitative bacterial estimation is easily performed. A great deal of investigation, therefore, has been focused on establishing the utility of other diagnostic techniques. Two alternative tests that depend on the detection of bacterially derived products (unconjugated bile acids and short-chain fatty acids) may be performed on duodenal aspirates: (1) determination of conjugated and deconjugated bile acid profiles in duodenal fluid[127] and (2) assay of duodenal fluid for the presence of short-chain, volatile fatty acids (ie, acetic, propionic, butyric, isobutyric, valeric, and isovaleric).[128,129] Each test may be helpful, if decidedly abnormal, but neither has been evaluated fully.

Duodenal aspiration is relatively invasive, and sedation or physical restraint is usually required in children to permit the passage of an aspiration tube through the oral cavity into the upper small bowel. Because fluorography is often used to establish localization of the catheter tip, a small dose of radiation is frequently required. The string test is not an accurate substitute for duodenal intubation.[130] Accurate, noninvasive diagnostic methods are, therefore, an attractive alternative. Although the optimal noninvasive diagnostic probe for use in children has not yet been defined, a number of options are available. Measurement of elevated urinary indican, produced by the conversion of dietary tryptophan to indican by intraluminal bacteria, is perhaps the simplest technique. Specificity is low, however, because indicanuria is not limited to bacterial overgrowth in the small intestine.[131]

Konishi and colleagues used a newly synthesized conjugate of monophosphated ursodeoxycholic acid with 5-aminosalicylic acid (5-ASA) monophosphate to diagnose SBBO in rats with surgical blind loops.[132] They found an elevation of urinary acetylated 5-ASA in rats 24 hours after oral administration.[132] This test has potential for future use in humans, with the intent of eliminating the need for obtaining duodenal aspirates.

Provided that the technique is available, a relatively simple quantitative estimate of serum conjugated and free bile acids may be helpful. Total serum bile acids are often elevated in patients with SBBO, and almost all of the increase is represented by free bile acids, which are normally present in trace amounts.[133] Individual bile acid profiles show that deoxycholate is uniquely elevated, a finding that distinguishes SBBO from ileal resection, which is also associated with high serum levels of free bile acids.[133,134]

A number of studies have examined the utility of various breath tests as diagnostic tools (Table 38.5-6). Measurement of carbon 14 in expired air following oral ingestion of an appropriate substrate, which is conjugated to the radioisotope, appears to be an excellent alternative for noninvasive diagnostic purposes. ^{14}C-labeled bile acids, such as glycocholate, were the first substrates to be used.[135] Use of the substrate by luminal bacteria releases ^{14}C, which then equilibrates within the tissues of the host and is excreted from the lungs as $^{14}CO_2$ in expired air. A negative test may occur if the bacteria are not able to deconjugate bile acids.[136] In fact, several different breath tests may be required to detect SBBO because of the different metabolic capabilities of the contaminating flora.[137] False-positive results may occur in patients with mucosal inflammation affecting the distal ileum. Several reports indicate that ^{14}C-labeled D-xylose is superior to ^{14}C–bile acid as substrate.[138,139] Improvement in the ^{14}C–D-xylose breath test may be possible by incorporating transit time markers.[140] Another group showed that combination of the lactulose breath hydrogen test with urinary *p*-aminobenzoic acid (PABA) collection following ursodeoxycholic acid–PABA increases the chance of diagnosing SBBO.[141] Urinary cholyl-PABA excretion is a reliable test in adult patients with SBBO and correlates well with the D-xylose breath test.[142] Unfortunately, the use of ^{14}C is not satisfactory for use in diagnostics for children. Stable isotopes, such as ^{13}C-substrate,[143] have been used to study children. For

TABLE 38.5-6 BREATH TESTS FOR USE IN THE DIAGNOSIS OF SMALL BOWEL BACTERIAL OVERGROWTH

RADIOISOTOPES
^{14}C-glycocholate
^{14}C–D-xylose
STABLE ISOTOPES
^{13}C-conjugates
BREATH HYDROGEN
Fasting levels
Lactulose
Lactose
Glucose

example, in six patients with known SBBO, 50 mg of ^{13}C-xylose produced a maximum breath $^{13}CO_2$ with 100% sensitivity but only 67% specificity.[144]

Measurement of hydrogen (H_2) levels in samples of expired air provides an alternative approach that is currently applicable to the pediatric population. Mammalian cells do not produce H_2, whereas many prokaryotes produce it as a by-product of substrate use. The commensal colonic bacteria are generally excellent H_2 producers. The H_2 is absorbed and distributed throughout the body and is subsequently expired in the breath. Provision of a nonabsorbable sugar, such as lactulose, supplies substrate to the colonic microbial flora and results in an increase in levels of expired H_2.[145] If a colonic type of microflora is present in the small intestine, an early H_2 peak is observed following lactulose challenge (Figure 38.5-4). Absorbable carbohydrates, including lactose[146] and D-glucose,[147] may also prove useful as substrates in testing for SBBO by measurement of breath H_2. However, the glucose breath hydrogen test correlated poorly with duodenal cultures in one study of 40 cirrhotic patients.[148]

Perman and colleagues reported that elevations in the fasting level of breath H_2 in children correlate with the presence of SBBO.[149] Previous meals containing nonabsorbable carbohydrates[150–152] and endogenous glycoproteins,[153] however, may elevate fasting breath H_2 and cause false-positive results. In children, however, a breath H_2 level of greater than 42 ppm was seen only in those with SBBO.[149]

Breath H_2 testing has shortcomings, however, which may limit its effectiveness. For instance, Douwes and colleagues reported that 9.2% of 98 healthy school-age children who were tested were non-H_2 producers.[154] Children with diarrhea and low fecal pH have an altered intestinal flora that may not yield H_2 in expired air samples.[155–157] Concurrent use of medications, particularly antibiotics,[158] also affects bacterial fermentation of test sugars, which can lower breath H_2 levels. A clear separation of "early" and "late" H_2 peaks following lactulose ingestion can be affected by the rate of gastric emptying and by intestinal transit time. In practice, two distinct peaks are often not documented.[159] Riordan and colleagues showed that the lactulose breath test was only 16% sensitive and 70% specific, and the double peak was frequently missing in cases of proven SBBO.[160] The same group also found that sensitivity for the rice-based breath hydrogen test was only 33% and did not provide a suitable alternative to culture of duodenal aspirates.[161] One study in adults found that a single resting breath hydrogen test was unreliable.[162] Several reports indicate that the normal microbial flora of the oral cavity also can contribute to the fermentation of carbohydrate substrates and produce modest elevations in the levels of H_2 in expired air.[163,164]

Breath H_2 tests require careful attention to technical details, which are sometimes difficult to reproduce. End-expiratory samples are most representative, but they are often difficult to obtain in toddlers and preschool-age children. Flow-through appliances that allow collection through a face mask are available and appear to be more readily tolerated.[165,166] Storage of collected samples in appropriate sealed containers is also critical for accurate results.[167,168] Although breath tests are more convenient, Corazza and colleagues documented their inferiority compared to jejunal cultures; the glucose breath test yielded a sensitivity of just 62% and a specificity of only 83%.[169]

Comparative studies in adults suggest that ^{14}C–D-xylose is the most appropriate substrate for use in breath testing.[138,159] The comparative sensitivity and specificity of other noninvasive diagnostic assays that are more suitable for use in children have not been clearly defined. However, comparison of a 1 g ^{14}C–D-xylose breath test with the 50 g hydrogen glucose breath test showed that the hydrogen glucose breath test was slightly more sensitive for detecting bacterial overgrowth.[170]

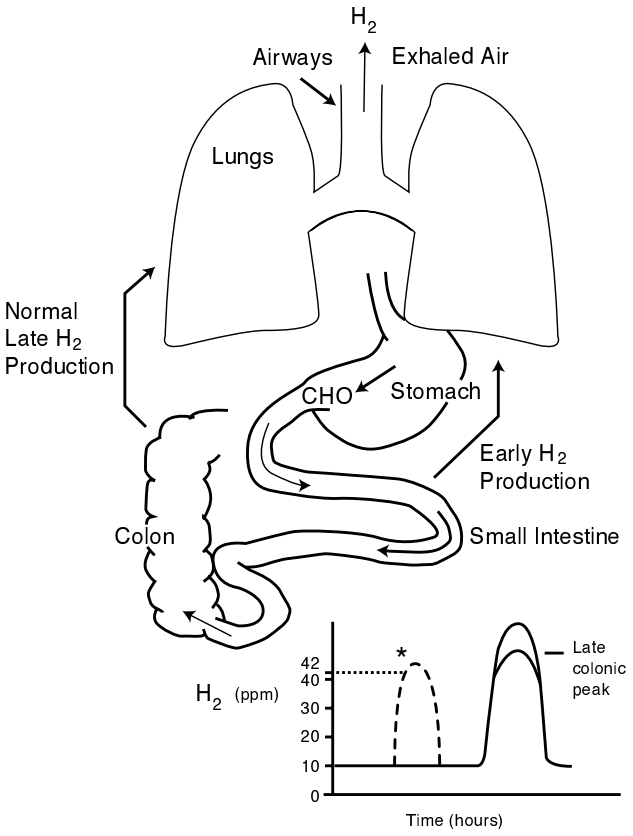

FIGURE 38.5-4 Conceptual framework for breath hydrogen testing. CHO denotes ingesting carbohydrate. *An early peak and an elevated baseline of hydrogen measured in expired breath samples are both suggestive of small bowel bacterial contamination.

TREATMENT

Correction of the Underlying Disease

As illustrated in Table 38.5-2, there are multiple causes of SBBO, some of which are potentially treatable by surgery. Reports of surgical correction include an ileal carcinoid tumor causing obstruction,[171] a large Meckel diverticulum,[172] and an ischemic jejunal stricture following blunt trauma to the abdomen.[173] Gastrointestinal, gastrocolic, and jejunocolic fistulae and intestinal strictures that occur following radiation or surgery or in Crohn disease are also amenable to surgery. The fact that some cases of SBBO can be cured by surgical intervention emphasizes the importance of investigating each patient carefully for such lesions.

Other cases may be improved by treatment of the primary disease, such as, for example, by employing the use of corticosteroids in patients with symptoms of active Crohn disease. When disordered motility is the primary problem, as in diabetes mellitus, scleroderma, and intestinal pseudo-obstruction, pharmacologic agents occasionally may prove effective. Cisapride, a prokinetic agent, can, for example, improve motility patterns in some patients with diabetic neuropathy[174] and certain forms of intestinal pseudo-obstruction.[175] The long-acting somatostatin analog octreotide stimulates propagative phase 3 motor activity in the duodenum of patients with scleroderma, which can result in decreased symptoms of SBBO and reduce bacterial counts in the proximal bowel.[176] SBBO associated with scleroderma has also been treated successfully with antibiotics.[177]

Supportive Therapy

Careful attention should be given to ongoing nutritional and metabolic complications. Nutritional deficits should be anticipated and prevented by using appropriate supplements. Energy intake may be limited by anorexia, abdominal pain, and malabsorption. Easily digestible nutritional supplements, which are low in fat, may be required to maintain normal growth and development. Medium-chain triglycerides have been advocated[56,171,178] and may be helpful. In intractable situations, such as occur in the pseudo-obstruction syndromes, enteral nutrition with elemental formulae or parenteral nutrition should be employed to maintain growth. Because the course of these diseases can be unpredictable, marginal improvement may eventually allow an adequate oral intake to meet nutritional requirements.

Clinical evidence of vitamin deficiency is rare, but fat-soluble vitamin deficiencies have been reported in patients with SBBO, including a striking case of neurologic deterioration owing to vitamin E deficiency,[179] night blindness owing to vitamin A deficiency,[180] and osteomalacia owing to vitamin D deficiency.[101,181] Patients with steatorrhea should receive fat-soluble vitamins. A good rule is to follow the recommendations for cystic fibrosis outlined in Chapter 68, "Liver Biopsy Interpretation."

Vitamin B_{12} deficiency is rare in children because of the time required to deplete body stores. It is correctable by monthly injections of cyanocobalamin. Anemia may also require treatment with supplemental iron to correct iron deficiency secondary to enteric losses.[73] Treatment with iron may unmask a coexistent and unrecognized macrocytic anemia. B vitamins, vitamin K, and folic acid are normally not depleted by bacteria, which may, indeed, add to host supplies. Tabaqchali and Pallis reported a very interesting case of nicotinamide deficiency that developed in an elderly patient with multiple jejunal diverticula, steatorrhea, and severe protein malnutrition several weeks after apparently successful treatment with protein infusions and antibiotics.[102] The sudden elimination of nicotinamide-producing bacteria in the upper intestine may have precipitated pellagra. The possibility of sudden loss of a vitamin source should be considered whenever evidence of deficiency appears during treatment.

Antibiotic Therapy

A variety of antibiotics have been used to treat SBBO successfully, but large studies comparing different antibiotics and protocols do not exist. The specific mechanisms by which antibiotics reverse different pathophysiologic events, such as disaccharide intolerance, protein-losing enteropathy, and steatorrhea, have not been entirely explained. Goldstein and colleagues stressed the importance of culturing intestinal contents to determine which antibiotic was most effective in treatment.[182] They found that antibiotics effective against aerobes reduced both aerobic and obligate anaerobic bacterial counts. Aerobes lower oxygen tension and maintain low oxidation-reduction potentials (E_h), thereby allowing anaerobes to thrive. When broad-spectrum antibiotics are successful, they may work by altering the intestinal microecology to reduce the growth of a critical organism. Beeken and Kanich also advocate the measurement of antibiotic sensitivities to plan treatment of SBBO in patients with Crohn disease.[183] Bouhnik and colleagues state that this should be done in all patients with SBBO.[184] On the other hand, because no single organism is responsible for all of the abnormalities that occur in SBBO, other investigators believe that isolation of organisms and testing for antibiotic sensitivity are not necessary.[178]

Table 38.5-7 summarizes a number of studies in which the antibiotic treatment of SBBO was evaluated by at least one objective parameter. Antibiotics have rarely been compared for effectiveness in the same group of patients. Barry and colleagues showed, however, that metronidazole was superior to kanamycin when evaluated in five patients with pseudo-obstruction.[185] In the table, total aerobic and anaerobic bacteria counts were reported in four separate studies in which jejunal cultures were obtained.[71,185,186,187] In three studies, bacterial counts decreased using antibiotic therapy, but in the fourth study, which used metronidazole,[186] only 1 of 12 patients had lower bacterial counts, although the drug was clinically effective in most patients. A similar discrepancy between bacterial counts and clinical outcome was observed in rats with experimentally induced SBBO treated with chloramphenicol.[15] Although total anaerobic counts were unchanged, *Bacteroides* species virtually disappeared, suggesting that the effectiveness of antibi-

TABLE 38.5-7 RESULTS OF ANTIBIOTIC TREATMENT OF SMALL BOWEL BACTERIAL OVERGROWTH

UNDERLYING CAUSE	NUMBER OF CASES	SYMPTOM OR LABORATORY TEST	ANTIBIOTIC (NUMBER IMPROVED)	INTESTINAL BACTERIA (NUMBER REDUCED)	REFERENCE
Scleroderma	4	Stool fat	Tetr (3) (Kan, Neo, Sulf—fail)	ND	194
Jejunoileal bypass	5	Diarrhea, pain	Metr (5) (Kan—fail)	Anaerobic (5); aerobic (0)	185
Billroth II	12	Diarrhea, pain, vomiting	Metr (6); cotrimoxazole (1)	Total (1/3)	186
Pseudo-obstruction	1	Indoxyl sulfate excretion	Tetr (1) (Neo—fail)	ND	195
Postanastomosis	1	Stool fat	Tetr (1)	*E. coli* (1)	187
Billroth II	1	^{51}Cr clearance	Broad spectrum (2)	Aerobic (2)	71
Pelvic radiation	1	Stool fat		Anaerobic (2)	
Jejunoileal bypass	12	Liver steatosis, diarrhea	Metr: steatosis (12); diarrhea (9)	ND	111
None	9	Breath H_2, abdominal pain	Linc (4); Trimeth (2); Amox (1); Flucox (1); Sulf (1)	ND	104
Malnutrition	14	Breath H_2, diarrhea	Metr (11)	ND	196
Mixed	18	Breath $^{14}CO_2$ (bile acid)	Tetr (12)	ND	31
Mixed	21	Breath H_2, symptom score	Rifax (9/10); Chlor (2/11)	ND	190
Mixed	10	Breath H_2, stool number	Norflox (9); Amox-Clav (6); Saccharo (0)	ND	191

Amox = amoxicillin; Chlor = chlortetracycline; Clav = clavulanic acid; Fluclox = flucloxacillin; Kan = kanamycin; Linc = lincomycin; Metr = metronidazole; ND = not done; Neo = neomycin; Norflox = norfloxacin; Rifax = rifaximin; Saccharo = *Saccharomyces boulardii*; Sulf = sulfisoxzole; Tetr = tetracycline.

otics against that organism may be crucial. The antibiotic responses shown in Table 38.5-7 are generally consistent with the crucial role of anaerobes, particularly *Bacteroides*, in the pathogenesis of SBBO. Patients improve on tetracycline, metronidazole, trimethoprim-sulfamethoxazole, lincomycin, and broad-spectrum antibiotics, whereas kanamycin and neomycin, two antibiotics to which *Bacteroides* is generally resistant, have proven ineffective. *Bacteroides* also plays a crucial role in experimentally produced SBBO. Welkos and colleagues showed that kanamycin and penicillin lowered the number of aerobic bacteria in rats with self-filling blind loops but did not reverse vitamin B_{12} malabsorption.[188] By contrast, metronidazole greatly diminished *Bacteroides* counts and corrected the vitamin B_{12} malabsorption. Subsequently, *Bacteroides* species were shown to bind to intrinsic factor–vitamin B_{12} complex much more avidly than to five aerobic bacteria and seven other anaerobic bacterial species.[188]

These and other studies suggest that the most appropriate therapeutic approach is to choose an antibiotic that is effective against *Bacteroides*, such as tetracycline, metronidazole, chloramphenicol, or lincomycin. Of these four antibiotics, metronidazole is the least likely to cause untoward side effects in children and is probably the antibiotic of choice for initiating treatment. An initial course of 2 to 4 weeks duration may be followed by clinical improvement lasting many months. If relapse occurs, a second course of the same antibiotic for a longer period (4–8 weeks) may be tried. Relapse or persistence of steatorrhea, vitamin B_{12} malabsorption, or other complications may be amenable to relatively continuous antibiotic administration, accompanied by periodic alternation with broad-spectrum antibiotics, such as trimethoprim-sulfamethoxazole or gentamicin. Chloramphenicol and lincomycin should be reserved for cases in which other antibiotics have failed.

A nonabsorbable derivative of rifamycin, rifaximin, was shown to be effective in treating SBBO.[189] Using breath hydrogen following a 50 g glucose load as an indicator of SBBO, rifaximin for 7 days normalized breath tests in 70% of subjects compared with chlortetracycline, which normalized breath tests in only 27%.[190] Norfloxacin and amoxicillin–clavulanic acid decreased diarrhea and improved breath hydrogen tests in a randomized crossover trial of 10 patients with SBBO.[191]

Increasingly, probiotics are becoming a therapeutic tool for use in a variety of gastrointestinal disorders. One study failed to show improvement in 17 patients with SBBO following treatment with *Lactobacillus fermentum*.[192] In contrast, there was some success reported in eight hemodialysis patients with SBBO by using the probiotic agent *Lactobacillus acidophilus*.[44] In another study, 22 patients with proven SBBO were treated with either a combination of *Lactobacillus casei* and *Lactobacillus acidophillus* (n = 12) or placebo (n = 10).[193] Probiotic treatment improved stool frequency and glucose breath hydrogen test results but did not improve the patients' other symptoms. Use of *Saccharomyces boulardii* failed to provide clinical benefit in an open trial of 10 patients with SBBO.[191]

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CHAPTER 39

GASTROINTESTINAL MANIFESTATIONS OF IMMUNODEFICIENCY

1. *Primary Immunodeficiency Diseases*

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The importance of the intestine as an immune barrier is highlighted by the intimate proximity of the gut-associated lymphoid tissue (GALT) to the luminal surface of the gastrointestinal (GI) tract, an external environment rich in microbial pathogens and dietary antigens. Thus, it is not surprising that there is a strong clinical relationship between immunodeficiency (ID) states and significant GI disorders.[1,2] ID states are usually classified as primary or secondary disorders. The best known among the latter acquired type is the ID resulting from infection by human immunodeficiency virus (HIV) type 1 and its outcome, acquired immune deficiency syndrome (AIDS) (see Chapter 39.2, "HIV and Other Secondary Immunodeficiencies"). Past decades have seen enormous progress in the field of primary ID. More than 70 different primary IDs are now described in the world's literature. This chapter presents an approach to the GI complications of primary ID. Most primary IDs are diagnosed in infants and children and, therefore, are managed by pediatricians and pediatric gastroenterologists. Advances in the treatment of these diseases have also been impressive. Antibody replacement, cytokine, and humanized anticytokine are now possible through recombinant technologies. The ability to achieve lifesaving immune reconstitution of patients with lethal combined ID by administering rigorous T cell–depleted allogeneic related haploidentical bone marrow stem cells has extended this option to virtually all such infants if diagnosed before untreatable infections develop. Finally, the past few years have witnessed the first truly successful gene therapy. Therefore, it is imperative that pediatric gastroenterologists have a high index of suspicion for these problems in that such patients will present to them in consultation early. Early diagnosis and treatment of patients are vital, allowing most pediatric patients affected by primary ID now to survive into adulthood.[3]

INTESTINAL MUCOSAL IMMUNE SYSTEM

The mucosal membranes that line the respiratory, digestive, and urogenital tracts, as well as the conjunctiva, ear, and ducts of exocrine glands, are constantly exposed to environmental factors. Complex protective systems have been elaborated to defend the host against pathogens, toxins, and allergens. There is a wide array of innate and specific acquired immune mechanisms that normally operate in forming a mucosal barrier to protect the host.[1]

The fetal intestine plays an important role in the ontogeny of both the cellular and humoral immune systems. Postnatally, the human intestine develops histologic characteristics of a secondary lymphoid organ. Lymphoid follicles, germinal centers, small lymphocytes, and plasma cells are abundant. This association demonstrates the critical role of the immune system in maintaining GI homeostasis. Not only are GI abnormalities frequently encountered in patients with ID syndromes, they are also occasionally severe enough to become the patient's presenting complaint. Furthermore, primary intestinal diseases may secondarily cause substantial losses of immune system components so as to induce a secondary ID.

Nonimmune host defense factors, in addition to the major contribution by the GALT, are known to play an important role in mucosal protection against the abundant, potentially noxious antigens and pathogens present in the gut lumen. These include bactericidal fluids such as gastric

acidity and proteases, intestinal motility, the mucus-glycocalyx, and the normal intestinal flora.[1,2] These adjunctive mucosal defense mechanisms may explain why major deficiencies in either the humoral or cell-mediated immune system can exist without the presence of significant GI symptoms.

Recurrent, persistent, severe, or unusual infections are the hallmark of ID states. Chronic diarrhea, often associated with malabsorption and concomitant failure to thrive, is characteristic of several of the ID syndromes. Paradoxically, autoimmune diseases and/or excessive production of immunoglobulin (Ig)E antibodies also characterize many ID syndromes. As discussed below, there is also an increased incidence of malignancies in patients with certain ID states.[2]

COMPONENTS OF THE IMMUNE RESPONSE

An immune response consists of two components: a specific response to a particular antigen and a nonspecific augmentation of the effect of that response (Table 39.1-1). Specific immune responses may be divided into humoral and cellular responses. Humoral responses result in the generation of antibodies reactive with a given antigen. In contrast, cell-mediated responses involve the induction of specific effector cytotoxic cells or the secretion of cytokines that trigger inflammation (Table 39.1-2). Thymectomized animals, or congenitally athymic animals (including humans), have grossly impaired cell-mediated responses yet are able to generate some antibodies. In contrast, children incapable of making antibodies may be able to mount cell-mediated responses. They appear to handle many viral and fungal infections and can even reject grafts. The thymus-dependent cells (T cells) and the antibody-producing lymphocytes (B cells) are the key cells involved in specific immune responses (Figure 39.1-1). The latter may be considered to involve two phases. First is the recognition phase, involving antigen-presenting cells (APCs) (dendritic cells, macrophages, and B cells) and T cells that recognize the antigen as foreign. The effector phase then ensues, in which antibodies (B-cell products) and effector T cells eliminate the antigen.[1]

The nonspecific effector component of the immune response includes certain factors that can augment the effects of antibody, and some of these factors are older, in evolutionary terms, than antibody production itself. The major factors are phagocytic cells (macrophages and polymorphonuclear leukocytes), which remove antigens (including bacteria), and complement, which can either directly destroy an organism or facilitate its phagocytosis.

Humoral immunity depends not only on antibody synthesis but also on effector mechanisms that eliminate antigen bound to antibody. Microorganisms coated (ie, opsonized) with IgG antibodies are readily bound and ingested by phagocytic cells. Complement-dependent lysis of bacteria needs a functioning complement pathway and a complement-fixing antibody. Thus, specific immunity requires nonspecific effector mechanisms for its efficient operation.

The primary ID syndromes are a heterogeneous group of relatively rare diseases that result in failure to manifest an efficient immune-mediated inflammatory response, accompanied by repeated bacterial, fungal, and viral infections of variable severity (see Table 39.1-1). An ID may involve any of the components of the immune system, including lymphocytes, phagocytic cells, and complement proteins, as described in detail below.

CELLULAR BASIS OF THE SPECIFIC IMMUNE RESPONSE

T Cells

The progenitors of immunocompetent cells are derived from the lymphoid stem cell, whose nature still remains unclear. Within the thymus, bone marrow precursor T cells are induced to express thymus-associated surface antigens (glycoproteins) that serve as differentiation markers, as well as antigen receptors. A network of epithelial cells in the thymic cortex and in Hassall corpuscles secretes soluble thymic "factors" needed for functional T-cell maturation. Each T cell is committed to a given antigen, which it recognizes by one of two types of T-cell receptors (TCRs), depending on the cell's lineage. The T cells have either TCR1, composed of γ and δ chains (determined early in ontogeny), or TCR2, another heterodimer of α and β chains. The TCR2 cells predominate in adults, although 10% of T cells in epithelial structures bear TCR1. The TCR is closely associated on the cell surface with the CD3 protein responsible for transmitting the antigen recognition signal inside the cell (transduction). Nearby accessory molecules, such as lymphocyte function antigen (LFA)-1, CD2, CD4, and CD8, are responsible for increased leukocyte adhesion. The CD4 and CD8 proteins,

TABLE 39.1-1 COMPONENTS OF THE IMMUNE RESPONSE AND TYPICAL INFECTIONS IN IMMUNODEFICIENCY STATES

	SPECIFIC IMMUNITY		NONSPECIFIC IMMUNITY	
Effector mechanisms	B lymphocytes	T lymphocytes	Macrophages Polymorphonuclear leukocytes	Complement
	↓	↓	↓	↓
Defence mechanisms	Antibody	Cellular immunity	Phagocytosis	Lysis
Typical infections (when impaired)	Enteroviruses Pyogenic bacteria	Viruses Fungi Bacteria Protozoa	Bacteria Staphylococci Gram negative (*Klebsiella*, *Serratia*) Fungi	Certain viruses Pyogenic bacteria *Neisseria*

TABLE 39.1-2 LYMPHOCYTE SUBGROUPS, CYTOKINE PROFILES, AND THEIR FUNCTION

CELL POPULATION	CYTOKINES	FUNCTIONS
Th1	IL-2, IFN-γ, TNF	Initiation and augmentation of inflammatory reactions Enhance MHC expression
Th2	IL-3, IL-4, IL-5, IL-6, IL-10, IL-13	Enhance B-cell antibody production Inhibit Th1 cytokine production
Tc	IFN-γ	Enhance MHC expression; activation of NK cells; lysis of antigen target
Ts	Suppressor factor(s)	Suppress Th and Tc cells
B	Antibody: IgM, IgG, IgA, IgE, IgD, IL-10	Neutralization, opsonization, cell lysis Inhibit Th1 cytokine production

IFN = interferon; Ig = immunoglobulin; IL = interleukin; MHC = major histocompatibility complex; NK = natural killer; Tc = cytotoxic T cell; Th = T helper cell; TNF = tumor necrosis factor; Ts = suppressor T cell (may not be a distinct subpopulation).

present (predominantly) on TCR2 cells, recognize histocompatibilty antigens. Class II molecules show restriction for the antigen receptor of $CD4^+$ T cells and class I molecules for the TCR of $CD8^+$ T cells.

Lymphocytes initiate specific immune responses and possess immune memory. Some are involved in recognition, whereas others carry out effector functions. Effector T lymphocytes have several different functional activities. They may cause the death (cytotoxicity) of antigenic cells or initiate inflammation in response to an antigenic stimulus (delayed-type hypersensitivity). Other T cells have a regulatory rather than an effector role. The T-lymphocyte subpopulations can be classified into helper (Th) and suppressor (Ts) or cytotoxic (Tc) groups. Two types of Th cells, defined by their cytokine secretion patterns, have been described in the mouse (see Table 39.1-2). The Th1 cells generally secrete cytokines, which activate other T cells (interleukin [IL]-2), natural and cytotoxic T cells (interferon [IFN]-γ), and other inflammatory cells (tumor necrosis factor [TNF]). The Th1 cells are thus particularly effective against intracellular infections with viruses and organisms that grow in macrophages.[1] On the other hand, Th2 cells secrete those cytokines that activate B cells (IL-4, IL-5) or induce T cells and hematopoietic cells (IL-6) to grow and differentiate. Several other cytokines are secreted by both types of Th cells. The Th2 cells are excellent helpers for antibody production and, in the absence of Th1 cells, will induce high IgE levels owing to IL-4 production. They are well adapted for defense against parasites.

Although Th1 cells can provide help for antibody production, excessive Th1 activation inhibits B-cell activation. The Th1 cells induce a strong delayed-type hypersensitivity

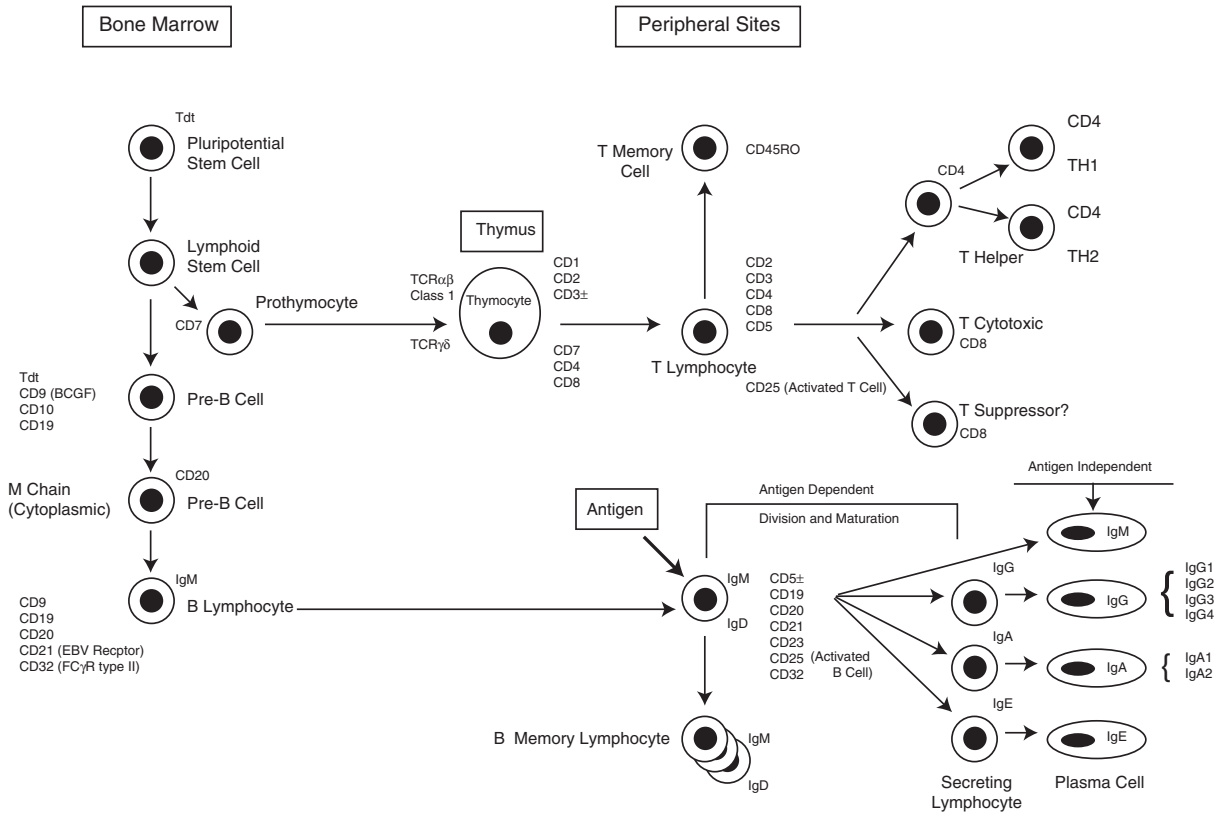

FIGURE 39.1-1 Maturation of T and B lymphocytes. Ig = immunoglobulin.

reaction, and IgE production is inhibited at all levels of Th1 activation owing to IFN-γ production. T-cell functions of help or suppression may depend on various stimuli, resulting in different cytokines being produced, with predominantly activating or inhibitory effects. Only Th cells that have responded to antigen presented by macrophages can subsequently help B cells already committed to that antigen. The effects of Th cells are balanced by those of functional Ts cells. Such Ts cells carry the characteristic surface glycoprotein CD8. Suppression by T cells is only partly understood. These cells are activated by an antigen and release factors that mediate suppression, which may be antigen specific or nonspecific. The $CD8^+$ T cells also include Tc cells, which lyse cells infected with virus in a specific manner. The Tc cells recognize viral antigens together with major histocompatibility complex (MHC) class I molecules. All endogenous antigens (including viral antigens) are presented in the context of MHC class I antigens. This combination probably activates $CD8^+$ T cells and certainly provides target cells for virally induced T-cell cytotoxicity and, consequently, a potential mechanism for autoimmune damage. Cytotoxic T cells play a role in graft rejection, in which Tc cells mature and are able to lyse target cells carrying the MHC class I molecules of the stimulating cells.

Antigen-Presenting Cells

Antigen is processed by specialized cells and then carried and "presented" to lymphocytes. Such specialized cells are known as APCs (Figure 39.1-2). The T cells cannot recognize antigen without such processing. Because activation of T cells is essential for most immune responses, antigen processing is a crucial step. The efficiency of T-cell activation is enhanced by the secretion of cytokines such as IL-1 by the APCs previously activated by antigen. The most efficient APCs are the interdigitating dendritic cells found in T-cell regions of lymph nodes. APCs are able to move about, and increased numbers are found in inflamed sites. Their recruitment is due to the adhesion molecules expressed on their surface that bind to receptors in the target sites. Follicular dendritic cells trap immune complexes that contain antigen and process and express it closely associated with MHC class II molecules on their surface. Class II molecules themselves determine the responsiveness of an individual to a particular foreign antigen because they interact with the antigen before T-cell help can be triggered. Macrophages in gut, Kupffer cells in liver, and astrocytes in brain, as well as activated B cells and intestinal epithelial cells, are also able to present antigen to T cells. Additional stimuli are provided by the binding of adhesion molecules on the cell surfaces of lymphocytes and APCs. For example, the LFA-3 on the APC binds to CD2 on the Th, giving an additional signal for activation. Likewise, intercellular adhesion molecule (ICAM)-1 binds to LFA-1 on the T-cell surface. Other cell to cell interactions, such as T-cell effector with a B lymphocyte or Tc cell and its target, are also enhanced by these molecules. Following the interaction of the TCR with antigen presented in association with MHC molecules, T cells become acti-

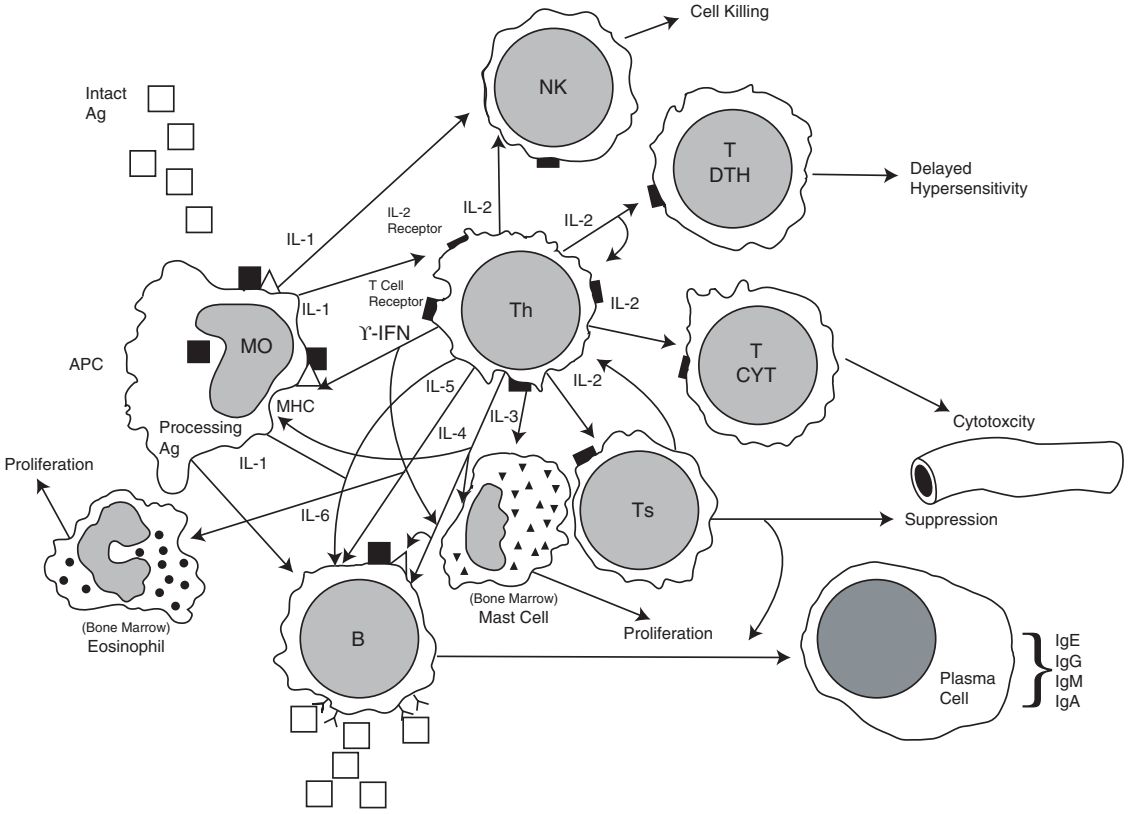

FIGURE 39.1-2 Cytokines and the immune response. Ag = antigen; APC = antigen-presenting cell; B = bursal cell; CYT = cytotoxicity; DTH = delayed-type hypersensitivity; IL = interleukin; γ-IFN = γ-interferon; MHC = major histocompatibility complex; Mo = macrophage; NK = natural killer cell; Th = T helper cell; TS = T suppressor cell.

vated to produce cytokines, such as IL-2, and to express IL-2 receptors. The subsequent interaction of IL-2 with its receptor is a critical step in immune regulation and is required for many effector and regulatory T-cell functions (see Figure 39.1-2).

B Cells

The B-lymphocyte development begins within the fetal liver and subsequently continues in the bone marrow (see Figure 39.1-2). Precursor cells give rise to a rapidly dividing population of pre-B cells that lack Ig receptors but produce cytoplasmic heavy chains. The next differentiation stage is characterized by immature surface Ig–bearing B lymphocytes that express IgM, which are already committed to the specificity of the antibodies that they and their plasma cell progeny will secrete. Most B lymphocytes then further mature and acquire surface IgD. During subsequent development of isotype diversity, one of the subclasses of IgG (1 to 4), IgA (1 to 2), or IgE is expressed by separate subpopulations of B cells, which then lose their surface IgM and IgD (see Figure 39.1-1). The maturation sequence of B cells fits with the kinetics of an antibody response; the primary response is mainly IgM and the secondary response is predominantly IgG. During this diversification process, B lymphocytes acquire other cell-surface receptors that allow them to respond to antigens and to T-cell help by proliferation and differentiation to plasma cells. Simultaneously, a population of memory cells is produced, which expresses the same Ig receptor. This clonal expansion helps account for the increased secondary response.

The initiation and completion of specific immune responses involve a complex series of genetically restricted interactions between APCs and T-cell subpopulations for cell-mediated immunity and between these cells and B cells for antibody response. The Th and Ts lymphocytes exert positive and negative regulatory effects, respectively, on B-cell responses. Similarly, B cells and antibodies can affect the activities of functionally distinct subpopulations of T cells through specific receptors. A minority of B cells can respond directly to antigens, referred to as T-independent antigens. They have repeating identical antigenic determinants and provoke predominantly IgM antibody responses. Such substances may also provoke nonspecific proliferation of other memory B cells and are therefore known as polyclonal B-cell mitogens. Such antigens include bacterial polysaccharides and endotoxin.

BASIS OF THE NONSPECIFIC IMMUNE RESPONSE

Macrophages

Macrophages and monocytes represent the mononuclear phagocytic system and are derived from stem cells closely related to lymphocytes in the bone marrow. Each lineage, either for lymphocytes or macrophages, has a different colony-stimulating factor. Once differentiated, functional differences between polymorphonuclear leukocytes, mononuclear phagocytes, and lymphocytes are evident. Most polymorphonuclear leukocytes develop in the bone marrow andy emerge only when mature, whereas macrophages differentiate principally in various tissues, including the gastrointestinal mucosa. Tissue macrophages are heterogeneous cells, which have as their major function the phagocytosis of invading organisms and antigens by lysosomal granules containing acid hydrolases and degradative enzymes. To be functional, macrophages must be activated by cytokines or substances that bind to their surface receptors (such as IgG:Fc receptors) or endotoxin (bacterial polysaccharides) to its receptor, or by soluble mediators such as C5a. Their activation results in release of TNF-α or IL-1, IL-6, and other cytokines (monokines), which then further amplifies the immune response and can cause further damage in already inflamed tissues (see Figure 39.1-2).

Neutrophils (Polymorphonuclear Leukocytes)

Neutrophils and macrophages constitute the main phagocytic cells.[1] In response to chemotactic agents (anaphylotoxins, C3a, C5a), cytokines released by Th1 cells, and mast cell products (kallikrein), neutrophils migrate out of blood vessels into tissues by the expression of adhesion receptors. Organisms adhere to the surface of phagocytic cells and activate the engulfment process. They are then taken inside the cells, where they fuse with cytoplasmic granules. Neutrophils are able to kill the microorganisms and degrade the substances that they internalize. These processes occur in association with their "respiratory burst" and superoxide production.

Natural Killer Cells

Natural killer (NK) cells are considered important in immunosurveillance against tumors, as well as viruses. Their cell lineage is not completely known, but there is some overlap with T cells. IL-15 is important in inducing precursors to develop into NK cells. They can kill target cells in the absence of any antibody or antigenic stimulation. Agents such as mitogens and IFN can nonspecifically activate NK cells.

Complement

The serum proteins of the complement system provide an important means of removing or destroying foreign antigens. The lysis of whole invading microorganisms and the opsonization of microorganisms and immune complexes are key functions of the complement pathway. This multicomponent-triggered enzyme cascade attracts phagocytic cells to microbes. Microorganisms coated with Ig and/or complement are more easily recognized by macrophages and more readily bound and phagocytosed through IgG:Fc and C3b receptors. All complement components are acute-phase proteins whose synthetic rates are increased by injury or infection. Most components are synthesized by hepatic macrophages. Complement activation occurs in two phases: cleavage of C3, the most abundant component, followed by activation of the "attack" or lytic sequence. The critical step is enzymatic cleavage of the C3 component by "C3 convertase." The classic and alternate pathways, both of which can generate C3 convertases in response to different stimuli (antigen-antibody complexes including IgM or

IgG, with bacterial cell wall antigens and endotoxin, respectively), achieve the cleavage of C3a. The next component (C5) is activated, yielding C5a, a potent chemotactic agent for neutrophils. The C3a and C5a components act on mast cells, inducing the release of mediators such as histamine, leukotriene B_4, and TNF-α. The influx of leukocytes and increase in vascular permeability constitute major components of the acute inflammatory response.

HEMOLYMPHATIC CYCLE

Peyer patches (PPs) are intramucosal lymph nodes made of B follicles separated by T areas and topped by an area rich in B cells, T cells, and macrophages called "the dome." The dome is overlaid by a particular epithelium, deprived in the small intestine of villi and containing unusual epithelial cells, the M cells. These cells have neither brush border nor basement membrane. They do not synthesize the secretory component necessary for the transport of IgA. They form cytoplasmic folds into which lymphocytes and macrophages can worm to come in close contact with the intestinal lumen. These properties enable M cells to be the elective entrance for intraluminal antigens, either soluble or particular, in their native form, as can be demonstrated by electron microscopic studies. After crossing M cells, antigens can then be trapped and digested by macrophages, which are numerous under the dome epithelium. It is likely that antigen-pulsed macrophages can then migrate into the B- and T-cell areas, where cellular interactions initiating the intestinal immune response will take place (see Figure 39.1-2).

Intraluminal antigenic stimulations indeed induce proliferation of B and T cells in the B and T areas of PPs, respectively. Interestingly, 70% of B blasts differentiated in the PP microenvironment bear membrane IgA, in marked contrast with other lymphoid organs, in which such cells are in a very small minority.

Hemolymphatic cycle T and B blasts are able to leave the PPs using subserosal lymphatics. They migrate toward the mesenteric lymph nodes, and then via the thoracic duct, they get into blood. Having circulated, blasts, which have arisen in the PPs, selectively migrate back into the intestinal mucosa. The hemolymphatic cycle thus allows dissemination of the immune response initiated at one intestinal site to the entire intestinal mucosa (Figure 39.1-3). This cycle has been well demonstrated in rodents by comparing migration patterns of blasts obtained from various lymphoid organs. Its existence in humans has been inferred but not proven.

During their circulation, T and B blasts undergo progressive maturation. B blasts lose their membrane IgA and acquire intracytoplasmic IgA. They transform into fully mature plasma cells when they have settled in the intestinal mucosa. Similarly, T cells stop dividing and acquire various intracytoplasmic and membrane markers, which reflect their mature and activated state.

MUCOSAL HUMORAL IMMUNITY

SECRETORY IGA

The mucosal surface of the GI tract normally represents an extensive and efficient barrier protecting the host internal milieu, preventing penetration by pathogenic organisms and potentially noxious luminal antigens and toxins.[1] An important component of the host mucosal defense at the gut epithelial surface (GALT) is the presence of intestinal antibodies, most notably secretory IgA. A deficiency in secretory intestinal antibody may impair mucosal barrier function, resulting in increased uptake of macromolecular antigens that could then contribute to the pathogenesis of intestinal or systemic disease states.[1,2] The interaction of intestinal antibodies with antigens, enterotoxins, or bacteria can prevent their attachment to epithelial cell mem-

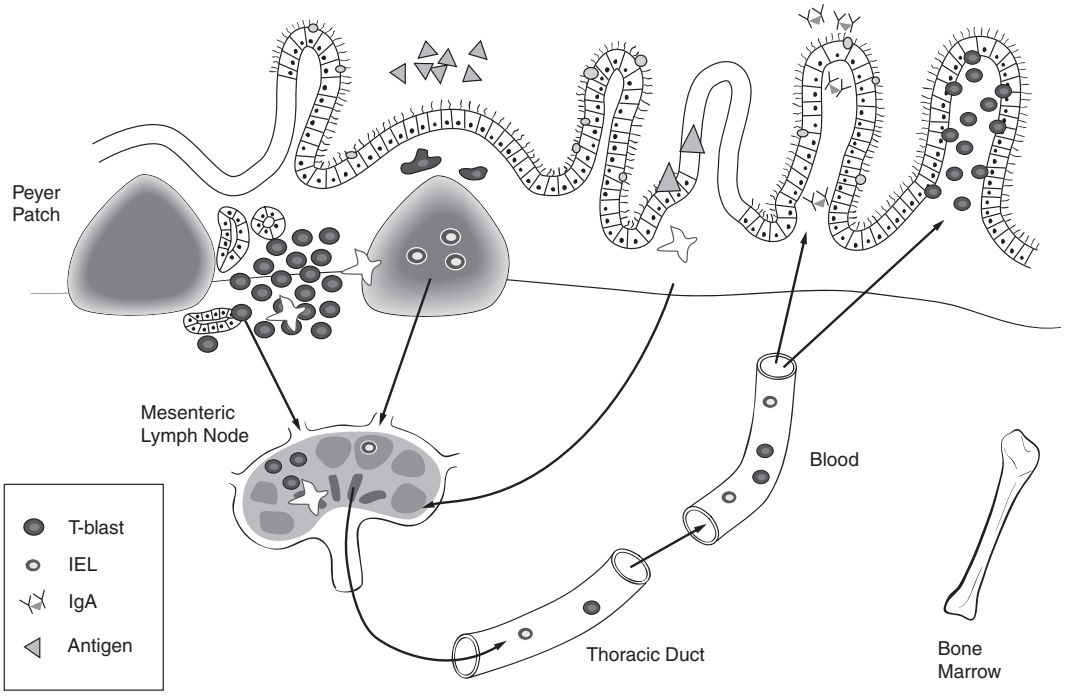

FIGURE 39.1-3 Hemolymphatic cycle of thymodependent T cells and IgA plasma cells. IEL = intraepithelial lymphocyte; IgA = immunoglobulin A.

branes, inhibiting antigen uptake or penetration by pathogens. The formation of antigen-antibody complexes on the surface of the small intestine may also facilitate the operation of other nonimmunologic host defense mechanisms. Thus, under normal conditions, the mature mucosal immune system (GALT) limits antigen uptake and eliminates pathogens.

These theories have been supported by studies in patients with common variable hypogammaglobulinemia, who were shown to have markedly increased absorption of dietary antigens.[4] An increased incidence of intestinal infections is noted in children with defects of the humoral system, such as *Campylobacter jejuni* enteritis.[5] Although symptoms were similar to those seen in normal children, the clinical course in immunodeficient patients tended to be prolonged and more often unimproved by antibiotic therapy. Chronic diarrhea is the second most common infectious complication of antibody deficiency syndromes.

Implication for Mucosal Immunization

The mucosal immune system is primarily protected by secretory IgA antibodies. Resident T cells produce large amounts of transforming growth factor-β, IL-4, and IL-10. These factors, also elaborated by intestinal epithelial cells, then promote the B cells to "switch" to IgA production. Site-specific vaccination by oral immunization leads to antibody production primarily in the small bowel but little in the colon. There is concomitant antibody production by the mammary and salivary glands. Intranasal immunization apparently gives rise to an antibody response in the upper airways and salivary glands, without provoking an immune response in the gut.[1]

CLASSIFICATION OF PRIMARY ID DISEASES

ID in children may be classified as primary or secondary. Primary ID diseases may be attributable to a wide variety of inherited defects in the development and function of the various components of the host immune system, reviewed above. The primary IDs have been classified (Table 39.1-3) by the World Health Organization.[3] The GI manifestations of primary IDs are reviewed below and classified according to the predominant type of ID: humoral, cellular, or combined defects.

PREDOMINANTLY ANTIBODY DEFICIENCIES

As a group, antibody deficiencies represent the most common types of primary IDs in human subjects. Often symptoms do not appear until the latter part of the first year of life, as passively acquired IgG from the mother decreases to below protective levels. As with the T-cell IDs, the spectrum of antibody deficiencies is broad, ranging from the most severe type of antibody deficiency with totally absent B cells and serum Igs to patients who have a selective antibody deficiency with normal serum Ig. In addition to the increased susceptibility to infections, a number of other disease processes (eg, autoimmunity and malignancies) can be involved in the clinical presentation. Fortunately, the availability of intravenous serum Ig has made the management of these patients more complete. Recently, molecular immunology has led to identification of the gene or genes involved in many of these antibody deficiencies. This has led to a better elucidation of the B-cell development and differentiation pathways and a more complete understanding of the pathogenesis of many of these antibody deficiencies.

X-Linked Agammaglobulinemia

X-linked agammaglobulinemia (XLA) is a congenital disorder that was first described by Bruton in 1952 as the congenital inability to form antibodies.[6] Patients were typically infants or young children with recurrent, severe bacterial infections. Since the discovery of the defective gene in XLA in 1993,[7] it has been shown that a significant number of male patients with sporadic or acquired hypogammaglobulinemia actually have XLA.[8–10] In addition to the virtual absence of serum Igs of all classes, an inability to produce antibody after antigen stimulation characterizes XLA. In almost all cases, circulating mature B cells are absent, and no plasma cells are detected in lymphoid tissues, including the gut.

Genetics. Mutations in Bruton tyrosine kinase gene (*BTK*), a B lymphocyte–specific kinase, disrupt intracellular signaling pathways, resulting in maturational arrest of B lymphocytes at the pre-B-cell stage. The abnormal *BTK* gene, a member of a family of proto-oncogenes that encode protein tyrosine kinases, was mapped to the proximal part of the long arm of the X chromosome.[7,8] The mutation results in the inability of tyrosine kinase to function in intracellular signaling involved in the production of Igs by B cells. Female carriers can be detected, and prenatal diagnosis of affected or unaffected male fetuses can be accomplished using closely linked probes and restriction fragment length polymorphism analysis. A similar condition, likely owing to another defect, has been described in females.[11]

Pathophysiology. Cell-mediated immunity is normal, and a normal number of pre-B cells are found in the bone marrow. However, plasma cells and blood lymphocytes bearing surface Ig (CD23, CD19, and CD20) or reacting with anti–B-cell monoclonal antibodies are absent or present only in very low numbers.[9] The underlying defect lies within the early B-lineage cells, reflecting an intrinsic maturation block in pre-B- to B-cell differentiation.[1] The maturational block may be in the transition between terminal deoxynucleotidyl transferase–positive, Cu^- pre-B, and Cu^+ pre-B cells.

Clinical Presentation. The afflicted infants generally remain well during the first 6 months of life by virtue of maternally transmitted Ig. The typical patient is a male presenting thereafter with severe and repeated respiratory infections or meningitis owing to extracellular pyogenic, often gram-positive, encapsulated organisms (such as staphylococci, pneumococci, streptococci, *Neisseria*, *Haemophilus*, or *Mycoplasma* species), unless given pro-

TABLE 39.1-3 CLASSIFICATION OF IMMUNODEFICIENCIES

ANTIBODY DEFICIENCIES
X-linked agammaglobulinemia
Non–X-linked hyper-IgM syndrome
Ig heavy-chain gene deletions
κ-Chain deficiency
Selective deficiencies of IgG or IgA subclasses or IgE class:
γ1 (IGHG1); γ2 (IGHG2); partial γ3 (IGHG3); γ4 (IGHG4); α1 (IGHG1); α2 (IGHG2); ε (IGHE)
Antibody deficiency with normal Igs
Common variable immunodeficiency
IgA deficiency
Transient hypogammaglobulinemia of infancy
Autosomal recessive agammaglobulinemia

T-CELL DEFICIENCIES
Purine nucleoside phosphorylase deficiency
CD3γ deficiency
CD3ε deficiency
70 kD Syk-family protein tyrosine kinase ZAP-70 deficiency

COMBINED IMMUNODEFICIENCIES
Severe combined immunodeficiencies (SCIDs)
T-B + SCID
X-linked γc chain deficiency
Autosomal recessive Jak3 deficiency
T-B-SCID
RAG1 deficiency
RAG2 deficiency
Adenosine deaminase deficiency
Reticular dysgenesis
Other SCIDs
X-linked hyper-IgM syndrome
CIITA, MHC-II transactivating protein deficiency
RFX-5, MHC-II promoter X box regulatory factor 5 deficiency
RFXAP, regulatory factor X-associated protein deficiency
TAP-2 deficiency
Other well-defined immunodeficiency syndromes
Wiskott-Aldrich syndrome
Ataxia-telangiectasia
DiGeorge syndrome

PHAGOCYTIC IMMUNODEFICIENCIES
Severe congenital neutropenia
Cyclic neutropenia
Leukocyte adhesion defect 1 (deficiency of β chain [CD18] of LFA-1, Mac-1, p150,50)
Leukocyte adhesion defect 2 (failure to convert GDP mannose to fructose)
Chediak-Higashi syndrome
Specific granule deficiency
Shwachman syndrome
X-linked chronic granulomatous disease (CGD) (cytochrome *b* 91 kD)
Autosomal recessive CGD deficiency of $p22^{phox}$
Autosomal recessive CGD deficiency of $p47^{phox}$
Autosomal recessive CGD deficiency of $p67^{phox}$
Neutrophil G6PD deficiency
Myeloperoxidase deficiency
IFN-γ receptor deficiency

COMPLEMENT DEFICIENCIES
C1q
C1r
C1s
C4
C2
C3
C5
C6
C7
C8α
C8β
C9
C1 inhibitor
Factor I
Factor H
Factor D
Properdin

OTHER PRIMARY IMMUNODEFICIENCY DISEASES
Primary CD4 deficiency
Primary CD7 deficiency
IL-2 deficiency
Multiple cytokine deficiency
Signal transduction deficiency

CONGENITAL OR HEREDITARY DISEASES ASSOCIATED WITH IMMUNODEFICIENCY
Chromosomal abnormalities
Bloom, Seckel, Dubowitz, ICF, Turner, Nijmegen, and Down syndromes
Fanconi anemia
Abnormalities in chromosomes 1, 9, 16, and 18
Multiorgan system abnormalities
Partial albinism
Congenital dyskeratosis
Cartilage hair hypoplasia
Agenesis of the corpus callosum
Hereditary metabolic defects
Transcobalamin-2 deficiency, biotin-dependent carboxylase deficiency
Acrodermatitis enteropathica
Type I orotic aciduria, mannosidosis, methylmalonicacidemia
Intractable diarrhea, associated with small for gestational age facial dysmorphy, and trichorrhexis
Hypercatabolism of Ig
Familial hypercatabolism of Ig
Intestinal lymphangiectasia
Others
Chronic mucocutaneous candidiasis
Hypo- or asplenia
Graft-versus-host disease

CGD = chronic granulomatous disease; CIITA = MHC class II transactivator; GDP = guanosine diphosphate; G6PD = glucose-6-phosphate dehydrogenase; ICF = immunodeficiency, centromere instability, and facial dysmorphism; IFN = interferon; Ig = immunoglobulin; IL = interleukin; LFA = lymphocyte function antigen; phox = phagocyte oxidase; RFX = regulatory factor X; RFXAP = regulatory factor x-associated protein; TAP-2 = antigen-peptide-transporter 2.

phylactic antibiotics or gammaglobulin therapy. The IgG, IgA, and IgM are far below the 95% confidence limits for appropriate age- and race-matched controls (usually less than 100 mg/dL total Ig). Polymorphonuclear functions are usually normal if IgG antibodies with intact Fc functions are provided. However, patients with this condition can have transient, persistent, or cyclic neutropenia.[10] In such cases, chronic fungal infections or *Pneumocystis carinii* pneumonia may be seen.

In addition to recurrent bacterial infections, patients may have persistent viral infections, particularly with hepatitis or enteroviruses, despite normal T-cell function. From the GI point of view, the child characteristically presents with chronic diarrhea or a malabsorptive syndrome associated with a protein-losing enteropathy.[12] In general, GI manifestations are much less notable than those encountered in patients with late-onset common variable immunodeficiency (CVID) discussed below. Giardiasis,

bacterial overgrowth syndrome (not correlating with diarrhea), nonspecific colitis, and chronic rotavirus infection are other well-recognized complications of XLA.[13,14] Recurrent fissuring necrosis of the small bowel resembling Crohn disease has been described.[12] The important role of antibody in protecting against these infectious and inflammatory complications is highlighted by the favorable response of these patients to replacement therapy with Ig.[14] The XLA has also been reported in association with growth hormone deficiency.[15]

IgA Deficiency

Selective IgA deficiency (IgAD) is the most common primary ID, with an incidence of about 1 in 400 to 600 in whites.[16] Most subjects are asymptomatic, but some may suffer from frequent respiratory and GI infections. Patients who suffer from frequent infections usually have a defect in antibody responses toward polysaccharides, which is often associated with IgG2 deficiency. Some IgA-deficient patients are also prone to develop more severe ID, called CVID, which is associated with decreased IgG and sometimes IgM production, as well as partial T-cell defect. In a few cases, IgAD may reveal a severe disease such as ataxia-telangiectasia. Although the majority of patients are entirely well, the literature is replete with reports associating IgAD with many conditions, including recurrent infections and various autoimmune diseases.[17]

Genetics. The occurrence of IgAD is consistent with autosomal inheritance. In some families, this appears to be dominant, with variable expression. The defective expression of regulatory factors important for IgA-immunocyte differentiation has been suggested to have its origin in certain human leukocyte antigen (HLA)-gene rearrangements. Molecular genetic studies suggest that the susceptibility genes for IgAD and CVID may reside in the MHC class III region on chromosome 6.[18] Genetic predisposition to develop IgAD has been shown to be linked to at least one locus on 6p21. Normally, there is a differential distribution of IgA subclasses throughout the body (Table 39.1-4). The IgA in bone marrow plasma cells and serum is predominantly IgA1. In contrast, IgA in secretions and intestinal plasma cells contain equal amounts of IgA1 and IgA2. Most IgA-deficient patients lack both serum and secretory IgA1 and IgA2. However, in some patients with serum IgAD, IgA2-producing plasma cells may be plentiful in the bowel. An IgG subclass deficiency and IgE deficiency may also be seen in patients with "selective" IgAD, thus reflecting a more generalized abnormality in the terminal differentiation of B cells in such patients. This mixed defect is particularly characteristic of those patients with GI symptoms.[19] Susceptibility to infection among children with selective IgAD has been linked with associated IgG subclass 2 or 4 deficiency.[17] The identification of an IgG subclass deficiency may theoretically lead to therapeutic options in that an associated IgG subclass deficiency may benefit from replacement therapy. However, the risks of blood product administration in such patients, as mentioned above, preclude their routine use.[20]

Pathophysiology. The basic defect leading to selective IgAD is unknown. Most IgA-deficient patients have immature B cells that express membrane-bound IgA, with IgM and IgD coexpression.[21] These B cells resemble those in umbilical cord blood and are not easily induced to become mature IgA-secreting plasma cells, suggesting a B-cell maturation arrest.[21] In some patients, the defect involves the secretory component as well. Of possible etiologic and clinical importance is the presence of antibodies to IgA in the serum samples of as many as 44% of patients with selective IgAD.[22] However, it is uncertain whether these antibodies prevent development of the IgA system or whether lack of tolerance resulting from IgAD permitted the production of antibodies to exogenous determinants that are immunologically related to IgA. Patients deficient in IgA may thus have severe or even fatal anaphylactic reactions after intravenous administration of IgA-containing products.[19] For this reason, parenteral administration of blood or blood products, including serum Ig, is potentially hazardous. Only extensively washed normal donor erythrocytes or blood products from other IgA-deficient individuals should be administered to such patients.

Clinical Presentation. There is an association between IgAD and a wide variety of GI disorders (Table 39.1-5). The majority of patients do not, however, suffer from significant clinical symptoms. Other host defense mechanisms likely compensate and protect the mucosal barrier, including an increase in IgG,[23] IgM-secreting cells,[24] and various nonimmune factors, discussed above.[1,2] As would be anticipated in the deficiency of the primary Ig of mucosal secretions, there is a high rate of infections of the respiratory, GI, and urogenital tracts. Bacterial pathogens are similar to those seen in other types of antibody deficiency syndromes, with no evidence of undue susceptibility to viruses. *Giardia lamblia* infestation is a common

TABLE 39.1-4 SECRETORY AND SERUM IGA

CHARACTERISTICS	SECRETORY	SERUM
Molecular form	Polymeric	Monomeric
Subclasses	IgA1 = IgA2	IgA1 > IgA2
Origin	Mucosal tissues	Bone marrow
IgA deficiency	Decreased or normal IgA2	Decreased

Ig = immunogloblin.

TABLE 39.1-5 GASTROINTESTINAL MANIFESTATIONS OF IGA DEFICIENCY

None
Giardia lamblia infestation (possibly recurrent)
Nodular lymphoid hyperplasia
Nonspecific enteropathy ± bacterial overgrowth ± disaccharidase deficiency
Increased incidence of circulating antibodies to food antigens
Food allergies
Gluten-sensitive enteropathy
Pernicious anemia/atrophic gastritis/increased risk of gastric cancer
Idiopathic inflammatory bowel disease (Crohn disease, ulcerative colitis)

Ig = immunogloblin.

problem in these patients. The prevalence of selective IgAD among patients with Crohn disease also appears to be significantly increased. Although there is an increased prevalence of both serum IgG antibodies against food antigens and circulating immune complexes in IgA-deficient patients, an association with food allergy is not clearly established.[25] As with CVID (discussed below), there is a frequent association of IgAD with collagen vascular and autoimmune diseases.[18] Finally, an increased risk of GI malignancy has been associated with IgAD.[26]

Celiac Disease in IgA-Deficient Patients. In addition to the high prevalence of chronic diarrhea and steatorrhea, there is a 10- to 20-fold increased incidence of celiac disease (CD) among IgA-deficient patients.[27–29] Indeed, selective IgAD and CD are frequently associated and share the ancestral haplotype HLA-8.1, which is characterized by a peculiar cytokine profile. As in nonimmunodeficient patients, the classic syndrome of chronic diarrhea, failure to thrive, and malnutrition is less common than more subtle, irritable bowel–like presentations of the disease.[28–30] In a prospective study of 65 consecutively diagnosed IgA-deficient children, routine jejunal biopsies revealed a diagnosis of CD in 7.7%.[27] Serum antigliadin or antiendomyseal antibodies (IgA) are often falsely negative. The IgG antigliadin antibodies and IgG antibodies against tissue transglutaminase are useful to screen for CD in such cases.[31,32]

CD in the IgA-deficient patient cannot be distinguished clinically, radiologically, or by laboratory means from CD in otherwise normal individuals. The only differentiating feature is that immunohistochemical staining of small intestinal biopsies reveals a lack of IgA-producing plasma cells. Patients with IgAD may also have chronic diarrhea and villous atrophy on jejunal biopsy without a concomitant gluten-sensitive enteropathy. The clinical differentiation depends on response (symptoms and biopsy) to a gluten-free diet (Figure 39.1-4). Even among treated patients on a gluten-free diet and those without CD, immunohistochemical studies have revealed a significant increase in $CD25^+$ cells in the surface epithelium and lamina propria of jejunal biopsy specimens among individuals with IgAD.[33] The increase in $CD25^+$ cells, along with an increase in the mitotic rate of crypt epithelial cells, was taken as evidence in favor of mucosal T-cell activation in IgA-deficient subjects.[33]

Treatment. Currently, there is no specific treatment for IgAD beyond the vigorous treatment of infections with appropriate antimicrobial agents.[34] Even if serum IgA were to be replaced, it could not be transported into external secretions because the latter is an active process involving epithelial cells and locally produced IgA. Selective IgAD contraindicates Ig administration. Only the minority of IgA-deficient patients who develop severe or frequent infections in association with IgG2 deficiency or impaired antibody response are candidates for prophylactic intravenous Ig substitution. Ig preparations containing particularly low amounts of IgA are required to avoid adverse effects related to anti-IgA alloantibodies.

Hyper-IgM Syndrome

Genetics and Pathophysiology. The hyper-IgM syndrome is a rare, inherited ID disorder resulting from defects in the CD40 ligand/CD40-signaling pathway.[35,36] CD40 is a member of the TNF receptor superfamily, expressed on a wide range of cell types, including B cells, macrophages, and dendritic cells. CD40 is a receptor for CD40 ligand, a molecule predominantly expressed by activated $CD4^+$ T cells. CD40-CD40L interaction induces the formation of memory B lymphocytes and promotes Ig isotype switching, as demonstrated in mice knocked out for either the CD40L or the CD40 gene and in patients with X-linked hyper-IgM syndrome.[37,38] X-linked hyper-IgM (XHIM) is caused by mutations in the CD40 ligand gene, whereas autosomal recessive hyper-IgM is caused by defects in the CD40-activated ribonucleic acid (RNA)-editing enzyme, activation-induced cytidine deaminase, which is required for Ig isotype switching and somatic hypermutation in B cells. This lack of inter-

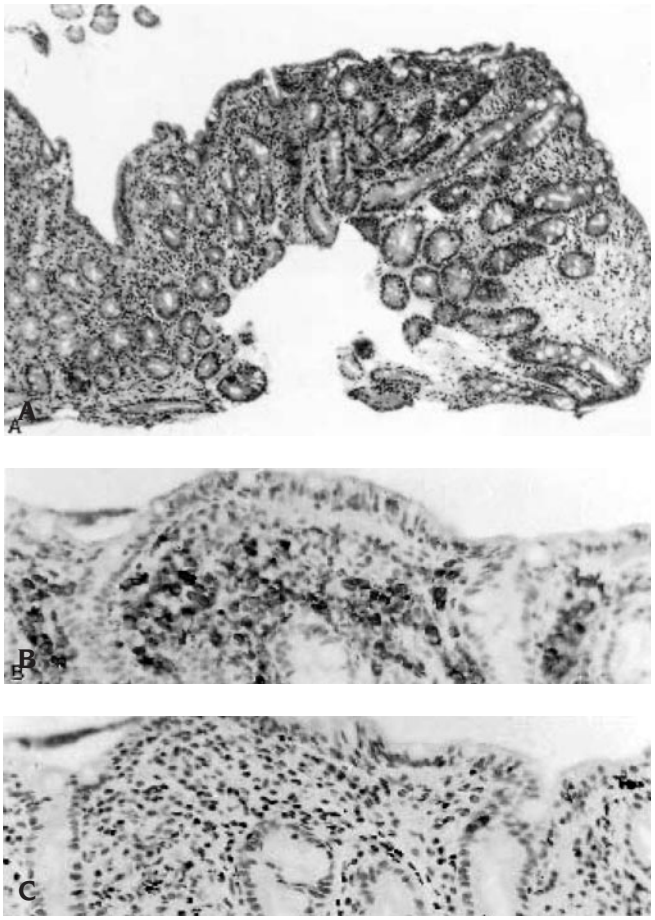

FIGURE 39.1-4 Gluten-sensitive enteropathy in association with autosomal recessive agammaglobulinemia. The patient was a 13-year-old female who presented with chronic diarrhea and growth failure that responded to a gluten-free diet. *A*, Jejunal biopsy shows a moderate to severe villous atrophy, crypt hyperplasia, and chronic inflammatory changes. (hematoxylin phloxine saffron stain; ×120 original magnification). An abundance of immunoglobulin (Ig)M-containing plasmocytes (*B*) and an absence of IgA-containing plasmocytes (*C*) can be seen (immunoperoxidase stain; ×300 original magnification). Courtesy of P. Russo, MD.

action between T and B cells is thought to lead to the unregulated production of IgM. This is accompanied by an inability to switch from IgM- to IgG- and IgA-secreting cells unless cocultured with a "switch" T-cell line or anti-CD40 plus IL-2, -4, or -10.[38]

Clinical Presentation. The clinical presentation resembles that in XLA, with recurrent pyogenic infections including otitis media, sinusitis, pneumonia, and tonsillitis in the first 2 years of life.[39] Chronic diarrhea and liver involvement are common. Both, at times, may be caused by infection with *Cryptosporidium parvum*.[40] Mouth or rectal ulcers, neutropenia, and *P. carinii* pneumonia are frequent presentations.[40] Unlike XLA, however, lymphoid hyperplasia is often seen in the hyper-IgM syndrome. Serum IgA, IgG, and IgE levels are usually very low, whereas a markedly elevated polyclonal IgM is typically present. Although lymphocyte counts and in vitro proliferative response to mitogens were reported to be normal, a defective response to antigens was observed.[40] Thus, additional defects of cell-mediated immunity may be presumed to be present in CD40 ligand mutations.

As described with other antibody deficiencies, an association with autoimmune disorders is quite frequent. Sclerosing cholangitis requiring liver transplant has been reported in 4 of 56 cases.[40] In that series, 23% of patients with XHIM syndrome died of infections or liver disease, whereas 6% underwent bone marrow transplant.[40] Successful bone marrow transplant was reported to promote complete recovery from *C. parvum* infection with gastroenteritis and sclerosing cholangitis.[41] XHIM syndrome is thus a severe ID, with significant cellular involvement and a high mortality rate without bone marrow transplant.

Transient Hypogammaglobulinemia of Infancy

Postnatally, there is a physiologic decrease in the serum IgG concentration as maternally derived IgG is catabolized. A nadir is reached between the third and sixth months. In premature infants, the amount of transplacentally acquired Ig is considerably less; thus, serum IgG concentrations are even lower. Intrinsic Ig synthesis follows as the neonate begins to respond to antigenic stimuli, with the appearance of IgM first, followed by IgG and IgA much later.

Transient hypogammaglobulinemia is primarily a deficiency of serum IgG. Normal antibody responses are demonstrable after antigenic stimulation to tetanus, polio, and pneumococcal vaccines.[42] Circulating B cells are normal in number, but the B-cell response to T cell–dependent stimulation with pokeweed mitogen is decreased, probably secondary to a lack of T-cell help.[43] Infants with this disorder usually have recurrent respiratory infections. Some may present with chronic diarrhea and malabsorption. The disease resolves spontaneously before the age of 4 years, often between the ages of 1 and 2 years.

IgG Subclass Deficiency

Patients may have deficiencies of one or more subclasses of IgG, despite normal or even elevated total serum IgG levels.[44] There is little value in measuring IgG subclasses in patients under 1 year of age owing to wide variations among normal infants.[45] Most of those with absent IgG2 are also IgA deficient,[46] whereas a minority evolve toward CVID.[47] Although many patients are asymptomatic, others resemble Ig-deficient patients. Chronic diarrhea is a common mode of presentation. Nonspecific colitis is a frequent problem among infants with IgA, IgG2, and IgG4 deficiency.[19] Some of these patients will not respond to protein and polysaccharide antigens in various vaccines, as opposed to cases of transient hypogammaglobulinemia. A recent study suggested that hypogammaglobulinemia may be associated with chronic intestinal pseudo-obstruction.[48] The children had a history of recurrent infections requiring gammaglobulin infusions. However, it is unclear as to whether the IDs in chronic intestinal pseudo-obstruction are primary or secondary to the intestinal problem.

Common Variable Immunodeficiency

CVID is one of the most frequent primary IDs.[42,45] CVID is characterized by reduced serum levels of all switched Ig isotypes (IgG, IgA, IgE), predisposing patients to recurrent infections of their respiratory and GI tracts. In addition to serious infection, CVID is associated with a number of comorbid disorders, including a variety of autoimmune diseases and neoplasms.[49] Many patients are diagnosed as adults, and delay in the recognition of the antibody defects is common.[50,51] This congenital ID, inherited in an autosomal recessive way, has not fully elucidated pathogenesis in spite of over 40 years of studies.[52,53]

Genetics. Although the genetic basis of CVID is not completely known, a large proportion of CVID patients possess the same HLA haplotypes as in IgAD. These two disorders have been reported in the same family.[54] In addition, certain patients with IgAD later manifest a CVID. There is also a high incidence of autoimmune disease and malignancies in both of these disorders. It has thus been suggested that IgAD and CVID share a common genetic basis. Studies suggest that the susceptibility genes are in the class III MHC region on chromosome 6. A small number of HLA haplotypes are shared by individuals with CVID and IgAD, consistent with a common genetic basis.[54,55] However, not all members of a pedigree with these susceptibility genes will manifest an ID.[54] Thus, this suggests that environmental factors trigger the disease expression in genetically susceptible individuals.

Pathophysiology. CVID represents a heterogeneous group of familial or sporadic diseases characterized by B-cell dysfunction, low levels of serum Igs, and a failure of B cells to differentiate into mature plasma cells.[3,49–53] Although the antibody deficiencies in CVID may be as profound as those in XLA, circulating Ig-bearing B lymphocytes and lymphoid cortical follicles are present in two-thirds of cases. However, when present, B lymphocytes from CVID patients do not differentiate into Ig-producing cells or plasma cells, despite pokeweed mitogen (PWH) stimulation or coculture with normal T cells.[51,53] Although T-cell subsets are usually present in normal quantities, T-cell functional abnormalities

have also been described and are thought to either cause or exacerbate the B-cell defects.[51–53] A decreased proliferative response to phytohemagglutinin (PHA) or anti-CD3 monoclonal antibody activation has been noted, as has lower IL-2 secretion.[50] These abnormalities are restored by addition of exogenous IL-2 or by using phorbol myristate acetate (PMA), a protein kinase activator, as a mitogen. The IL-2 receptor expression is also impaired in CVID when patients' peripheral blood mononuclear cells are incubated with anti-CD3 antibodies. However, IL-2 and IL-2 receptor messenger RNA expression by peripheral blood mononuclear cells are normal, suggesting a possible defect at the post-transcriptional level.[53] The defective IL-2 production in CVID is also reversed by normal allogeneic macrophages, reflecting a potential defect of macrophage activation in selected patients with this disorder.[52,53] The T cells from CVID patients have been found to be deficient in a number of cytokine genes.[56] In most cases, however, the defect is intrinsic to the B cell, with abnormal terminal differentiation.[53] The pattern of expression of Ig genes after PWM stimulation varies among CVID patients, suggesting that B-cell defects may occur at different stages of maturation in such patients.[54]

Classification. Patients with CVID are no longer classified as having a predominantly antibody- or cell-mediated immune defect.[3] Nevertheless, such a distinction may be useful clinically. The first category is similar clinically to XLA and is characterized by low levels of IgG and of the other Ig classes. It can occur sporadically at any age, but familial cases have been identified, primarily with autosomal recessive inheritance. The peak age at onset is in the second decade of life.[42] Peripheral B cells do not synthesize Ig normally when stimulated by mitogens, as described above. Although CVID patients generally have a decreased number of Ig-secreting cells in GALT, this is not a consistent finding. CVID patients with predominant cellular defects may have profound deficiencies of total T cells and T-cell subsets. Despite the numeric deficiency, there is usually a normal helper (CD4) to suppressor (CD8) cell ratio, in contrast to AIDS, as described in Chapter 39.2. Peripheral lymphoid tissues are hypoplastic, with paracortical lymphocyte depletion. The thymus is typically very small, and no Hassall corpuscles or thymic epithelium is present. Thrombocytopenia and neutropenia are seen in some cases.[42,45]

Clinical Presentation. There is a great deal of clinical variability in this syndrome.[51] Patients may present late in infancy and childhood, whereas others present earlier and are indistinguishable from those with severe combined immunodeficiency (SCID) syndrome. Children with this disorder typically present with a history of recurrent otitis media, bronchopulmonary infections, and chronic diarrhea with malabsorption.[49,51] A number of GI symptoms and signs are associated with CVID (Table 39.1-6). The majority of patients develop significant malabsorption, which is often due to giardiasis. Such patients usually have mild steatorrhea, and small bowel biopsy reveals mild to moderate villous atrophy.[57] These abnormalities may sometimes respond favorably to treatment with metronidazole. There is little evidence demonstrating an increased incidence of parasites other than *Giardia* in CVID patients from industrialized countries.[58] Although bacterial overgrowth is commonly recognized, its presence does not correlate with achlorhydria, diarrhea, steatorrhea, lactose intolerance, vitamin B_{12} malabsorption, or the presence of *Giardia*. Other specific pathogens noted in CVID patients include *Rotavirus*, *Campylobacter jejuni*, and *Campylobacter fetus*. In some patients with early onset of symptoms, as with SCID patients, respiratory tract infections are severe, and malnutrition is often secondary to diarrhea and malabsorption. The GI manifestations include oral, esophageal, and perianal candidiasis.

Gastrointestinal Involvement. Nonspecific enterocolitis in the absence of microbial pathogens is also commonly encountered in CVID.[58] The entire small and large intestine may be involved, contributing to the chronic diarrhea and malabsorption (Figure 39.1-5). In some cases, small bowel biopsies in CVID may contain foamy histiocytes in the lamina propria, resembling Whipple disease. In others, biopsies are similar to those observed in chronic granulomatous disease (CGD), with numerous apoptotic bodies in the crypts or poorly formed granulomas, resembling Crohn disease. Patients with CVID thus manifest a spectrum of abnormalities in the GI tract, with patterns superficially resembling graft-versus-host disease (GVHD) or inflammatory bowel disease, as well as Whipple disease or collagenous colitis.[58,59]

Gastritis may be observed in a large number of patients with hypogammaglobulinemia and CVID. Gastritis is associated with pernicious anemia–like syndrome without antibodies to intrinsic factor, gastric parietal cells, or thyroglobulin.[60]

Nodular lymphoid hyperplasia is often encountered in the bowels of CVID patients who do have B cells. However, nodular lymphoid hyperplasia is also seen in selective IgAD, as well as in normal individuals. Malignancy is frequent, including GI tumors and generalized lymphomas.[57,61] Those with gastritis carry an increased risk of gastric carcinoma.[61] The association with lymphoreticular malignancies and CVID has recently been attributed to an increased radiation-induced chromosomal instability.[62]

Management and Outcome. Patients frequently require nutritional support in the form of enteral elemental diets or total parenteral nutrition. In our experience,

TABLE 39.1-6 GASTROINTESTINAL MANIFESTATIONS OF COMMON VARIABLE IMMUNODEFICIENCY

Nodular lymphoid hyperplasia
Infectious enterocolitis (bacterial, viral)
Giardia lamblia infestation
Bacterial overgrowth
Nonspecific enteritis or colitis
Pernicious anemia/atrophic gastritis/gastric cancer
Gluten-sensitive enteropathy

bone marrow transplant can be curative in such cases. One report on eight childhood cases of CVID focused on the autoimmune manifestations that may dominate the clinical course, leading to significant morbidity and mortality.[63] These included idiopathic thrombocytopenia, hemolytic anemia, secretory diarrhea, arthritis, chronic active hepatitis, parotitis, and Guillain-Barré syndrome. Most patients also had lymphadenopathy, splenomegaly, growth failure, and delayed puberty, reflecting the multisystemic nature of CVID.[63] Patients who have been treated with intravenous gammaglobulin are susceptible to an aggressive progression of hepatitis C, which can respond to IFN-α therapy.[64]

The clinical and immunologic status of 248 CVID patients was reported with survival 20 years after diagnosis, being only 64% for males and 67% for females.[50] Parameters associated with mortality were lower levels of serum IgG, weaker T-cell responses to mitogens, and, particularly, a lower percentage of circulating B cells. Of interest is a report that a subset of CVID patients with excessive suppressor cell activity benefited from therapy with cimetidine.[65] This H_2-receptor antagonist reduced suppressor cell activity, presumably allowing for endogenous Ig production. Improved B-cell differentiation has also been reported with 13-*cis*-retinoic acid,[66] as well as with ketoprofen,[67] the latter, however, in vitro only.[66–68]

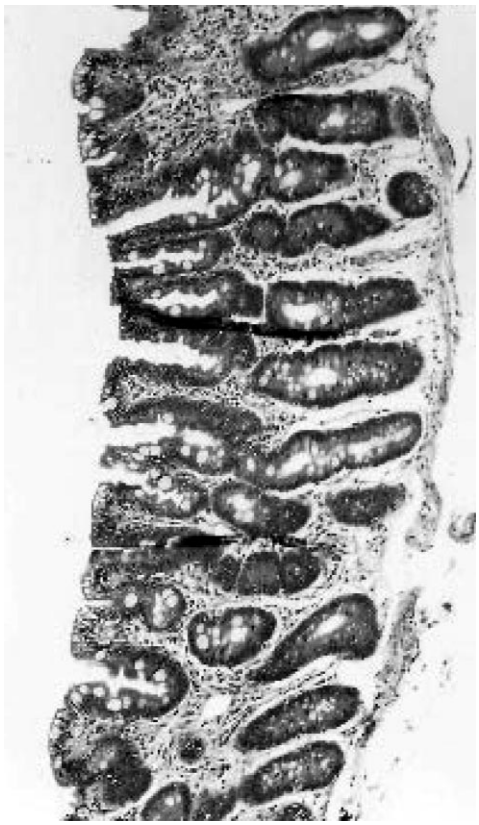

FIGURE 39.1-5 Nonspecific enteropathy of common variable immunodeficiency can be seen in this small bowel biopsy specimen from a 3-year-old boy with chronic diarrhea and failure to thrive who was unresponsive to a gluten-free diet or prednisone. There is irregular, partial villous atrophy with acute and chronic inflammatory cell infiltrates of the lamina propria (hematoxylin phloxine saffron stain; ×120 original magnification). Courtesy of P. Russo, MD.

Hyper-IgE Syndrome and IPEX Syndrome

Intractable diarrhea associated with absence of islets of Langerhans and neonatal insulin-dependent diabetes mellitus has been reported in the hyper-IgE syndrome.[69,70] Affected male infants usually die of overwhelming infection. More recently, the association of diabetes mellitus, severe enteropathy, and endocrinopathy involving boys has been shown to be related to mutations of the *FOXP3* gene, which is the human equivalent of mouse scurfy.[71] This syndrome of immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) is one of a group of clinical syndromes that present with multisystemic autoimmune disease, suggesting a phenotype of immune dysregulation.[72,73] Several mutations of *FOXP3* have been reported in patients with IPEX.[74,75] *FOXP3*, the gene responsible for IPEX, maps chromosome Xp11.23-Xq13.3 and encodes a putative deoxyribonucleic acid (DNA)-binding protein of the forkhead family. Data indicate that the *FOXP3* gene is expressed primarily in the CD4+CD25+ regulatory T-cell subset, where it may function as a transcriptional repressor and key modulator of regulatory T-cell fate and function.[76]

Clinically, IPEX manifests most commonly with severe persistent diarrhea despite complete bowel rest, early onset of insulin-dependent diabetes mellitus, thyroid disorders, and eczema (see Chapter 44.3, "Autoimmune Enteropathy"). IPEX can be differentiated from other genetic immune disorders by its genetics, clinical presentation, characteristic pattern of pathology, and, except for high IgE, absence of substantial laboratory evidence of ID. Immunosuppression may provide temporary benefit for some patients but does not allow complete remission. Remission has been observed after allogeneic bone marrow transplant, but the long-term outcome is uncertain.[77]

DEFECTS AFFECTING BOTH T AND B CELLS

Severe Combined Immunodeficiency

The SCID syndromes are hereditary disorders characterized by a profound deficiency of both T- and B-lymphocyte function, resulting in the virtual absence of immune function from birth, and by the onset of severe, life-threatening infections in the first months of life.[1,3,78,79] The frequency of all types of SCID is 1 in 50,000 to 75,000. A great diversity of genetic, enzymatic, hematologic, and immunologic features (see Table 39.1-3) characterizes this large category of syndromes, all of which manifest clinically as a severe congenital ID.

Genetics. Over half of the cases derive from mutations in the γ_c chain of the receptors for the cytokines IL-2, -4, -7, -9, and -15. Among these, IL-7R is the most crucial for lymphocyte differentiation. In the X-linked form, the abnormal gene produces a truncated γ chain of the IL-2 receptor on T cells.[80] Among autosomal recessive cases, approximately half have a genetic deficiency in the purine degradation enzymes (see Table 39.1-3), purine nucleoside phosphorylase (PNP), or adenosine deaminase (ADA) (Figure 39.1-6).[81,82] This results in the accumulation of metabolites

(deoxyguanosine triphosphate and deoxyadenosine triphosphate, respectively) toxic to lymphoid stem cells. A few cases involve mutations in the *RAG* genes that catalyze the introduction of DNA double-strand breaks.

Where a biochemical defect has been defined, recognition of heterozygote carriers and prenatal diagnosis become possible. For example, in ADA deficiency, prenatal prediction of affected fetuses can be made by measuring ADA activity in amniotic fibroblasts obtained at amniocentesis.

Clinical Presentation. The affected infant presents within the first few months of life with severe infections, chronic diarrhea, malabsorption, and failure to thrive. There may be a history of a neonatal hyperpigmented rash secondary to GVHD, resulting from transplacentally acquired maternal lymphocytes. GVHD may also occur after transfusion of nonirradiated blood products or subsequent to allogenic bone marrow transplants. Intractable diarrhea and recalcitrant oral and perineal thrush are common presentations. The diarrhea may begin slowly and become massive, watery, bloody, and mucopurulent. The pathophysiologic mechanisms underlying the intractable diarrhea are poorly understood. These patients are extremely susceptible to viral infections and often succumb to overwhelming varicella, measles, Epstein-Barr virus (EBV), herpes, or cytomegalovirus infection. It is worthwhile investigating stools for viral particles in these patients in that viruses—singly or in concert—may play an important role in the pathogenesis of the diarrhea.[83] Some viral agents, such as rotavirus, adenovirus, and picornavirus, which normally cause self-limited diarrhea, may cause a chronic enteropathy in SCID.[84] These patients are also susceptible to systemic infections caused by organisms such as *Candida albicans*, *P. carinii*, and *Listeria monocytogenes*. Enteropathogenic bacteria such as *Salmonella* and *Escherichia coli* can also cause chronic infections in these patients.[85] Death usually occurs within the first 1 to 2 years of life unless immunologic reconstitution can be achieved, either by bone marrow transplants, enzyme replacement therapy, or gnotobiotic isolation in the absence of the above.[86]

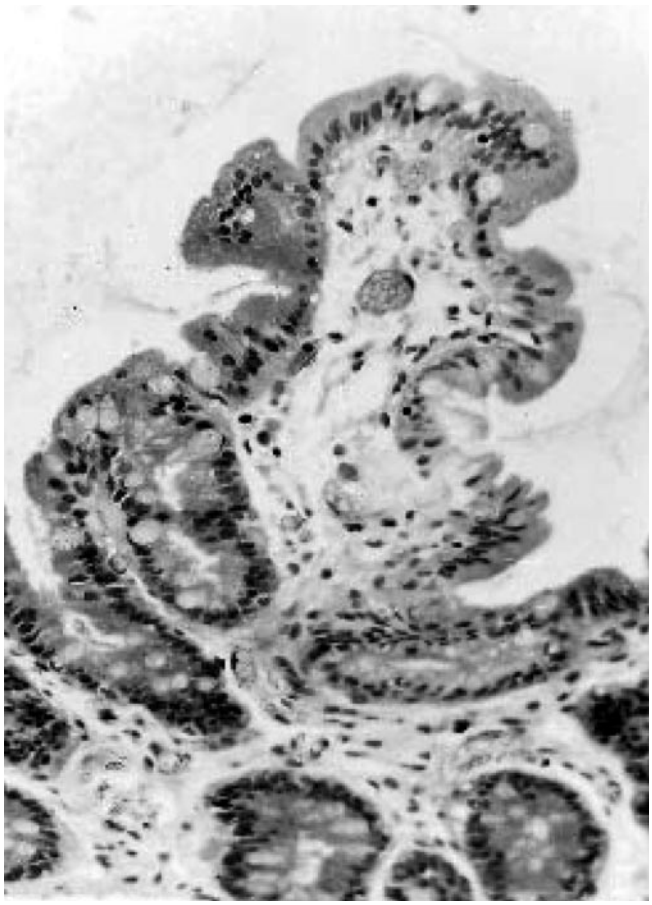

FIGURE 39.1-6 Intestine in severe combined immunodeficiency. The patient presented with intractable diarrhea, failure to thrive, and persistent oral candidiasis during infancy. Investigations confirmed adenosine deaminase deficiency. The jejunal and rectal biopsy specimens (not shown) demonstrate a markedly hypocellular lamina propria with absence of plasmocytes (hematoxylin phloxine saffron stain; ×300 original magnification). Courtesy of P. Russo, MD.

Histologic Features. Histologic features of the intestinal mucosa in SCID include the absence of plasma cells, blunted villi, and the presence of periodic acid–Schiff–positive macrophages in the lamina propria (see Figure 39.1-6). Most patients have a paucity of lymphoid tissue and profound lymphopenia with few mature T cells and low levels of Igs. Despite the uniformly profound lack of T- or B-cell function, many patients may have elevated percentages of B cells. There is, however, marked heterogeneity among SCID patients, even in groups with similar inheritance patterns or ADA deficiency. The SCID cases with Omenn syndrome are exceptional for the presence of lymphoid hyperplasia and hepatosplenomegaly.[87] Cutaneous anergy and failure to reject transplants are typical, and peripheral eosinophilia is not uncommon. As noted above, these patients are very susceptible to GVHD; thus, bone marrow transplant is better performed after removal of T cells from the donor marrow using monoclonal antibodies.[88]

Management. The management of patients with SCID involves not only appropriate antimicrobial therapy but also education to avoid potentially infectious situations. Immunization with live vaccines or bacille Calmette-Guérin (BCG) and conventional blood transfusions must be avoided in patients with proven or suspected defects in cellular immunity.[89] Live vaccines can lead to disseminated infection, and blood transfusions may result in GVHD unless the blood is first irradiated.

Grafting of viable immunocompetent cells offers the only hope of permanent restoration of immune responsiveness. Bone marrow transplant is the treatment of choice for all forms of SCID and some of the other defects. Replacement of missing factors is a logical approach to treatment but has achieved only limited success. Cytokine replacement was encouraging in the short term. IL-2 binding to polyethylene glycol has been used in some patients with defective cellular immunity. This induced an increase in Ig synthesis in vitro, as well as a

clinical response. However, the treatment is limited by the toxicity of IL-2. Common side effects are mild bone marrow suppression and abnormal liver function. The most serious side effect is the vascular leak syndrome. IL-2 provokes massive release of IL-1 and TNF-α, both mediators of enhanced vascular permeability.

Enzyme replacement is beneficial to patients with ADA or PNP deficiency (see Table 39.1-3). Frozen, irradiated red blood cells may also provide a source of PNP. An ADA replacement is successful provided that a chemically modified enzyme with a prolonged in vivo life is used. Transfection of the missing gene involved in SCID into benign retroviral vectors has led to hopes that gene therapy may be another practical approach. Both ADA and PNP deficiency forms of SCID meet the theoretic requirements for gene transfer. The gene for ADA has been identified on chromosome 20, and the DNA has been cloned. To transfer the ADA gene to patient cells, a modified retrovirus vector called SAX has been prepared. Treatment has involved repeated apheresis to collect circulating T cells, culture of these cells with anti-CD3 and IL-2 to induce T-cell expansion, gene transfer by SAX, and reinfusion of cells into the patient. Treated patients with ADA-SCID have shown transient ADA activity in T cells and some clinical benefit. Stem cells have been transfected and reinfused to provide a renewable source of "normal" cells. Half of the ADA-deficient SCID patients respond to transfusions of normal erythrocytes containing the enzyme. Others more severely affected also require treatment with the enzyme modified by polyethylene glycol, which prolongs its half-life. These patients are excellent candidates for gene therapy. Some patients have been successfully treated with periodic infusions of their own T cells or umbilical cord blood cells that have been transfected with the ADA gene linked to a retroviral vector.

Outcome. In a series of 117 patients with SCID, 22 died before transplant could be performed.[90] Among the various subtypes, infants with ADA deficiency and Omenn syndrome (large numbers of oligoclonal T cells and eosinophilia)[87] had the highest rate of mortality before transplant could be performed. The survival rate among recipients of HLA identical bone marrow grafts was significantly higher (80%) than that among recipients of HLA-haploidentical T cell–depleted bone marrow (56%).[90] Of the latter group, 35% had a persistent requirement for Ig administration after bone marrow transplant.

MHC Class II Deficiency

MHC class II deficiency is a rare primary ID disorder characterized by defects in HLA class II expression, inconsistent class I molecule expression, and a lack of cellular and humoral immune responses to foreign antigens. Clinical onset occurs early in life, with recurrent infection and severe protracted diarrhea.[91] Small bowel biopsies show moderate to severe villous atrophy. The diagnosis is usually performed by using HLA-DR immunostaining. [91] The prognosis is poor, with death usually occurring at a mean age of 4 years. Bone marrow transplant should thus be considered early in life.

OTHER PRIMARY ID DISORDERS

IFN-γ deficiency has been associated with neonatal intractable diarrhea and weight loss owing to *C. parvum*.[92] This illustrates the importance of specific cytokine deficiencies in the recovery from cryptosporidiosis and other GI disorders.

IMMUNODEFICIENCY ASSOCIATED WITH OTHER DEFECTS

Wiskott-Aldrich Syndrome

Wiskott-Aldrich syndrome is an X-linked recessive condition characterized by a triad including thrombocytopenia (small, dysfunctional platelets), severe eczema, and ID. The ID involves an inability to respond to polysaccharide antigens, later to all antigens, and deficient cellular immunity leading to repeated opportunistic infections.

Genetics. A gene on chromosome 16 encodes sialophorin, whereas the abnormal gene responsible for Wiskott-Aldrich syndrome has been closely linked to the novel hypervariable locus DXS255 on the proximal arm of the X chromosome.[93,94] The responsible gene on the X chromosome codes for a protein that functions in signal transduction.

Clinical Presentation. Patients present in the first few months of life with the above clinical picture, often accompanied by bloody diarrhea.[3,95] Although other GI complications are not prominent, malabsorption and nonspecific colitis may be encountered.

The median survival has been shown to be less than 6 years, with more than 50% of patients dying of infection, 27% with hemorrhage, and 5 to 12% with tumors, almost all of which involve the lymphoreticular system.[96] In younger patients, infections are caused by pneumococci and other bacteria with polysaccharide capsules. They characteristically present with otitis media, pneumonia, meningitis, and/or sepsis. Later, infections with agents such as *P. carinii* and herpesvirus become more frequent. Such patients produce specific antibodies poorly but have normal numbers of B lymphocytes and plasma cells, along with normal or increased rates of globulin synthesis. Their T cells exhibit a progressive decrease in number and function. Patients with this defect have an impaired humoral immune response to polysaccharide antigens. Studies of Ig metabolism have shown an accelerated rate of synthesis as well as hypercatabolism of albumin, IgG, IgA, and IgM. This results in highly variable Ig concentrations, even within the same patient. The predominant pattern is a low serum IgM, elevated IgA and IgE, and normal or slightly low IgG concentration. Lymphocyte responses to mitogens are depressed, and cutaneous anergy is frequently noted. There are low percentages of CD3 T cells, as well as CD4 and CD8 subsets. There is defective expression of the sialoglycoprotein CD43 on all leukocytes and platelets owing to its instability on cell surfaces in this syndrome.[94]

Optimal therapy requires bone marrow transplant, which appears to correct all of the problems with the exception of thrombocytopenia.

Ataxia-Telangiectasia

Ataxia-telangiectasia is a chromosomal instability disorder marked by progressive ataxia, oculocutaneous telangiectasias, and variable ID because of low IgA and IgG. This multisystem hereditary disease is associated with a complex ID, impaired organ maturation, x-ray hypersensitivity, and a high incidence of neoplasia.[97]

Pathophysiology and Genetics. The synthesis of antibodies and certain Ig subclasses appears to be disrupted owing to abnormal B-cell and Th-cell function in these patients. Individuals affected have various disorders of cell-mediated immunity, including the inability to produce antigen-specific cytotoxic lymphocytes against viral pathogens.[98] Moderately depressed proliferative responses to T- and B-cell mitogens are noted. Reduced percentages of total T cells and T cells of the helper phenotype, with normal or increased percentages of Ts cells, are found. Studies of Ig synthesis have revealed Th-cell and intrinsic B-cell defects. The thymus is very hypoplastic, with poor organization. This disease is considered a model of aberrant gene control, with persistently increased production of α-fetoprotein and carcinoembryonic antigen. However, these tests are not reliable markers of malignancy in ataxia-telangiectasia because they are elevated in the absence of cancer.[99] Cells of affected patients have an increased sensitivity to ionizing radiation, defective DNA repair, and frequent chromosomal abnormalities.[98] Breakpoints involve the genes that code for the TCR and Ig heavy chains, thus explaining the combined T- and B-cell abnormalities. The inheritance follows an autosomal recessive pattern. The abnormal gene has been mapped to the long arm of chromosome 11[98] and codes for a protein with similarity to DNA-dependent protein kinases and functions in DNA repair.

Clinical Presentation. Cerebellar ataxia becomes apparent at the time the child begins to walk, usually progressing until he or she is confined to a wheelchair, typically early in the second decade. Oculomotor abnormalities consist of nystagmus and difficulty in initiating voluntary eye movements. Oculocutaneous telangiectasia first appears as dilated venules on the conjunctiva between the ages of 3 and 6 years. Patients present with repeated sinopulmonary infections and progressive bronchiectasis (80% of cases).[98] Common viral exanthema and smallpox vaccinations have not usually resulted in untoward sequelae, although fatal varicella infection has been described.[98] GI disease is not a characteristic feature in these patients unless secretory IgA is also deficient, reported in 50 to 80% of cases.[98]

Outcome. The patients are at increased risk of developing malignancies, including non-Hodgkin lymphoma, lymphocytic leukemia, Hodgkin disease, and adenocarcinoma of the stomach. No satisfactory treatment has yet been found to treat this immune disorder and to prevent malignancy.

DiGeorge Syndrome

Thymic dysplasia is observed in several primary ID states, the most frequent of which is SCID (reviewed above).[100] DiGeorge syndrome is a rare syndrome characterized by a triad including hypocalcemia, congenital heart disease, and T-cell lymphopenia from thymic hypoplasia.

Pathophysiology and Genetics. Thymic dysplasia results from the failure of formation of the third and fourth pharyngeal pouches early during embryogenesis.[101] Other structures forming at the same time are also frequently affected, resulting in anomalies of the great vessels (right-sided aortic arch), esophageal atresia, bifid uvula, congenital heart disease (interrupted arch or truncus arteriosus, atrial and ventricular septal defects), and dysmorphic facial features. The thymic hypoplasia or aplasia is associated with a cellular immune deficit and severe infections. Defect is due to a deletion of a large region on chromosome 22, and definitive diagnosis is possible using a fluorescent DNA probe on patient cells.

Clinical Presentation. Clinically, the syndrome is characterized by absent T-lymphocyte function, cardiovascular abnormalities, and hypoparathyroidism.[101,102] Patients present with hypocalcemic tetany early in life, congenital cardiac abnormalities, and dysmorphic features, including a shortened philtrum, micrognathia, ear anomalies (low-set, notched lobes), an antimongoloid slant to the eyes, and hypertelorism. Those with the complete syndrome may resemble patients with SCID in their susceptibility to infection with low-grade or opportunistic pathogens (fungi, viruses, and *P. carinii*) and to GVHD from nonirradiated blood transfusions. It has become apparent that a variable degree of hypoplasia is more frequent than total aplasia of the thymus and parathyroid glands. Some affected children may grow normally, and such patients are referred to as having partial DiGeorge syndrome.

Concentrations of serum Igs are nearly normal for age, but IgA may be diminished, and IgE is sometimes elevated. The T-cell percentages are decreased, with a relative increase in the percentage of B cells. Despite low $CD3^+$ T cells, the proportions of CD4 and $CD8^+$ cells are usually normal. Proliferative response of lymphocytes may be absent, reduced, or normal, depending on the degree of thymic deficiency.[102] The GI manifestations in the children who survive the hypocalcemic seizures may include esophageal atresia, GI candidiasis, and intractable diarrhea. It has recently been reported that early transplant of thymus tissue, before the development of infectious complications, can promote successful immune restitution.[103]

X-Linked Lymphoproliferative Disease

X-linked lymphoproliferative (XLP) syndrome is a rare, often fatal, primary ID that has profound and damaging effects on the immune system of affected children. It is characterized by a dysregulated immune response, most commonly to Epstein-Barr viral infection.[104–106] In this syndrome, also known as Duncan disease or Purtilo syndrome, the affected patient typically develops a chronic,

often fatal, infectious mononucleosis, progressive hypogammaglobulinemia, aplastic anemia, or malignant B-cell lymphoma following EBV infection.[106,107] Although this severe susceptibility to EBV appeared to be transmitted as an X-linked recessive trait, cases have been reported in female patients.

Genetics. The defective gene in this syndrome has been identified as a signaling lymphocyte activation molecule (SLAM)-associated protein (SAP).[108,109] It is a T and NK cell–specific protein containing a single SH2 domain encoded by a gene that is defective or absent in patients with XLP syndrome. The SH2 domain of SAP binds with high affinity to the cytoplasmic tail of the hematopoietic cell-surface glycoprotein SLAM and five related receptors. SAP regulates signal transduction of the SLAM family receptors by recruiting SRC kinases.[110,111]

Pathophysiology. These patients generally appear to be healthy prior to EBV infection.[107] However, immunologic studies have demonstrated elevated IgA or IgM and/or variable IgG subclass deficiencies prior to EBV infection.[112] Subsequent to EBV infection, circulating B-cell population and Igs decrease. The predominant T cell in the peripheral circulation becomes the NK cell. Subsequently, a proliferative B-cell disorder (lymphoma) may develop in approximately 35% of patients.[113,114]

There is a marked impairment in the production of antibodies to the EBV nuclear antigens, whereas titers of antibodies to the viral capsid antigen are extremely variable. Antibody-dependent cell-mediated cytotoxicity against EBV-infected cells has been low in most affected individuals, and NK function is also depressed. There is also a deficiency in long-term T-cell immunity to EBV.[114] Studies of lymphocyte subpopulations have revealed elevated percentages of cells of the suppressor phenotype (CD8). Ig synthesis in response to polyclonal B-cell mitogen stimulation in vitro is markedly depressed.[107] Thus, both EBV-specific and nonspecific immunologic abnormalities occur in these patients.

Clinical Presentation. The clinical spectrum is variable, as typified by the following cases. In our experience, school-age children have presented with aplastic anemia, followed by a fulminant infectious mononucleosis with renal and hepatic failure, resulting in death within weeks. Another case presented with a history of a fever of unknown origin of several years duration. Massive hepatosplenomegaly was noted on physical examination. Liver biopsy revealed microabscesses focally without granulomas, resembling a septic hepatitis. Multiple cultures were negative for bacterial, fungal, and viral pathogens. Splenectomy revealed a lymphoproliferative disorder and erythrophagocytosis. Specific antibody titers to EBV were demonstrated to increase significantly. The patient was treated with antiviral agents but eventually died of a lymphoma. Most patients present in the preschool-age group with a severe, often fatal (80%), mononucleosis owing to severe hepatitis.[107] The majority of those who survive the primary infection progress to a combined type of ID with hypogammaglobulinemia and/or lymphomas.

DEFECTS OF PHAGOCYTIC FUNCTION

A number of genetically determined defects affecting polymorphonuclear and/or mononuclear phagocytes have been described (see Table 39.1-3). Neutrophil function involves cell migration in response to chemotactic stimuli, adherence, endocytosis, and killing or destruction of ingested particles. Cell motility depends on the integrity of the cytoskeleton and the contractile system, whereas directional motility is adhesion molecule receptor mediated. Endocytosis depends on the expression of membrane receptors (eg, for IgG, C3b, IC3b) and on the fluidity of the membrane.

Defects in intracellular killing of ingested microorganisms result from failure of the "respiratory burst," which is critical to production of superoxide radicals, oxygen singlets, hydroxyl radicals, and hydrogen peroxide. The organisms cultured from the lesions of patients with this type of defect are generally catalase producing and typically include *Staphylococcus*, *E. coli*, fungi, and other opportunistic organisms. Patients with defective endocytosis and killing tend to have chronic infected granulomas, especially of the lymph nodes, liver, and lung. Patients whose neutrophils fail to adhere normally to surfaces have a biosynthetic defect of a 94 kD glycoprotein (CD11/CD18).[115] These molecules are present on the surfaces of all leukocytes and play a critical adhesive role in cell-microbe, cell-cell, and cell-surface interactions.

In a classic case, a patient with a neutrophil functional defect has had multiple invasive bacterial (especially *Pseudomonas*, *Serratia*, and *Staphylococcus aureus*) and fungal (*Aspergillus* and *Candida* species) infections, beginning during the first year of life. The early infections involve primarily the skin and portals of entry: impetigo, paronychia, periodontitis, sinusitis, and perirectal abscesses. Later infections involve deeper structures: lymphadenitis, pneumonia, osteomyelitis, and splenic and hepatic abscesses. Table 39.1-3 summarizes the primary defects of phagocytic function.[3] Interestingly, several of these phagocyte disorders present with Crohn disease–like involvement of the GI tract and perianal area, as described below.

Chronic Granulomatous Disease

CGD is a primary ID that affects phagocytes of the innate immune system and is characterized by a greatly increased susceptibility to pyogenic infections with catalase-positive organisms of the respiratory tract, skin, and soft tissues.[116–119]

Genetics. CGD is caused by mutations in any one of four genes that encode the subunits of phagocyte reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, the enzyme that generates microbial proinflammatory oxygen radicals. Of the 410 CGD mutations identified, 95% cause complete or partial loss of protein. The CGD phenotype has two major forms of inheritance: X-linked recessive and autosomal recessive.[116] The cytochrome has 92 and 22 kD subunits. In the X-linked form of the disease, mutations

occur in the larger of these subunits. In the majority of cases, the GP92 mutation of the phagocyte oxidase (phox) permits no cytochrome production. In a $P92^{phox}$ variant that permits low levels of superoxide production, the condition can be improved with IFN-γ. The 30% of CGD patients with the autosomal recessive disease have mutations of the smaller $P22^{phox}$ cytochrome subunit and the cytosolic $P47^{phox}$ and $P67^{phox}$ components of the total NADPH-oxidase system. Recent CGD studies have revealed that recombination events between the $P47^{phox}$ gene and its pseudogenes not only cause the absence of $P47^{phox}$ but also predict the generation of a novel fusion protein.[119]

Pathophysiology. The neutrophils of patients with CGD demonstrate normal chemotaxis, engulfment, and degranulation, but their ability to kill microorganisms is impaired owing to defective oxidative burst capacity. Neutrophils and monocytes from these patients, activated in vitro by phagocytosis with a variety of particulate and soluble stimuli, fail to consume the oxygen needed for the production of superoxide anions, hydrogen peroxide, and hydroxyl radicals. The reduced form of NADPH oxidase is found exclusively in phagocytes and is dormant unless activated. The NADPH is the physiologic electron source; a flavin and a phagocyte cytochrome *b* are also postulated to function in a short electron transport chain that transfers a single electron to molecular oxygen to form the superoxide anion. The failure to produce superoxide anions in CGD can result from abnormalities in the components of the oxidase itself, as well as in its activation pathway.

Clinical Presentation. The clinical presentation of CGD comprises recurrent infection, multifocal abscesses affecting the skin and liver, lymphadenopathy, hepatosplenomegaly, chronic lung disease, and persistent diarrhea. Patients with CGD are particularly susceptible to infections with microbes that produce catalase, allowing them to survive destruction by endogenous peroxide. The most common pathogen is *S. aureus*. Others include gram-negative bacilli and fungi such as *Aspergillus fumigatus* and *C. albicans*.

The GI tract involvement, present in the majority of cases, may be present initially and recurrently, causing substantial morbidity and mortality.[118] Steatorrhea and vitamin B_{12} malabsorption are often present. Jejunal biopsy usually reveals normal villi. However, lipid-filled pigmented foamy histiocytes are present in the lamina propria throughout the GI tract.

Diagnosis. The sine qua non for the diagnosis of CGD is the demonstration of an absent or greatly diminished respiratory burst capacity. This defect can be demonstrated by measuring superoxide (O_2^-) production in response to both soluble (PMA) and opsonized particulate stimuli (zymosan). In the majority of cases, there is either no detectable O_2^- generation or production at rates between 0.5 and 10% of controls.[102] An alternative method for measuring respiratory burst activity is the commonly employed nitroblue tetrazolium (NBT) test. Neutrophils able to produce a normal oxidative burst reduce the NBT, causing a change in color from clear to blue. In the most common forms of CGD, no NBT reduction is observed in any of the cells. In some of the variant forms, however, a high percentage of cells may contain small amounts of formazan. The NBT test is helpful in classifying variant forms of CGD. Historically, the major classification criteria for CGD depended on the cytochrome *b* spectrum. Its determination can be accomplished using intact neutrophils or in subcellular fractions by Western blot analysis using antibodies to the two subunits of cytochrome *b*.[120]

Enterocolitis. Patients with CGD often present with an enterocolitis greatly resembling Crohn disease.[118,121] Manifestations typically include vomiting, diarrhea, abdominal pain, weight loss, and fever. Disordered intestinal motility, ulceration, obstruction, and infection (eg, abscesses) can occur anywhere along the GI tract, from the mouth to the anus. The other similarities to Crohn disease include physical findings (most notably perianal abscesses and fistulae), endoscopic appearance, and radiographic abnormalities. Granulomas and giant cells are found quite frequently in colonic biopsies (Figure 39.1-7). The mechanism of granuloma formation in CGD is unknown. It has been postulated that the defective respiratory burst in phagocytes results in persistent inflammation because chemoattractants are not oxidatively inactivated. Delayed clearance of microorganisms may also explain these inflammatory changes. Similar hypotheses have been proposed to explain granuloma formation in Crohn disease.

A recent study of colonic mucosal biopsies of patients with CGD showed that the inflammatory infiltrate differed from the normal controls by an increase in eosinophils and macrophages.[121] There was a paucity of neutrophils compared with ulcerative colitis. Expression of HLA-DR was increased in the epithelium and vascular endothelium compared with normal controls. Moreover, patterns of expression of the adhesion molecules (ICAM-1, vascular cell adhesion molecule 1 [VCAM-1], E-selectin) differed significantly in CGD from those in other inflammatory bowel disease: ICAM-1 was more strongly expressed in the lamina propria, VCAM-1 was more patchily expressed, and E-selectin was present only in the small vessels.[121]

Gastritis. In addition to frequent hepatic and perirectal abscesses, patients with CGD may develop a granulomatous narrowing of the gastric antrum, with symptoms suggestive of gastric outlet obstruction.[117–119] It is important to consider a diagnosis of CGD in patients presenting with an unexplained annular narrowing of the antrum.[122–124] The differential diagnosis includes pyloric stenosis, peptic ulcer disease, eosinophilic gastroenteritis, or Crohn disease. However, tissue examination, the NBT test, and analysis of CD68-positive cells are diagnostic.[118,123]

Management and Outcome. Management depends on the extent of intestinal involvement and its complication:

- Antimicrobial therapy and drainage of abscesses lead to clinical improvement.[118]

- The use of steroids can hasten the resolution of colitis or gastric outlet obstruction and is recommended by several groups.[118,125]
- Sulfasalazine may be helpful to manage colonic disease.
- Malnourished patients may require parenteral nutrition. The gastric outlet obstruction often can be managed medically with broad-spectrum antibiotics and continuous enteral alimentation. Nutritional support and antimicrobial agents may obviate the need for surgery,[124] with symptomatic resolution of the obstruction after 2 to 4 months of therapy.
- IFN-γ was reported to be active on CGD phagocyte superoxide generation, NADPH-oxidase kinetics, and expression of the gene for the phagocyte cytochrome *b* heavy chain.[126] In vitro treatment with IFN-γ increased the respiratory burst activity of polymorphonuclear neutrophil (PMN) leukocytes and macrophages from patients with CGD type IA variant (X-linked; A designates a form in which phagocytes exhibit decreased but detectable superoxide production). Phagocytes from classic type I, IIA, and II CGD did not respond to IFN-γ. In vivo studies demonstrated similar responses. IFN-γ appears to upregulate expression of cytochrome *b* genes by increasing their transcription or through post-translational stabilization of messenger RNA.[127] These studies support the reported potential efficacy of IFN-γ in the treatment of these patients.[118] The safety and effectiveness of long-term recombinant human IFN-γ therapy has been reported in CGD.[128] Thirty patients received recombinant IFN-γ three times weekly for an average of 2.5 years. The rate of serious infection was 0.13 per patient-year, compared with 1.10 in untreated patients.[128] Fever (23%), diarrhea (13%), and flu-like illness (13%) were the most common adverse effects of recombinant IFN-γ. No serious adverse effects or impairments in growth or development were observed.

Finally, it is critically important for the clinician to consider the possibility of CGD in patients with a "Crohn-like" disease in whom a history of recurrent infections and abscesses is noted. The intestinal and perianal manifestations are remarkably similar, although the treatments differ.

Leukocyte Adhesion Molecule Deficiency 1

This immune disorder is characterized by the inability of phagocytes to adhere to vascular endothelium and migrate into tissues owing to an absence of CD11/CD18 β_2 integrins on the phagocyte surface. Leukocyte adhesion molecule deficiency 1 is a rare inherited adhesion molecule disorder that is manifested by recurrent and often fatal bacterial infections.[116]

Pathophysiology. The leukocytes of affected individuals are characterized by absent or deficient expression of plasma membrane glycoproteins that are members of the leukocyte integrin family. The LFA-1 (CD11a/CD18) serves as an adhesion-promoting molecule, facilitating lymphocyte blastogenesis, cellular cytotoxicity (cytotoxic T lymphocyte, NK, and K), and lymphocyte endothelial cell adhesion. The Mac-1 (CD11b/CD18) is the receptor for C3b1 (CR3), an adhesion-promoting molecule facilitating PMN aggregation, PMN/macrophage (Mp) adhesion to substrates, and PMN/Mp chemotaxis. The P150,95 (CD11c/CD18) is a less well-defined glycoprotein that may promote adhesion of PMN and Mp to substrates and also bind C3bi.[116,128] Leukocytes in affected individuals have defective migration and adherence, resulting in an increased susceptibility to infections. There are at least two variants of CD11/CD18 leukocyte glycoprotein deficiency.[116,129] The degree of CD11/CD18-deficient expression (ranging from 10% of normal to totally absent) correlates closely with the severity of the clinical manifestations and the magnitude of the in vitro cellular abnormalities. In vitro leukocyte abnormalities include a defect in adhesion by unstimulated or PMA-stimulated cells. Neutrophils fail to demonstrate

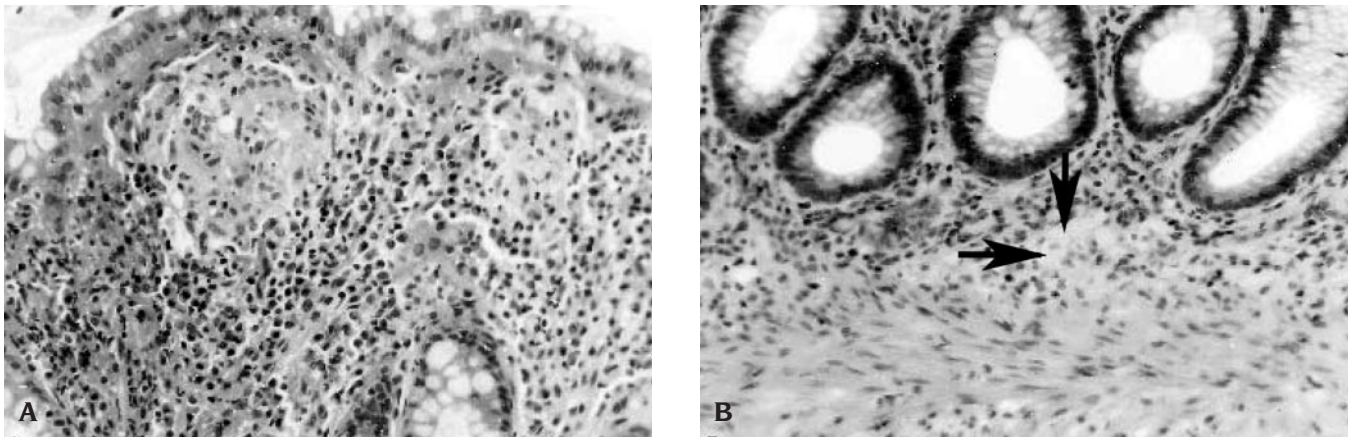

FIGURE 39.1-7 Long-standing colitis in a patient with chronic granulomatous disease. His severe colitis and perianal involvement resembling Crohn disease were resistant to usual therapy (salazosulfapyridine, 5-acetylsalicylic acid, prednisone, 6-mercaptopurine) but responded to total parenteral nutrition and complete bowel rest. *A*, Rectal biopsy specimen shows two granulomas in the superficial part of the mucosa and a dense chronic inflammatory reaction peripherally. *B*, Foamy macrophages (*arrows*) are seen near the muscularis mucosae (hematoxylin phloxine saffron stain; ×300 original magnification). Courtesy of P. Russo, MD.

aggregation in response to stimulants (eg, C5a, PMA). Impairment of directed motility is demonstrated in vitro in response to chemoattractants. A severe defect in CR3 aggregation activity is noted. The NBT for respiratory burst activity is impaired, as is secretion of granular contents by neutrophils and monocytes when induced by particulate stimuli. Lymphoid cells present a diminished blastogenic activity to mitogen (PMA). There is also an impairment in cytotoxic activity mediated by T lymphocytes, NK cells, and K cells.[129]

Clinical Presentation. Infants frequently present weeks after birth (2–3 weeks), with delayed umbilical cord separation and cellulitis of the umbilical stump (omphalitis). Other tissue infections such as cellulites, perirectal abscesses, and necrotizing bowel infections are characteristic. Stomatitis or pharyngitis is present in 40%, and gingivitis or periodontitis is present in 56% of patients.[130,131] The oral and perineal manifestations of this disorder may be mistaken for Crohn disease. We have seen a patient presenting with an ischiorectal abscess and distal ileocolitis, greatly resembling Crohn disease.[130] GI tract involvement has been reported in very few patients, including appendicitis, peritonitis, ischemic ileitis, and necrotizing enterocolitis.[130,131]

Bacterial septicemia is a common complication and may frequently be fatal. Common bacterial pathogens include *S. aureus*, group A β-hemolytic *Streptococcus*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *E. coli*. Severe viral infections (viral meningitis or fatal enteroviral infection) and oral candidiasis have also been described.

Management and Outcome. No specific therapy has been shown to ameliorate the clinical manifestations of the disorder. Antibiotic therapy has proved to be successful in most situations, but patients have often died from bacterial sepsis. Leukocyte adhesion molecule deficiency 1 is uniformly fatal within the first 10 years of life, and bone marrow transplant is the only effective cure. It rapidly reversed the intractable Crohn-like ileocolitis in one of our young patients.[130] Because the *CD18* gene has been cloned and sequenced, this disorder is a leading candidate for gene therapy.[132]

Other Disorders of Neutrophils

In addition to CGD and the CD11/CD18 adhesion molecule deficiency, other hereditary errors of neutrophil number and function are notable for their association with GI manifestations. These include glycogen storage disease type 1B and the Hermansky-Pudlak syndrome. Both of these disorders are also characterized by a nonspecific colitis that greatly resembles inflammatory bowel disease. In the former disorder, treatment with human recombinant granulocyte colony-stimulating factor has been shown to improve both the neutropenia and the colitis. Finally, Shwachman syndrome is another multisystem congenital disorder associated with cyclical neutropenia. The primary GI manifestations, exocrine pancreatic insufficiency, and failure to thrive are discussed in detail elsewhere in this book.

OTHER DISORDERS AT TIMES ASSOCIATED WITH ID

A number of other clinical disorders are associated with various forms of primary ID (see Table 39.1-3). The discussion is limited to those conditions with prominent GI manifestations.

Chronic Mucocutaneous Candidiasis

Chronic mucocutaneous candidiasis is a syndrome characterized by *Candida* infection involving the esophageal and buccal mucosa, skin, and nails. It is frequently associated with an endocrinopathy (Addison disease, hypoparathyroidism, hypothyroidism) and pernicious anemia.[133] This condition may result from a variety of causes, including a primary defect in cell-mediated immunity to *Candida* (autosomal recessive). Patients may present with esophageal candidiasis in the presence or absence of oral involvement. Therefore, any patient with mucocutaneous candidiasis who has dysphagia, odynophagia, or hematemesis should be suspected of having *Candida* esophagitis, even if oral involvement is not evident. These individuals are at risk for development of esophageal stricture and thus require aggressive treatment for chronic *Candida* infection. The use of antacids of H_2 antagonists may worsen the esophageal involvement with *Candida* by reducing gastric acidity.

Patients with familial chronic mucocutaneous candidiasis may also present with a chronic indeterminate colitis. The colitis may be unresponsive to medical management, including sulfasalazine, steroids, elemental diet, parenteral nutrition, 6-mercaptopurine, and cyclosporine. The unrelenting colitis eventually required colectomy, without subsequent recurrence of disease in a patient. Interestingly, his mother, who is also affected by chronic mucocutaneous candidiasis, has primary biliary cirrhosis.

Autoimmune Polyendocrinopathy-Candidiasis-Ectodermal Dystrophy

This autosomal recessive disease is characterized by a variety of clinical manifestations occurring in variable combinations. The polyendocrinopathy may include failure of parathyroid glands, the adrenal cortex, pancreatic beta cells, gonads, gastric parietal cells, and thyroid gland. Other manifestations may include hepatitis, chronic mucocutaneous candidiasis, dystrophy of the dental enamel and nails, severe alopecia, vitiligo, and keratopathy.[134,135] In one series of 68 patients from 54 families, 60% initially presented with oral candidiasis, 9% with malabsorption, and 3% with keratopathy.[136] A malabsorptive syndrome has been reported in up to 24% of patients with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy or type I polyglandular autoimmune syndrome.[137] The steatorrhea has been attributed to a number of causes. Our experience is similar to that recently reported by Ward and Scirè and their colleagues, who reported that intestinal infections (including bacterial overgrowth) may play a role but that exocrine pancreatic insufficiency is the major factor.[135,137] The malabsorptive syndrome and accompanying

decreased absorption of calcium and vitamin D may aggravate the severe hypocalcemia in those patients who have a polyendocrinopathy. Use of pancreatic enzymes dramatically improved symptoms and the hypocalcemia. However, control of the various autoimmune manifestations of the disease was only achieved using cyclosporine.[135]

GRAFT-VERSUS-HOST DISEASE

Hematopoietic stem cell transplant is the treatment of choice for a number of primary ID involving cellular immunity. Bone marrow transplant is also largely used in the treatment of leukemia. However, GVHD has long been regarded as a serious complication of this procedure.[138] GVHD can present as two, but not mutually exclusive, clinical syndromes: acute GVHD and chronic GVHD.

Acute GVHD is a distinctive syndrome of dermatitis, hepatitis, and enteritis occurring within 3 months of allogeneic bone marrow transplant. Although GVHD may affect any organ, intestinal GVHD is particularly important because of its frequency, severity, and impact on the general condition of the patient. Severe diarrhea is common and usually associated with symptoms of protein-losing enteropathy. The GI tract plays a major role in the amplification of systemic disease because GI damage increases the translocation of endotoxins, which promote further inflammation and additional mucosal damage. Translocation may be complicated by septic shock and multivisceral failure. Clinical symptoms, together with timing after bone marrow transplant, make the diagnosis easy. Data in adults suggest the usefulness of transabdominal ultrasonography, color Doppler imaging, and endoscopy in the diagnosis of acute GVHD.[139,140] Intestinal biopsies show villous atrophy, apoptotic enterocytes within glands, and lamina propria infiltration. Progressive elucidation of the mechanisms of GVHD has shown that donor T cells are critical for the induction of GVHD because depletion of T cells from bone marrow grafts effectively prevents GVHD. The standard regimen that is used to prevent GVHD classically includes cyclosporine plus short-term methotrexate. Corticosteroids can be added to this regimen, but adverse effects have to be considered. Tacrolimus is a more potent alternative to cyclosporine. Mycophenolate mofetil can be used as part of a combination therapy. Systemic antibacterial therapy, including eradication of intestinal bacteria, prevents the intestinal translocation of lipopolysaccharide and avoids the subsequent increase of inflammatory cytokines.

Chronic GVHD is a more pleiotropic syndrome, involving skin, liver, lung, and intestine, suggesting a sclerodermatous-like syndrome that develops after 3 months, and includes diffuse collagen deposition resulting in fibrosis, production of autoantibodies, and ID. Digestive symptoms include nausea, vomiting, food intolerance, diarrhea, and failure to thrive. The clinical presentation and endoscopic findings are nonspecific, and there is a broad differential diagnosis, including bacterial, fungal, viral, and parasitic infections. Intestinal lesions are poorly described in the literature, primarily being described in the esophagus.[141] A recent study compared the histologic features of chronic GVHD and control children.[142] Chronic GVHD with intestinal involvement was usually multisystemic (88.2%) and preceded by acute GVHD in 88.2% of cases. Histologic features from duodenal and/or colonic biopsies included (1) villous atrophy; (2) glandular lesions, mainly apoptotic with variable intensity; and (3) lamina propria infiltrate with cytotoxic T lymphocytes ($CD3^+$, $CD8^+$, $TiA1^+$, granzyme B^-). Differential diagnosis of GVHD includes cytomegalovirus colitis and *C. parvum* infection. In chronic GVHD, the apoptotic process could be related to Tc lymphocytes, probably with other cells, such as the enterocytes via the Fas/Fas ligand pathway. The outcome of chronic GVHD is usually severe, especially in cases of GI involvement, and requires an intensive immunosuppressive treatment that renders the host vulnerable to opportunistic infections.[142] Long-term parenteral nutrition is often necessary to maintain growth and nutritional status. For all of these reasons, histologic confirmation is recommended to avoid inappropriate treatment of patients.

CONGENITAL OR HEREDITARY DISEASES ASSOCIATED WITH ID

Chediak-Higashi Syndrome

This syndrome is due to defective lysosomal granule formation in a variety of cells, resulting in phagocytic dysfunction, partial albinism, and mild neurologic impairment. Phagocytosis occurs, but lysosomal fusion with the phagosomal membrane is deficient, with subsequent impaired bacterial killing. The gene responsible presumably functions in intracellular granule trafficking.

Affected individuals suffer from pyogenic infections, which can be fatal. From the GI point of view, Crohn disease–like involvement of the bowel has been observed.

Acrodermatitis Enteropathica

Metabolic or transport disorders, such as acrodermatitis enteropathica, result in hypogammaglobulinemia and abnormal cell-mediated immunity. This autosomal recessive disease, characterized by an eczematous rash, alopecia, chronic diarrhea, malabsorption, and recurrent sinopulmonary infections, can be mimicked by acquired conditions, resulting in severe zinc deficiency, such as Crohn disease and intractable diarrhea of infancy. The symptoms and immunologic abnormalities respond to zinc supplementation. Hypogammaglobulinemia may occur in some patients with more advanced disease; it is not known whether this is due to a protein-losing enteropathy or a direct effect on lymphocytic function.

Intestinal Lymphangiectasia

This intestinal disorder may be classified as ID because it is responsible for a combination of lymphopenia and hypogammaglobulinemia (IgA, IgG). Symptoms of protein-losing enteropathy suggest the diagnosis of intestinal lymphangiectasia. The T lymphocytes are lost from the intestine or into chylous effusions, and this may be associated with a failure to manifest delayed-hypersensitivity skin reactions. Susceptibility to infections is variable, but

rarely severe. Diagnosis requires confirmation by intestinal biopsy. Lymphangiography may show other lymphatic abnormalities in familial cases. As with other protein-losing enteropathies, serum IgM tends to remain within normal limits, whereas the serum IgG and IgA levels may fall to very low levels. This pattern may be seen in patients with primary hypogammaglobulinemia, although the serum IgA is usually much lower. However, it differs from that seen in the hypogammaglobulinemia secondary to lymphoproliferative disease, in which the serum IgM is usually the first Ig class to fall.

There is usually a good response to a fat-restricted diet and supplementation with medium-chain triglycerides. This reduces the lymphatic flow in the intestine, thus reducing the pressure driving the gut losses. Steroids are helpful when the lymphangiectasia is due to local inflammation, such as in Crohn disease, and may also have a short-term palliative effect in malignant infiltration.

Facial Dysmorphy, Intractable Diarrhea, and ID

These patients present with diarrhea starting in the first 6 months of life (less than 1 month in most cases). They are small for gestational age and have an abnormal phenotype, including facial dysmorphism with prominent forehead, broad nose and hypertelorism, and a distinct abnormality of hair, trichorrhexis nodosa. These patients have defective antibody responses despite normal serum Ig levels and defective antigen-specific skin tests despite positive proliferative responses in vitro.[143] Small bowel biopsy specimens show moderate or severe villous atrophy with variable mononuclear cell infiltration of the lamina propria and absence of epithelial abnormalities. Histologically, there are no specific abnormalities. Prognosis of this type of intractable diarrhea of infancy is poor because most patients have died between the ages of 2 and 5 years, some of them with early onset of liver disease.[143] The cause of this diarrhea is unknown, and the relation between low birth weight, dysmorphism, severe diarrhea, trichorrhexis, and ID is unclear (see Chapter 43.3, "Congenital Enteropathy Involving Intestinal Mucosa Development").[144,145] Among the congenital forms of hair dysplasia, trichorrhexis nodosa is very common and can be present in several pathologic conditions.[146–148]

Intestinal Atresia Associated with ID

Intestinal atresia, which is a common congenital defect, has been reported to be associated with ID.[149–151] However, the first report involved three siblings from healthy, nonconsanguineous parents, with multilevel intestinal atresias.[149] One sibling had documented SCID, whereas the clinical histories of the two other siblings strongly suggested a congenital ID syndrome. All patients died before 2 years of age. One last sibling was born in 2001 with the same defects and documented SCID. He survived $2^1/_2$ years with short-bowel syndrome on total parenteral nutrition but finally died from sepsis. This rare syndrome appears to have an autosomal recessive mode of transmission. Other cases have been reported, such as two affected siblings born 18 months apart and a third child with duodenal atresia.[151] One additional child with multiple intestinal atresia was diagnosed for ID after a post-transfusion GVHD. In case of multiple GI atresias, attention should be given to possible associated immune disorder, and irradiation of blood products is recommended pending evaluation of immune sytem status. Donor immune reconstitution after liver-intestine transplant was recently reported in a child with multiple intestinal atresia and SCID.[152] This child did not experience intestinal graft rejection but only a mild GVHD. It is postulate that this child engrafted a donor intestine–derived immune system and is incapable of rejecting transplanted organs.

Congenital Hypothyroidism

Bidirectional interactions between the immune and endocrine systems have been well described, particularly in relation to the growth hormone and adrenal axes. More recently, an association between congenital hypothyroidism and ID was described.[153] Severe and persistent lymphopenia was associated with bronchiectasis and chronic diarrhea. It was proposed that the prolonged thyroid hormone deficiency might be related to the impaired cellular immunity.[153]

DIAGNOSIS OF PRIMARY ID

Primary ID states are relatively rare compared with those that occur secondary to various diseases or their treatment.[42,154] Patients with primary ID have an increased susceptibility to infections, as well as to diverse GI problems, as reviewed above. A systematic approach to investigating children with suspected ID is necessary.[42,155] Early treatment may prevent otherwise inevitable and devastating complications or death. By classifying them into disorders of antibody production, cell-mediated immunity, combined humoral and cellular immunity, phagocytic function, and complement components, a logical approach to their diagnosis can be developed.

Clinical Analysis

In general, one should consider a possible diagnosis of primary ID on the basis of the pattern or type of infection rather than on their frequency alone. Multiple benign viral infections of the upper respiratory tract are of much less significance than a single episode of *P. carinii* pneumonia or recurrent staphylococcal and gram-negative infections. The pattern of infection may often give a clue to the component of the immune system that is most likely affected, as summarized in Table 39.1-1. Most patients with a significant ID present in the first year of life. Infants with predominantly cellular or combined defects present slightly earlier than those with humoral ID. In the latter group, maternally acquired antibodies serve to protect the child for the first 5 months or so. The age at presentation is, however, quite variable and is influenced by the timing of exposure to infectious organisms. By virtue of their associated GI manifestations, pediatric gastroenterologists are most likely to be consulted for the primary ID detailed in this chapter.

Microbiologic Investigations

In patients who are febrile or severely ill, a septic workup should include blood cultures for aerobic and anaerobic bacteria, as well as fungi. Serologic diagnosis of viral or other infections may be unreliable owing to the inability to produce specific antibodies, as discussed above. Thus, efforts should focus on attempts to isolate infectious organisms from respiratory secretions, urine, stool, blood, and cerebrospinal fluid. The polymerase chain reaction may be useful to enhance the sensitivity for the detection of viral genomes. Perhaps more than any other possibility, HIV infection must be extensively ruled out in cases of suspected primary ID. The immunologic and GI manifestations (reviewed in Chapter 39.2) bear an overlap with many of the cellular immune defects described above. The diagnosis should be excluded by identification of HIV antigens and virus isolation, in addition to standard serologic methods.

Digestive and Nutritional Assessment

Patients with chronic diarrhea should have serum albumin, blood urea nitrogen, and electrolyte levels measured. If malabsorption or sinopulmonary disease is present, a sweat test and nasal ciliary biopsy should be considered to exclude cystic fibrosis and the immotile cilia syndrome, respectively. Micronutrient deficiencies of vitamins, minerals, and trace metals should be assessed in cases with malnutrition or chronic diarrhea.

Immunologic Assessment

Screening tests employed for suspected ID are summarized in Table 39.1-7. Detection of an ID requires careful assessment of the patient's ability to develop and express B-cell, T-cell, and combined B- and T-cell functions. Both the amplification of the immune response (cytokine production, complement factors) and effector mechanisms (phagocytosis and inflammatory response) require investigation. Evaluation starts with enumeration of immune cell populations. This should include T- and B-cell, granulocyte, and monocyte counts. Quantitative measurement of Ig concentrations is necessary. Serum total Ig levels lower than 2 g/L are abnormal. The humoral immune response can be examined by screening for natural antibodies to ubiquitous antigens (A and B isohemagglutinins, heteroantibodies to sheep erythrocytes, bactericidins against *E. coli*). Specific antibody response to well-tolerated active immunizations (diphtheria and tetanus toxoids, killed poliovirus antigens, and *Haemophilus* conjugates) can be analyzed. Live vaccines are prohibited. The B-cell markers (CD19, -20, and -22) can be examined by immunofluorescence and flow cytometry.

T-cell function can be assessed by skin testing for delayed hypersensitivity to antigens, which generally reveals positive results in healthy individuals, such as *Candida*, *Trichophyton*, streptokinase or streptodornase, and mumps. Response to active skin sensitization may be assessed with dinitrochlorobenzene. The T-cell function can also be examined by in vitro reactivity of peripheral blood mononuclear cells to PHA, other mitogens, and common antigens. Anti-CD3 is a good indicator of general T-lymphocyte reactivity, as is the one-way mixed lymphocyte reaction (see Table 39.1-7). Enumeration of T cells and their subsets is achieved by surface markers by flow cytometry. In vitro assays for complement components (classic and alternate pathways) can be evaluated immunochemically and functionally in those patients with relevant symptoms. Specific testing of bactericidal and other functions of polymorphs is available (see Table 39.1-7). Patients suspected of having CGD should have phagocytic function evaluated by the semiquantitative reduction of NBT dye and the stimulation of superoxide production. Additional phagocyte testing includes chemiluminescence following stimulation with PMA or dichlorofluorine, as well as by quantifying the capacity of cells to ingest and kill catalase-positive microorganisms. In vitro analysis of inflammatory response capacity can be examined by measuring chemotaxis, chemokinesis, and cytokine production. Adhesion molecule expression can also be determined. Specific details are reviewed in detail elsewhere.[3] Specific investigations that may provide clues to certain primary IDs include thrombocytopenia with small platelets in the Wiskott-Aldrich syndrome, α-fetoprotein in ataxia-telangiectasia, and a deletion on chromosome 22 in DiGeorge syndrome.

TABLE 39.1-7 INITIAL LABORATORY SCREENING FOR IMMUNODEFICIENCY

CBC, WBC and differential, platelets (count and size)
Serum protein electrophoresis
Chest radiography for thymic evaluation
Quantitative serum Igs
IgG, IgM, IgA, IgE, and IgG subclasses
Flow cytometry
Quantitation of total T cells (CD2, CD3)
T-cell subsets (CD4, CD8)
B cell1s (CDI9, CD20)
NK cells (CD16, CD56, CD57)
HLA-DR (to rule out bare lymphocyte syndrome)
Metabolic bursts (to rule out CGD), including NBT testing
In vitro proliferative response to mitogens (PHA, Concanavalin A) and antigens (MLR)
Isohemagglutinin titers
Antibody titers (to documented immunizations) (diptheria, tetanus, rubella, measles)
C3, C4, CH50
If indicated
Sweat Cl^- (to rule out cystic fibrosis)
α_1-Antitrypsin
Celiac disease screening

CBC = complete blood count; CGD = chronic granulomatous disease; HLA = human leukocyte antigen; Ig = immunoglobulin; MLR = mixed leukocyte reaction; NBT = nitroblue tetrazolium; NK = natural killer; PHA = phytohemagglutinin; WBC = white blood count.

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2. HIV *and Other Secondary Immunodeficiencies*

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Gastrointestinal symptoms are common manifestations of human immunodeficiency virus (HIV) infection in children because the digestive system represents an important point of contact with infectious organisms and an important reservoir of lymphocytes. Symptoms include diarrhea, failure to thrive, poor appetite, malabsorption, vomiting, and dysphagia. Alterations to gut morphology and function may be caused by a variety of infectious and noninfectious processes that become increasingly severe as immunosuppression deepens. Children with HIV become susceptible to infection with opportunistic organisms and with virulent pathogens that also infect immunocompetent children (eg, cryptosporidiosis). These virulent infections often have a worse outcome in HIV-infected children. Treatment of gastrointestinal disease in children with HIV infection may often be complex and difficult and require a multidisciplinary approach because nutritional consequences can be severe. However, recent advances in antiviral therapies have resulted in marked improvements in survival and morbidity, mainly owing to preservation and improvement of immunologic function. As a result, in industrialized countries, there has been a decrease in the number and severity of opportunistic infections caused by enteropathogens. This chapter discusses disease of the gastrointestinal tract in children with HIV infection.

HISTORY OF HIV INFECTION

The first indication that a new disease was emerging appeared in the *Morbidity and Mortality Weekly Report* in 1981 with a report of an increase of unusual opportunistic infection in young men in San Francisco, California.[1] This report indicated that *Pneumocystis carinii* pneumonia, which until then had been found only in severely immunocompromised people undergoing chemotherapy, had been diagnosed in apparently healthy men. Further investigation revealed that this unexplained breakdown of the immune system was occurring in homosexual men who had had contact with each other. The affected individuals were found to have depletion of CD4 cells, the cells that control and direct adaptive immune responses. It was not until 1984 that the virus responsible for this immune problem was identified and called human T lymphotropic virus type III, later renamed HIV. By 1985, it was also apparent that other problems related to immunodeficiency were emerging in Africa. Molecular epidemiologic analysis now suggests that HIV developed as a mutant form of simian immunodeficiency virus (SIV), which had crossed from monkeys to humans several decades previously. Its successful spread in human populations is attributable to several features, including that it destroys lymphocytes and disturbs their control, thus reducing the host's potential for controlling HIV itself, its potential for sexual spread, and the fact that infected individuals apparently remain well for many years before the immune failure leads to opportunistic infections. This stage of advanced immune failure is called the acquired immune deficiency syndrome (AIDS), but it is important to emphasize that defining this stage in children is often difficult.[2]

EPIDEMIOLOGY OF HIV

Since the first descriptions of the virus in the early 1980s, HIV has been detected in every continent and has caused millions of deaths. In sub-Saharan Africa, HIV has reshaped whole populations, cutting swaths through the middle years of the population and leaving the elderly and the children. Adults in middle life constitute the most economically active sector of society, and their loss leads to breakdown of families and negative effects on development.

The impact on children is twofold.[3] First, children themselves become infected by vertical transmission from their mothers. This accounts for the great majority of HIV infection in children, although some cases arise by transmission through blood transfusion or through use of unsterile hypodermic needles. Second, children suffer through the subsequent death of their parents, and in many less developed countries, there are difficulties in caring appropriately for these orphaned children. The HIV epidemic has been most severe in sub-Saharan Africa,[4] and it has been estimated that 90% of HIV-infected children live in Africa.[5] It is not yet clear how many children will become infected with HIV in the emerging epidemics in Asia or Eastern Europe.[6]

Transmission from mother to child may occur through transplacental infection, exposure at the time of birth, or breastfeeding. Statistical modeling, based on viral load studies, suggests that the majority of infections, at least in industrialized countries, occur during or soon after birth.[7] However, there is no doubt that in less developed coun-

tries, a high proportion of infections in children are attributable to breastfeeding, implying that the gastrointestinal tract is permeable to HIV in neonates, although the precise mechanism is still to be elucidated. In Africa, the risk of mother-to-child transmission has been estimated at 25 to 45%, whereas in industrialized countries, the risk is probably 10 to 39%. The majority of this excess risk is probably attributable to breastfeeding in populations where breastfeeding is the norm,[5] although there is evidence that exclusive breastfeeding is less likely to be associated with transmission.[8] Recent trials indicate that transmission can be reduced by short-course antiretroviral therapy; currently, the most widely used drug is nevirapine.[9]

HIV-1 VIROLOGY AND IMMUNOPATHOGENESIS

HIV-1 is a retrovirus that is closely related to primate retroviruses, such as SIV, and another human lymphotropic virus, HIV-2. HIV-1 is a ribonucleic acid (RNA) virus that is composed of a cylindrical virion core containing two copies of single-stranded RNA, together with integrase, protease, and reverse transcriptase enzymes. The core is surrounded by a spherical envelope, which is also studded with glycoprotein spikes (p120, p41) and coreceptors (chemokine receptors), which are important for viral attachment and entry into cells. The HIV-1 genome contains structural (*gag*, *pol*) and viral enzyme genes together with other genes (*rev*, *vpr*, *vpu*, *vif*, and *nef*) that are implicated in viral replication and pathogenesis.

HIV-1 entry into cells is mediated through the attachment of virus to CD4 molecules, which are present on the surfaces of certain cells (CD4+ T cells or T helper cells, some monocytes and macrophages).[10] The process of attachment and entry is aided by chemokine receptors such as CCR5 and CXCR4. Once attachment has occurred, fusion of the viral envelope and cell wall occurs with incorporation of virus into the host cell. The viral cycle begins with the generation of a deoxyribonucleic acid (DNA) transcript of viral RNA genome mediated by viral reverse transcriptase. Double-stranded proviral DNA is incorporated into the host genome assisted by viral integrase. Incorporated viral DNA is then "read" as part of the process of protein synthesis within the host cell. Viral products are subsequently assembled and cleaved by viral proteases into individual virions. Viral budding occurs at the cell surface, where the cell wall lipid bilayer contributes to the formation of the viral envelope together with viral envelope proteins. Budding and formation of virions often result in host cell death.

The hallmark of HIV-1 infection is the loss of cell-mediated immunity, predominantly CD4+ T cells or T helper cells.[11] T helper cells are key orchestrators of the immune response and are responsible directly or indirectly for the induction of a wide array of immune functions. A decrease in CD4 cells results in an inverted CD4-to-CD8 ratio, which is usually less than 1.0. The degree of immunosuppression in children with HIV is determined by the age-specific CD4 T-lymphocyte count and percentage (Table 39.2-1). T-lymphocyte function may also be impaired with loss of mitogen responses and cutaneous anergy to antigens. Cytotoxic T-cell and natural killer cell function has also been shown to be diminished with HIV infection.

In addition to T-lymphocyte dysfunction, B-cell dysfunction may occur with polyclonal B-cell activation, hypergammaglobulinemia, and circulating immune complexes.[12] B-cell activation or dysregulation may actually precede CD4 depletion, with dramatic rises in immunoglobulins G, A, and M. Despite polyclonal activation and elevated immunoglobulins, specific antibody production is inadequate for host protection. This "functional hypogammaglobulinemia" underlies the 10- to 50-fold increase in the risk of bacteremia and bacterial infection in adults and children.[13]

Decline of T helper cells over time results in progressive immunoparesis and subsequent risk of opportunistic infections, such as intracellular bacteria and parasitic infections. In addition to opportunistic infections, there is also an increased risk of malignancies such as non-Hodgkin lymphoma (NHL), Kaposi sarcoma (KS), and leiomyosarcoma, although these conditions are less commonly seen in children compared with adults. Table 39.2-2 summarizes the AIDS-defining conditions.[14]

CLINICAL FEATURES IN RELATION TO PATHOLOGY

The clinical manifestations of HIV infection related to the gastrointestinal tract in children can usually be attributed to infections (opportunistic or virulent; see above) or malignancy. Because nutritional failure (failure to thrive) is so important in children, we consider this alongside the clinical features of these other processes.

Infections of the Gastrointestinal Tract

Infectious causes of gastrointestinal tract disease can be divided into major categories based on microbiologic classification. Several different parts of the gastrointestinal tract may be affected, often simultaneously, and often multiple infectious agents may be present. Eight organisms that infect the gastrointestinal tract are classified as AIDS-

TABLE 39.2-1 AGE-SPECIFIC CD4+ T-LYMPHOCYTE COUNT AND PERCENTAGE OF TOTAL LYMPHOCYTES

DEGREE OF IMMUNOSUPPRESSION	AGE < 12 MO		AGE 1–5 YR		AGE 6–12 YR	
	CELLS/μL	%	CELLS/μL	%	CELLS/μL	%
No suppression	≥ 1,500	≥ 25	≥ 1,000	≥ 25	≥ 500	≥ 25
Moderate suppression	750–1,499	15–24	500–999	15–24	200–499	15–24
Severe suppression	< 750	< 15	< 500	< 15	< 200	< 15

Adapted from Centers for Disease Control and Prevention.[14]

TABLE 39.2-2 AIDS-DEFINING CONDITIONS

Serious bacterial infections
Candidiasis (pulmonary, esophageal)*
Coccidiomycosis
Cryptococcosis
Cryptosporidiosis*
Cytomegalovirus*
Herpes simplex virus*
Histoplasmosis
HIV encephalopathy
Isosporiasis*
Kaposi sarcoma
Lymphoma
Mycobacterium avium-intracellulare complex (disseminated/ extrapulmonary)*
Mycobacterium tuberculosis
Pneumocystis carinii pneumonia
Salmonella septicemia*
Toxoplasmosis (cerebral)
Wasting syndrome

*May affect the gastrointestinal tract.
HIV = human immunodeficiency virus.

defining conditions, including cytomegalovirus (CMV), herpes simplex virus, *Histoplasma capsulatum*, *Isospora belli*, *Mycobacterium avium-intracellulare* complex (MAC), and *Salmonella* spp (see Table 39.2-2).[14] Many other organisms can produce gastrointestinal infection, and these can be broadly divided into the major classes of bacteria, viruses, fungi, and parasites. The clinical features and treatment of these infections are summarized in Table 39.2-3. Each class of infection is discussed below.

Viruses. Acute viral intestinal infections of childhood cause a similar spectrum of disease in HIV infection and are usually self-limited illnesses with no increase in severity associated with immunosuppression (eg, rotavirus[15]). Acute diarrhea is the most common presentation and can be due to any of several viral agents, including rotavirus, adenovirus, astrovirus, calicivirus, and small round-structured viruses.[16] CMV is a common coinfection with HIV; however, its significance in pediatric gastrointestinal tract disease is unclear. Esophageal, hepatic, and large bowel involvement have all been described with CMV infection.[17] Herpes simplex virus has also been associated with erosive esophagitis, indistinguishable from CMV esophagitis.

As the gastrointestinal tract constitutes a major component of the total lymphocyte population, this region is also an important target for HIV infection. Lymphocyte populations in the gastrointestinal tract show similar depletion of CD4 lymphocytes, particularly in the lamina propria. An enteropathy, with partial villous atrophy and crypt hyperplasia, associated with HIV has also been described in the absence of opportunistic infection, but there is controversy as to whether this is due to HIV itself or to undetected opportunistic agents.[18] The consequences of this enteropathy are variable: in some instances, these findings are associated with severe malabsorption, but in other cases, there may be no symptoms associated with these findings.

Bacteria. Several bacterial enteric pathogens associated with acute infectious diarrhea in immunocompetent children also cause disease in HIV-infected children, notably *Campylobacter* spp, *Shigella* spp, and *Salmonella* spp.[19,20] Intestinal MAC infection, however, usually occurs in the more severely immunosuppressed children and is characterized by severe chronic diarrhea, malabsorption, and wasting. Infection with these agents is most often through

TABLE 39.2-3 INFECTIONS OF THE GASTROINTESTINAL TRACT IN CHILDREN WITH HIV INFECTION

INFECTION TYPE	CLINICAL PRESENTATION	TREATMENT
BACTERIA		
Shigella spp	Acute/persistent diarrhea	TMP-SMX, fluoroquinolone, ceftriaxone, cefotaxime
Nontyphi *Salmonella*	Acute/persistent diarrhea, septicemia	TMP-SMX, ampicillin, fluoroquinolone, ceftriaxone
Campylobacter spp	Acute/persistent diarrhea	Erythromycin, fluoroquinolone
MAC	Persistent diarrhea, malabsorption	Macrolide, ethambutol, rifabutin
VIRUSES		
Rotavirus	Acute diarrhea	—
Adenovirus	? Ribavirin/cidofovir	
CMV	Esophagitis, colitis	Ganciclovir, foscarnet, cidofovir
HSV	Esophagitis, perianal disease	Acyclovir
PARASITES		
Cryptosporidium parvum	Acute/persistent diarrhea	Paromomycin, immunoglobulin*
Isospora belli	Acute/persistent diarrhea	TMP-SMX
Giardia intestinalis	Acute/persistent diarrhea	Metronidazole, albendazole
Microsporidia	Acute/persistent diarrhea	Albendazole
Cyclospora cayetanensis	Acute/persistent diarrhea	TMP-SMX
Strongyloides stercoralis	Acute/persistent diarrhea; hyperinfection syndrome	Thiabendazole, albendazole
FUNGI		
Candida albicans	Esophagitis	Fluconazole, itraconazole, amphotericin

CMV = cytomegalovirus; HSV = herpes simplex virus; MAC = *Mycobacterium avium-intracellulare* complex; TMP-SMX = trimethoprim-sulfamethoxazole.
These anti-infective agents should be used in standard pediatric doses except for TMP-SMX for isosporiasis or cyclosporiasis, when double the usual dose should be given, and albendazole, which should be given for 4 weeks. Only one species of microsporidian (*Encephalitozoon intestinalis*) is likely to respond well to albendazole, and very little information is available to confirm its efficacy in children.
*Anti-*Cryptosporidium* immunoglobulin is unlikely to be available outside a research setting.

contaminated food or water, contact with infected animals, or person-to-person transmission through the fecal-oral route. Infection with these enteropathogens can result in prolonged diarrhea, malnutrition, recurrence after apparently successful treatment, and extraintestinal infections.[21]

Parasites. Cryptosporidial infection is associated with protracted watery diarrhea in immunocompetent children,[22,23] and in HIV-infected children, there is more severe anorexia and weight loss and higher mortality.[24] Infection may be acquired by person-to-person contact or through contaminated water supply or food. *Isospora belli* and *Cyclospora cayetanensis* infections may also cause a similar clinical picture, but these appear to be less common in HIV-infected children[24] than in HIV-infected adults in the same setting.[25] Acquisition of infection may take place from person to person or by contaminated food or water. Giardiasis may present with an acute diarrheal illness characterized by abdominal cramps and bloating or a more chronic protracted diarrheal illness leading to malabsorption and failure to thrive, but there is little evidence that giardiasis is more common or more severe in HIV infection. Again, transmission usually occurs by person-to-person transmission through the fecal-oral route or through ingestion of contaminated food or water. There is uncertainty surrounding the importance of microsporidia in HIV-related intestinal disease in children. A study in Thailand suggested that microsporidiosis was more common in HIV-related diarrhea than in HIV-unrelated diarrhea,[26] but in Uganda, the prevalence of microsporidiosis was the same in children with and without diarrhea,[27] and similar observations were made in Tanzania.[28] It is important to realize that diagnosis of microsporidiosis is difficult, and no consensus yet exists as to its epidemiology.

Strongyloides stercoralis infection may be asymptomatic in HIV-infected individuals but may also cause a severe hyperinfection syndrome characterized by a Löffler-like syndrome with eosinophilia, pulmonary infiltrates, rash, and diarrhea.[29] This is uncommon. Other helminths are not associated with disease in HIV.

Fungi. Oral thrush owing to *Candida albicans* is a common presentation in children with HIV infection, sometimes causing feeding difficulty. More troublesome symptoms of dysphagia and retrosternal chest pain are associated with *Candida* esophagitis, which may be clinically indistinguishable from CMV and HSV esophagitis without endoscopic clarification.[30]

Failure to Thrive and Nutritional Problems

Many children with HIV/AIDS experience wasting and/or failure to thrive during the course of their disease.[31] In developing countries, weight loss is one of the most common presentations of HIV infection and is often associated with diarrhea.[32] The etiology of failure to thrive in this population may often be unclear and, in many cases, multifactorial. The most important factors include reduced oral intake, malabsorption in the gastrointestinal tract, increased energy use with HIV infection, and psychosocial stressors. In many instances, more than one factor may be responsible but may also exacerbate other factors. Poor energy intake is an important cause of failure to thrive, particularly when owing to infections of the upper gastrointestinal tract, which limit oral intake (eg, esophagitis), and poor energy intake is often a consequence of anorexia related to intestinal infection. HIV encephalopathy may also cause neurologic disease, resulting in difficulty in swallowing or incoordination and therefore a reduction in oral intake. More recently, newer HIV therapies have been associated with significant gastrointestinal toxicities, including severe taste aversion, which has resulted in nutritional problems in some children. Malabsorption is another important factor in children who fail to thrive, either directly owing to HIV enteropathy or secondary to enteropathogen infection (see above). Increased energy use may also play an important role in failure to thrive, mainly through increased metabolic rate and high cellular turnover/inflammation owing to HIV infection and other coinfections; however, the exact importance of this factor has been difficulty to measure, and further research is needed in this field. Psychosocial factors are important causes of failure to thrive in children with HIV infection, particularly in the setting of perinatally acquired infection, where other family members may also be infected. Illness of both a child and his/her parent may have profound influences on the individual. HIV infection in this setting may also be associated with other social factors, such as poverty and intravenous drug use, that may impact on the HIV-infected child.

Malnutrition was a serious health problem in children in tropical populations long before the HIV pandemic, but the interaction of intestinal infection, nutritional impairment, and HIV has escalated the problem. HIV appears to induce changes in small intestinal mucosal function, and these changes are exacerbated in the presence of opportunistic infection. Whether these changes explain the increased severity of malnutrition in HIV-infected children remains to be elucidated. These children have severe malnutrition and high mortality rates, particularly in the presence of specific infections, such as cryptosporidiosis.[24] Treatment of the most severely affected children can be very challenging.

Gastrointestinal Malignancy

There is an increased risk for the development of malignancies in HIV infection, including KS, NHL, and leiomyosarcoma.[33] However, there are clear differences between malignancies in children and adults with HIV infection. For example, KS is relatively unusual in children and leiomyoma/myosarcoma is relatively more common.[34] All of these malignancies can affect the gastrointestinal tract and may occur without severe immunosuppression. The most common tumors seen are NHL, which may affect extranodal sites such as the gastrointestinal tract, liver, and central nervous system. NHL may behave aggressively and present with nonspecific symptoms such as weight loss, fever, and fatigue, which are not dissimilar to symptoms seen with other opportunistic infections.[35] Lymphoprolif-

erative disorders represent a spectrum of disease, of which NHL is the most malignant and aggressive form of disease. At the benign end of the spectrum of lymphoproliferative disorders are mucosa-associated lymphoid tissue (MALT) tumors. Several different agents have been implicated in the pathogenesis of lymphoproliferative disorders, including Epstein-Barr virus and *Helicobacter pylori* infection.[36,37] MALT tumors may occur in the lung, stomach, and salivary glands and may be slow-growing, indolent tumors. KS is a hemangiosarcoma that has been associated with human herpesvirus 8 infection.[38] KS is reasonably uncommon in children, although there has been an increased incidence in children with HIV in sub-Saharan Africa.[39] Typically, KS causes mucocutaneous lesions, including lesions in the mouth and gastrointestinal tract. Lymphadenopathic KS may also occur, affecting specific regional lymph nodes such as the inguinal areas. Less common forms of KS include visceral organ involvement such as the spleen and lungs.[40] Smooth muscle tumors (leiomyoma and leiomyosarcoma) are rare in children, although the risk is estimated to be 10,000 times higher in children with HIV infection. Smooth muscle tumors are not classified as AIDS-defining conditions (unlike NHL and KS). Epstein-Barr virus infection has been associated with smooth muscle tumors and may have a pathogenic role. After NHL, smooth muscle tumors are the second most common malignancy in children with HIV and in some surveys represent 17% of all reported tumors.[41] Leiomyosarcomas may occur in the gastrointestinal tract, spleen, retroperitoneal space, adrenal glands, and lungs.

INVESTIGATION

Children presenting with chronic gastrointestinal symptoms need to be thoroughly evaluated to determine the etiologic agent and institute appropriate therapy and symptom relief. Stepwise diagnostic testing is essential to exclude common pathogens initially and search for more unusual pathogens, using, in some cases, more invasive testing modalities. Initial investigation involves obtaining at least two stool samples for bacterial culture to exclude bacterial enteropathogens. Microscopy using specific stains (saline, iodine, trichrome, acid-fast, and fluorescein-conjugated stains) should be performed to detect specific parasites. *S. stercoralis* infection usually requires identification of the rhabditiform larvae in feces or duodenal fluid. Serodiagnosis may be less useful due to cross-reactivity with other filaria and reduced antibody responses, particularly in more immunocompromised individuals. Stool samples should also be analyzed by acid-fast staining to detect MAC infection. In children who have diarrhea and fever, blood for bacterial culture, mycobacterial culture, and CMV culture/polymerase chain reaction may be useful. Often repeated blood sampling may be necessary to identify bacteremia or viremia. If a diagnosis is not established using standard culture and microscopic techniques, then upper and lower gastrointestinal endoscopy may need to be performed to obtain biopsy specimens. This may be particularly important to detect MAC and CMV infection because stool examination is relatively insensitive. Samples obtained by endoscopy need to be sent for histology, mycobacterial culture, and virologic examination using immunohistochemistry and/or electron microscopy if available. Radiologic investigations may be useful in determining the anatomic site and extent of disease. Barium swallow studies may be useful in supporting the diagnosis of *Candida* esophagitis in patients with dysphagia. Barium radiographs may also demonstrate large intestinal changes, such as mucosal thickening and ulceration, as seen in CMV colitis; however, endoscopic investigation may need to be performed to confirm the diagnosis. Computed tomography may be useful in demonstrating bowel wall thickening and luminal narrowing, as well as intra-abdominal masses, such as lymphadenopathy or malignancies. Major intra-abdominal lymphadenopathy should be investigated further and may require biopsy at laparotomy or laparoscopy to detect tuberculosis, NHL, or MAC.[42] In children from tropical populations, tuberculosis should always be considered in a child with unexplained abdominal symptoms because treatment is so dramatically successful.

MANAGEMENT

Secondary and Supportive Treatment

Treatment for children with HIV infection includes the prompt and aggressive control of acute infections, including opportunistic infections. These are summarized in Table 39.2-3. In children with acute diarrhea, replacement of fluid and electrolytes is initially important. Nutritional support and supplementation are frequently required, especially in children with chronic diarrhea. Enteral support should be used whenever possible, although parenteral support may sometimes be required (see Chapter 76, "Drug Therapy"). In children with difficult or dysfunctional swallowing, a feeding gastrostomy may be a useful method of enteral support.[43]

Prophylactic therapy using trimethoprim-sulfamethoxazole is often given to prevent *P. carinii* pneumonia, but it may have the desirable effect of reducing infections with *I. belli* or *C. cayetanensis*. At a community level in tropical populations, the most important intervention is vitamin A supplementation, which has marked benefits for HIV-infected and HIV-uninfected children.[44]

Anti-HIV Treatment

Initial antiviral therapy for children with HIV infection involved the use of monotherapy or dual therapy with drugs such as zidovudine and didanosine that belonged to the nucleoside analog family (Table 39.2-4). These agents inhibit the viral enzyme reverse transcriptase and thereby reduce viral replication. Although this approach appeared to have an initial beneficial effect, it soon became apparent that specific viral mutations developed against these drugs, rendering them ineffective.[45] Improved monitoring of HIV infection by measurement of HIV-1 viral load made it apparent that viral replication was an important prognostic marker and complete viral suppression was a key goal in permitting immune reconstitution.[46] The development of

TABLE 39.2-4 CLASSES OF ANTIRETROVIRAL DRUGS FOR CHILDREN

Nucleoside analog reverse transcriptase inhibitor
Zidovudine
Didanosine
Zalcitabine
Lamivudine
Stavudine
Abacavir
Non-nucleoside analog reverse transcriptase inhibitor
Nevirapine
Delavirdine
Efavirenz
Protease inhibitor
Ritonavir
Nelfinavir
Indinavir
Saquinavir
Amprenavir
Lopinavir

newer antiviral agents, including the more potent protease inhibitor group, has meant that effective and prolonged viral suppression is now possible. Combination therapy or highly active antiretroviral therapy (HAART) has dramatically altered the outlook for many children with HIV infection who have access to this form of treatment.[47] Both mortality and morbidity have improved by HAART, and some have suggested that HIV is now another chronic treatable disease of childhood.[48] Combination therapy usually involves the use of at least three different drugs from different classes (eg, two nucleoside reverse transcriptase inhibitors plus a non-nucleoside reverse transcriptase inhibitor or a protease inhibitor (see Table 39.2-4 for drug classes). However, complete eradication of HIV is not possible with current drugs, and children will probably have to remain on some form of lifelong therapy. Furthermore, HAART requires high levels of compliance for sustained viral suppression and the prevention of viral resistance and treatment failure. Obstacles to long-term successful treatment and compliance include poor drug tolerability, particularly taste, and long-term adverse effects (including abnormal lipid and glucose metabolism).[48] Despite these problems, HAART has been used effectively and safely in children in North America and Europe, with dramatic improvements in long-term survival. Treatment of children in these settings has involved a multidisciplinary team, often in specialist centers that have the expertise and experience in managing the many complex physical, psychological, and social issues that affect children with HIV infection. Newer therapeutic strategies are also being developed to boost anti-HIV immune response, which may, in the future, provide more durable immune reconstitution.

The use of HAART in resource-limited countries, which have the highest burden of HIV infection, has sadly been very limited or nonexistent. This has been primarily due to high drug costs, which make HAART inaccessible to the vast majority of HIV-infected individuals globally. However, HAART has been demonstrated to be effective and safe when used in even the most resource-limited settings, and recent initiatives in reducing drug costs will mean that access to HAART will improve.[49] In addition to providing affordable drugs, infrastructural development will also be necessary if treatment programs are to be successful.

Prevention of vertical HIV transmission from mother to child has been an important initiative in resource-limited countries with a high HIV burden. Zidovudine and nevirapine have both been used, the latter drug used as a single dose to mother and child with effective interruption of HIV transmission.[9]

OTHER SECONDARY IMMUNODEFICIENCIES

Secondary immunodeficiency may occur with a variety of different conditions (and their treatments), including hematologic malignancies, transplant, malnutrition, and autoimmune disorders, such as inflammatory bowel disease. Gastrointestinal manifestations are common in these children, with an increase in opportunistic and enteric infections, which may cause chronic relapsing illness similar to that seen with HIV infection in children. Particularly at risk of infection because of enteric pathogens are children receiving chemotherapy for malignancies and those who are immunosuppressed following bone marrow or solid organ transplant. In those children receiving chemotherapy, the main problems occur with neutropenia and gram-negative bacilli infection, particularly *Escherichia coli*. In children receiving immunosuppressive therapy following transplant, viral (CMV, adenovirus) and fungal (*Candida, Aspergillus*) infection may be particularly problematic, especially when immunosuppressive therapy is maximal. In both groups of children, *Clostridium difficile* has also been recognized as a cause of pseudomembranous colitis. Antibiotic therapy is a key factor in pseudomembranous colitis, particularly through disruption of normal bowel flora and subsequent colonization with *C. difficile*. Parasitic infections, especially *Cryptosporidium* and *Strongyloides*, may be important causes of chronic diarrhea and gastrointestinal disease. Gastrointestinal syndromes associated with CMV are an increasingly important problem in bone marrow transplant recipients, causing ulceration of the esophagus, stomach, and small and large bowel. Graft-versus-host disease (GVHD) is also an important cause of gastrointestinal disease in children receiving bone marrow transplant and may be difficult to differentiate clinically from other opportunistic infections and the effects of chemoradiotherapy. Acute GVHD may affect the distal small bowel and colon, presenting with profuse diarrhea, intestinal bleeding, and abdominal pain. It should also be borne in mind that any gastrointestinal problem in a child receiving corticosteroids or another immunosuppressive treatment might be related to an opportunistic infection or malignancy, and due consideration should be given to this. The approach to a child with gastrointestinal symptoms and secondary immunodeficiency will be similar to that for HIV infection, in which stepwise diagnostic testing is important to identify infectious agents. Endoscopy and biopsy may be required in some children, especially when GVHD is suspected. Management of these

children will require treatment of the infectious agent (where identified) and supportive treatment, particularly nutritional support.

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CHAPTER 40

INTESTINAL FAILURE

1. *Short-Bowel Syndrome and Intestinal Adaptation*

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Short-bowel syndrome constitutes a major clinical challenge for the pediatric gastroenterologist. It represents a complex disorder characterized by multiple disruptions of normal intestinal anatomy and physiology, complicated by a variety of nutritional, infectious, and metabolic alterations that challenge the art and science of medicine. There have been numerous attempts to anatomically define short-bowel syndrome. Most of these definitions related poorly to infants and children, who may develop short-bowel syndrome at any age, with different lengths of small intestine and different anatomic causes. Consequently, the functional definition of short-bowel syndrome is generally accepted.[1] Short-bowel syndrome is therefore defined by malabsorption in the presence of shortened small intestine. Malabsorption may include nutrients, fluid, or electrolytes, but nutrients appear to play the most important role in determining the presence of short-bowel syndrome and its consequences.

Great strides have been made over the last 30 years in the treatment of short-bowel syndrome. The development of parenteral nutrition; the continuing refinement of parenteral solutions, techniques, and catheters; the introduction of improved enteral feeding formulas; and the use of trophic factors have all greatly impacted on the prognosis of short-bowel syndrome.[2,3] This formerly fatal disorder is now often compatible with long-term and even normal life span, often with good quality of life.[4] Advances in intestinal transplant are currently changing the perspective of short-bowel syndrome. This chapter discusses the abnormal pathophysiology and the sequence of interventional steps the clinician must negotiate to allow patients with short-bowel syndrome to achieve full potential.

ETIOLOGY

In pediatrics, most patients with short-bowel syndrome present at or near birth. A few remaining patients may present at any age from a variety of causes. The patients can often be subdivided into those who were intially anatomically normal and those who were not.

Patients who begin with normal gastrointestinal anatomy constitute a large number of neonates with short-bowel syndrome. The majority of cases occur as a result of intestinal resection in infants, especially premature infants, who develop necrotizing enterocolitis (see Chapter 42, "Necrotizing Enterocolitis").[2,3,5–7] The actual cause of this condition is hotly debated, but it appears that ischemic injury of the small intestine results in nonviable bowel, which must often be resected. Ileal or proximal colonic resections are most common, and patients are frequently left with compromised intestinal function. Later in life, Crohn disease (see Chapter 41.1, "Crohn Disease"), volvulus with intestinal ischemia, tumors (see Chapter 45, "Intestinal Tumors"), and radiation enteritis secondary to radiation therapy for neoplastic diseases (see Chapter 8.2, "Radiation Enteritis") may result in short-bowel syndrome. Occasionally, Hirschsprung disease may involve the small bowel as well as the colon, and extensive resection may result in short-bowel syndrome (see Chapter 46.4, "Chronic Intestinal Pseudo-obstruction").

A number of patients have short-bowel syndrome owing to congenital anomalies (see Chapter 32, "Congenital Anomalies"). Atresia may occur anywhere in the small intestine and may be either isolated or multiple. Frequently, multiple atresias result from anomalies in the superior mesenteric artery and are known as "apple peel" or "Christmas tree" deformities of the small intestine. Some children are born with shortened small bowel, and a number of patients with gastroschisis will have short bowel either congenitally or as a result of resection for ischemia or bowel injury.

From a functional perspective, there is little difference between these two groups of patients. However, certain anatomic considerations, to be discussed later, may greatly impact on the patient's long-term prognosis and may affect the interventional steps required.

PHYSIOLOGIC ABNORMALITIES

Before one can begin to assess the functional abnormalities that occur as a result of short-bowel syndrome, it is impor-

tant to consider the differences in function of the proximal versus the distal small intestine. The jejunum is characterized by long villi, a large absorptive surface area, a high concentration of enzymes and transport carrier proteins, and an epithelium in which the tight junctions are relatively large, rendering the epithelium more porous to larger molecules. Consequently, the jejunum is the site for the greatest nutrient absorption in the small intestine. It is also relatively leaky, allowing free and rapid flux of water and electrolytes from the vascular to the intraluminal space. The ileum, on the other hand, is characterized by shorter villi, more lymphoid tissue, less absorptive capacity, and a tighter epithelium. The tight junctions are smaller, permitting less flux of fluid from the vascular space to the lumen; consequently, the ileum is a more efficient epithelium for the absorption of fluid and electrolytes. Nutrients are absorbed less rapidly than in the jejunum. The ileum also has certain capabilities that are not present in the jejunum, namely, the absorption of vitamin B_{12} and bile salts through site-specific receptors. In the normal small intestine, fluid and electrolytes flow from the plasma into the lumen to dilute the highly concentrated nutrients delivered into the duodenum. Rapid mixing, digestion, and subsequent carrier-mediated transport of monosaccharides, amino acids, and dipeptides occur predominantly in the jejunum. Although some of these functions occur in the ileum as well, its tighter epithelium is better suited to the reabsorption of the water and electrolytes. Consequently, the patient with a jejunostomy and major ileal resection will be extremely susceptible to fluid losses from osmotic diarrhea associated with high-carbohydrate feedings, whereas a patient with a jejunal resection may tolerate such feedings better with less fluid loss. Likewise, following an ileal resection, the patient may initially be able to absorb more nutrients, although absorption will adapt rapidly following jejunal resection as the ileum assumes jejunal function. The jejunum cannot develop site-specific carrier-mediated transport of vitamin B_{12} and bile salts; consequently, these will be malabsorbed permanently following ileal resection. The ileum is also the site of synthesis for many gastrointestinal hormones, especially those that affect small intestinal motility such as enteroglucagon and peptide YY. Resection of the ileum may impair regulation of gut motility by nutrients, especially fat (ie, the ileal brake). Also, the normal negative feedback mechanism for gastrin is lost, and hypergastrinemia is common. This may partially explain why acid-peptic disease and esophagitis are common in patients with short-bowel syndrome.

Resection of the ileocecal valve may have major effects on physiology following small bowel resection.[2,3] The ileocecal valve appears to have two functions. It serves as a barrier for reflux of colonic bacteria from the colon into the small intestine and may also play a role in regulating the exit of fluid and nutrients from the small intestine. Consequently, resection of the ileocecal valve will permit greater reflux of bacteria into the small intestine, resulting in bacterial overgrowth. The consequences of this functional abnormality are discussed later. In addition, following resection of the ileocecal valve, rapid transit of nutrients from the small intestine into the colon may exacerbate malabsorption and increase sensitivity to osmotic loads in the small intestine. The importance of the ileocecal valve has been questioned, and its perceived importance may actually be related to the value of the adjacent ileum.

The major consequence of resection of the small intestine is malabsorption, and at least initially, this is primarily due to reduction in the absorptive surface area, with a concomitant loss of digestive enzymes and transporters.[8] The normal consequences of malabsorption become more pronounced in patients with short-bowel syndrome. Malabsorption of rapidly digested carbohydrate, for example, produces tremendous osmotic diarrhea following resection of the ileum because reabsorption of water is impaired. Proteins are larger molecules and are generally ingested in smaller quantities, creating less osmotic diarrhea. Fats are extremely large molecules, and although they may be less well absorbed, their malabsorption produces little additional osmotic fluid loss from the small intestine. However, fat generally requires a greater mucosal surface area for absorption, and the coefficient of fat absorption may be less than that for proteins and carbohydrates. Fat-soluble vitamins are also malabsorbed in large quantities in short-bowel syndrome. In patients with extensive ileal resections, reabsorption of bile salts may be impaired to such a degree that the patient will become bile salt depleted. This will result in bile salt concentrations falling below the critical micellar concentration, preventing solubilization of fats and fat-soluble vitamins and exacerbating malabsorption. Because fat is not thought to be absorbed by a carrier-mediated saturable process, additional total fat absorption may take place by increasing dietary fat content, even though the coefficient of absorption is already relatively low and substantial fat is being malabsorbed. To state it another way, as fat intake increases, the percentage of fat absorption decreases, but the total fat calories absorbed increases. Conversely, additional calories in the form of carbohydrate and protein cannot be absorbed once transport carriers are saturated. The exception is the fermentation of some malabsorbed carbohydrate to short-chain fatty acids in the colon. Colonic absorption of these substances may result in the capture of some additional calories; consequently, preservation of the colon may be important for reasons other than control of fluid and electrolyte losses.[9] These advantages are perhaps of greater benefit to older children and adults than to infants.

Abnormalities in motility also occur following resection. Following ileal resection, transit time is faster through the jejunum. Gastric emptying is also more rapid following ileal resection but can be normalized if the colon can be retained.[10] Additional functional abnormalities may develop with time. Many of these result because of compensatory changes that occur within the small intestine. For example, motility may decrease and small intestinal transit time may increase as the intestine tries to increase nutrient contact time with the small bowel mucosa. The small intestine will dilate in an attempt to increase mucosal surface area. This will result in an increased bacterial content in the small intestine, which may result in a variety of problems, which are addressed later.

INTESTINAL ADAPTATION

Ultimately, successful medical management of short-bowel syndrome is dependent on stimulation of the process of intestinal adaptation. Adaptation is the process by which the small intestine functionally adapts to increased nutrient needs owing to loss of absorptive surface area.[11–13] Through adaptation, in response to a variety of stimuli, the small intestine is able to increase its absorptive surface area and functional capacity to meet the body's metabolic and growth needs. Increases in intestinal mass also occur in other conditions in which caloric needs are increased. Two such examples include lactation and diabetes mellitus. In lactating rats, an increase in intestinal mass per unit length of bowel can be demonstrated.[14] Morphologic changes in the small intestine also have been described in diabetic rats. It has been hypothesized that these changes occur primarily in response to increased nutrient intake, emphasizing the sensitivity of intestinal adaptation to variations in enteral nutrition.[15] Adaptation also occurs to allow for decreased nutrient needs or availability. An example is starvation, which, in the rat, results in shorter villi and reduced intestinal transport.[16,17] In many ways, adaptation can be considered a compensatory overexpression of the processes that maintain normal mucosal integrity and function in response to normal enteric feeding.

The proliferative status of the intestinal epithelium is an important determinant of adaptation. The cell production rate in the crypt is governed by several factors. One is the cell cycle time (the duration of the cell cycle or interval between two successive divisions of a proliferative crypt cell). In the human jejunum, this interval is about 48 hours and, in the rat, only about 11 hours.[18,19] When the cell cycle time decreases, the cell production rate increases. Starvation, which reduces the cell proliferation rate, increases the duration of the cell cycle.[20] Cell production rate is also influenced by the growth fraction, which is defined as that portion of the cell population devoted to proliferation. In the intestine, this is usually the bottom two-thirds of the crypt.[21,22] The larger the proportion of proliferative enterocytes, the greater the cell production rate. Disorders such as starvation decrease the crypt growth fraction, whereas inflammatory conditions, which necessitate increased cell production, increase crypt growth fraction.[23,24] The crypt cell population size also influences cell production. Proliferative epithelia are commonly associated with increased crypt size and therefore increased crypt cell population. A reduction in the crypt cell population size is common in hypoproliferative states such as starvation.[20,25] Finally, there are many more crypts than villi in the normal small intestine. In the human, there are roughly six crypts for every villus, and the ratio increases to 30:1 in the rat.[26,27] Changes in the ratio of the number of crypts to the number of villi may also influence the total production of epithelial cells.

CHANGES THAT OCCUR WITH ADAPTATION

The primary event in intestinal adaptation is hyperplasia of the mucosal epithelium.[28–32] Hyperplasia is preceded by increased crypt cell production, which results in increased crypt depth and subsequent lengthening of the intestinal villi. Some dilatation of the small bowel also occurs, with increased folding of the mucosa and gradually increasing surface area. Because this process is one of hyperplasia and not hypertrophy, it is associated with growth in cell number rather than cell size. This observation can be confirmed by concurrently measuring the changes in deoxyribonucleic acid (DNA), protein, and mucosal weight. Because all increases are in direct proportion to one another, an increase in cell number is suggested. Length of intestinal villi necessitates an increased rate of migration of cells from the crypt up the shaft of the villus and an increase in the rate of cell renewal. Cell number has been shown to be the primary determinant of villus and microvillus surface area.[33] Recent evidence suggests that significant increases in enterocyte proliferation, as well as apoptosis, occur following small bowel resection. Although increasing apoptosis might seem incongruous in epithelium destined to increase its surface area, the investigators speculated that as enterocyte proliferation is increased, the rate of enterocyte apoptosis must also increase to maintain homeostasis.[34] In most animal studies, an increase has been demonstrated in the absorption of almost all nutrients following completion of the adaptation process.[35–38] Disaccharide digestion, monosaccharide absorption, and absorption of trace metals, vitamins, fluid, and electrolytes all increase. In rats, within 8 weeks, enhanced glucose-dependent electrogenic sodium absorption can be demonstrated using the Ussing chamber technique.[39]

Ultrastructural analysis of the ileum after 60% proximal small bowel resection reveals that the general structure of a single enterocyte is not impaired after resection. All subcellular organelles appear to be morphologically intact. Resected animals demonstrate more dilated intracellular spaces in the tips of the villi. Microvillus surface area also appears to decrease as a function of time after proximal resection. There appears to be no significant difference in the relative areas of mitochondria, rough endoplasmic reticulum, and nuclei as a function of time following resection.[40]

The increased absorptive surface area does not immediately result in corresponding functional improvement. Measurement of digestive enzymes such as lactase, sucrase, and maltase in hyperplastic epithelium suggests some functional immaturity of the epithelium. Consequently, replicative enzymes such as thymidine kinase are often increased. This functional immaturity appears to gradually change with time as absorptive function improves.[35–38] Adaptive improvement in intestinal function appears to be a diverse process. Improved absorption of some nutrients occurs much more rapidly than that of others.[41] Some controversy exists regarding improvement in intestinal function because of the means of expressing data in many of the experimental animal studies.[11,12] In studies using tissue preparations such as intestinal sacs or rings, isolated enterocytes, or membrane vesicles, it is customary to express the data based on some parameter of mucosal mass. Typically, absorption per unit of DNA, protein, or mucosal weight is used. Because the hyperplastic epithelial cells are often

functionally immature, at least initially, these parameters suggest that absorption following adaptation is impaired. In reality, this apparent reduction in function is of less magnitude than the positive increase in mucosal mass. Therefore, when the data are appropriately expressed per centimeter of small intestine or related to small intestinal absorptive surface area, intestinal function appears improved.

Careful analysis of transport kinetics reveals some functional changes in hyperplastic mucosa. No changes in Michaelis constant occur, suggesting no alterations in the thickness of the unstirred layer. However, maximum velocity for substrates may be reduced by 50% when expressed per unit of mucosal surface. Nonetheless, reduced enterocyte function is still more than canceled out by the larger absorptive surface area.[42,43] It is likely that hyperplastic epithelial membranes contain fewer transport proteins than are found in comparable normal ileum. Digestive enzyme function also varies significantly from study to study. In one study, specific activity of sucrase appeared to increase, but lactase decreased following resection. Messenger ribonucleic acid (mRNA) analysis suggested that sucrase changes were pretranslationally regulated, but the decrease in lactase activity was a post-translational event.[44] Unfortunately, most studies evaluating functional changes have been performed roughly 2 to 3 weeks postoperatively in an experimental model. Whether or not further maturation of absorptive function occurs with time is largely unknown, but clinical experience would suggest that it does.

Although functional adaptation per unit length of bowel accompanies morphologic changes, functional adaptation may also occur independently of villus hyperplasia.[45] In some studies, glucose uptake has been observed to increase, even in the absence of increases in intestinal mass.[46] Differences in adaptation rates of glucose and certain amino acids have also been observed. Changes in administration of nutrient concentrations can likewise cause adaptive changes in the bowel without changing morphology. For example, increasing dietary carbohydrate content may result in a marked increase in glucose transport without changing villus architecture or mucosal mass. In the proximal small intestine, mucosal mass is greater, and villus length is increased over the distal small intestine. There is a proximal to distal gradient in transport of several nutrients, especially glucose. The gradient is more pronounced with glucose absorption than with intestinal mass.[46] This suggests that there is a functional adaptive response to the high concentration of glucose in the proximal bowel, which occurs independently of morphologic adaptation. The effect of dietary substrate on nutrient transport appears to be quite substrate specific. For example, a diet high in carbohydrate and low in protein will stimulate enhanced glucose transport, whereas amino acid transport may be reduced. Likewise, a diet high in fructose may enhance fructose absorption but not glucose absorption, whereas diets high in glucose will stimulate glucose transport independently of fructose transport. These observations suggest that nutrients may act directly on intestinal cells to induce the synthesis or suppress the degradation of transport proteins.

Functional adaptation, when it occurs independently of morphologic adaptation, may progress rapidly.[15,47] Increased glucose transport may occur within 1 to 3 days of a change in dietary carbohydrate, whereas morphologic adaptation in the resected model occurs over 1 to 3 weeks. Synthesis of carrier proteins and brush border digestive enzymes is seen rapidly in response to large quantities of intraluminal nutrients.

Most of the data concerning intestinal adaptation following bowel resection have been derived from animal studies, mainly in dogs and rats. In the rat, the mucosal adaptation process occurs very rapidly, with an increase in cell replication in the crypts being visualized as early as 24 to 48 hours.[30] By 2 to 3 weeks, the villus hyperplasia process, at least from a morphologic standpoint, appears to be nearly completed. Few data of a corresponding nature exist in humans, primarily owing to the difficulty in performing such studies, but clinical experience suggests that the adaptation process proceeds more slowly. The adult patient with short-bowel syndrome will likely reach his potential much more rapidly than a child will. Previous studies concerning intestinal adaptation in 3-week-old versus 8-week-old rats suggest that older animals have greater potential for intestinal adaptation.[48] Older animals are able to increase their intestinal mass by a greater proportion than are smaller animals. It was assumed that the small animals were already under substantial stimulation just to meet the needs for growth alone and therefore had little potential to adapt beyond their natural capacity. In very old animals, the response appears to develop more slowly, although the capacity to adapt remains intact.[49] Another factor likely responsible for what appears to be delayed adaptation in children versus adults is the linear growth of the bowel. Children with very short bowels have a great capacity to increase bowel length when resection is performed as a neonate.[50] Extensive linear growth is observed in the normal small intestine during the first year of life and continues beyond that point to some degree. However, it is not clear whether there is much opportunity to enhance linear growth beyond that which normally occurs. Clinical experience has taught us that pediatric patients following neonatal small bowel resection may not reach their full adaptive potential until beyond the fifth year of life.

Whereas most data have been derived from animal studies, some data regarding functional adaptation are available on humans. In human children, following an extensive resection, a marked increase in glucose absorption was observed using the intestinal perfusion technique. Sucrose hydrolysis appeared to be increased in the same proportion as glucose absorption. In addition, studies of human children have demonstrated intestinal hyperplasia (ie, increased numbers of enterocytes per unit length of villus and a suggestion of increased villus length), although this was not statistically significant.[51] Elevation of brush border enzyme-specific activities, however, was not observed in the human children, although it is likely that overall absorption was enhanced because of the increased mucosal surface area. The hydrogen peptide transporter PEPT-1 has been shown to be up-regulated in the colonic mucosa of

patients with short-bowel syndrome.[52] This appears to occur independently of morphologic adaptation.

ROLE OF ENTERAL NUTRITION IN ADAPTATION

The adaptation process is highly dependent on enteral nutrition. In certain animal models, atrophy can occur in a small intestine deprived of nutrient contact by either bypassing the small bowel surgically or by using parenteral nutrition.[53] Following bowel resection in dogs, intravenous feeding results in slight mucosal atrophy, whereas enteral nutrition stimulates mucosal hyperplasia.[53] The mechanism by which enteral nutrients stimulate adaptation is complex. Nutrient effects have been broken down into three major categories: (1) direct stimulation of hyperplasia through contact of the epithelial cells with intraluminal nutrients, (2) stimulation of secretion of trophic gastrointestinal hormones, and (3) stimulation of the production of upper gastrointestinal secretions, which themselves are trophic to the small intestine.

Demonstration of the direct effect of nutrient contact with the intestinal epithelium is fairly straightforward. Normally, jejunal villi are longer than ileal villi, probably because they are exposed to higher concentrations of nutrients. When a segment of ileum is transposed into the proximal jejunum, ileal mucosal mass increases rather markedly, to the point where the length of the villi in the transposed ileum may actually exceed the length of adjacent villi in the jejunum.[54] The high concentration of nutrients in the jejunum appears to stimulate maintenance of jejunal villus length. Ileal villi appear to be equally, if not more, sensitive than jejunal villi to nutrient concentration. It is important to note, however, that although the length of small intestinal villi may be altered by luminal extrinsic factors, such factors are not required for the establishment of a villus height gradient. Grafts of fetal duodenum develop longer villi than grafts of fetal ileum without the influence of luminal factors.[55]

Enteral nutrition is important not only in stimulating intestinal adaptation but also in stimulating regeneration of the mucosa following injury. In a group of patients with protracted diarrhea of infancy, the combination of parenteral and enteral nutrition was superior to parenteral nutrition alone in stimulating intestinal recovery of disaccharidases.[56] Further studies demonstrated a more rapid resolution of malabsorption and diarrhea following enteral nutrition alone versus parenteral nutrition in the same disorder.[57] Enteral nutrition is also important in maintaining the mucosal mass. Maintenance of mucosal mass and glucose absorption is much improved following enteral versus parenteral nutrition. Direct contact with nutrients appears to be important in maintenance of normal glucose transport.

Several factors may contribute to nutrient-sensitive epithelial proliferation. Whereas it has been hypothesized that increased intraluminal nutrient content may provide increased fuel for proximal small intestinal enterocytes, it is more likely that trophic factors are secreted locally and act through a paracrine mechanism to stimulate increased epithelial cell production. The best evidence in support of this hypothesis comes from studies that show that nonmetabolized substances that require active transport are just as capable of stimulating adaptation as glucose.[58] Therefore, functional workload appears to be the major stimulus for adaptation. Certain enteral nutrients may be more effective stimulants than others, either because they require greater work for digestion and absorption or because they preferentially stimulate the release of trophic factors. For example, hydrolyzable disaccharides are capable of greater mucosal stimulation than constituent monosaccharides on a weight-to-weight basis.[59] The hydrolysis of the disaccharide, coupled with subsequent absorption of the monosaccharide components, appears to exert a greater functional workload on the intestinal mucosa. The effect of sucrose on mucosal proliferation can be abolished by inhibiting the sucrase enzyme with acarbose. Likewise, lactulose, which is a nonabsorbable, nonmetabolized carbohydrate, requires no workload from the intestine and consequently results in no mucosal hyperplasia.[59]

The second mechanism by which enteral nutrition stimulates intestinal adaptation is through the stimulation of secretion of trophic gastrointestinal hormones. This can be demonstrated using Thiry-Vella fistula models. Thiry-Vella fistulae have an intact blood supply but through the creation of proximal and distal ostomies are excluded from the flow of enteric contents (Figure 40.1-1). After the placement of a Thiry-Vella fistula, enteral feeding stimu-

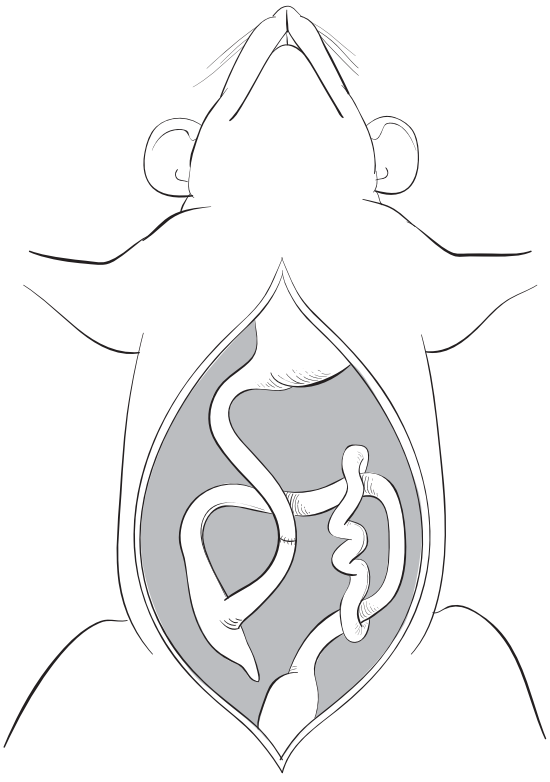

FIGURE 40.1-1 A Thiry-Vella fistula. A segment of distal jejunum and proximal ileum has been removed, its blood supply left intact, and ostomies created at both proximal and distal ends of the fistula.

lates intestinal adaptation not only in the small intestine but also in the fistula. Intravenous feeding conversely results in atrophy in both Thiry-Vella fistulae and intact small bowel.[60] Comparable findings have been observed in self-emptying blind loops, where mucosal mass in animals, who had been given an 85% jejunoileal bypass, markedly exceeded animals given a 25% bypass. This observation suggests that the defunctionalized bowel was responding to a greater adaptive stimulus produced in the animals with less functioning small intestine.[61] Trophic gastrointestinal hormones circulating via the bloodstream into defunctionalized bowel stimulate adaptation, even in the absence of direct contact with the intraluminal nutrients.

Additional evidence of the importance of the role of hormones in adaptation is derived from studies using parabiotic animals (Figure 40.1-2). In these experiments, one animal in a pair undergoes partial small intestinal resection, and blood is cross-circulated between the two animals. Increased mucosal cell proliferation is subsequently observed, not only in the resected animal but also in its parabiotic partner.[62]

The third mechanism by which intestinal adaptation can be stimulated by enteral nutrition is through the stimulation of gastrointestinal secretions. This can be demonstrated by transplant of the ampulla of Vater to the distal small intestine. Mucosal hyperplasia distal to the transplanted ampulla occurs in response to enteric feedings.[63,64] Pancreatic and biliary secretions enter the distal bowel through the ampulla in much higher concentrations than are normally present in the ileum and consequently stimulate villus hyperplasia distally. Diversion of biliary and pancreatic secretions into self-emptying ileal loops also induces villus hyperplasia.[63]

HORMONAL REGULATION

Hormonal regulation of intestinal adaptation is presently an area of intense investigation. Numerous hormonal mediators have been postulated to be important in this process (Table 40.1-1). Enteroglucagon, so named because of its structural similarity to glucagon, has been extensively studied. Patients with enteroglucagon-secreting tumors develop massive mucosal hyperplasia, which resolves once the tumor is removed.[65] Enteroglucagon is produced in highest concentrations in the distal small intestine, which is the segment that has the greatest potential for adaptation. Enteroglucagon levels and enteroglucagon mRNA levels tend to be increased following intestinal resection or jejunoileal bypass, situations in which mucosal hyperplasia is active.[66-68] Unfortunately, attempts to more directly link enteroglucagon with intestinal adaptation have been disappointing. Immunoneutralization of endogenous enteroglucagon by monoclonal antibodies failed to obliterate the adaptive response.[69] Direct administration of glucagon and enteroglucagon to animals does not result in mucosal hyperplasia.[70] Furthermore, the hormone appears to have antiproliferative properties when studied in an in vitro cell culture system.[69] Recently, precursors to enteroglucagon have received some attention and may be the means through which enteroglucagon stimulates intestinal hyperplasia.[71] Proglucagon-derived peptides may be responsible for the effects previously attributed to enteroglucagon. Ileal

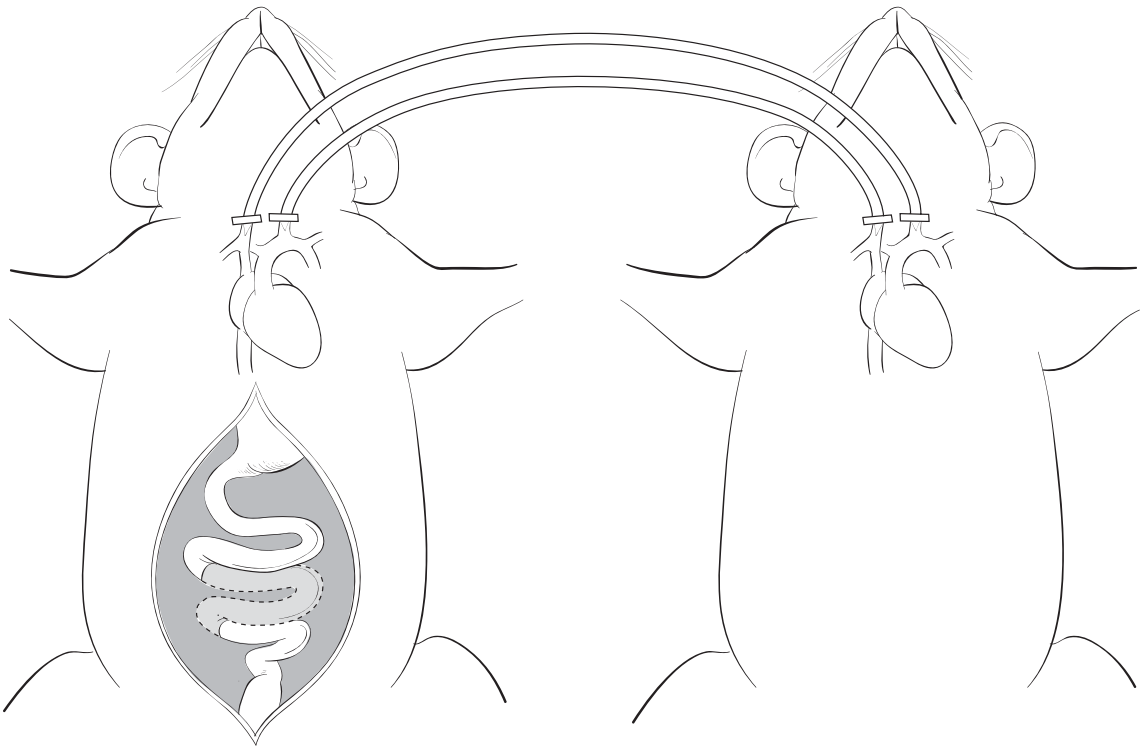

FIGURE 40.1-2 Parabiotic animals. Blood is cross-circulated between the two animals, one of which has undergone partial small intestinal resection.

TABLE 40.1-1 HORMONES THOUGHT TO BE IMPORTANT IN INTESTINAL ADAPTATION

Enteroglucagon
Gastrin
Secretin
Cholecystokinin
Epidermal growth factor
Insulin-like growth factor I
Peptide YY
Glucagon-like peptide 2

proglucagon mRNA levels rise rapidly following resection.[72] Glucagon-like peptide 2 (GLP-2) now appears to be the most likely hormonal candidate for adaptation.[73,74] Recent clinical data have demonstrated that GLP-2 improves intestinal absorption and nutritional status in short-bowel patients with impaired postprandial GLP-2 secretion. This specifically included patients in whom the terminal ileum and colon had been resected. Patients with an intact terminal ileum and colon appear to have adequate GLP-2 secretion, and further adaptation was not achieved in these patients when GLP-2 was administered.[75]

Gastrin levels are highly elevated following intestinal resection. Gastrin is also known to be trophic to the stomach and the proximal small intestine.[76,77] However, hypergastrinemia appears to result in hyperplasia only in the very proximal small intestine and is not likely a major hormonal factor in stimulating intestinal adaptation more distally. Neurotensin is a hormone found mainly in the central nervous system but also in the distal small intestine. Its major role appears to be related to regulation of gastrointestinal motility but has been found to stimulate hyperplasia in the small intestine. It also has an apparent important regulatory function in the growth of colonic mucosa.[78] Secretin and cholecystokinin, when infused into parenterally fed dogs and rats, may prevent mucosal hypoplasia.[79,80] It is possible that the effects of secretin, cholecystokinin, and neurotensin can be explained by the stimulatory effect that these hormones have on pancreatic or biliary secretions.[81]

Epidermal growth factor (EGF), present in breast milk, is known to stimulate proliferation in gut epithelium, primarily in the stomach.[82] It appears to stimulate ornithine decarboxylase (ODC) activity in the small intestine, resulting in polyamine synthesis and subsequent mucosal proliferation.[83] Numerous EGF receptors are present in the small bowel, and the hormone is known to stimulate DNA synthesis.[84] In one study, no differences were detected in ileal EGF receptor mRNA or protein expression following small bowel resection, which led the investigators to conclude that ileal EGF receptor expression was not mandatory for intestinal adaptation.[85] The hormone is produced in high concentrations in both the salivary and Brunner glands and therefore could stimulate proliferation predominantly in the proximal small bowel, where mucosal surface area is normally the greatest. It may therefore play a role in the maintenance of normal gut mass in the physiologic state. Pharmacologic inhibition of the EGF receptor attenuates proliferation and other normal adaptive responses in the small intestine. This observation provides additional evidence for the requirement of a functional EGF receptor as a mediator of the postresection adaptation response.[86]

Recently, interest has developed in insulin-like growth factor I (IGF-I), so named because of its structural similarity to insulin. Otherwise known as somatomedin C, this growth factor appears to be responsible for many of the effects of growth hormone. Initial studies were conducted using a growth hormone analog, plerocercoid growth factor, produced by a tapeworm.[87] This was found to be an effective stimulant of intestinal adaptation in a rat model. Subsequent studies using growth hormone itself produced equivocal results. Studies showing a positive response were hampered by lack of control of nutrient intake.[88,89] Further studies in rats using IGF-I and a truncated analog des-IGF-I following small bowel resection demonstrated augmentation of the adaptation process.[90] Studies using a transgenic mouse model have further demonstrated the importance of IGF-I and growth hormone in regulating intestinal mass.[91] At the present time, it appears that IGF-I plays a role in the adaptation process. In a rat model of short-bowel syndrome, sandostatin has been shown to decrease cell proliferation and inhibit structural adaptation following massive resection.[92]

It is likely that additional hormonal substances may also be involved in intestinal adaptation. Peptide YY levels may be elevated by administration of menhaden oil, which also stimulates adaptation. Serum concentrations of this hormone are markedly elevated in patients with short-bowel syndrome.[93] Because this hormone reduces gastrointestinal motility and increases nutrient contact with the intestinal epithelium, peptide YY might potentially play a role in intestinal adaptation through this mechanism. Leptin, a hormone produced by adipose sites that plays an important role in the regulation of body fat and satiety, has recently been shown to increase small intestinal carbohydrate absorption beyond the normal adaptive response following small bowel resection.[94] As yet undefined, growth factors of 4,500 and 1,500 D have been isolated, which appear to be associated with nutrient-induced gut adaptation.[95] Antitrophic hormones probably also exist. Transforming growth factor-β1 has been shown to induce stem cell quiescence in the intestinal mucosa of the rat.[96] Sandostatin, for example, has been shown to impair gut adaptation.[92] The hormone ghrelin has been shown to be decreased in patients with short-bowel syndrome. The significance of this finding is unknown.[97]

PROSTAGLANDINS

Prostaglandins may also play some role in regulating intestinal epithelial cell proliferation.[98] Inhibition of prostaglandin synthesis has been shown to reduce the mitogenic effect of some gastrointestinal hormones, and, conversely, prostaglandins themselves have been shown to be trophic to numerous cell types.[99] 15,15-Dimethyl prostaglandin E_2 increases mucosal mass and intestinal length in rats. The effects are much more pronounced in the gastric antrum, however. Administration of another prostaglandin analog,

16,16-dimethyl prostaglandin E_2, results in stimulation of intestinal adaptation following resection.[100,101] Likewise, inhibition of prostaglandin synthesis using aspirin adversely affects intestinal adaptation of the ileum but not in the proximal small intestine.[100] Kollman-Bauerly and others have demonstrated that specific blockage of the cyclooxygenase-2 enzyme results in inhibition of adaptation, whereas selective inhibition of the lipoxygenase enzyme actually increases adaptation.[102] This phenomenon occurs specifically in the presence of diets containing large quantities of arachidonic acid and provides further evidence for the important role of prostaglandins in intestinal adaptation.[103] The stimulatory effects of the exogenous prostaglandins are observed primarily in the proximal small intestine.

POLYAMINES

The role of polyamines in intestinal adaptation has also been a subject of major interest. Polyamines are polycationic compounds that are present in all prokaryotic and eukaryotic cells.[15,104] The polyamine putrescine is formed from the decarboxylation of ornithine by ODC, and this constitutes the rate-limiting step in polyamine biosynthesis (Figure 40.1-3). Spermine and spermidine are subsequently synthesized. The activity of the enzyme ODC is low in resting and nondividing tissues. Polyamines are present in high concentration in rapidly proliferating tissues such as the small intestinal epithelium.[105] They appear to be essential for normal cell growth and differentiation. During adaptive hyperplasia, both polyamine content and ODC increase as intestinal proliferation increases. Increases also occur during other proliferative states, such as recovery from intestinal injury, lactation, and poststarvation refeeding.[106-109] A rise in ODC activity constitutes one of the earliest cellular events observed during the transition of cells from quiescence to active proliferation.[110]

Polyamines are known to stimulate mucosal hyperplasia. Blocking polyamine synthesis by blocking ODC reduces adaptation.[111] Likewise, blocking polyamine degradation using aminoguanidine increases intestinal adaptation.[112] In addition to the trophic effects, polyamines also appear capable of inducing maturation of sucrase isomaltase synthesis and sodium or glucose transport, probably mediated by both transcriptional and post-transcriptional events.[113]

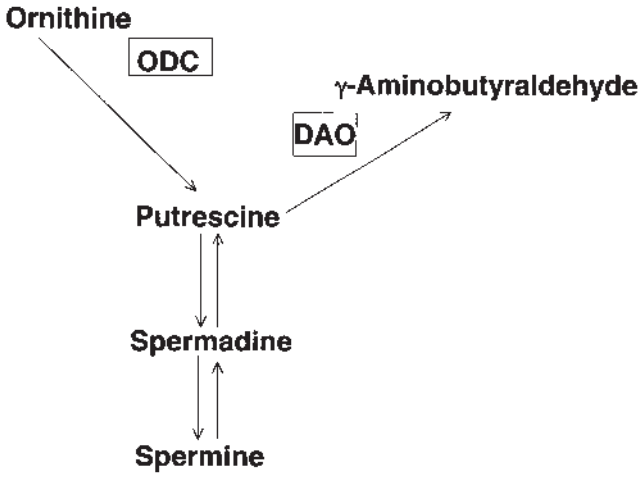

FIGURE 40.1-3 A diagram showing the synthesis and degradation of polyamines. DAO = diamine oxidase; ODC = ornithine decarboxylase.

INTRACELLULAR REGULATION

Studies have also been conducted to test the hypothesis that specific genes are transcriptionally regulated in response to loss of functioning bowel surface area to both initiate and maintain a compensatory response. Using ribonucleic acid from remnant ileum of resected rats, 40 complementary DNAs were found that were more abundant in experimental intestinal segments compared with control. Eleven clones were subsequently identified, which were thought to be proteins likely to be involved in the adaptation process. Because several potentially important genes are apparently up-regulated in the adaptation process, it appears that the genetic regulation of adaptation is complex, including genes that may help to augment nutrient trafficking, heat shock genes that maintain normal cellular function, and genes that themselves are likely to mediate the proliferative response.[114]

It is likely that protein phosphorylation by kinases may be important in controlling cell-signaling mechanisms that regulate cell proliferation and differentiation. Certain tyrosine kinases appear to be uniquely expressed in the proliferating small intestine.[115]

APPLICATIONS OF ADAPTIVE PROCESS

Manipulation of the adaptation process through diet has some potential in the treatment of short-bowel syndrome. Certain nutrients are more capable of stimulating adaptation than others (Table 40.1-2). Most studies to evaluate the function of nutrients on adaptation have been done in experimental models, primarily in rats. Complex diets tend to induce more adaptation than elemental diets, probably because they demand a greater functional workload for assimilation.[116] However, hydrolyzed casein appears to be more trophic in stimulating adaptation than whole protein.[117] Two studies have demonstrated that long-chain triglycerides are more trophic than medium-chain triglycerides.[118,119] High-fat diets also appear to more rapidly reverse starvation-induced mucosal atrophy than diets containing predominantly carbohydrate or protein.[120] Diets containing higher percentages of long-chain triglycerides but deficient in essential fatty acids are clearly less trophic than diets containing adequate quantities of essential fatty acids. This is at least partially attributable to the induction of essential fatty acid deficiency.[121] Menhaden oil, a highly unsaturated fish oil containing ω-3 fatty acids, has been shown to be more trophic than safflower oil, which is high in essential fatty acids, or beef tallow, and a highly saturated fat source.[122] The beneficial effects of menhaden oil could not be attributed to enteroglucagon secretion but were associated with a small but significant increase in peptide YY levels.[123] The effects of menhaden oil could also be due to

TABLE 40.1-2 NUTRIENTS THAT MAY STIMULATE ADAPTATION MORE THAN OTHERS

Long-chain fats
Omega-3 fatty acids
Short-chain fatty acids
Fiber
Glutamine?

its high eicosapentaenoic acid content because arachidonic acid is also a major stimulant of adaptation and is likely to be mediated through prostaglandins.[103] Other lipids that may be important in adaptation include short-chain fatty acids. Short-chain fatty acids, when added to parenteral nutrition solutions, reduce atrophy associated with lack of enteral feeding and may ultimately improve adaptation following resection.[124] The addition of short-chain triglycerides to a chemically defined diet enhances both jejunal and colonic adaptation compared with a control diet containing medium-chain triglycerides.[125]

There has been increasing interest in the use of glutamine in the treatment of a variety of gastrointestinal disorders, primarily because of its apparent role as an important nutrient for the small intestinal mucosa. Administration of glutamine has been shown to reduce bacterial translocation in the small intestine and to prevent mucosal atrophy in certain animal models. Consequently, many have assumed that glutamine should be an important stimulator of intestinal adaptation. Intravenous glutamine appears to have some trophic effect on the small intestine.[126] Animal studies have been unable to demonstrate a trophic effect of oral glutamine. Even when used in pharmacologic quantities, glutamine produced less hyperplasia than either glycine or glucose.[127] One controlled study demonstrated that 8 weeks of treatment with oral glutamine and a high-carbohydrate, low-fat diet did not significantly improve intestinal morphology, gastrointestinal transit, D-xylose absorption, or stool losses in patients with short-bowel syndrome.[128] An extensive review of the literature regarding the effect of growth hormone and glutamine in short-bowel syndrome confirms the likely lack of effective therapy in inducing gut adaptation.[129]

Fiber may also enhance adaptation. Its effects are likely to be most important in the colon and are probably mediated through short-chain fatty acid production. Supplementation of an elemental diet with pectin, which is metabolized to short-chain fatty acids in the colon, improves adaptation in the jejunum, ileum, and colon following resection.[130–132] Furthermore, mucosal atrophy associated with parenteral nutrition can be reversed by parenteral administration of short-chain fatty acids.[124]

CLINICAL MANAGEMENT

The management of short-bowel syndrome is a multistage process (Figure 40.1-4).[133–135] It begins with a period of total parenteral nutrition, which is rather short in duration and is characterized primarily by stabilization of fluid and electrolytes. Immediately following bowel resection, all nutrients must be given parenterally because of a transient ileus. Initially, patients tend to have large-volume fluid and electrolyte secretion, and gastric fluid and ostomy losses may be relatively high. In this setting, it is often easiest to begin the patient on a standard parenteral nutrition solution containing all appropriate macro- and micronutrients, as well as appropriate fluid and electrolyte concentrations for metabolic needs. The excessive fluid losses from gastric tubes, gastrostomies, and diarrheal or ostomy fluid can be replaced based on the electrolyte content of these secretions. It is preferable to measure the volume of these secretions every 2 hours and replace them using a separate fluid and electrolyte solution based on actual measurement of electrolytes in the ostomy losses. Losses tend to be high in sodium content, and solutions with at least 80 to 100 mEq/L of sodium are commonly needed to maintain fluid and electrolyte homeostasis. Once ostomy losses drop, fluid replacement is reduced accordingly. In this setting, use of dual pumps, one for the parenteral nutrition solutions and the other for the replacement solutions, is needed. Despite the apparent increase in cost of the double-infusion system, reduction in wastage of parenteral solutions and frequent changes in laboratory monitoring will more than compensate for this additional expense. When fluid and electrolyte losses have decreased, continuous enteral infusion is started. Most commonly, this is done using an elemental diet.[136,137] The initial rate is quite slow, and the concentration is rapidly increased up to 0.67 kcal/mL in infants or 1 kcal/mL in older patients and adults (ie, full-strength enteral feeding solutions). Once this is done, the volume of enteral feedings can be gradually increased as the volume of parenteral feedings is decreased without overloading the patient with fluid.

The continuous enteral infusion is gradually advanced based on several parameters. If stool losses increase by more than 50% and are greater than about 40 to 50 mL/kg/d, or stool or ostomy output is strongly positive for reducing substances, advances in enteral feeding should be withheld until these parameters improve. In patients with an intact colon, a decrease in the stool pH below 5.5 is also indicative of carbohydrate malabsorption and suggests that further advancement of enteral feedings would result in a significant increase in osmotic diarrhea.

The use of continuous enteral infusion is often controversial. The major advantages to the use of continuous enteral infusion include tolerance of enteral feedings, better control of enteral caloric administration, and a reduction in emesis.[1] By using continuous enteral infusion, small intestinal carrier proteins can be continuously saturated, permitting optimal use of limited gastrointestinal function. The increased administration of enteral calories has the potential added benefit of additional stimulation of intestinal adaptation, although this has not been proven in the laboratory. Administration of extra calories reduces the parenteral nutrition need and, in theory, will also reduce the risk of liver disease, which accounts for a major percentage of morbidity and mortality in short-bowel syndrome.

Despite the inconvenience of continuous enteral feeding, portable pumps and backpacks have allowed children

FIGURE 40.1-4 A diagram of the clinical management decisions in short-bowel syndrome. bx =biopsy; EGD = esophagogastroduodenoscopy; EM = electron microscopy; SBS = short bowel syndrome; TPN = total parenteral nutrition.

to have reasonably normal mobility. Solid feedings can be initiated and fed around the nasogastric tube without difficulty, and small bolus feedings are often tolerated. Institution of solid feedings at the usual time and continued administration of small bolus feedings at least two to three times a day are important because they teach the infant how to suck and swallow and will lessen the likelihood of feeding difficulties once the tube feedings are discontinued. In theory, this will also reduce the risk of speech delay as a result of disuse of muscles of mastication and swallowing.

Elemental or chemically defined diets have been used extensively in short-bowel syndrome.[136–138] In pediatrics, truly elemental diets (ie, diets in which amino acids rather than peptides are administered) are not commonly used

and are probably not optimal for treatment of patients with short-bowel syndrome. Predigested or partially elemental formulas appropriate for use in pediatrics are available from several manufacturers. These usually consist of a protein hydrolysate, most commonly casein, a carbohydrate source that may contain one or more rapidly absorbable carbohydrates, and fats, which are usually a mixture of medium- and long-chain triglycerides. Adult elemental formulas are often inappropriate for use in pediatrics because they may be deficient in vitamins, minerals, and fatty acids and are often very high in carbohydrate content, resulting in increased osmotic diarrhea. These formulas can be modified somewhat, if an amino acid formula is desired or needed in infants, by the addition of extra vitamins, minerals, and fat.[137,138] In addition, increased fat content in the pediatric formulations may be beneficial because of the stimulatory effect of fat on adaptation in the small intestine. Long-chain fats are more trophic to the small intestine than medium-chain fats (Figure 40.1-5). Furthermore, highly unsaturated fats derived from fish oil or formulas high in arachidonic acid may prove to be even more effective in stimulating intestinal adaptation (Figure 40.1-6).[139] In reality, the form of the protein, whether amino acid, enzymatic hydrolysate, or intact protein, makes little difference from an absorptive standpoint in most patients with short-bowel syndrome. Perhaps the primary value for use of these formulas in small infants is to reduce the risk of the development of allergic disease, which is more common in children with enhanced mucosal permeability and is often the case in children with short-bowel syndrome. These patients tend to have small bowel bacterial overgrowth and frequently have had inflammatory processes in their small intestine. After the first year of life, there is probably little advantage in the use of a hydrolysate formula. Careful attention to the fat content of the formula is probably more important. Recent experience suggests that high-fat enteral feeding formulas may offer a significant advantage in patients with short-bowel syndrome provided that the fat is given primarily as long chain.[140] This is probably because fats exert a greater

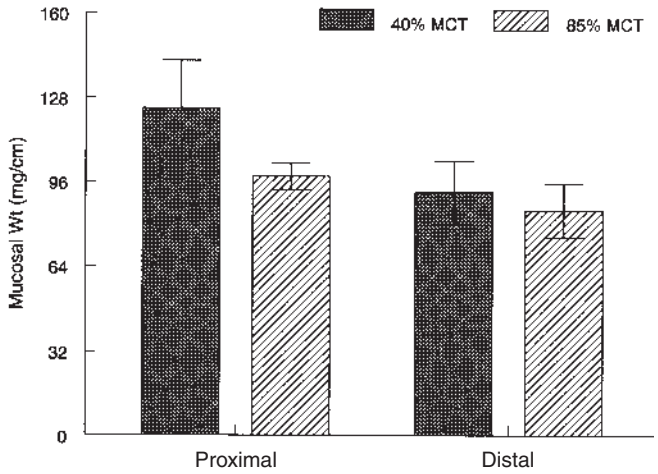

FIGURE 40.1-5 Mucosal weight in rats fed diets containing either 40% or 85% of their total lipid concentration as medium-chain triglycerides (MCT) for 3 weeks following 85% jejunoileal resection.

FIGURE 40.1-6 Mucosal protein concentration in rats fed menhaden, safflower oil, or beef tallow for 3 weeks following 85% jejunoileal resection.

trophic effect in the small intestine, produce a lower osmotic load, and are not an ideal substrate for the proliferation of excess bacterial flora in the gut lumen.

As parenteral nutrition is decreased and enteral nutrition is increased, the patient is gradually weaned to intermittent parenteral nutrition to be given over a portion of each day. This is important because the patient will likely be dismissed on home parenteral nutrition, and intermittent parenteral nutrition improves patient freedom.[136,141–143] In small infants, parenteral nutrition is often continued for a majority of the 24-hour day, but this interval can gradually be weaned as the patient's tolerance of enteral calorie increases and as the infant ages. In short-bowel syndrome, this is often a very gradual change, taking several months to years. If tolerated, the rate of enteral calories is increased and the duration of parenteral nutrition is decreased until the patient can actually be taken off parenteral nutrition on one or more nights per week. It is important to make isocaloric changes because the caloric density of the parenteral nutrition solution and the enteral nutrition solution may differ. Caloric intake is gradually increased based on the child's growth needs to ensure that the child parallels the 50th percentile, both for height and weight. Excessive caloric administration is often a problem at this stage of management because the child is incapable of regulating his own nutrient intake. It is the physician's responsibility to ensure that the patient's weight gain is appropriate for his length and is not excessive.

Fluid losses at this stage are often great. Ostomy output may have sodium concentrations of 80 to 100 mEq/L. Diluting elemental diets with oral electrolyte solution and increasing the rate of administration accordingly are often helpful in replacing these losses during the enteral feeding stage.[144]

Early in the course of therapy, the child should be prepared for home parenteral nutrition. This reduces the cost of long-term management in patients with short-bowel syndrome and decreases family stresses and nosocomial

infections. It has now become the standard of care in patients with short-bowel syndrome, and prolonged hospitalizations are rarely necessary.

Solid feedings should be introduced early in patients with short-bowel syndrome, especially infants. Chronic tube feeding, if given throughout the first year of life, may result in protracted feeding refusal later. Small children, especially infants, tolerate the osmotic effects of carbohydrates, both simple and complex, rather poorly and, consequently, do much better when fed diets or foods higher in fat content. Starting meat as the initial solid because of its high-fat and -protein and low-carbohydrate content is often beneficial. Fats stimulate gut adaptation, are a poor substrate for bacterial overgrowth in the small bowel, and produce little adverse osmotic effect. Older children and adults do better with more complex carbohydrate and more fiber in their diet. In either instance, an appropriately balanced diet with small frequent feedings is often advantageous. Increasing dietary fat and lowering dietary carbohydrate content may also reduce the substrate for bacterial overgrowth and, to some degree, reduce the need for pharmacologic intervention.

CHRONIC COMPLICATIONS

The real challenge in short-bowel syndrome comes from managing the many chronic complications that arise. Many of these are complications of parenteral nutrition, including catheter-related problems, sepsis, and total parenteral nutrition liver disease. Others are unrelated to the parenteral nutrition, such as small bowel bacterial overgrowth, or they occur when the parenteral nutrition has stopped, such as micronutrient deficiency.

Bacterial Overgrowth

Bacterial overgrowth is perhaps the least recognized complication of short-bowel syndrome but also one of the most treatable. Bacterial overgrowth is defined as increased bacterial content in the small intestine.[145] Normal small bowel bacterial counts vary from 10^3 proximally to greater concentration in the ileum. A high concentration of gastric acid normally limits the number of bacteria that successfully enter the small intestine. Bacteria are subsequently eliminated from the small intestine through the combination of normal antegrade peristalsis and mucosal immune factors. In short-bowel syndrome, many of these factors, especially anatomy and motility, are disrupted. It is not uncommon for bacterial content of the proximal small intestine to exceed 10^5. When motility is slowed, the bowel is dilated, and the ileocecal valve is absent, bacterial overgrowth is almost universally present. Reduction in gut-associated lymphoid tissue following resection might also impair the immune response to these bacteria.[3] A wide variety of organisms are present, mainly facultative bacteria and anaerobes. These bacteria deconjugate bile salts, resulting in rapid reabsorption of bile acids, depleting the bile salt pool, which subsequently impairs micellar solubilization and results in steatorrhea and malabsorption of fat-soluble vitamins. Bacterial overgrowth also causes mucosal inflammation, which further exacerbates nutrient malabsorption (Figure 40.1-7). Additionally, bacteria may compete with the host for vitamin B_{12} and perhaps other nutrients. Bacterial overgrowth should be considered when a patient experiences bloating, cramps, diarrhea, or gastrointestinal blood loss in the face of seemingly adequate gut length. It is also a common cause of clinical deterioration in a previously stable patient with short-bowel syndrome.

Diagnosis of bacterial overgrowth is classically based on demonstration of increased bacterial content by small intestinal aspiration and culture of the fluid, although this is usually not practical and is unnecessary. Screening for bacterial overgrowth can often be accomplished through the use of breath hydrogen determination. Markedly elevated fasting breath hydrogen levels or a rapid rise in breath hydrogen following oral administration of glucose (2 g/kg up to a maximum of 50 g) is suggestive of bacterial overgrowth provided that the transit time through the small intestine is not so rapid as to produce immediate entry of malabsorbed glucose into the colon. Glucose is the ideal substrate for this test because it is absorbed rapidly in the small bowel and rarely makes it to the colon, where it could produce a false-positive test. Urine indican is also an indicator of bacterial overgrowth and may be used as another simple screening test for small bowel bacterial overgrowth. Small intestinal biopsies demonstrating inflammatory changes often suggest bacterial overgrowth, especially when the small intestine is dilated, motility is poor, or a partial obstruction exists.

Two other complications of bacterial overgrowth include D-lactic acidosis and small bowel colitis. D-Lactic acidosis results because bacteria produce both D- and L-lactate, but only L-lactate is well metabolized by most humans.[146-149] Consequently, the malabsorbed carbohydrates are broken down to lactic acid by the bacteria. D-Lactate then accumulates in the bloodstream, resulting in neurologic symptoms varying from disorientation to frank coma. Bacterial overgrowth may also result in the development of colitis or ileitis, with large ulcerations that

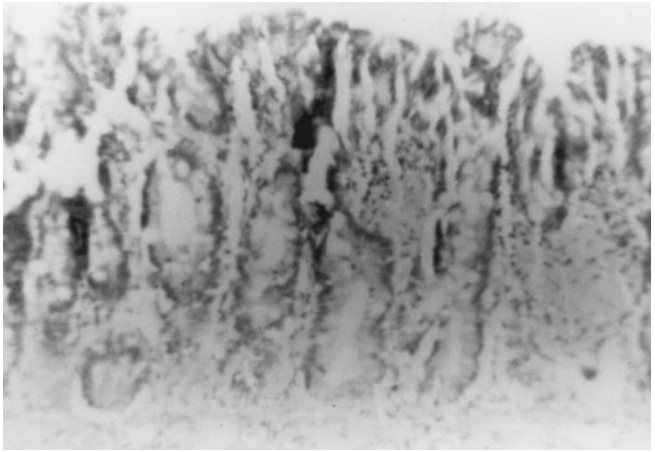

FIGURE 40.1-7 Photograph of a small intestinal biopsy from a patient with short-bowel syndrome and documented severe bacterial overgrowth showing inflammation and villus destruction (hematoxylin and eosin; ×400 original magnification).

appear to be similar to those in Crohn disease. Granulomas are not identified.[148] The presence of arthritis and other rheumatologic symptoms in some of these patients suggests the possibility that the disorder may be immune complex related, possibly owing to absorbed bacterial antigens.[149,150] This form of colitis occasionally responds to antimicrobial therapy, although sulfasalazine and other immunosuppressive medications are often efficacious. A short course of corticosteroids often produces marked improvement in patients with small bowel bacterial overgrowth–induced enterocolitis.

Bacterial overgrowth can usually be treated with broad-spectrum antibiotics given intermittently, usually the first 5 days of each month (Table 40.1-3). Oral metronidazole, 10 to 20 mg/kg/d, either alone or in combination with trimethoprim-sulfamethoxazole, is an effective combination. Oral gentamicin may also be used and is minimally absorbed. Several other combinations are often helpful. Occasionally, patients are refractory to therapy, and the antibiotics must be given continuously. In this event, the antibiotics should be rotated periodically to prevent overgrowth with resistant organisms. Probiotic therapy has been shown to reduce the risk of bacterial translocation in experimental short-bowel syndrome.[151] However, the experience with probiotic therapy in small bowel bacterial overgrowth is limited, and the addition of exogenous flora to an already overgrown small bowel ecology is likely to produce mixed results.

In some children, the absence of an ileocecal valve results in severe overgrowth in the distal small intestine. This is especially true as children age and learn to defecate less frequently. Encouraging frequent voluntary defecation may result in clinical improvement in many patients. Daily saline enemas or occasionally enteral lavage with polyethylene glycol solutions is required to reduce bacterial content in some patients. The use of antimotility agents such as loperamide in patients with short-bowel syndrome may exacerbate bacterial overgrowth and may be contraindicated in patients whose gastrointestinal motility is already delayed.

Watery Diarrhea

Excessive fluid secretion occurs in many patients with short-bowel syndrome. Often this is simply a result of excessive osmotic load in the small intestine when large quantities of carbohydrates are fed. This occurs especially after bolus feeding. However, elevated serum gastrin levels are often present in patients with short-bowel syndrome and may be partially responsible for enhanced fluid secretion. Rarely, this responds to the administration of histamine$_2$ receptor antagonists. Somatostatin analogs have been used in a limited number of such patients, with varying results.[150,152,153] Subjects improve initially, but the favorable response is often transient, and exacerbation of fat malabsorption may negate the benefits of the drug.[154] Cholestyramine is occasionally administered to patients with watery diarrhea. This resin binds bile acids, especially following ileal resection, where increased concentrations of bile acids in the colon may cause secretion and watery diarrhea. However, in the case of massive ileal resection, patients may have bile acid insufficiency, and cholestyramine may, in fact, exacerbate steatorrhea by further reducing effective bile acid concentration (Table 40.1-4). In patients with radiographically demonstrated rapid small bowel transit, the addition of loperamide may be beneficial. However, in the presence of slow transit and small bowel bacterial overgrowth, it is likely to be contraindicated.

TABLE 40.1-3 TREATMENT FOR BACTERIAL OVERGROWTH

Antibiotics
Intermittent
Continuous cyclical
Surgery
Tapering
Lengthening
Prevention of colonic stasis
Frequent bowel movements
Saline enemas
Enteral lavage

Nutritional Deficiency States

Once patients are off parenteral nutrition, the physician no longer has control over the patient's nutritional status. The compromised small intestinal function becomes a major problem in ensuring adequate nutrient stores. Usually, macronutrients such as proteins, carbohydrates, and fats can be absorbed in adequate quantities, but micronutrients such as minerals, trace elements, and vitamins are frequently deficient. Malabsorption of fat-soluble vitamins, especially A, D, and E, is common. A variety of trace metal deficiencies have also been demonstrated in short-bowel syndrome, with iron and zinc being most common. A low serum zinc level, especially in association with a low serum alkaline phosphatase level, suggests zinc deficiency. Zinc deficiency may result in poor growth and impaired intestinal adaptation, and administration of exogenous zinc is important in such patients.[155] Selenium absorption may also be impaired.[156] Deficiencies of minerals also may exist, especially calcium and magnesium. Extra vitamin D and calcium may correct calcium deficiency, but magnesium deficiency is more difficult to manage because enterally administered magnesium often results in osmotic diarrhea. Some magnesium salts are better tolerated than others.[157] Other micronutrients, such as carnitine, choline, and taurine, may also be important.

The ileum is solely responsible for bile acid and vitamin B_{12} malabsorption. In the case of ileal resection, the proximal small intestine will not develop the ability to absorb vitamin B_{12}; consequently, such patients should be periodically monitored for vitamin B_{12} deficiency. Occasionally, parenteral administration of vitamin B_{12} may be

TABLE 40.1-4 EFFECTS OF BILE SALT MALABSORPTION

Mild = secretory diarrhea
Severe = fat malabsorption
Loss of calories
Loss of fat-soluble vitamins

required. Vitamin B_{12} deficiency may take years to develop, and periodic attention to this possibility is advisable.

Parenteral Nutrition–Induced Liver Disease

Parenteral nutrition–induced liver disease is currently the major cause of death in children with short-bowel syndrome. This disorder is especially common in children receiving long-term parenteral nutrition because the incidence increases in inverse proportion to age.[2,3,5,8] The mechanism by which parenteral nutrition causes liver injury is unknown. Toxicity of amino acids, competition of amino acids with bile acids for transport across the canalicular membrane, production of toxins in the unused bowel, excess nutrient administration, toxic substances in the parenteral nutrition solution, and nonstimulation of gastrointestinal hormones that normally control biliary secretions have all been postulated. Aggressive administration of enteral feedings, hopefully to ensure at least 20 or 30% of total daily caloric intake through the enteral route, prevention of bacterial overgrowth in the small intestine, and reduction of catheter-related sepsis all appear to be important in protecting patients from parenteral nutrition–induced liver disease (Table 40.1-5). In addition, the new parenteral solutions, especially designed for small infants, may provide some protection. In some instances, reduction or cessation of intravenous lipid administration may result in improved hepatic function or reduction in serum bilirubin concentration.[158]

Biliary disease may also occur in children who are dependent on parenteral nutrition.[159] As many as 20% of infants receiving parenteral nutrition may develop cholelithiasis. Malabsorption of bile acids, altered bilirubin metabolism, and gallbladder stasis are likely to be important factors in cholelithiasis. In some centers, early cholecystectomy is advocated in patients on long-term parenteral nutrition.[159]

Bone Mineralization

Adequate intestinal absorption of calcium, magnesium, and phosphorus is necessary for normal bone mineralization. Vitamin D, through its active metabolite 1-hydroxyvitamin D_2, is critical in the successful absorption of these elements. Normally, only about 30% of calcium is absorbed each day from the diet. About 65% of dietary phosphorus is absorbed. Inadequate dietary calcium and phosphorus intake and poor absorption of these elements appear to contribute to a high incidence of impaired bone mineralization in patients with short-bowel syndrome. Careful attention to detail in management is often able to overcome these problems.[160,161]

Catheter-Related Complications

Complications relating to chronic indwelling central venous catheters are common.[162] In one series, patients required replacement of the central venous catheter approximately every 200 days, with septic episodes typically occurring more frequently than once per year. Complications were highest in infants under 1 year of age. Catheter thrombosis is also common. Central venous catheter infections may result either from poor catheter care technique or from bacterial overgrowth, with subsequent seeding of the bloodstream with bacteria from the small intestine. The former appears more common, even in patients in whom enteric organisms are found to cause catheter infection, and a careful analysis of catheter care techniques should always be the first step in patients with frequent central venous catheter infections.

SURGICAL OPTIONS

Patients with short-bowel syndrome, especially those with bacterial overgrowth, commonly have anastomotic strictures. Further resection of already shortened small intestine may be avoided by using tapering enteroplasty, strictureplasty, and even serosal patching.[163] Relieving a tight anastomosis or stricture often improves flow of luminal contents through the small intestine and reduces bacterial overgrowth. This may occasionally produce dramatic clinical improvement provided that the patient has normal small bowel motility and previously demonstrated bacterial overgrowth.

A number of procedures have been designed to slow small intestinal transit.[164-169] These include reversed segments of bowel or colon interpositioned to slow the delivery of nutrients through the small intestine and creation of valves that produce a partial obstruction to disrupt the normal flow of contents. All of these procedures may increase bacterial overgrowth and in patients with preexisting bacterial overgrowth will likely do more harm than good.

Increasing the length of bowel through using the intestinal lengthening or Bianchi procedure has recently been popularized.[170-175] This procedure involves transecting the bowel longitudinally, preserving the blood supply to both sides of the small intestine, thereby creating a segment of bowel twice the length and half the diameter of the original segment. This procedure should be performed only when the small intestine is dilated because it allows reduction of the diameter of the dilated bowel without the loss of surface area. It is primarily a means of reducing bacterial overgrowth, although progressive intestinal dilatation will eventually result in increased absorptive surface area. In a series of 13 pediatric patients, marked improvement in absorptive capacity was achieved as measured by decreased parenteral fluid and nutrient requirements.[172] Long-term follow-up has been more disappointing. Although the procedure is successful only in the jejunum and ileum, attachment of the antimesenteric side of the small intestine to the liver or abdominal wall has been proposed, allowing vasculature to grow into the bowel from that surface and the subsequent lateral division of the small intestine; this has been partially successful in at least one patient.[176]

TABLE 40.1-5 PREVENTION OF TOTAL PARENTERAL NUTRITION LIVER DISEASE

Aggressive use of enteral feedings
Prevention of catheter sepsis
Prevention of bacterial overgrowth

TRANSPLANT

Recent reports have suggested that children have been able to survive rather massive small intestinal resections during the neonatal period. Advances in parenteral nutrition and long-term management have made it possible for some children with under 15 cm of small intestine, even in the absence of ileocecal valve, to eventually become independent of parenteral nutrition. The presence of a well-functioning ileocecal valve appears to improve the prognosis in short-bowel syndrome. As a general rule, patients with greater than 25 cm of small intestine at the time of neonatal resection and who have an ileocecal valve or those with greater than 40 cm of small bowel at the time of neonatal resection who do not have an ileocecal valve have a reasonable prognosis of becoming independent of parenteral nutrition.[2,3,5,8,177] These numbers apply only to patients resected during the neonatal period because substantial growth in the small intestine occurs during the first few months of life. Application of these numbers to older children results in excessive optimism. Nonetheless, children can often adapt despite massive resection, and predicting success or failure is difficult.[172,178] Despite aggressive treatment of bacterial overgrowth, aggressive enteral feeding to stimulate the greatest amount of adaptation, and the appropriate use of intestinal lengthening procedures and other forms of surgical therapy, many patients will still never become independent of parenteral nutrition. If a patient resected as a neonate is still dependent on parenteral nutrition beyond 4 to 5 years of age, it is unlikely that the child will survive without lifelong parenteral nutrition. This form of therapy is expensive, averaging about $100,000 per year and frequently costing much more.

At the present time, small intestinal transplant is being advocated by some for children with short-bowel syndrome (see Chapter 40.2, "Small Bowel Transplant"). To date, several hundred transplants have been performed, including predominantly either combined liver and bowel or isolated intestinal transplants.[179-184] Most data now suggest that survival beyond 3 years is little better than 50% in patients undergoing combined liver and bowel transplant. Patients with isolated small bowel grafts may exhibit longer survival. Intestinal graft loss primarily owing to rejection but also to lymphoproliferative disease reduces long-term good results to nearly the same level.[185] Infection appears to be a greater problem in patients with small intestinal transplant than with liver transplant, probably owing to a breakdown in the intestinal mucosal barrier during episodes of allograft dysfunction and rejection. Subsequent translocation of bacteria and fungi into the bloodstream is likely to remain a significant problem. Diagnosis of rejection is difficult, and histologic assessment of rejection is still somewhat elusive as pathologists continue to struggle with criteria for diagnosis. Clinical parameters, such as increased ostomy output or diarrhea that cannot be attributed to feeding changes or infection, should alert the clinician to the possibility of rejection. Enteroscopy with multiple biopsies is important in making this diagnosis and should be done visually and site directed because rejection appears to be a patchy lesion, at least initially.

The key to successful small bowel transplant appears to be aggressive immunosuppression. Tacrolimus, which permits greater immunosuppression relative to its side effects, may be successful in preventing rejection that was previously not treatable with cyclosporine. The increased need for immunosuppression, however, raises concern about development of post-transplant lymphoproliferative syndrome. This Epstein-Barr virus–driven malignancy is a major problem following intestinal transplant. It may respond to reduction or cessation of immunosuppression, but graft loss is at risk when this is done. There is some question as to whether intestinal graft rejection may be somewhat less with liver and bowel transplants than isolated intestinal transplants because of the protective effect conferred by the liver on rejection in the small intestine, but through the use of tacrolimus and other new immunosuppressants, both procedures appear to be feasible.

The ultimate usefulness of intestinal transplant awaits greater experience, but concern about post-transplant lymphoproliferative syndrome, late-onset complications such as rejection and other malignancies, and infectious complications associated with intestinal transplant suggests that greater experience should be sought before recommending this procedure in patients who are otherwise stable on parenteral nutrition.[183,186] In patients with irreversible total parenteral nutrition liver disease, the combined liver and bowel transplant appears to be a potential option, and because of donor shortage, patients should be referred for transplant once liver disease is considered irreversible. Patients with impaired central venous access or patients with early progressive liver disease may be potential candidates for isolated intestinal transplant. Patients with direct hyperbilirubinemia of 12 to 15 mg/dL and fibrosis, but no cirrhosis on liver biopsy, have reverted to normal liver function following isolated intestinal transplant. In a limited number of carefully selected patients, isolated liver transplant may be useful in infants with end-stage liver disease associated with short-bowel syndrome provided that adequate bowel length is available to stimulate subsequent bowel adaptation.[187] Further experience will need to be obtained before appropriate indications for intestinal and combined liver and intestine transplant can be developed.[177]

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2. Small Bowel Transplant

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In many ways, small bowel transplant is the logical extension of treatments such as intravenous feeding (also known as parenteral nutrition [PN]), which have become accepted in the management of chronic irreversible intestinal failure. However, small bowel transplant has taken a long time to become established. The first intestinal transplants were carried out as technical experiments in dogs in the 1950s.[1] This was followed up in some quite desperate cases in the 1960s in humans, but all failed because of the intensity of rejection, which led to sepsis and multiorgan failure within a few days. In the 1970s, intravenous feeding was refined, and it became possible to maintain children and adults for years with daily infusions of carefully balanced solutions of amino acids, dextrose, and lipids.[2–4] These developments in intravenous feeding improved the nutritional state of potential transplant recipients and have probably contributed to the gradually improving results of small bowel transplant.[5,6] The other key development has been improvements in immunosuppression, specifically, the widespread availability from 1991 of tacrolimus, a drug derived from a saprophytic fungus that targets cytotoxic T lymphocytes and inhibits clonal expansion of those immune cells responsible for the recognition of new antigens.

There are some specific clinical issues to consider when contemplating small bowel transplant that distinguish it from other solid organ transplants: (1) the volume of tissue transplanted is large and contains a vast number of lymphocytes in the lamina propria and Peyer patches, and (2) the small bowel allograft is contaminated with billions of bacteria. This means that graft-versus-host disease is a possibility in patients with underlying immunodeficiencies[7] and that a large exposure to immunosuppression is routinely needed to prevent rejection. The presence of bacteria means that when moderately severe rejection develops, causing the intestine to become more permeable, then translocation of bacteria to the systemic circulation may occur via mucosal ulceration at a time when additional immunosuppression is needed to deal with rejection.[8] This clinical paradox goes some way to explaining the high complication rate and modest survival of 50 to 65% that obtain after small bowel transplant.[9–11]

INDICATIONS

Although many adult patients request small bowel transplant in preference to the inconvenience of long-term PN, the current survival rates of small bowel transplant mean that the transplant is reserved for patients who have developed life-threatening complications related to the administration of PN. It is well known that cholestasis and progressive liver disease are poor prognostic factors in patients requiring long-term PN (Figure 40.2-1),[12–14] but in addition to liver disease, unstable venous access and recurrent life-threatening episodes of infection (caused by bacterial overgrowth or contamination of the feeding catheter) constitute appropriate indications for transplant, as detailed in the consensus statement produced by Kaufman and colleagues[15] and Table 40.2-1.

These indications apply to adults and children, but the prognosis in children with short gut may be unpredictable with regard to the possibility of long-term adaptation of residual gut function. It is very important that transplant teams have pediatric gastroenterologists advising on the feasibility of improving intestinal function to make a judgment about the type and necessity of transplant (Table 40.2-2).[16,17] Furthermore, patients with disease other than short gut as the underlying cause of intestinal failure may be stabilized without resorting to small bowel transplant.[18,19] It is noteworthy that patients managed by a nutritional care team have usually required fewer replacements to their central lines and experienced less liver disease at an early age than do patients looked after by a nonspecialist team.[20,21] Given the relatively high morbidity and mortal-

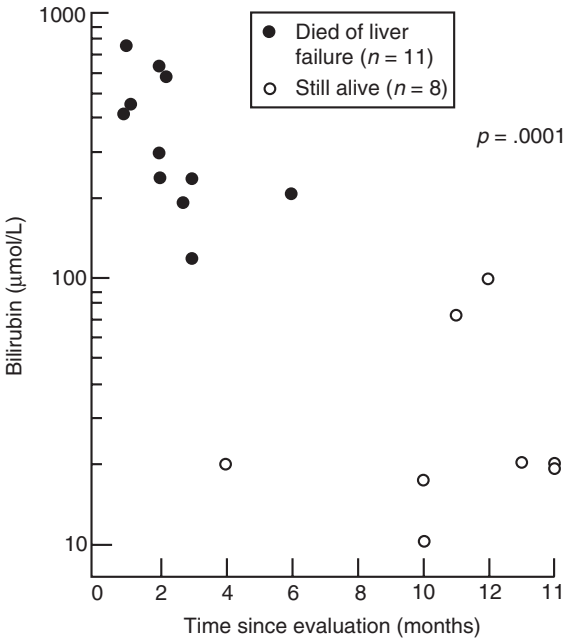


FIGURE 40.2-1 Graph showing plasma bilirubin at time of assessment and subsequent duration of survival. Adapted with permission from Beath SV et al.[20]

TABLE 40.2-1 INDICATIONS FOR INTESTINAL TRANSPLANT

LIFE-THREATENING COMPLICATIONS ARISING FROM PARENTERAL NUTRITION THERAPY	TYPE OF TRANSPLANT
Impending loss of venous access, ie, when 2 of the 4 available sites have been lost in infants or 3 of 6 in older children Recurrent sepsis (especially if metastatic, eg, brain abscess or infective endocarditis, if unusually severe, resulting in multiorgan failure)	Isolated bowel transplant
Erratic fluid balance requiring hospitalization Congenital intractable epithelial disorder, eg, microvillous inclusion disease and tufting enteropathy Some cases of short-bowel syndrome	Isolated bowel transplant or combined liver and bowel transplant depending on severity of hepatic complications of parenteral nutrition
Irreversible liver disease: hyperbilirubinemia persisting beyond 3–4 mo of age and features of portal hypertension, splenomegaly, prominent superficial abdominal veins	Combined liver and intestinal transplant

Adapted from Kaufman SS et al.[15]

ity, it is also important to identify patients who would not benefit from intestinal transplant, for example, those with severe congenital or acquired immunodeficiency or nonresectable malignancies (Table 40.2-3).

SURGICAL CONSIDERATIONS

Considerable surgical ingenuity and innovation have been required to make intestinal transplant a practical reality, although the type of transplant that is recommended, either small bowel transplant combined with liver and/or other organs or isolated small bowel transplant, is based on medical criteria. The original surgical concept of the abdominal viscera as a "bunch of grapes" and the mesentery as the "stem" is described in early operations by Starzl and Deltze[22–24] and has been modified in the late 1990s to lessen the risk of postoperative biliary leaks and strictures.[25]

The majority of intestinal transplants are done in children, 12% of whom are less than 12 months old (Table 40.2-4).[26] The infants who require combined liver and bowel transplants have often had previous abdominal surgery, which renders the abdominal cavity even smaller and more difficult to work in. This means that there is frequently a size mismatch between donors who tend to be adolescents or adults and the desperately ill babies who need the transplant. Consequently, the waiting list mortality of 50 to 60% for combined liver and small bowel transplant has been one of the highest for any solid organ transplant.[27,28] Such a high mortality has stimulated further innovations in surgical technique, including the development of the reduction en bloc technique.[29] Up to a 5:1 size mismatch between the donor and the recipient

TABLE 40.2-2 CLINICAL STAGE AND TYPE OF TRANSPLANT

CLINICAL STAGE	TRANSPLANT TYPE	NOTES AND NONINTESTINAL TRANSPLANT OPTIONS
No jaundice, no portal hypertension, normal abdominal sonogram, no venous access problems, fluid balance satisfactory	None indicated	No need for transplant; review enteral feeds and intestinal anatomy/stomas (if short gut); ensure nutritional care team involvement, shared care follow-up with pediatric gastroenterologist
No cholestasis, minimal portal hypertension (borderline enlarged spleen), modest fibrosis only in liver biopsy, only two satisfactory central veins still patent Recurrent episodes of systemic sepsis (requiring intensive care) Irreversible intestinal failure with poor prognosis, eg, microvillous inclusion disease	Isolated small bowel transplant	A good opportunity to re-evaluate tolerance of enteral feeding (if short gut) and assess hygiene related to feeding catheter Review prescriptions of motility agents and ursodeoxycholic acid Liver disease may be progressive, so delay may result in combined liver bowel transplant being needed
Patient with short gut with potential to adapt Jaundice (bilirubin greater than 100 µmol/L or 6 g/L) Severe fibrosis and marked portal hypertension, disturbance to coagulation and intermittent ascites/encephalopathy	Isolated liver transplant	Patient is so ill that liver transplant within weeks is needed; the resolution of portal hypertension may be accompanied by improvement in adaptation of residual short gut
Patient with pseudo-obtruction or mucosal disorder in which jaundice, hepatic fibrosis, impaired coagulation, ± ascites and coagulopathy have developed	Combined liver and bowel transplant	Combined liver-bowel transplant recommended, but if patient is less than 10 kg, donor organs may not be available quickly enough, so sequential liver and small bowel transplants may be carried out
Jaundice (bilirubin greater than 100 µmol/L or 6 g/L), fibrosis bordering on cirrhosis, evidence of progressive liver disease, ultrashort gut with no sign of adaptation, dysmotile gut, irreversible intestinal failure		Prevent further deterioration, review enteral feeds, review prescription for motility agents, ursodeoxycholic acid; consider selective decontamination of intestinal tract

TABLE 40.2-3 CONTRAINDICATIONS TO INTESTINAL TRANSPLANT

Patients with the following conditions should not normally undergo intestinal transplant because they are unlikely to derive an obvious benefit:
Profound neurologic disabilities
Life-threatening and other noncorrectable illnesses not directly related to the digestive system
Severe or acquired immunodeficiency
Nonresectable malignancies
Multisystem autoimmune diseases
Those with insufficient vascular patency to guarantee easy central venous access for up to 6 mo following transplant

Adapted from Kaufman SS et al.[15]

may be accommodated by removing the right lobe of the donor liver en bloc with removal of the middle section of small bowel allograft (the distal ileum is preserved for use in the recipient and is exteriorized to allow for close inspection of the small bowel allograft).[29] The vascular anastomoses are made by joining the hepatic veins to the inferior vena cava, where it joins the right atrium, and the celiac axis to the infrarenal aorta.

In contrast, the operative procedure in isolated small bowel transplant is relatively straightforward, with care being needed to anastomose the donor portal vein to the recipient portal vein to avoid kinking and torsion. If hepatic fibrosis is present, then anastomosing the portal vein to the inferior vena cava is preferred to avoid the possibility of portal hypertension affecting the small bowel allograft. The celiac axis is anastomosed to the infrarenal aorta. The proximal end of the graft may be anastomosed to jejunum in short gut patients or to the stomach in patients with a history of dysmotility. The distal end of the small bowel graft may be brought out as an end stoma if the patient has a nonfunctioning large bowel (eg, Hirschsprung disease) or connected to residual large bowel via a loop ileostomy. Whatever reconstruction is done, it is essential to allow exteriorization of the small bowel allograft to allow for regular biopsy for early detection of rejection (Figures 40.2-2 and 40.2-3).[10]

RESULTS

Since 1992, the world experience of intestinal transplant has been collected by the Intestinal Transplant Registry, which publishes updated results every 2 years on its Web site and in journals.[9,26] In the past decade, the number of transplants has grown from 5 to approximately 100 per year. Information on the age, underlying cause of intestinal failure, type of transplant (isolated bowel, combined liver and bowel transplant, multivisceral transplant), type of immunosuppression used, autonomy from PN, major complications resulting in graft removal, and survival are collected and published regularly (see Tables 40.2-4 and 40.2-5).

According to the 2001 report from the Intestinal Transplant Registry, three-fifths of all intestinal transplant candidates received combined grafts of liver and bowel (of which 13.6% were multivisceral grafts).[26] The overall graft survival is 50% at 3 years post-transplant, although survival is significantly better in centers that have experience of carrying out at least 10 transplants (Figures 40.2-4 and 40.2-5). A number of factors that influence survival have been identified by the transplant registry and in large series in single centers (Table 40.2-6).

The survival curves from the Intestinal Transplant Registry[26] show that the early mortality occurs in the first 6 weeks and is around 20%. This is related to the poor preoperative condition of the patients who develop multiorgan failure while still in the intensive care unit. The multiorgan failure is often triggered by a systemic inflammatory response to bacteremias and viral pathogens such as respiratory syncytial virus. By 6 weeks postoperatively, most patients have stabilized, and 70 to 80% of the original cohort of transplant recipients are discharged at this point free of PN but on high levels of immunosuppression. Over the next 6 to 18 months, there is a gradual reduction in survival caused by infection (56.8% of all deaths are predominantly caused by infection). The current worldwide overall survival rate of adults and children in 55 centers is 335 patients of 651 transplanted patients.[26]

TABLE 40.2-4 DEMOGRAPHIC DETAILS OF TRANSPLANT RECIPIENTS WORLDWIDE TAKEN FROM INTESTINAL TRANSPLANT REGISTRY RECORDS UP TO MID-2001

PARAMETER	DETAIL	% (*n*)
Age of recipients (yr)	0–1	12
	1–13	46
	13–16	2
	16–55	40
Underlying cause of intestinal failure in children	Short gut	62
	Pseudo-obstruction	18
	Mucosal disorder	10
	Other	10
Status at time of transplant (adult and children)	At home	47.9
	Hospitalized	52.1
Type of transplant in children	Isolated small bowel	41.8 (151)
	Combined liver and small bowel	44.5 (243)
	Multivisceral	13.6 (32)

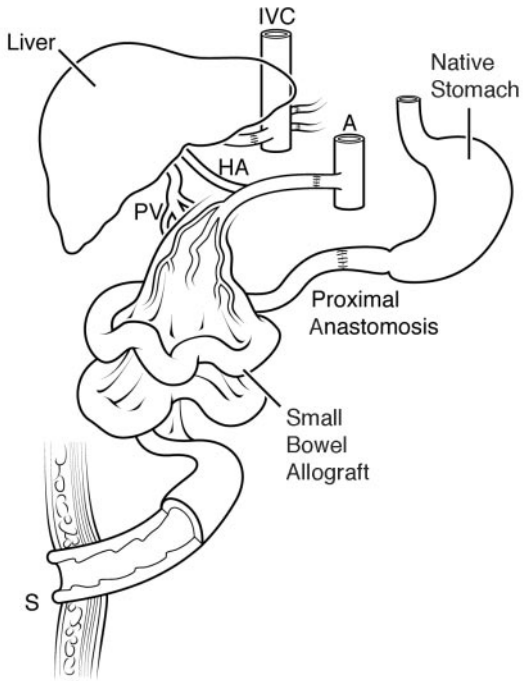


FIGURE 40.2-2 Combined liver bowel en bloc diagram. A = aorta; HA = hepatic artery; IVC = inferior vena cava; PV = portal vein; S = stoma.

COMPLICATIONS

TECHNICAL

These correlate with the complexity of the surgery and tend to occur in the first 10 days postoperatively. They include perforation of native intestine and/or small bowel allograft, prolonged ileus, biliary obstruction or leaks, traumatic pancreatitis, and hemorrhage (see Chapter 40.3, "Aspects of Surgery"). The signs of intra-abdominal pathology may be subtle, being masked by the effects of high-dose steroids, so there is a low threshold for second-look laparotomy.

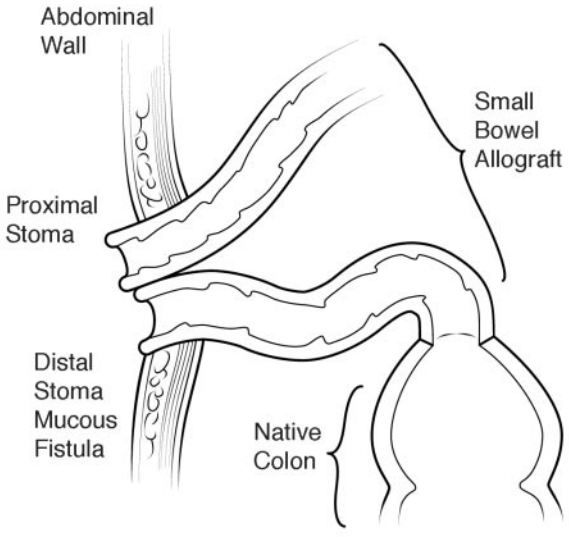
Chylous ascites as a result of interruption to lacteals may also develop if feeds high in long-chain fats are administered in the first few weeks postoperatively.

INFECTIONS

Common Bacterial and Viral Infections. There is an increased risk of all infections, including common respiratory pathogens such as *Streptococcus pneumoniae*, respiratory syncytial virus, and parainfluenza virus.[30] These require aggressive treatment, including antibiotics, ribavirin, and respiratory support such as ventilation, depending on the diagnosis and severity of infection. *Pseudomonas* has also been observed and may be difficult to detect without bronchoalveolar lavage. Adenovirus is also associated with severe infection affecting respiratory and gastrointestinal tracts; diagnosis may be difficult because the symptoms and pathology of the intestine may mimic rejection (diarrhea and apoptosis).[31,32]

Opportunistic Infections. Less common but of particular note in transplant recipients are opportunistic infections with *Pneumocystis carinii* and vancomycin-resistant enterococcus (VRE). *Pnemocystis* may be prevented by continuing treatment with cotrimoxazole until immunosuppression is reduced (in practice, usually at least for 1 year postoperatively). VRE is a low-virulence pathogen analogous to methicillin-resistant *Staphylococcus aureus* and is often present in the bowel of healthy individuals. However, when VRE colonizes the biliary tree or infects the abdominal cavity in immunosuppressed patients, the infection may be life threatening, and early treatment with the new class of antibiotic, quinupristin-dalfopristin, is recommended.[33]

Herpesviruses. One of the major groups of infectious agents relevant to pediatric intestinal transplant recipients is the herpesviruses, in particular cytomegalovirus (CMV) and Epstein-Barr virus (EBV). CMV is the cause of major sys-



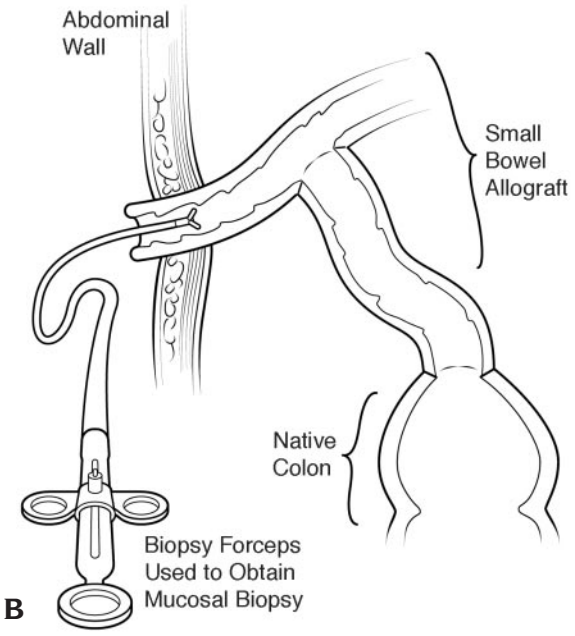


FIGURE 40.2-3 Stoma types and biopsy. *A*, Simple loop ileostomy. *B*, Bishop-Koop stoma.

TABLE 40.2-5 PRINCIPAL REASONS FOR GRAFT REMOVAL AFTER SMALL BOWEL TRANSPLANT IN CHILDREN

REASON	GRAFT REMOVAL (%)
Rejection	65.3
Thrombosis	15.3
Sepsis	4.2
Lymphoma	2.7
Other	12.5

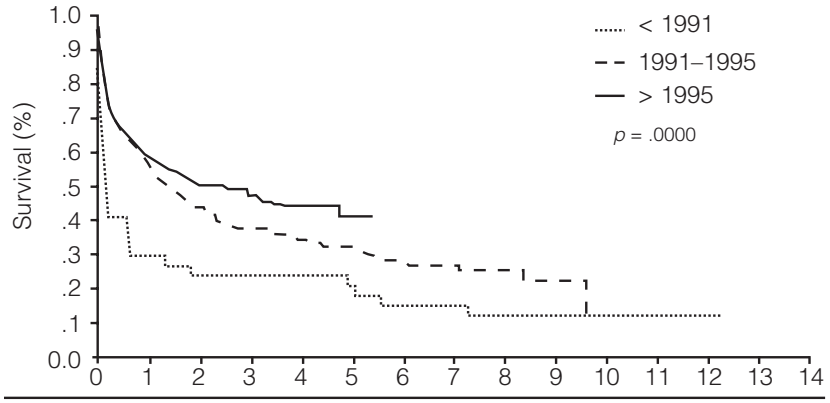
Adapted from the Intestinal Transplant Registry.[26]

temic illness, including fever, rash (Figure 40.2-6), gastrointestinal bleeding, and small bowel perforation, and transplant teams generally avoid using CMV-positive donors. Prophylaxis (ganciclovir and hyperimmunoglobulin) against CMV infection is usually given even in apparently low-risk patients for CMV because the consequences of infection are so severe. EBV appears to be more severe if it is acquired as a novel infection within months of transplant, which means that children are at greatest risk of developing the lymphomas and hematologic malignancies that are associated with this virus.[34] The use of tacrolimus, which selectively inhibits cytotoxic T lymphocytes as the principal immunosuppressant, may aggravate the progression from glandular fever to post-transplant lymphoproliferative disease (PTLD).

PTLD. Depending on the presence of risk factors (young age, no previous immunity to EBV, dependence on tacrolimus-based immunosuppression, EBV-positive donors), the incidence of PTLD ranges from 10 to 50% in small bowel transplant recipients.[34,35] In some cases, the virus is probably transplanted with the donor organs and infects the naive recipient, especially when older donors have been used. Infection with EBV is extremely common in the population, and it would be difficult to exclude donors who are EBV positive without increasing the waiting list mortality of transplant candidates. Thus, the main tactics used in reducing PTLD are early detection, stepwise reduction of immunosuppression, especially tacrolimus, and adjuvant treatment if the tumor fails to regress. For example, selective B-cell lysis (rituximab) or EBV-specific cytotoxic T cells harvested from healthy blood donors and partially human leukocyte antigen (HLA) matched to the small bowel transplant recipient have been used with success.[36,37] Some transplant centers have used chemotherapy (cyclophosphamide, prednisolone, doxorubicin, and vincristine). Even with this variety of treatments, the mortality after the onset of PTLD is high at around 50% of patients with established PTLD, especially in children.

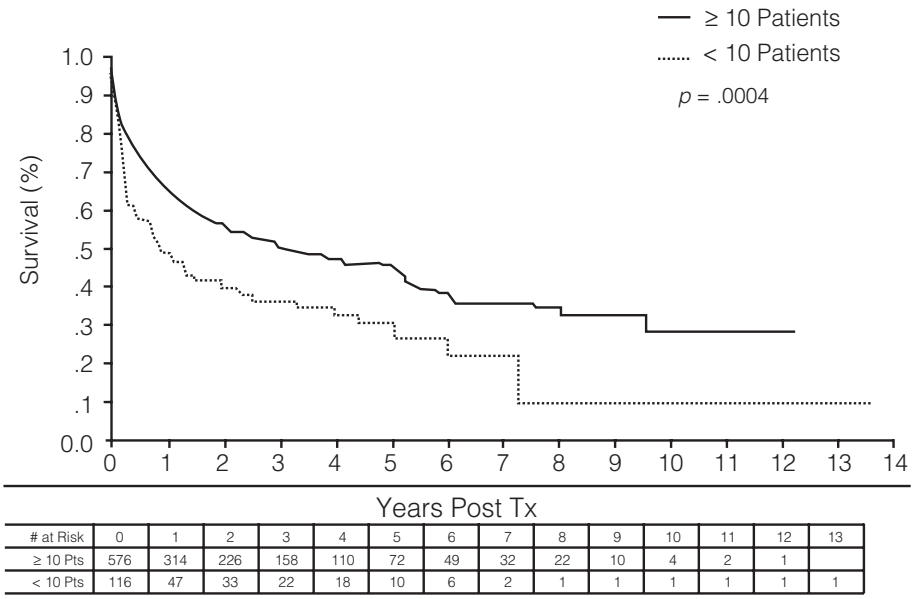
REJECTION

Acute Cellular Rejection. Acute cellular rejection of the small bowel allograft is common and is the major cause of graft loss but not death because it is often possible to remove the small bowel graft and recommence PN (see Table 40.2-5). Clinical signs of rejection of the small bowel allograft (malaise, fever, increased stomal output, or diarrhea) occur most frequently at 7 to 14 days postoperatively, although the histologic process will have started 1 to 2 days earlier. Specific laboratory markers for rejection, such as tissue enzymes, are lacking in small bowel transplant, and reliance is made on serial histologic assessments of mucosal biopsies taken in context with clinical features. The histologic signs of rejection in the small bowel mucosal biopsy have been graded into four levels of severity depending on the degree of apoptosis, destruction of crypts, and inflammation of the lamina propria (Table 40.2-7).[38–40] Rejection may progress rapidly from mild systemic upset and a moderate increase in stomal output by about 50% to a severe secretory diarrhea associated with sloughing of the mucosa and gram-negative bacteremia, all within 1 week. Prompt recognition and treatment with a bolus of high-dose intravenous steroid (eg, methylprednisolone 20–50 mg/kg) and broad-spectrum antibiotics, followed by a general increase in tacrolimus and other agents, such as mycophenolate or sirolimus, are usually effective in restoring normal graft function.[41]



# at Risk	0	1	2	3	4	5	6	7	8	9	10	11	12	13
> 1995	34	10	8	8	8	7	5	5	4	4	4	1	1	
1991–95	167	94	73	62	57	54	41	24	14	3				
< 1995	491	212	137	84	42	5								

FIGURE 40.2-4 Graph showing graft survival 3 years after small bowel transplant. Adapted from the Intestinal Transplant Registry.[26]



# at Risk	0	1	2	3	4	5	6	7	8	9	10	11	12	13
≥ 10 Pts	576	314	226	158	110	72	49	32	22	10	4	2	1	
< 10 Pts	116	47	33	22	18	10	6	2	1	1	1	1	1	1

FIGURE 40.2-5 Graph showing patient survival in centers with experience of 10 small bowel transplants compared with less experienced centers. Adapted from the Intestinal Transplant Registry.[26]

Centrilobular Rejection in Liver. In children who have received a combined liver and bowel transplant, acute cellular rejection of the liver allograft independently of the small bowel allograft is unusual. However, with increasing experience, some transplant groups have observed centrilobular changes rather than the more usual portal tract inflammation occurring months or years after the transplant. There is often a history of only minor changes in liver enzymes. The centrilobular changes usually respond to increases in immunosuppression and are thought to represent a chronic rejection process in the liver modified by cytokines emanating from the intestinal allograft.[42]

Chronic Rejection of Small Bowel Allograft. Chronic rejection of the small bowel is poorly understood, but it appears to be a vascular phenomenon starting in the mesenteric vessels that become inflamed and impede blood flow. There is little to observe in the mucosa of the allograft, although the patient will tend toward diarrhea alternating with episodes of obstruction. This pattern of symptoms has been labeled "distal ileal obstruction syndrome"[43] and may require resection of the affected bowel if recognized, but some patients go on to complete graft failure. There are no convenient noninvasive means of diagnosing chronic rejection, but selective angiography of the celiac axis and biopsy of mesenteric vessels at the time of laparotomy have been used.

Drug Toxicity

Compared with liver transplant, approximately twice as much immunosuppression is needed to achieve tolerance of the small bowel allograft. Inevitably, adverse effects occur, the most frequent being hypertension, bone marrow toxicity, and nephrotoxicity (Table 40.2-8). The majority of patients require antihypertensive medications such as nifedipine, and many become neutropenic in the first 6 months postoperatively. These effects are mainly related to tacrolimus, although the neutropenia may be induced by other drugs, including azathioprine, mycophenolate mofetil, sirolimus, cotrimoxazole, and ranitidine. Tacrolimus induces upregulation of endothelin receptors, producing constriction in arterioles in the systemic circulation and in the kidneys, and this probably explains the hypertension and the long-term effects on glomerular filtration rates. In an attempt to

TABLE 40.2-6 FACTORS ASSOCIATED WITH REDUCED REJECTION RATES AND/OR IMPROVED SURVIVAL AFTER SMALL BOWEL TRANSPLANT

	FACTOR	*p* VALUE	REFERENCE
Associated with reduced rejection (no effect on 12-mo survival)			
Type of immunosuppression	Tacrolimus and sirolimus	.002	52
	Exposure to anti–interleukin-2 receptor antibodies	.02	49
Associated with improved survival			
Experienced transplant center	More than 10 intestinal transplants performed	.0004	26
Age of recipient	Older than 3 yr	.0288	30
Liver function preoperatively	Absence of early liver failure (before 24 mo age)	.0161	30
Preoperative status	At home (compared with being hospitalized)	.011	30

Adapted from Intestinal Transplant Registry,[26] Kato T et al,[30] Sudan D et al,[49] and Fishbein TM et al.[52]

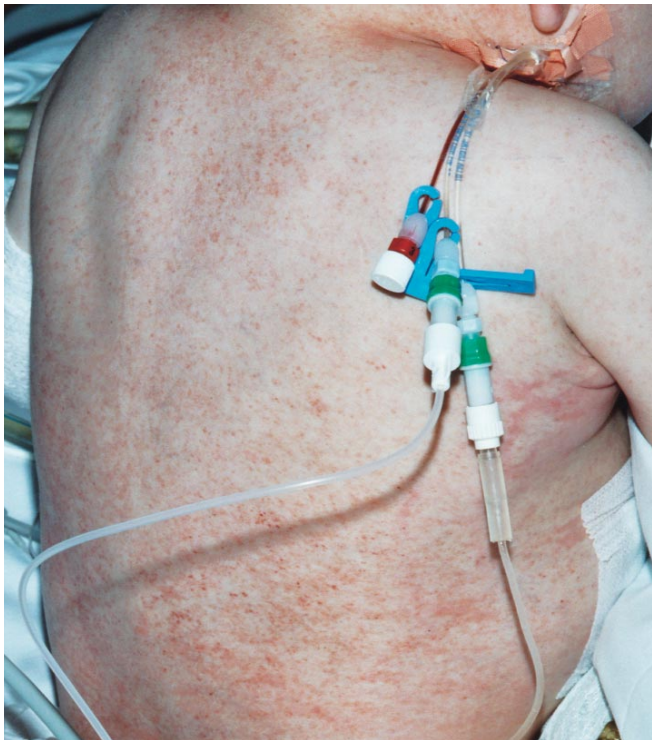


FIGURE 40.2-6 Photograph of cytomegalovirus rash.

reduce the effects of tacrolimus-induced nephrotoxicity, it has become common practice to use prostaglandin infusions for 5 to 10 days immediately after small bowel transplant.[44] Apart from the effects on renal vasculature, tacrolimus also affects the tubular handling of magnesium, resulting in the loss of magnesium in urine and low magnesium levels in blood. Low magnesium may contribute to another important side effect of tacrolimus, neurotoxicity, which is manifested by tremor and even convulsions. Patients may also complain of limb pain, the so-called reflex sympathetic dystrophy. Less common in children is the onset of insulin-dependent diabetes mellitus caused by tacrolimus, which may develop shortly after the transplant or some years later. Apart from diabetes mellitus, which seems to be an idiosyncratic response in children, all of these effects of tacrolimus are dose related and have prompted the use of alternative drugs such as sirolimus and mycophenolate mofetil, which are not neurotoxic or nephrotoxic.

Steroids are also used at higher doses and for a longer time than in other solid organ transplants and can result in impaired linear growth, especially in the first 6 months, after which growth velocity seems to improve again as steroids are reduced.

INNOVATIONS IN SMALL BOWEL TRANSPLANT

In addition to the innovations in surgical technique described above, the intensity of rejection provoked by the small bowel allograft has driven the search for alternative ways to induce tolerance.

Bone Marrow Infusions

The two observations that repeated blood transfusion in renal allograft recipients reduced rejection rates and that long-term partly tolerant recipients of liver transplants have a chimera of immune cells derived from the donor liver led to the hypothesis that more exposure to donor immune cells would further enhance tolerance. To test this idea, bone marrow from the same small bowel transplant donor was harvested at the same time and routinely infused during reimplantation. The short- and medium-term results have not produced the hoped for tolerance, although long-term results on tolerance at 5 to 10 years after transplant are not yet available.[45]

Conditioning Regimen

The experience of bone marrow transplant has been applied to the small bowel transplant field, and the relatively mild conditioning agent alemtuzumab (Campath-1H, Berlex Laboratories, Montville, NJ) has been used with some initial success. The results from one large center indicate an 80% 12-month survival rate, but no long-term results are yet available.[46] There are theoretic concerns about opportunistic infections, particularly in young recipients, but this manipulation of the immune system may prove to be important if the medium-term results match those of the first reports.[30,47]

CD25 Blockade

The humanized monoclonal antibodies basiliximab and daclizumab bind specifically with the CD25 receptor car-

TABLE 40.2-7 SMALL BOWEL ALLOGRAFT: GRADES OF ACUTE CELLULAR REJECTION

GRADE	MUCOSAL BIOPSY HISTOLOGY	CLINICAL FEATURES
1 Mild	2–5 apoptotic bodies per 10 crypts, a few inflammatory cells scattered in lamina propria	Increase in bowel frequency or ileostomy output may go up by 50%
2 Moderate	5–10 apoptotic bodies per 10 crypts, infiltration of crypts by lymphocytes, obvious increase in inflammatory cells in lamina propria	Secretory diarrhea, malaise, low-grade fever
3 Severe	Greater than 10 apoptotic bodies per 10 crypts, some loss of villi, destruction of crypts in places, ulceration may be seen, numerous inflammatory cells in lamina propria	Secretory diarrhea associated with thirst and dehydration, protein-losing enteropathy, lassitude, fever ± peripheral edema, gram-negative septicemia
4 Exfoliative	Extensive ulceration, lamina propria replaced by inflammatory cells, complete loss of crypts in places	Prostration, fever, gastrointestinal bleeding, systemic inflammatory response syndrome

Adapted from Lee RG et al,[38] Roberts CA et al,[39] and White FV et al.[40]

TABLE 40.2-8 IMPORTANT TOXIC EFFECTS OF IMMUNOSUPPRESSANT DRUGS COMMONLY USED AFTER SMALL BOWEL TRANSPLANT

DRUG	TOXIC EFFECTS
Tacrolimus (Prograf, Fujisawa Benelux, Houten, The Netherlands)	Vasoconstriction, hypertension, reduced glomerular filtration, elevated urea and creatinine, hypomagnesemia, tremor and convulsions, impaired glucose tolerance, neutropenia, reflex sympathetic dystrophy, hypertrophic cardiomyopathy
Sirolimus (Rapamune, Wyeth Laboratories, Madison, NJ, USA)	Generalized suppression of bone marrow, thrombocytopenia, elevated serum triglyceride and cholesterol, increased incidence of thrombosis,* impaired wound healing*
Mycophenolate mofetil (CellCept, Roche, Basel, Switzerland)	Diarrhea, vomiting, gastritis and gastrointestinal bleeding, neutropenia, generalized suppression of bone marrow
Azathioprine (Imuran, GlaxoWellcome, Uxbridge, UK)	Agranulocytosis, generalized suppression of bone marrow, myalgia, arthralgia, hepatotoxicity
Basiliximab/daclizumab (Simulect, Novartis/Zenapax Roche)	Fever, lymphopenia, pulmonary edema, anaphylaxis
Steroids, ie, prednisolone, methylprednisolone, hydrocortisone	Sodium and water retention, hypertension, impaired glucose tolerance, impaired bone mineralization, impaired linear growth, muscle wasting, risk of hypotensive collapse if sudden withdrawal, impaired wound healing

*It is not clear if there is a causal link between these events and the drug.

ried on T lymphocytes. The CD25 receptor normally recognizes interleukin-2, a lymphokine released by cytotoxic T cells, which amplifies the rejection process. These monoclonal antibodies have been widely used in renal and liver transplant and have a half-life of 4 to 8 weeks.[48] They are used to reduce the severity of rejection episodes at a time when the patient is most vulnerable, which is a useful outcome particularly in small bowel transplant, although it does not appear to reduce the length of stay.[49]

Sirolimus

Sirolimus has been used predominantly in renal transplant since 1990. It acts on the T-cell receptor of rapamycin in immune cells, inhibiting the cell cycle at G_1, resulting in an antiproliferative effect that is most pronounced in lymphocytes but can extend to other hematologic cell lines (megakaryocytes, granulocytes).[50] This is potentially beneficial in patients at risk of PTLD. Because sirolimus acts on a different point from the cell cycle (G_1) compared with the calcineurin inhibitors, which act at G_0 (G_0 is the resting phase), the two agents can be used in combination, although this appears to be a very potent combination.[51] Early reports from some transplant centers suggest that rejection after small bowel transplant is significantly reduced in those patients receiving sirolimus, but this may just be a cohort effect.[52]

Ex Vivo Irradiation

This is a radical idea designed to remove mature T and B cells from the allograft by irradiation after harvesting and before reimplantation and repopulating the graft by infusing stem cells and other precursors from the donor's marrow. The irradiation also reduces the bacterial contamination of the graft, but access to radiation sources at a time convenient to the operative procedure while maintaining the aseptic condition of the organ has posed practical difficulties, and it is too early to know if this kind of immunomodulation will induce tolerance and better outcomes for small bowel transplant.[10,53]

None of these treatments have become established practice, but in the next few years, it is possible that one or more may produce a genuinely positive improvement in the long-term results of small bowel transplant.

POSTOPERATIVE GRAFT FUNCTION AND DIETETIC MANAGEMENT

Oral fluids are introduced as soon as the ileus resolves, and if no major complications have occurred, enteral feeding will have been introduced from day 7. Typically, the allograft switches from a state of ileus to secretory diarrhea around day 5,[54] and the stoma becomes very productive, with as much as 500 mL/kg/d of stomal output in extreme cases. The differential diagnosis for the secretory state at this point includes recovery from the effects of preservation procedures during harvesting and reimplantation, sepsis, and rejection. Provided that rejection and sepsis are accurately diagnosed and treated if present, the secretory diarrhea lessens such that by day 14 to 21, most patients are in balance with oral intake and stomal output.

Different transplant teams have varying approaches to establishing enteral nutrition, but the common theme is avoidance of long-chain fats in the first 6 weeks. Many teams also use a hydrolyzed protein source. At Birmingham Children's Hospital, we use a modular feed as a means of choosing individual components such as glucose polymer, which incorporates calories without increasing the osmolality of the feed excessively. The feed is introduced as relatively calorie dense (0.8 kcal/mL) but low volume (eg, 5 mL/h) and is increased stepwise over 21 days so that full enteral sufficiency is achieved by day 30 postoperatively, and PN is discontinued.[55] Previously, we have used hydrolyzed whey protein[56] but now use a whole-protein source, with no increase in allergy or intolerance (Table 40.2-9).

TABLE 40.2-9 DIETETIC MANAGEMENT AFTER SMALL BOWEL TRANSPLANT

POSTOPERATIVE DAY	FEED COMPONENTS	COMMENT
0–7	Balanced glucose and salt solution, eg, Diorolyte or Rehidrat*	Sips of sterile water allowed
7–30	Start 5 mL/h modular feed containing whole protein (eg, Maxipro†), medium-chain triglyceride (eg, Liquigen†), and glucose polymer (eg, Maxijul†) made up to produce a calorie density of about 0.8 kcal/mL L-Glutamine† added (0.25% of total protein) and pectin to thicken the feed	Volume is increased as rapidly as patient will tolerate; patient is encouraged to take feed by bottle or cup as well as by nasogastric tube
30–90	A small amount of long-chain fat (Calogen†) is allowed (up to 25% of total lipid), adjustments made to carbohydrate content, glutamine discontinued, total volume between 500 and 1,200 mL/d	Low-fat diet is encouraged; discharge advice about avoidance of live yogurts, sugary "pops," and tap water, which may contain cryptosporidia
90–onward	Modular feed discontinued in favor of a normal standard feed with whole protein and long-chain fat, eg, Nutremi‡	Not all patients need feed supplements unless prone to dehydration or averse to eating food

*Searle, High Wycomb, Bucks, UK.
†Scientific Hospital Supplies, Liverpool, UK.
‡Nutricia Clinical, Wiltshire, UK.

The motility of the small bowel allograft depends on the intrinsic nervous system and is sensitive to local factors such as nutrient content and luminal distention rather than autonomic nervous control.[57] This means that there is no vagally mediated postprandial suppression of motility, and drugs such as loperamide (50–200 μg/kg/dose) are needed to slow transit. Care must also be taken with the carbohydrate content of the feed, which, if excessive, can induce an osmotic diarrhea that could be mistaken for rejection.

Improving allograft function allows modification to the dietetic support with time, such that by 6 months postoperatively, most patients are on a normal family diet supplemented by nutritionally complete calorie-rich drinks that can be taken by cup or beaker or by nasogastric feed in children with an aversion to eating. Most patients seem to transfer from PN to enteral feeding with no loss in growth velocity provided that the small bowel allograft is functioning well.[55,58]

LONG-TERM ISSUES AND SMALL BOWEL TRANSPLANT

Rehabilitation

Successful rehabilitation depends on a multidisciplinary team, which, ideally, should be composed of individuals with the following skills: play therapy, teaching school-age children while they are in hospital, physiotherapy, dietetics, stoma care, and liaison work. In the United Kingdom, specialist nurses increasingly take on a key coordinating role to ensure that local community teams, including the family doctor and schoolteachers (for older children), are aware of the child's needs after transplant and that supplies of immunosuppression (eg, stoma bags) are arranged before discharge. Depending on the extent of provision of community facilities, physiotherapy and speech therapy initiated in hospital can be continued at home. This is important because many children who are recipients of small bowel transplants have had a life of emergency hospitalizations, poor experience of eating, and restrictions to their physical and psychological development. Integration in mainstream schooling is possible, but susceptibility to infection, especially varicella-zoster, rotavirus, and adenovirus, remains high.

Monitoring

The aim of monitoring is to detect and treat complications at an early stage. The principal complications are rejection, infection, and drug toxicity, so the monitoring regimen revolves around blood tests, assessment of small bowel function by measuring growth, and recording significant symptoms such as diarrhea or increases in the volume stomal output, mucosal biopsy, measurement of blood pressure, and screening for the onset of PTLD, which may include endoscopy and detailed imaging such as computed tomography or magnetic resonance imaging (Table 40.2-10). The blood tests are performed once per month, when the patient is stable (from about 6 months postoperatively), and mucosal biopsy at the same time as the screening test for PTLD is done every 3 to 6 months depending on the patient's risk factors and symptoms.

QUALITY OF LIFE

Although the long-term survival is not yet known, it is encouraging to note that quality of life in the medium term seems to improve. It is difficult to judge the quality of life in young children,[59,60] but some attempts have been made to do so either by objective evaluations of hospital attendance, the number and use of appliances such as indwelling central lines and stoma bags,[61] or a semistructured questionnaire using instruments such as the General Health Questionnaire.[62] Children and families who are recipients of small bowel transplant do not have a normal quality of life, and it is clear that many of them have adapted to their predicament by developing obsessive or neurotic traits, but their scores are similar or superior in some cases to those of children on home PN.

TABLE 40.2-10 MONITORING TESTS GROUPED ACCORDING TO COMPLICATION

TEST	REJECTION	INFECTION	TOXICITY
Clinical	Weight loss, fever, lassitude, loss of appetite, stomal output or stool frequency doubles or triples	Fever, obtundation, stomal output or stool frequency increases but not usually as much as in rejection, enlarged painful lymph nodes	Hypertension, tremor, recurrent infections, glucose intolerance, bruising (thrombocytopenia)
Blood tests	Nil specific Urea and creatinine often increased Low levels of tacrolimus (and sirolimus) may be noted	Blood culture EBV and CMV PCR Pattern of lymphocyte subsets to detect over suppression of immune system	Tacrolimus (± sirolimus) levels Magnesium Urea and creatinine, full blood count Cholesterol triglyceride
Histology	Mucosal biopsy may show apoptosis, infiltration of crypts by lymphocytes, increase in inflammatory cells in lamina propria, ulceration; low-grade rejection may be detected despite mimimal symptoms; allows for modification of immunosuppression	Adenovirus and other enteric infections may also induce apoptosis but regenerative features common as well In situ hybridization can detect worsening EBV infection; allows for modification of immunosuppression	Gastric erosions may be seen with high levels of immunosuppression, also malignant transformation of lymphoid tissue by EBV
Other monitoring tests	Liver biopsy, endoscopically aided mucosal biopsy (with zoom facility)	Stool and urine culture, throat swab Culture of internal sites such as liver tissue, ascitic fluid, thoracic or abdominal fluid collections, cerebrospinal fluid, bronchoalveolar lavage Imaging to evaluate possible malignancy (CT or MRI)	Bone marrow aspirate, bone density studies, upper and lower intestinal endoscopy

CMV = cytomegalovirus; CT = computed tomography; EBV = Epstein-Barr virus; MRI = magnetic resonance imaging; PCR = polymerase chain reaction.

COST-EFFECTIVENESS

The relatively small numbers distributed across many transplant centers have made it difficult to produce cost-effectiveness studies of the robustness of the ones published for renal replacement therapy. However, it is noteworthy that medical insurance companies in the United States will now fund small bowel transplant. In the United Kingdom, a small study following the outcome of 50 children referred for small bowel transplant is under way, and although it is too early to report the outcome of this, it is clear that the cost of managing intestinal failure with or without transplant is much higher than previously calculated (M. Buxton, personal communication, 2003).

SUMMARY AND CONCLUSION

In the past 10 to 15 years, there have been major advances in the surgery of small bowel transplant and management of rejection and specific opportunistic infections. Small bowel transplant has become an established treatment for chronic intestinal failure in children who cannot remain on intravenous feeding long term because of progressive liver disease, pulmonary disease from recurrent feeding catheter embolization, and/or impaired venous access.[15,63] There has been a steady increase in the number of transplants performed annually worldwide to around 100, of which approximately 65 are in children, the majority of whom have a history of short gut and liver failure.[26] The current survival at 3 years postoperatively is around 50%, although the results in the larger centers are better, and this may improve further with the availability of new approaches to immunomodulation.[11,30] Quality of life and cost-effectiveness studies in countries with well-financed health care systems suggest that this treatment will continue to be of benefit to children who are experiencing complications related to the long-term administration of intravenous feeding solutions.

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3. Aspects of Surgery

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Short-bowel syndrome (SBS) is the spectrum of malabsorption that occurs after resection of a major portion of the small intestine for congenital or acquired lesions.[1,2] A useful definition is based on the need for intervention. Patients requiring total parenteral nutrition (TPN) support for more than 1 to 3 months after major resection can be defined as having SBS.[3] Other definitions are based on residual bowel length. For instance, small bowel resection greater than 75% of small bowel length is considered SBS.[1] Variation in the definition between institutions produces differences in outcome between published series. The Canadian Association of Paediatric Surgeons defines SBS as the need for TPN greater than 42 days after bowel resection or a residual small bowel length of less than 25% expected for gestational age.[4] This is the definition used at The Hospital for Sick Children in Toronto. Unfortunately, patient characteristics such as gestational age, region of bowel resected, functional capacity of the remaining intestine, and the presence or absence of the ileocecal valve all contribute to the difficulty in determining the critical length of bowel required to avoid SBS.[5]

The most common causes of pediatric SBS are neonatal conditions such as necrotizing enterocolitis, extensive aganglionosis, intestinal atresia, midgut volvulus, and abdominal wall defects.[3,6] In older children and teenagers, Crohn disease and trauma are more common causes.[7] Accurate estimates of incidence and outcome in children with SBS remain difficult owing to variation in the definition of SBS between institutions, difficulty of tertiary care referral centers to accurately determine their catchment population, and problems ensuring complete follow-up of the whole cohort. A recent population-based study at our institution determined the incidence of neonatal SBS to be 24.5 per 100,000 live births, with a much higher incidence in babies born before 37 weeks gestation compared with term newborns (353.7 in 100,000 live births vs 3.5 in 100,000 live births).[8]

The major features of SBS are dehydration secondary to diarrhea, malabsorption of macro- and micronutrients, malnutrition, and failure to thrive.[3,5] After intestinal resection, the residual small bowel undergoes intestinal *adaptation*, which is the gut's attempt to optimize its absorptive capacity. The adaptation process may take several months or years to complete. During that time, the infant is either partially or totally dependent on parenteral nutrition. A multitude of complications ensue with long-term hospitalization and prolonged parenteral nutrition, including central line complications, multiple systemic infections, cholestasis, liver failure, failure to thrive, and the resultant effects on family dynamics.[2,6]

The advances in medical management of SBS over the last 30 years, most importantly TPN, have led to improved survival.[9,10] Parenteral nutrition permits survival after massive small bowel resection and provides the patient with time and opportunity to achieve intestinal adaptation. Spontaneous adaptation occurs in approximately 75% of SBS patients and allows them to be weaned from TPN.[2] Reasons for continued TPN dependency include bowel dysmotility, bacterial overgrowth, insufficient adaptation, and very short bowel length. These patients may benefit from surgical procedures that improve intestinal function to optimize adaptation and increase the absorptive surface area.[11] This chapter summarizes the surgical techniques available to the surgeon who is treating this complicated group of patients.

ROLE OF SURGERY IN SBS

The surgeon has an important role in the natural history of all patients with SBS. The surgeon is present at the beginning of every case in which their perioperative and operative decisions have a direct impact on whether a patient will develop SBS. Often they are making the best of a poor situation. The surgeon is also intimately involved during the subsequent adaptation process to perform adjunctive operative procedures, as necessary, to optimize a patient's chance for TPN independence. Ultimately, if adaptation is insufficient and the patient develops end-stage liver disease, the surgeon will perform an intestinal or liver transplant.

Initial Resection

Primary prevention of SBS should be a high priority. Thompson outlined how it may be possible to avoid extensive bowel resection if surgeons intervene early in cases of intestinal ischemia, mesenteric emboli or clots, and complete bowel obstruction.[12] Intraoperative determination of intestinal viability and the use of second-look laparotomies can potentially preserve what looked like bowel of questionable viability. A conservative attitude regarding resection in Crohn disease and the use of strictureplasties may minimize the chance of SBS in these patients.

One of the most difficult intraoperative decisions for a surgeon to make when faced with a patient who requires massive intestinal resection is whether to proceed with a resection that will result in SBS. In general, we have a fairly aggressive approach to resection, but the overall mortality rate of SBS remains high. The wishes of the family and the existence of comorbidities such as prematurity, bronchopulmonary dysplasia, or other structural anom-

alies are all important considerations that will influence one's decision.[12,13]

Once the decision is made to proceed with massive bowel resection, the goal should be to preserve all bowel length that is possible, including the ileocecal valve. The use of internal stents for patients with multiple intestinal atresias or patchy necrotizing enterocolitis requiring multiple resections is helpful.[13] It is important to document the length of residual small and large bowel remaining with the patient. Stomas are frequently required because of intra-abdominal contamination, inflammation, or hemodynamic instability. Placing the end stoma beside the mucous fistula, at the same stoma site, is a simple way of making subsequent stoma closure more straightforward. Bowel continuity can be re-established without the need for a full laparotomy and lysis of adhesions. In my experience, patients tolerate this approach better and have faster resolution of their ileus. The early treatment of SBS is focused on the acute surgical emergency. Adjunctive SBS procedures should not be performed at the time of the initial resection. Intestinal adaptation will often be adequate enough to preclude the need for surgical therapy.[12]

Reoperation

During the adaptation phase, surgical intervention is reserved for the management of significant surgical complications or to provide central venous or enteral access (eg, gastrostomy) to allow adaptation to continue.[14]

It is difficult to know the precise point at which adjunctive surgical procedures should be performed in SBS patients. Patients who plateau and fail to make progress with TPN weaning should be considered for surgery. Also, patients who develop complications such as TPN cholestasis, recurrent line sepsis, or bacterial overgrowth may be candidates. Three factors guide the decision of which operation to perform: the underlying intestinal function, the length of the residual bowel, and the caliber of the intestinal remnant.[14]

Before surgery occurs, patients require a complete assessment of their general medical condition and comorbid diseases. It is important to know if the patient has end-stage liver disease because this would be a contraindication for surgery. A referral for intestinal and liver transplant may be more appropriate. All previous operative notes should be reviewed, and a small bowel follow-through should be obtained to assess motility, bowel length, and caliber.

At the time of the adjunctive surgical procedure, a liver biopsy should be performed to determine the extent of TPN cholestasis. Prophylactic cholecystectomy should also be considered at the time of reoperation. Patients with a history of ileal resection and long-term parenteral nutrition are at risk for cholelithiasis. Twenty percent of infants on long-term parenteral nutrition develop gallstones secondary to lack of enteral feeding and gallbladder stasis.[15] Patients with SBS are three times more likely to develop gallstones because the loss of bile salts from ileal resection permits cholesterol in bile to precipitate.[16] The incidence of complications from cholelithiasis is not known in children; however, it is probably prudent that patients with cholelithiasis at the time of reoperation have a cholecystectomy because it can usually be performed quickly and safely.[17]

The primary objective of surgery for SBS is to improve intestinal function, optimize bowel motility, and increase the mucosal absorptive surface area.

TECHNIQUES TO IMPROVE INTESTINAL FUNCTION

Lysis of Adhesions, Strictureplasty, or Segmental Intestinal Resection

Children with a history of gastrointestinal pathology are at risk of developing mechanical bowel obstruction. The etiology may be stenosis secondary to the late effects of bowel ischemia or inflammation, as seen in patients with necrotizing enterocolitis or Crohn disease. In addition, previous intra-abdominal surgery or inflammation produces adhesions that may be the source of obstruction. Bowel obstruction causes proximal bowel dilatation, dysmotility, and bacterial overgrowth. Dilated bowel from primary or secondary dysmotility conditions, such as adaptation with bacterial overgrowth, can be very difficult to distinguish from mechanically obstructed bowel with proximal dilatation. Mechanical obstruction may be corrected by lysis of adhesive bands, strictureplasty if bowel length is short, or segmental resection for patients with adequate bowel length.[18]

Re-establishing Intestinal Continuity

Diverting stomas are often necessary at the time of laparotomy for children suffering acute, life-threatening conditions. The presence of peritoneal contamination, extensive inflammation, hemodynamic instability, or other systemic factors that might affect anastomotic healing (eg, malnutrition or immunosuppression) often preclude the desire to perform a primary anastomosis.

There are several advantages to reversing stomas and re-establishing gastrointestinal continuity. The primary benefit of stoma closure is that it increases the mucosal surface area available for nutrient absorption. Nutrient absorption is improved because the longer intestinal length increases transit time and permits nutrients more time for contact with the absorptive mucosa. If the colon is present, the patient benefits because the colon is the major site of water reabsorption and therefore affects the volume of stool output. It also enhances carbohydrate absorption because bacteria ferment unabsorbed carbohydrates into short-chain fatty acids, acetate, butyrate, and proprionate, which are then absorbed by colonocytes. These substances are then used as caloric substrate. This salvage mechanism may provide up to 5 to 10% of calories.[16]

Diverted intestine undergoes mucosal atrophy; therefore, re-establishing intestinal continuity facilitates the adaptive response in the distal bowel.[19] Multiple mechanisms and mediators have been proposed for the adaptation response, but no single factor adequately explains all of the changes that occur in intestinal structure and function. There is a role for luminal nutrients, gastrointestinal secretions, and humoral factors, and delivery of these substances to the previously diverted segment enhances adaptation.[20]

The main disadvantage to stoma closure is diarrhea and the resulting perineal irritation. Elevated stool volume and frequency result from inadequate absorption in the proximal bowel but also from colonic mucosal inflammation caused by the irritating effects of bile acid delivery to the large bowel, producing secretory diarrhea and steatorrhea.[11] Usually, aggressive application of barrier products to the perineum can protect the skin, but it is important to begin applying these immediately after surgery because it can be difficult, and more painful for the patient, to treat a rash that is already well established.

Patients with a colon in continuity are at risk of calcium oxalate nephrolithiasis. Oxalate is absorbed because the delivery of bile acids to the colon prevents oxalate excretion in the stool. The circulating oxalate can then precipitate with calcium and cause renal calculi.[3]

Determining the optimal time for stoma closure can be difficult. These patients often have significant comorbid conditions in addition to their gastrointestinal disorders. Predicting which patients will tolerate stoma closure without significant diarrhea and perineal complications is often not possible. Even though high stoma output often initially gets converted to large stool volumes, most patients usually benefit from the longer intestinal surface area. The goal should be to reverse stomas early once the patient is deemed able to tolerate it from a technical and physiologic perspective. Maximizing the percentage of calories received from the enteral route helps decrease the risk of parenteral nutrition–associated cholestasis.[21] Experience from the Intestinal Care Center in Pittsburgh suggests that patients who have had more than 30% of their colon resected and who have stoma output totaling greater than 40 cc/kg/d are more likely to suffer significant perineal complications.[14]

TECHNIQUES TO IMPROVE INTESTINAL MOTILITY

Intestinal Tapering and Plication

An increase in intestinal caliber is the normal physiologic response to extensive bowel resection. Bowel dilatation slows intestinal transit and increases mucosal absorptive area. At a point, however, this normal adaptive mechanism becomes pathologic. The dilated segments become dysmotile, and fecal stasis, bacterial overgrowth, and malabsorption ensue. Oral antibiotic therapy can help suppress overgrowth, but some patients develop refractory bacterial overgrowth and require surgical intervention.

Dysmotile segments of bowel must be distinguished from mechanically obstructed bowel with proximal dilatation. Mechanical obstruction can be corrected by release of adhesive bands, strictureplasty, or resection of the obstructing segment. Functionally dysmotile segments should be streamlined by tapering or plication to improve peristalsis and decrease bacterial overgrowth. Streamlining has the advantage of preserving bowel length compared with resection of dilated segments.[18]

Tapering enteroplasty involves partial resection of the antimesenteric border of the dilated segment to reduce the diameter of the intestinal loop (Figure 40.3-1). The blood supply arising from the mesenteric side is not disturbed. The disadvantage is that some absorptive surface area is lost, which is already limited in these patients. There is also a risk of postoperative leak from the suture line, but this is not significant. To ensure an adequate and uniform lumen, an appropriately sized Foley catheter or chest tube can be inserted into the bowel lumen through a small enterotomy at the beginning of the dilated segment. It can be gently held in position along the mesenteric border of the bowel with a Babcock clamp, and then the excess bowel can be excised with a stapler or free hand.

Plication also streamlines the bowel, but no bowel wall is resected. The dilated bowel wall is inverted into the lumen, and the serosal surfaces are imbricated (Figure 40.3-2). Therefore, mucosal surface area for absorption is maintained, and motility is improved.[22] Unfortunately, the inverted bowel may cause bowel obstruction because it blocks the bowel lumen. Also, the suture line may fail, resulting in repeat dilatation and dysmotility.[11] The serosa can be removed along the antimesenteric bowel wall at the site of the imbrication in an attempt to prevent suture line failure.

Antiperistaltic Small Intestinal Segments

Reversed small bowel segments to slow intestinal transit and increase absorption have been used mostly in adults. A segment of small bowel is placed in the opposite direction of normal intestinal flow or peristalsis. The reversed peristalsis in the interposed segment slows the movement of intestinal content. The difficulty is determining how long the reversed segment should be because long segments can stop intestinal flow altogether, producing intestinal obstruction.

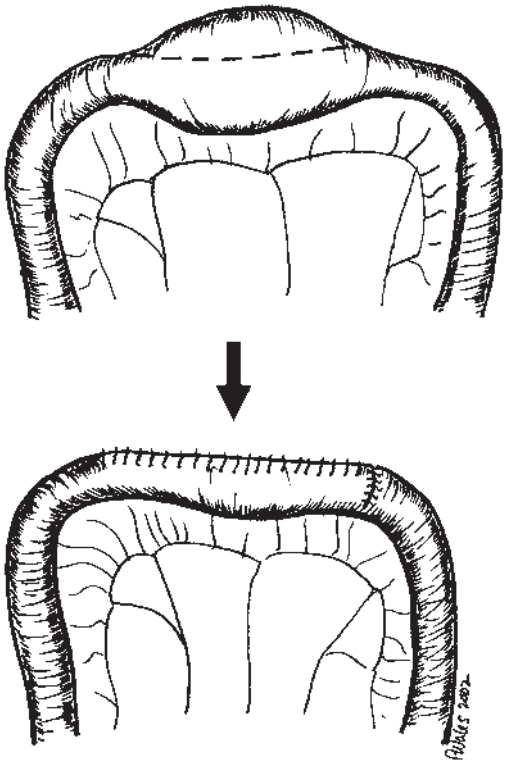

FIGURE 40.3-1 Tapering enteroplasty. The antimesenteric portion of the dilated segment of bowel is removed. A portion of the absorptive surface is lost.

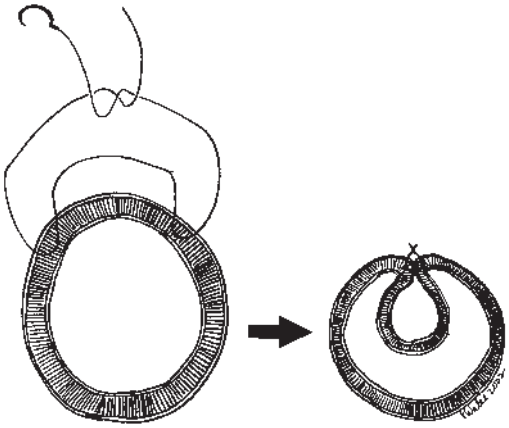

FIGURE 40.3-2 Intestinal plication. A portion of the dilated bowel wall is inverted into the bowel lumen and secured by seromuscular sutures.

Experience with reversed small bowel segments occurred in the 1970s when they were employed to slow peristalsis in patients with postvagotomy diarrhea.[23] The indication for reversed intestinal segments was expanded to include patients with SBS. In a canine model of beagle puppies with extensive small bowel resection, there was a short-term improvement in water, lipid, and nitrogen absorption.[24] Panis and colleagues described reversed small bowel segments in a case series of eight adult patients.[25] A median length of 12 cm was used for the reversed segment. Complications occurred in six of eight (75%) patients, and one patient died. Four patients (50%) weaned off parenteral nutrition, and the other four had decreased TPN requirements. In general, adult series have shown a favorable response to reversed segments in approximately 70% of patients, but the experience in children has shown a lack of sustained benefit.[23,26]

Neonates and infants are less favorable candidates for reversed small bowel segments because of their potential for intestinal growth (lengthening). Growth of a reversed segment may ultimately produce complete bowel obstruction.[26] In adults, the ideal length of the reversed segment is approximately 10 to 15 cm; in infants, it is 3 cm.[14] This procedure has limited use as a definitive procedure for patients with SBS.

Colonic Interposition

The frequency of colonic peristaltic contractions is lower than that in small bowel. Proximal placement of an isoperistaltic segment of colon between two ends of small bowel may prolong intestinal transit and permit more effective absorption of nutrients and electrolytes. Animal studies performed in the 1970s in both rats and canines were successful in showing delayed intestinal transit and improved growth.[27,28]

As with reversed small bowel segments, pediatric data show some favorable outcomes, with the ability to wean parenteral nutrition, but the results are variable.[29,30] The proposed advantage of isoperistaltic colonic interposition is fewer obstructive complications than witnessed in reversed small bowel segments, but the overall human experience is low.[23]

Creation of Intestinal Valves

The ileocecal valve has two functions. It slows transit of small bowel content into the colon and prevents reflux of colonic bacteria into the ileum. Colonic bacteria in the small bowel induce mucosal inflammation and malabsorption. Wilmore published his landmark article in 1972 in which he demonstrated the relationship between residual intestinal length, the presence or absence of the ileocecal valve and gut adaptation, and parenteral nutrition independence.[31] As a result, numerous procedures have been developed over the years to create intestinal valves that will slow intestinal transit and minimize colonic reflux in SBS patients. In most cases, the valve represents a short intussusception created by "telescoping" a proximal segment of bowel into the distal segment and suturing the seromuscular layers together (Figure 40.3-3).[32] Serosal stripping can be performed between the two walls of intestine being sutured to try to reduce the chance of valve failure.

An intestinal valve produces a partial obstruction with proximal dilatation of the small bowel. The partial obstruction increases absorption time, but, more importantly, it induces adaptation and dilatation that could permit a subsequent lengthening procedure. Georgeson and colleagues presented a series of six patients who received valve construction followed by an intestinal lengthening procedure 3 to 9 months later.[33] There were no complications associated with the valve. Three patients were weaned from parenteral nutrition. Other reported cases of intestinal valves in children have had mixed results.[11]

The major limitation is to determine the optimal valve length to slow intestinal transit without inadvertently causing a mechanical bowel obstruction. The variability between patients makes standard recommendations difficult. It has been reported that valve length in children should be less than 3 cm.[14] Creation of a valve requires approximately 8 cm of bowel; therefore, this procedure may not be feasible for many patients with SBS.[26]

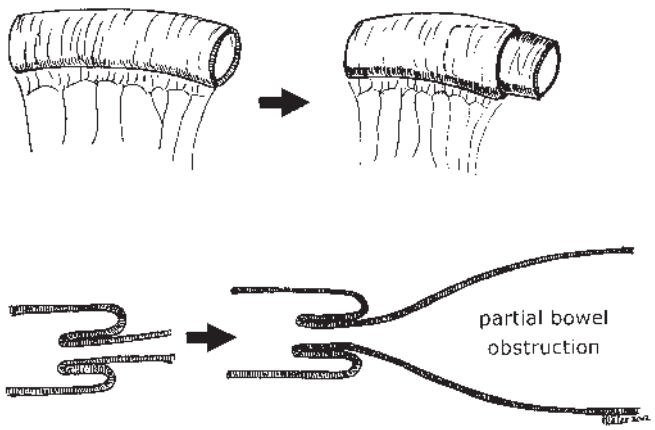

FIGURE 40.3-3 Creation of intestinal valves. A segment of bowel is telescoped into the distal bowel and secured with sutures. This will slow transit and induce a partial bowel obstruction. The proximal bowel dilates, and an intestinal lengthening procedure becomes possible.

Reversed Electrical Intestinal Pacing

Hyperperistalsis is common in SBS. The premise of reversed electrical intestinal pacing is based on the cardiology experience in which the heart is paced to treat arrhythmias. Overriding the intrinsic intestinal pacemaker could slow intestinal transit in SBS patients with hyperperistalsis to optimize water and nutrient absorption.

The normal intestinal pacemaker is located in the foregut within the duodenum. The electrical wave is propagated toward the ileum to generate peristalsis. Canine experiments have demonstrated better absorption of water and electrolytes, but manipulation of the duodenal pacemaker requires duodenal transsection.[34,35] Hence, no humans have received this approach because of the perceived high morbidity.

More recently, entrainment of intestinal slow waves in dogs has been achieved by Lin and colleagues.[36] Four pairs of intraluminal intestinal electrodes placed within the jejunum permitted intestinal pacing by entraining the intestinal slow waves. This treatment has not been evaluated in human trials, but it may be helpful in both SBS patients and in patients with primary motility disorders.

TECHNIQUES TO INCREASE ABSORPTIVE SURFACE AREA

As previously mentioned, the underlying problem in SBS is inadequate mucosal surface area for absorption. In addition, the adaptive response or partial mechanical obstruction from strictures produces proximal bowel dilatation, stasis, and bacterial overgrowth. The common thread between all intestinal lengthening procedures is the exploitation of the dilated segment of bowel to gain gut length. More importantly, however, the bowel is tapered, which improves peristalsis and prevents the sequelae of recurrent bacterial overgrowth.[26]

Longitudinal Intestinal Lengthening Procedure (Bianchi)

Bianchi first described the longitudinal intestinal lengthening procedure (LILP) in 1980 using a porcine model.[37] The procedure is based on the small bowel's dual blood supply that enters the bowel wall within the two layers of the mesentery. The bifurcated blood supply enters each hemicircumference of the bowel wall. The two leaves of the mesentery that contain the blood vessels are separated on the mesenteric surface of the bowel. The bowel is divided longitudinally, between the two layers, so that each parallel lumen has its own single source of blood supply.[26] The two newly created bowel segments are then anastomosed end to end, creating one intestinal segment that is twice as long and half the diameter of the original (Figure 40.3-4). The absorptive surface area may increase as the newly created loops slowly dilate over time. LILP is indicated for TPN-dependent patients with very short and dilated bowel.

Bianchi reported outcome in 20 patients who received a LILP.[38] At 6 years follow-up, 9 patients were alive. Seven of nine survivors were independent of parenteral nutrition. Survival was influenced by a small bowel length greater than 40 cm and no liver dysfunction at the time of the procedure. Weber reported 16 children who received a Bianchi procedure.[39] Eighty-eight percent were TPN independent; however, some of the children in that series had good residual small bowel length to begin with; therefore, it is possible that some of the patients would have adapted spontaneously and weaned off TPN without a lengthening procedure. Thirty-one percent required reoperation for complications. Thompson and colleagues published a series on long-term outcome after LILP in 16 patients with residual small bowel lengths ranging from 23 to 120 cm. Forty percent of the patients were independent of parenteral nutrition at both 1 and 5 years postsurgery.[40]

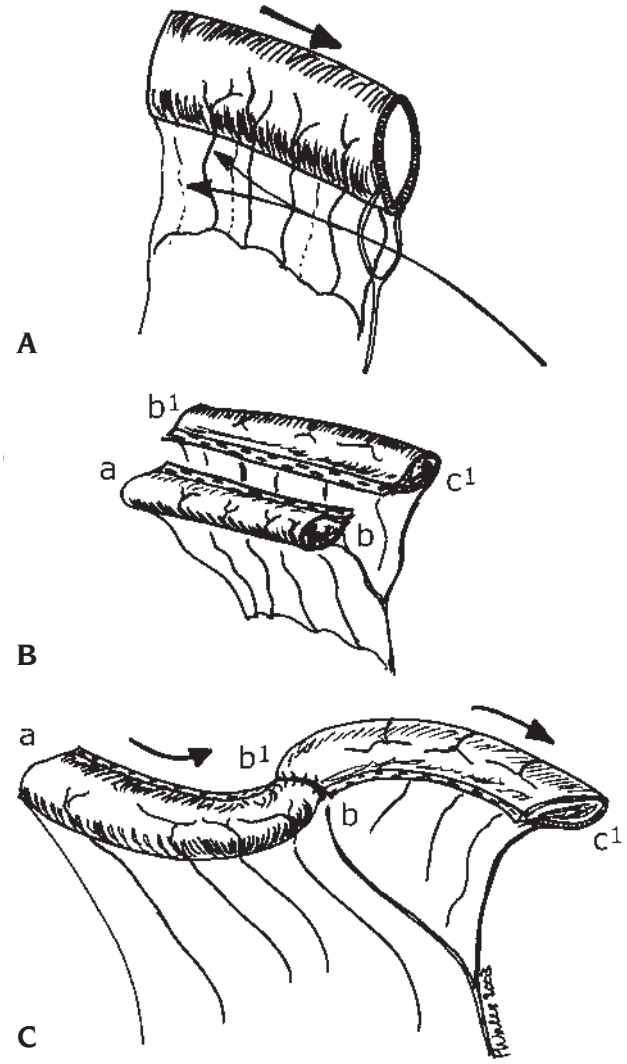

FIGURE 40.3-4 *A*, The mesenteric blood vessels within the two leaves of the mesentery are separated under the dilated bowel segment. *B*, The dilated bowel is divided longitudinally within the mesenteric leaves to create two parallel loops of bowel half the diameter of the original loop. *C*, The two bowel segments are anastomosed end to end to create a bowel segment double the length of the original. The bowel mesentery needs to be amenable to mobilization for this to be feasible. The bowel at "a" is the most proximal portion and the bowel at "c" is the most distal. The end of the reduced loop at "b1" is anastomosed to the proximal end of the other loop at "b2" to create an isoperistaltic segment of tapered and lengthened bowel.

Complications from this procedure include adhesive small bowel obstruction, anastomotic strictures or leak, and recurrent bowel dilatation. Patients who develop recurrent dilatation are candidates for the Kimura procedure or preferably the serial transverse enteroplasty (STEP).[26,41] The Bianchi procedure requires mobility in the mesentery to be able to anastomose the two tapered segments end to end. If a patient has a short, tethered, or thickened mesentery, a Bianchi procedure may not be technically feasible.[40,42]

Kimura Procedure (Isolated Bowel Segments)

The Kimura procedure is an alternative bowel-lengthening technique that may be used when the mesentery is very short, tethered, or absent.[43] It is a two-staged approach that consists of the initial coaptation of the antimesenteric surface of a dilated bowel segment to host organs (abdominal wall or liver) and then secondary longitudinal division of the bowel to create two bowel loops, one from the mesenteric half and one from the antimesenteric half.[42]

The serosal surface of the antimesenteric bowel wall is deseromyotomized to expose the submucosa. It is then sutured to the abdominal wall or liver that has had its peritoneum removed. Over a period of months, collateral blood supply develops from the host organs into the antimesenteric surface of the bowel. The bowel is then amenable to horizontal division into two parallel structures. The two bowel segments are then anastomosed end to end, creating a lengthened, tapered intestinal segment analogous to the Bianchi procedure.

No large reports of the Kimura procedure in humans have been published. This operation is not considered a first-line procedure for bowel lengthening. One major concern with this procedure is the limited access to the abdominal cavity owing to the nonanatomic placement of the bowel. Repeat laparotomy is frequent in this patient population; therefore, bowel parasitized to the abdominal wall makes these operations more difficult. Another criticism of the Kimura procedure is the lack of nutrient blood flow through the portal system, but in patients who have received this procedure, no problem has been noted.

Serial Transverse Enteroplasty

The STEP has been the most exciting addition to the surgical management of SBS in several years. The results from porcine experiments were first published in March 2003, and the first case report in a human was published in June 2003.[41,44] The STEP procedure satisfies the objectives of other bowel-lengthening procedures such as lengthening and tapering, but it has the advantages of being easy to perform and not requiring bowel anastomoses; it can theoretically more than double the intestinal length and can be applied as a primary or secondary procedure, such as after a Bianchi operation.

The procedure is performed by first dividing all of the abdominal adhesions to have full bowel mobility (Figure 40.3-5). The antimesenteric border of the dilated bowel is marked with a surgical pen to prevent twisting of the bowel during placement of the surgical stapler (Figure 40.3-6). A reusable gastrointestinal anastomotic (GIA) stapler is applied sequentially, from alternating and opposite directions through small mesenteric windows at the site of each staple line. The stapler is placed from the 90° and 270° positions (with 0° being the mesenteric border). The sequential stapler firings create a zigzag-like channel (Figure 40.3-7).[41] The diameter of the channel is determined by the surgeon and is commonly approximately 2 cm. The presence of normal caliber bowel can be used as an internal guide to channel size.

The blood supply to the intestine enters from the mesenteric side and travels along the bowel wall perpendicular to the long axis of the intestine; therefore, if the staple lines remain perpendicular to the long axis, the bowel segments should remain well vascularized.[41] As opposed to the Bianchi procedure, the mesentery is never in danger, and the bowel is never opened.

The Bianchi and Kimura procedures reduce the caliber of the bowel by 50%. With the STEP procedure, the extent of the tapering is dependent on the surgeon. The potential increase in bowel length depends on the extent of the bowel dilatation and the chosen size of the channel created. If the bowel segment is massively dilated, one could theoretically more than double the segmental length.[41]

The preliminary reports by Kim and colleagues establish the feasibility of the STEP procedure for bowel lengthening.[41,44] It could potentially be applied to other situations for which intestinal tapering is desired, but the loss of mucosal surface area should be avoided, such as in small bowel atresias with a very dilated proximal loop. The proximal end usually needs to be tapered or partially resected to deal with the size discrepancy between the proximal and distal bowel ends that are to be anastomosed. The STEP procedure deals with the size discrepancy and preserves surface area.

As with any new treatment modality, enthusiasm needs to be controlled until longer-term patient outcomes become known. We have performed two STEP procedures to date at The Hospital for Sick Children. Both children are early in their postoperative course. The first child was born at 30 weeks and suffered necrotizing enterocolitis requiring a bowel resection. She had recurrent episodes of bacte-

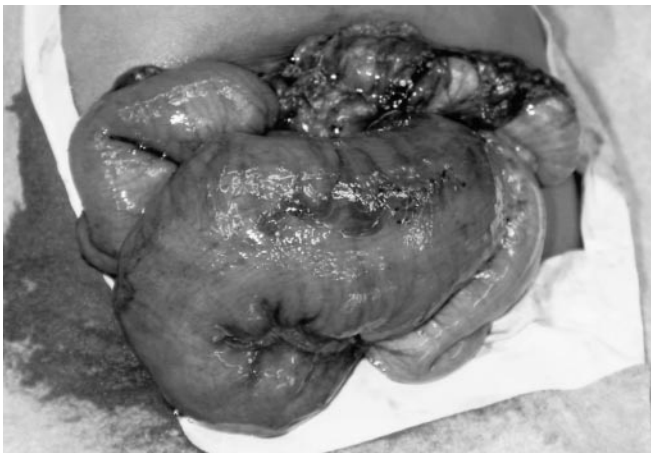

FIGURE 40.3-5 Patient with short-bowel syndrome and recurrent bacterial overgrowth. Note the very dilated loop of bowel that will be tapered and lengthened.

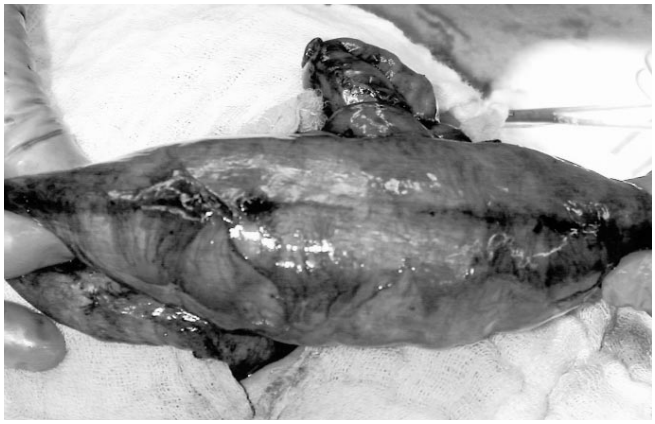

FIGURE 40.3-6 Serial transverse enteroplasty: marking of the antimesenteric border of dilated bowel with a surgical pen. This helps prevent twisting of bowel during application of stapler.

rial overgrowth from a very dilated intestinal loop and was suffering from TPN cholestasis. She underwent a STEP procedure at 12 months corrected age. She had a focally dilated loop of small bowel that was lengthened 60% from 25 to 41 cm. In the 2 months since the surgery, her stool consistency has changed from that of water to more particulate, and she has started to tolerate an increase in enteral feed volume and the introduction of a small amount of carbohydrate into her gut. To date, she has not had a recurrent episode of bacterial overgrowth.

The second patient was an ex-26-week gestational age male with recurrent necrotizing enterocolitis. He had carbohydrate intolerance and refractory bacterial overgrowth. He underwent a STEP procedure at 16 months corrected age and had his small bowel lengthened and tapered from 117 to 169 cm. He developed a gastrointestinal bleed from one of the staple lines postoperatively and subsequently an anastomotic leak from the terminal ileal staple line on the fifth postoperative day. A second laparotomy was performed to repair the bowel and create a diverting distal ileostomy. He is presently receiving 70% of his calories enterally and has not had another episode of sepsis to date.

Sequential Intestinal Lengthening Procedures

In 1994, Georgeson and colleagues reported their approach to nine infants and children with refractory SBS.[33] The authors employed a combination of several short-bowel operations described above, applied sequentially in patients with intestinal failure. For six patients who had a remnant bowel of normal caliber, an intestinal valve was created to induce partial bowel obstruction and proximal dilatation. After several months, a Bianchi procedure was performed. One patient had a Bianchi procedure initially, followed by a Kimura procedure for recurrent bowel dilatation. The outcome in the seven survivors was good after a range of 5 to 49 months of follow-up. Patients were tolerating a higher percentage of their calories enterally, and two patients were completely weaned from parenteral nutrition. This publication was a significant contribution because it emphasized the need for perseverance, flexibility, and creative solutions to this very complicated population of patients.

FUTURE CONSIDERATIONS

Tissue-engineered intestine is on the horizon as a possible treatment for SBS. Rat experiments have been performed and show promise.[45,46] A microporous biodegradable polymer is implanted with intestinal epithelial units for several weeks. The polymer is then implanted into adult rats that have received a 75% small bowel resection. The polymer is subsequently anastomosed to the native gut. The tissue-engineered intestine maintained patency for up to 36 weeks and was lined by columnar epithelium and goblet and Paneth cells; however, the unit's ability to absorb water and nutrients was poor.

CONCLUSIONS

SBS is a complex condition with significant morbidity and mortality. These patients require the expertise of a multidisciplinary team. The advent of TPN provided these acutely ill patients with a chance at survival, but it is also the cause of life-threatening complications. Fortunately, most children with SBS will adapt spontaneously over time. Children who remain TPN dependent are candidates for one of several adjunctive operations that promote adaptation by optimizing intestinal function, motility, and surface area. The choice of procedure must be individualized. Patients with a short and dilated bowel should receive an intestinal lengthening and tapering procedure such as the Bianchi or STEP. If the bowel is not dilated, then a colonic interposition is an option. Alternatively, creation of an intestinal valve to induce dilatation followed by a lengthening procedure could be beneficial. If bowel length is adequate but the bowel is dilated, then a simple tapering enteroplasty or plication would suffice.

Objective assessments of outcome from the various surgical techniques are lacking because of the relatively small number of patients at individual institutions and the lack of a consistent definition of SBS. All of these procedures, however, do provide an option that is of less magnitude than intestinal transplant.

FIGURE 40.3-7 Serial transverse enteroplasty. Zigzag pattern of bowel created after sequential and opposite firings of a surgical stapler across the dilated bowel loop. The channel size is determined by the surgeon. The normal loops of bowel can be used as an internal guide. A gastrointestinal contrast study is performed on the seventh postoperative day prior to initiation of enteral feeds.

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4. Outcomes

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Intestinal failure designates a state of intestinal tract function that is inadequate to fulfill the nutrient and fluid requirements of the body.[1] In children, intestinal failure often begins in the neonatal period. Implicit in the concept of intestinal failure is a prolonged and potentially permanent requirement for parenteral nutrition (PN). The outcome of intestinal failure is judged in two related ways. The first outcome measure is *recovery*. Recovery is generally synonymous with the ending of PN, although special enteral feeding techniques and nutrient supplements may remain necessary in individual patients. The second outcome measure is patient *survival*, particularly survival on PN.

Intestinal failure has two fundamental causes. Most commonly, intestinal failure results from anatomic short-bowel syndrome (see previous chapters). Second, intestinal failure may result from a critical loss of intestinal function in the absence of significant reduction in intestinal length. The distinction is somewhat artificial because anatomically short bowel is often dysfunctional. Intestinal failure from short-bowel syndrome is temporary in the majority of affected pediatric patients, whereas functional intestinal failure usually mandates permanent PN. In light of this difference, anatomic failure and functional intestinal failure are considered separately.

DURATION OF PN IN PATIENTS WITH ANATOMIC SHORT-BOWEL SYNDROME

The body undergoes physiologic changes to compensate for or adapt to intestinal loss. Adaptation may take the form of axial (longitudinal) intestinal growth, augmented colonic nutrient and fluid absorption, and possibly reduced metabolic expenditures. The magnitude and timeframe over which these changes occur have not been established with certainty in humans,[2] but they may eventually permit discontinuation of PN, especially in the growing child. In adults, PN may be successfully discontinued when as little as 25 to 35% of enteral calories are assimilated.[3,4] In children, tolerance of 50% assimilation of enteral calories is probably a more realistic requirement for ending PN, given the increased nutritional demands of growth and development. The corollary is that caloric intake that is 100% or more above the basal metabolic rate may be required for the patient to remain off PN.[3] Diarrhea may limit the ability to deliver this quantity of calories to a short gut. In most series, the average age at which PN that was started in infancy can be ended is around 18 months.[2,3,5,6] A need for PN beyond age 3 to 5 years in patients with neonatal short bowel predicts lifelong therapy.[6–8]

Length of Remnant Small Bowel

The most important determinant of PN duration in individual patients is postresection small-bowel length. A period of PN dependency is expected when more than 75% of the small bowel has been removed.[9–11] As small-bowel length approximates about 250 cm in the full-term newborn infant, prolonged PN is likely when no more than 60 to 70 cm of small bowel remains after resection surgery. Infants with 40 cm of small bowel have a 50% probability of coming off PN.[7] This percentage may be conservative because some reports include an early PN experience with death rates that are high by current standards. Children with at least 70 to 80 cm of small bowel remnant now have a 90 to 100% probability of ending PN.[6,12] It is generally thought that jejunal resection is less likely to require permanent PN than an equivalent magnitude of ileal resection.[13] However, anatomic detail regarding how much residual bowel is jejunum or ileum is lacking in most pediatric literature on this topic. Slower peristalsis, active bile salt and vitamin B_{12} absorption, and intrinsically greater ability of ileal enterocytes to proliferate and thereby increase nutrient uptake per unit of bowel length may be responsible for the greater adaptive capacity of the ileum.[10,11]

Colon and Ileocecal Valve

The colon is the key modifier of small intestinal length in determining PN dependence in short-bowel syndrome.[14] An intact colon, which implies salvage of the ileocecal valve (ICV) and some ileum, effectively doubles de facto digestive capacity in the pediatric short-bowel syndrome patient. In older patients, an intact colon may provide the absorptive capacity of as much as 80 cm of small bowel.[15] Thus, ending PN is commonly accomplished in pediatric patients who have 20 to 25 cm of small bowel[16–18] and may even be possible in some infants with as little as 10 to 15 cm of small bowel and an intact colon with ICV.[19]

Less clear is the impact of partial colon loss, including loss of the ICV, on the ability to end PN. Most[6,8,12,20] but not all[7,21,22] pediatric outcome studies fail to demonstrate an independent effect of the ICV on the duration of PN in those able to end therapy. Similarly, it has never been demonstrated that a limited colon resection is less deleterious to prognosis than a relatively long resection in pediatric patients,[8] although adult studies demonstrate that retention of half of the colon is functionally equivalent to about

50 cm of small bowel.[14] Most pediatric patients remaining on PN indefinitely have lost the ICV and some colon.

Pediatric patients uncommonly have a terminal enterostomy placed with the expectation that it is to be permanent. Rather, anastomosis to an intact or partially resected colon is the aim, given the potential for improved nutrient and fluid absorption following re-establishment of enterocolonic continuity.[23] Infants with remnant small bowel and/or a colon so short as to preclude anastomosis are expected to remain on PN indefinitely.[24] Patients with total colon Hirschsprung disease may undergo ileoanal anastomosis when attainment of continence postoperatively is realistic.[25] Relatively little information is available concerning the amount of remnant small bowel required to end PN when aganglionosis extends to the small bowel. In one study, resection of less than 50 cm of aganglionic ileum mandated PN for an average of 10 months (up to maximum of 35 months). Removal of greater than 50 cm of aganglionic bowel, considered equivalent to retention of up to about 100 cm of ganglionic bowel, required indefinite PN (ie, for more than 5 years) in 80% of affected patients.[26] Thus, patients with anatomic short bowel plus additional underlying functional intestinal impairments generally require longer remnant small-bowel length to obtain PN independence than do short-gut syndrome patients without functional impairments.

Age at Time of Intestinal Loss

The small bowel lengthens roughly twofold in the second half of gestation.[9] Numerous studies have demonstrated that prematurity favorably influences the ability to wean PN, presumably because the small bowel of premature infants has inherently greater growth potential than that of the full-term infant.[8,18,27] Conversely, studies of adult patients imply little or no intestinal lengthening following major resection,[15] which also probably applies to older children and adolescents because normal intestinal growth slows considerably by 3 to 4 years after birth.[6] Although PN has been successfully ended in children after 7 years of therapy,[7] the duration of PN in adults lasting more than 2 years predicts a 94% probability of lifelong PN.[28] Patients who develop short-bowel syndrome after infancy require approximately twice the length of small bowel than infants to wean from PN for any given magnitude of colon loss. Thus, by adulthood, 100 to 115 cm of small bowel ending as a jejunostomy is needed to obtain a 75 to 100% probability of ending PN. In this age group, possession of 60 to 65 cm of jejunum is usually required to permit ending of PN when anastomosed directly to partially resected colon, whereas only 30 to 35 cm of small bowel permits ending of PN when the colon and ICV are intact.[15,28]

Etiology of Short-Bowel Syndrome

Most studies fail to identify a definite effect of short-bowel etiology on the duration of PN.[8,12,22] However, there are some indications that necrotizing enterocolitis may give affected infants a PN-weaning advantage over those with congenital anomalies, possibly because of coassociated prematurity.[6,29] Disorders such as gastroschisis or proximal jejunal atresia may yield a less favorable prognosis than necrotizing enterocolitis because remnant small bowel may be relatively ischemic and atrophic, with resultant poor function and growth potential.[30] Pediatric recipients of an intestinal transplant appear to include a disproportionate number of patients with obstruction in utero.[31] An analogous example is Hirschsprung disease. Patients with this disorder who have lost the entire colon and some small bowel appear to be more likely to remain PN dependent than patients with other diagnoses who have comparable remnant small-bowel length and a terminal enterostomy.[26] Defective motility of remnant bowel in Hirschsprung disease may be responsible for this phenomenon. Similarly, infants with cystic fibrosis and short-bowel syndrome owing to meconium ileus may have a lesser probability of ending PN because of concurrent pancreatic deficiency, hepatobiliary disease, defective enterocyte transport, and increased caloric requirements.[32]

Ability to Resume Enteral Feeding after Resection

Studies consistently show that the sooner enteral feeding can be tolerated after a major neonatal resection, the greater (and earlier) the ultimate probability of ending PN.[12] Failure to tolerate enteral feeds in a nutritionally significant quantity by 12 weeks of age after neonatal resection portends an especially poor prognosis for achieving eventual independence from PN.[6] In contrast, infants with only 25 cm of small bowel who tolerate 50% of total delivered calories via the gastrointestinal tract by age 12 weeks have a 70 to 75% chance of ending PN. Presumably, early ability to establish (or re-establish) enteral nutrition is a surrogate marker for the overall quality of remnant bowel.

Managing Ongoing Complications of Short-Bowel Syndrome

Prognostic factors that are apparent at the onset of short-bowel syndrome are modified by subsequent clinical events. In short-bowel syndrome beginning in infancy, there are indications that amino acid–based formulas are tolerated better and permit faster weaning of PN than hydrolysate or intact protein formulas.[12] Similarly, use of breast milk appears to confer a weaning advantage to the PN-dependent infant. However, in adult patients with short-bowel syndrome, intact protein-based diets are equivalent to those with modified nitrogen sources.[11] The applicability of this information to children beyond the infant age range is unclear.

In younger patients, susceptibility to protein hypersensitivity may explain the superior performance of amino acid formulas. Protein hypersensitivity may be one of several factors that influence the tendency of pediatric patients to develop an active, and occasionally eosinophilic, enterocolitis.[8,33] Enteritis associated with short-bowel syndrome delays ending of PN up to 2 years compared with children without significant inflammation when gut anatomy is otherwise favorable for weaning.[8] Bacterial overgrowth may contribute significantly to mucosal inflammation in these patients.

The propensity for the short bowel to increase in diameter, in addition to length, over time contributes to ineffective peristalsis, stasis of intestinal succus, and secondary bacterial overgrowth. Reconstructive operations that taper dilated intestinal segments are intended to improve peristaltic efficiency and thereby improve feeding tolerance and hasten weaning of PN. When dilatation is severe enough, operative tapering may be combined with lengthening simultaneously to reduce the diameter of the affected segment by half while doubling its length. Numerous reports indicate that intestinal lengthening improves enteral feeding tolerance and permits ending of PN in some patients within 4 to 6 months after surgery.[34,35] Careful patient selection is important because the operation appears primarily to benefit patients beyond infancy who tolerate some enteral feeding and have mild or no cholestasis.[36,37]

PN-Associated Cholestasis

Premature infants receiving PN for intestinal failure are thought to be more likely to develop cholestasis than older children or adults,[38] although this claim has been disputed.[39,40] Mild and transient cholestasis has not been shown to prolong PN therapy in pediatric patients with short-bowel syndrome.[8,41] Cholestasis usually remits as PN is progressively reduced and then discontinued.[38,39] When sufficiently severe enough to produce portal hypertension, cholestasis slows the transition from PN to enteral feeding, assuming that patients do not succumb to complications of liver failure.[42] Deleterious effects on bowel motility and promotion of bacterial overgrowth are possible explanations.[43]

Factors that Do Not Predict Duration of PN in Pediatric Short-Bowel Syndrome

A high frequency of catheter-associated sepsis does not delay eventual ending of PN in those patients able to survive recurrent infection.[8,12] Furthermore, factors that might contribute to an unusually high frequency of catheter-related complications in home PN patients, such as the level of parental education and socioeconomic status, have also been shown not to affect the duration of PN.[8]

SURVIVAL WITH SHORT-BOWEL SYNDROME

The survival of pediatric patients who have received PN for short-bowel syndrome is about 85 to 90%, reflecting the high rate of adaptation to total enteral nutrition.[6,44] Survival of adults with short bowel appears inferior; about 20 to 40% are dead after 4 to 5 years of PN.[28,45] Patients with intestinal failure who are destined to die while receiving PN should be considered for intestinal transplant, either with or without concurrent transplant of other failing organs, most notably the liver.[46]

In adult patients with short-bowel syndrome, reduced survival reflects the causes of intestinal failure in this age group, rehabilitation from which is unlikely, including extensive intestinal infarction, inflammatory bowel disease, and radiation enteritis.[28] Furthermore, other systemic disorders, particularly vascular diseases, are important sources of mortality. Conversely, superior survival in children likely accrues to the disproportionate representation of young infants with marked intestinal growth potential who are destined *not* to require PN indefinitely. Nonintestinal comorbidities are typically absent in these patients, extremely premature infants with necrotizing enterocolitis being a key exception.[20,47]

When all infants diagnosed with short-bowel syndrome are considered, about 70 to 80% are eventually able to end PN therapy, meaning that 20 to 30% of pediatric patients with short-bowel syndrome require PN indefinitely.[6–8,12] Although death is uncommon in the entire cohort of pediatric patients who have experienced intestinal failure, about half of all pediatric patients who are destined to remain on PN indefinitely do succumb to complications directly associated with short-bowel syndrome or its treatment. The probability of death on PN is not a simple function of cumulative time on therapy.[27,39] The median age of death on PN, which resulted mainly from liver failure, in three recent studies was only 19 months,[6,8,19] which is similar to the duration of PN for all pediatric patients successfully ending this therapy.[8,12,22]

Death from Sepsis

During the first decade of PN therapy, the 1970s, sepsis was probably the most common cause of demise, sources of which included contamination of PN delivery systems.[7,48,49] Additional deaths accrued to metabolic abnormalities associated with PN and noninfective catheter complications that are uncommon at present.[50] Although a high frequency of infection has not been demonstrated statistically to delay the ending of PN, patients who die on PN also have the highest rates of infection.[44] Undoubtedly, these deaths included some patients who would have ended PN had fatal sepsis not supervened. Improved management of PN delivery, including central venous catheters and catheter-related sepsis, has contributed to the reduction in mortality unrelated to liver disease, as has the subsequent shift from hospital-based to home-based care.[51] Whether recurring sepsis is an independent risk factor for death or is just a marker of unusually severe and complex intestinal disease is not established.[48]

Death from Liver Failure

Currently, mortality from short-bowel syndrome in children results primarily from progressive PN-associated liver disease. Consequently, the majority of intestinal transplants in children are performed with a simultaneous liver transplant to forestall impending demise from liver failure and its complications.[31,46] Historically, the death rates of pediatric patients with short-bowel syndrome caused by liver failure have ranged from about 33 to 66%.[6,12,52]

The risk of fatal PN-associated liver disease in patients receiving indefinite PN is predictable based on factors that are often apparent at the time PN is initiated. Aggressive liver disease is not a function of duration of PN therapy, onset of which most often occurs comparatively early in the course of PN. In infants, hyperbilirubinemia often presents after an initial septic episode and gradually worsens over the next 9 to 24 months.[8,39,53,54] Plasma bilirubin greater

than about 10 mg/dL in this setting is particularly ominous and has been associated with death within 6 months.[55] In older patients, hyperbilirubinemia may appear after a few months to a decade or more after the start of treatment.[56–58] The probability of advanced disease is 50% after 6 years of PN in adults, and once jaundice supervenes, death comes quickly, with an average survival of only about 1 year.[57,58] In older PN-dependent patients, reported death rates directly attributable to liver failure vary from 7 to 22%.[56–58] The short interval between the onset of significant liver disease and the progression to fatal liver failure necessitates rapid consideration of intestinal transplant when liver disease becomes apparent. If performed early, cholestasis resolves following isolated intestinal transplant, obviating the need for simultaneous transplant of the liver.[59,60] The clinical risk factors for advanced liver disease follow.

Extreme Short Bowel. Patients receiving PN therapy that is predicted to be permanent based on unfavorable anatomy are at high risk of developing fatal liver disease. Pediatric patients who are able to wean from PN rarely are left with end-stage PN-associated liver disease.[61] In adults and presumably older children, predictors have included small bowel length less than 50 cm[56] and loss of all small bowel distal to the ligament of Treitz.[58] In infants with PN dependence dating to the neonatal period, most succumbing to liver failure have a small bowel length of no more than 35 cm.[8,39] Termination of small bowel as a stoma (ie, absence of enterocolonic continuity) may place pediatric patients at further risk.[12]

Sepsis and the Systemic Inflammatory Response. Infants with fatal liver failure have an increased frequency of confirmed early bacterial or fungal sepsis in association with bowel resection, on average within 30 days of birth.[39] The propensity for sepsis to be associated with liver disease is not surprising because the systemic inflammatory response is inherently prone to produce cholestasis.[57,62] Bacteria and their by-products from the short gut have been hypothesized to be an important source of liver injury.[34,63] The distinction between death from sepsis and death from liver failure is blurred by the fact that liver failure begets an increased vulnerability to sepsis, which is progressively likely to be fatal as liver disease worsens.[58,53]

Lack of Early Enteral Feeding. Tolerance of little if any enteral nutrition after major bowel resection is another risk factor for fatal PN-associated liver disease.[39] It is unclear whether a lack of enteral feeding itself represents the risk or whether delayed feeding reflects systemic sepsis and possibly intra-abdominal infection associated with the original indication for surgery.[64]

PN Quantity and Composition. Although total parenteral calorie intake greater than 70 to 80% of the calculated energy requirement is associated with an increased propensity for liver disease, an effect of PN calories independent of gut loss has not been shown.[65] In contrast, provision of greater than 1 g/kg/d of intravenous emulsified lipid is associated with fatal liver disease.[56] Although the effect may reflect direct hepatotoxicity of components in lipid emulsion,[66] extra lipid may also provide substrate to fuel the systemic inflammatory response and its deleterious effect on the liver.[67]

Death from Loss of Central Venous Access

It is axiomatic that relentless thrombotic occlusion of all sites of venous access will ultimately be fatal to those who depend on PN for survival.[68,69] Precise data regarding the incidence of death of pediatric patients with intestinal failure and this complication are lacking. The relative frequency of impending total loss of venous access may be inferred from the number of recipients of an isolated intestinal transplant (as opposed to a combined liver and intestinal or multivisceral graft), which amounts to 35 to 40% of the total.[31] This percentage is undoubtedly an overestimate of the number of patients with loss of access because early, reversible liver damage and recurrent, life-threatening, catheter-associated sepsis are also indications for isolated intestinal transplant.[46] Coincident congenital thrombophilic syndromes probably predispose the patient to catheter-associated vein occlusion.[70]

OUTCOME OF FUNCTIONAL INTESTINAL FAILURE

Functional intestinal failure is rare in comparison with anatomic short-bowel syndrome. There are two forms of functional intestinal failure: the congenital enteropathies and the pseudo-obstruction syndromes.

Congenital Enteropathy

As a group, these disorders preclude meaningful assimilation of calories and fluid because of the combination of severe malabsorption and secretory diarrhea.

Microvillus inclusion disease and tufting enteropathy are prototypes. Abnormalities of apical plasma membrane protein trafficking appear to cause microvillus inclusion disease,[71] whereas abnormal basement membrane proteins are present in tufting enteropathy in association with crowding of surface enterocytes.[72] These disorders generally present soon after birth.[73,74] Affected patients tolerate little or no enteral feeding. No treatments including anti-inflammatory agents or growth factors have proven beneficial. Early onset of progressive PN-associated liver failure is common and, combined with recurring sepsis, makes intestinal transplant the only viable option when present.[75] In the absence of liver disease or loss of venous access, PN may be continued indefinitely. There exist only rare reports of spontaneous improvement that permits ending of PN support.[76]

Congenital deficiency of mitochondrial respiratory chain enzymes resulting in defective cellular energy production represents a second cause of intestinal failure associated with intractable diarrhea.[77,78] Intestinal biopsies demonstrate villous atrophy. Age at onset is variable, frequently between infancy and adolescence. Multiorgan involvement is the rule, eventually affecting the liver, skeletal musculature, and central nervous system in vari-

ous combinations. Death at an early age is typical and is often caused by failure of other affected organ systems rather than supportive PN therapy.

Pseudo-obstruction

The second form of functional intestinal failure is that resulting from profoundly defective intestinal motility (ie, intestinal pseudo-obstruction). Pseudo-obstruction may result from various defects in visceral smooth muscle (ie, myopathic pseudo-obstruction) or from abnormalities of the enteric nervous system (ie, neuropathic pseudo-obstruction). One mitochondrial disease variant, the mitochondrial neurogastrointestinal encephalomyopathy syndrome (MNGIE), presents primarily with dysmotility rather than intractable diarrhea in association with combined neuromuscular dysfunction.[78,79] In contrast to MNGIE, in which extraintestinal neuromuscular involvement is prominent and familial with autosomal recessive inheritance, most pseudo-obstruction syndromes in childhood are sporadic and affect only the gastrointestinal and genitourinary tracts.[80]

Pseudo-obstruction syndromes in childhood take a more variable clinical course than the primary disorders of enterocyte function such as microvillus inclusion disease. Effective peristalsis may resume transiently, allowing for periodic interruption of PN.[81] Regions of the gastrointestinal tract may be differentially affected, such that palliation can be obtained with decompressing enterostomies and colostomies. The urinary tract is involved in about 85% of patients with myopathic pseudo-obstruction but only 10 to 15% of patients with neuropathic pseudo-obstruction, resulting in urinary bladder, ureteral, and pyelocalyceal dilatation; hydronephrosis; urinary stasis; and renal insufficiency.[80]

The prognosis of intestinal pseudo-obstruction in childhood is determined by the severity, the key criterion of which is the need for PN. About 15 to 40% of patients require total PN, and an additional 30% require partial PN.[82,83] Only 50% survival through childhood can be anticipated if continuous PN is needed.[80] Thus, PN-dependent pseudo-obstruction appears to carry a prognosis similar to extreme short-bowel syndrome in children. In contradistinction, studies in adults consistently demonstrate reduced survival of PN-dependent patients with motility disorders compared with those with nonmalignant short-bowel syndrome.[45,84]

Death largely results from septic complications of chronic venous catheterization or PN-associated liver disease. Patients with myopathic pseudo-obstruction appear to have more consistently refractory ileus and earlier and greater PN requirements, resulting in correspondingly higher mortality.[83]

CONCLUSION

The outcome of intestinal failure is good in children, reflecting the transient nature of PN dependence in the majority, most of whom have short-bowel syndrome. An indefinite need for PN is associated with a guarded prognosis, which reflects the severity of disease that leads to PN rather than PN itself. Most children who die as a consequence of intestinal failure, probably around 10% who have carried this diagnosis, will succumb owing to PN-associated liver failure and its complications. Interventions that facilitate the ending of PN and/or reversal of developing or impending liver failure, including intestinal transplant, promote survival.

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CHAPTER 41

INFLAMMATORY BOWEL DISEASE

1. *Crohn Disease*

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Crohn disease is a chronic inflammatory disorder of the gastrointestinal tract that manifests during childhood or adolescence in up to 25% of patients. In 2001, two independent groups of investigators concurrently identified nucleotide oligomerization domain (NOD2), subsequently renamed caspase recruitment domain protein 15 (CARD15), as a Crohn disease susceptibility gene.[1,2] This landmark discovery marked the first time that a gene in any complex disorder had been first localized by linkage studies[3] and then positionally cloned.[1] *NOD2/CARD15* participates in the innate immune system, which regulates the immediate response to microbial pathogens.[4] The recognition of *NOD2/CARD15* mutations in roughly one-third of patients with both sporadic and familial Crohn disease[1,2,5] clearly links genetic susceptibility and enteric bacteria, two factors long hypothesized as important in etiopathogenesis.[6,7]

Although yet to be translated into a therapeutic breakthrough, the identification of Crohn disease–associated *NOD2/CARD15* polymorphisms has refocused and inspired research efforts in many ways. The role of bacteria and the mechanisms by which *NOD2/CARD15* polymorphisms predispose individuals to chronic intestinal inflammation require precise elucidation. Other susceptibility loci continue to be intensely investigated. For the first time, it is possible to begin to explain the well-recognized clinical heterogeneity of Crohn disease on the basis of genotype.[8,9]

This chapter reviews the epidemiology, etiologic hypotheses, clinical manifestations, and current management of Crohn disease in children and adolescents. The emphasis is on the exciting recent progress in understanding factors involved in disease pathogenesis and on the significant evidence-based advances in medical treatment.

EPIDEMIOLOGY

Classic epidemiologic studies characterize disease with regard to person, place, time, and associations. Hypotheses as to etiologic factors are then generated to explain observed variations in incidence. There are, however, many barriers to ascertaining accurate incidence data concerning Crohn disease.[10] Most locations do not have true population-based registries, so surrogates such as hospitalization figures are sometimes used to estimate incidence. Differences in diagnostic facilities and disease definition can give the false impression of discrepant incidence rates. Improving case ascertainment leads to an artificial increase in incidence. Some patients now diagnosed as having Crohn disease may have been more likely labeled as having ulcerative colitis in the past. Despite these confounding factors, there is a general consensus about several epidemiologic trends.[10]

Trends in Time

What has come to be known eponymously as Crohn disease was first described as "regional ileitis" in 1932, although it was considered likely that the same pathologic and clinical entity had been the subject of published case reports even in the early nineteenth century.[11] The occurrence of Crohn disease increased sharply in all age groups in most Western populations between the 1950s and 1980s. Whether it has since leveled off or continues to rise is in dispute.[10]

Increases in childhood and adolescent Crohn disease have paralleled overall population trends. The incidence of Crohn disease doubled among children in Wales from 1983 to 1993.[12] A threefold rise in the incidence of Crohn disease among Scottish children between 1968 and 1983 and a continued rise at the same rate between 1981 and 1992 have been reported.[13,14]

Geographic Trends

Geographic variability of inflammatory bowel disease (IBD) is striking.[10] The worldwide distributions of Crohn disease and ulcerative colitis are similar; both are most prevalent in North America, northwestern Europe, especially Scandinavia, and the United Kingdom.[10] Countries in southern Europe, South Africa, and Australia have somewhat lower incidence rates. A carefully designed recent collaborative European study of prospectively accrued cases confirms that incidence rates for both ulcerative colitis and Crohn

disease are higher in northern than in southern regions, but the north-south gradient in incidence rates is more modest than previously suspected.[15] IBD is relatively rare in Asia, Africa, and South America. In continental Europe, the occurrence of ulcerative colitis including proctitis in adults still exceeds that of Crohn disease at a ratio of 2:1, whereas the incidence of Crohn disease has become relatively greater than that of ulcerative colitis in parts of North America and the United Kingdom.[10,16]

Recent studies reporting the incidence of pediatric-onset illness in countries where IBD is most common are summarized in Table 41.1-1.[12–14,17–21] Reported incidence rates may be significantly influenced by even slight variations in the definition of "childhood" because of the steep rise in age-specific incidence during the teenage years.[10]

Trends in Age and Gender

Peak incidences of Crohn disease are observed in late adolescence or young adulthood. A second smaller peak is observed in the sixth decade.[10] There is no convincing evidence that age at onset is decreasing. The incidence of pediatric Crohn disease generally exceeds that of pediatric ulcerative colitis, although among children very young at presentation, ulcerative colitis is diagnosed more commonly than is Crohn disease.[12,21,22]

For Crohn disease overall, the incidence among females exceeds that among males by 20 to 30%.[10] In contrast, however, studies restricted to pediatric-onset Crohn disease document a male-to-female preponderance.[12,21] As summarized in Table 41.1-2, demographic data from a recently completed survey of new diagnoses of IBD among patients less than 16 years in the United Kingdom and Ireland are strikingly similar to relatively population-based data from the urban center of metropolitan Toronto, Canada, concerning children and adolescents diagnosed with IBD between 1980 and 1996.[12,21]

Trends among Races and in Ethnic Groups

Both ulcerative colitis and Crohn disease affect whites more than Asians and African blacks. Individuals of Jewish descent appear to be particularly susceptible to IBD.[23,24] Compared with Sephardic or Oriental Jews, Ashkenazi Jews have an increased risk.[23,24] Their incidence rate for IBD varies tremendously around the world, in parallel with but always remaining three to four times greater than the local non-Jewish population risk.[24] The persistence of increased risk across different time periods and geographic areas provides indirect evidence that there is a genetic predisposition to developing IBD.

ETIOPATHOGENESIS

It is unlikely that a simple cause and effect relation accounts for the majority of Crohn disease cases. Rather, it is hypothesized that the chronic immune-mediated intestinal injury results from complex interactions between predisposing genetic factors and exogenous or endogenous triggers, likely microbial in origin.[6,7] The proposed multifactorial etiopathogenesis is summarized schematically in Figure 41.1-1. Impressive progress has been made in elucidating the genetic basis of Crohn disease susceptibility, but, to date, the precise role played by the intestinal microflora in triggering and perpetuating chronic inflammation remains elusive.

GENETIC SUSCEPTIBILITY

Observations from Family Studies

The familial occurrence of Crohn disease is well recognized.[25–29] A positive family history in a first-degree relative remains the major identified risk factor for its development.[29] Familial aggregation could be explained by shared environmental or by genetic factors. However, the relative rarity in spouses compared with first-degree relatives and, most convincingly, data from studies comparing disease concordance in monozygotic versus dizygotic twins argue for the importance of genetic factors.[30,31]

A high (44.4–58%) rate of concordance for Crohn disease among monozygotic twins versus dizygotic twins (0–3.8%) was reported in Swedish and Danish studies, respectively, based on unselected twin registries.[30,31] If only environmental factors were involved in Crohn disease, the observed concordance rates between dizygotic twins and monozygotic twins would be expected to be the same. Conversely, because monozygotic twins share identical genomic material and yet are not 100% concordant for Crohn disease, no combination of genes is sufficient for the development of Crohn disease. Rather, some environmental trigger is also required for the disease to be expressed.

TABLE 41.1-1 RECENT INCIDENCE DATA FOR INFLAMMATORY BOWEL DISEASE IN CHILDREN AND ADOLESCENTS

LOCATION	AGE CRITERIA	CALENDAR YEARS OF STUDY	INCIDENCE OF PEDIATRIC CD (PER 100,000/YR)	INCIDENCE OF PEDIATRIC UC (PER 100,000/YR)
Scotland[13,14]	≤ 16 yr at symptom onset	1981–1983	2.3	1.6
		1990–1992	2.8	
South Glamorgan, Wales[12]	< 16 yr at diagnosis	1989–1993	3.1 (95% CI 1.8–5.3)	0.7
Nord-Pas-de-Calais, France[17]	≤ 15 yr at diagnosis	1984–1989	2.1	0.5
Western Norway[19]	≤ 15 yr at diagnosis	1984–1985	2.5	4.3
Metropolitan Toronto, Canada[20]	≤ 17 yr at diagnosis	1991–1996	3.7	2.7
United Kingdom and Ireland[21]	< 16 yr at diagnosis	1998–1999	3.0	2.2*
Copenhagen County, Denmark[18]	< 15 yr at diagnosis	1962–1987	0.2	2.0

CD = Crohn disease; UC = ulcerative colitis.
*Provisionally labeled indeterminate colitis included with ulcerative colitis.

TABLE 41.1-2 COMPARISON OF DEMOGRAPHIC DATA AT TIME OF DIAGNOSIS OF CROHN DISEASE*

	GREATER TORONTO AREA, 1980–1996	UNITED KINGDOM AND IRELAND, 1998–1999
Total number of children diagnosed with IBD	787	739
Crohn disease		
Number (% of total)	486 (61.8)	379 (58)
Gender	62% male	62% male
Age at diagnosis (yr)	12.7 ± 2.56 (mean ± SD)	12.9 (10.8–14.3) Median (interquartile range: Q1–Q3)

IBD = inflammatory bowel disease.
*The Toronto, Canada, data concern children prospectively registered at the time of diagnosis in The Hospital for Sick Children pediatric inflammatory bowel disease database. The United Kingdom and Ireland data represent the results of 13-month surveillance of incident inflammatory bowel disease cases.[21]

The relative importance of genetic versus environmental factors, or heritability, is commonly expressed as λs, the prevalence in siblings divided by population prevalence.[29] For Crohn disease, the overall λs has been calculated in several studies worldwide to range between 10 and 35.[29] Hence the relative contribution of genetic factors to the pathogenesis of Crohn disease is greater than in schizophrenia, asthma, or hypertension and is at least equivalent to that identified in insulin-dependent diabetes.

Familial aggregation is more common among Jewish versus non-Jewish patients.[32,33] The age-adjusted empiric risks for developing IBD during a lifetime were calculated for first-degree relatives of Jewish and non-Jewish white probands with Crohn disease in a study from southern California.[32] The age-adjusted lifetime risks for siblings of Jewish and non-Jewish probands with Crohn disease were, respectively, 16.8% and 7.0%.[32]

Crohn disease and ulcerative colitis are observed to coexist in the same family with a frequency higher than just the co-occurrence by chance alone.[25–29,33] Nevertheless, concordance within a family for the type of IBD is much more common than discordance.[25–29,33] These observations indicate that the two diseases share at least some common etiologic factors. The occurrence of Crohn disease in offspring of couples both affected by ulcerative colitis supports the same conclusion.[34]

Genetic factors may be particularly important in the development of early-onset IBD. Polito and colleagues reported the proportion of patients with relatives affected with IBD according to the age at diagnosis in the proband.[35] Thirty percent of 177 patients diagnosed under age 20 years had a positive family history. The percentage decreased to 18% for those diagnosed between 20 and 39 years of age (n = 311) and to 13% among those diagnosed after age 40 years (n = 67).[35] Among a cohort of 770 probands with pediatric-onset IBD in Toronto, Canada, 16% had a first-degree relative (parent and/or sibling) who was also affected.[33] Thirty percent of the Jewish children with Crohn disease had one or more first-degree relatives with IBD.[33]

It has been observed that Crohn disease develops at a younger age in offspring in comparison with their affected parents.[36–38] Genetic "anticipation" refers to the tendency of a disease to develop at an earlier age and with increased severity in subsequent generations. In several monogenic neurodegenerative disorders, such anticipation is explained on a molecular basis by an increased number of trinucleotide repeats within the disease gene.[39] Although some investigators have proposed the same mechanism in Crohn disease,[36] earlier exposure to an environmental trigger and ascertainment biases are more plausible explanations.[37,38]

The number of genes that may confer susceptibility to IBD is still unknown. Initial segregation analyses led investigators to hypothesize that a major autosomal recessive gene may account for a proportion of the genetic predis-

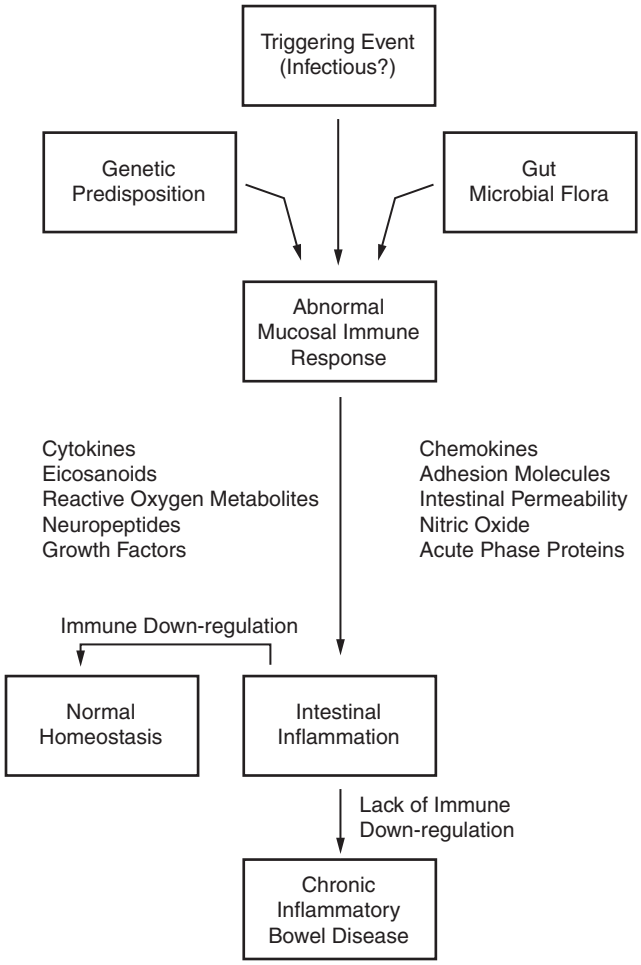

FIGURE 41.1-1 Multifactorial etiopathogenesis of Crohn disease. It is postulated that in a genetically susceptible individual, interacting environmental factors and the host intestinal microbial flora, in the presence of a yet unspecified triggering event, activate an aberrant immune response, the end result of which is chronic intestinal inflammation.

position to Crohn disease.[40] It is usually accepted, however, that IBD involves multiple susceptibility loci, as well as genetic heterogeneity (whereby different genes result in a similar disease phenotype), and both gene-gene and gene-environment interactions in a complex system.[29,41,42]

Genetic Studies in Crohn Disease

Two complementary strategies are used to search for the genetic determinants of a disease trait: linkage studies and candidate gene analysis. In the former, systematic screening of the entire human genome using highly polymorphic markers consisting of di- and trinucleotide repeats provides a strategy for localizing genes without prior hypotheses about their nature. The intrafamilial segregation of these microsatellite marker alleles is compared with segregation of the disease trait within a family. The markers have no direct relationship to disease pathogenesis, but when there is an excess of a marker allele shared among individuals with the disease, the disease gene and the marker locus are presumed to be transmitted together by virtue of being in proximity on the same chromosome.[43] Such linkage analyses have been successfully performed for numerous mendelian disorders, leading to the localization and further identification of the predisposing gene. However, the application of genome scanning to the study of complex disorders is relatively new and has much less often been fruitful.[43]

The candidate gene approach to identification of susceptibility genes requires determination of the frequency of different alleles at a polymorphic locus on a putative disease gene in patients versus ethnically matched controls (case-control studies). If a statistically significant difference is observed between the two groups, the allele that is more prevalent in the patient group is said to be associated with the disease. However, spurious associations may be generated by ignoring differences between the control and the disease groups that are not related to the disease. To overcome the bias of improper matching, tests that incorporate analysis of unaffected parents have been proposed. As an example, in transmission disequilibrium testing, the frequency with which an allele is transmitted from a usually healthy parent to an affected child is compared with the theoretically expected value of 0.5.[44] Significant departure from this expected value confirms an association between the allele and the disease.

Because the pathophysiologic processes leading to IBD are complex, the number of potential candidate genes is immense. Without direction from linkage studies, association studies would only very fortuitously successfully identify susceptibility genes. However, case-control candidate gene studies or transmission disequilibrium testing at loci identified by linkage studies can be complementary to genome scanning. Indeed, the application of the above-described strategies to Crohn disease has been rewarded with enormous recent advances.[1,2]

Identification of CARD15/NOD2 as a Crohn Disease Susceptibility Gene

Hugot and colleagues reported the first genome scan in families with two or more siblings affected with Crohn disease and thereby identified the pericentromeric region of chromosome 16 as a possible locus of a Crohn disease gene.[3] Linkage to this region, named IBD1, was subsequently confirmed in a multicenter replication study undertaken by the International Inflammatory Bowel Disease Genetics Consortium.[45] The eventual identification at this locus of *NOD2*, further renamed caspase recruitment domain protein 15 (CARD15) by an international nomenclature committee, as the first Crohn disease susceptibility gene marked the first time a gene in any complex disorder had been first localized by linkage studies and then positionally cloned.[1,3] At the same time, Ogura and colleagues successfully used a candidate gene approach to gene identification.[2] They selected *NOD2/CARD15* as a potential Crohn disease gene based on knowledge of its role in the recognition of bacterial components and on the gene location within the IBD1 region.

The 12-exon *NOD2/CARD15* gene encodes an intracellular protein, structurally related to the R proteins in plants, which mediate host resistance to microbial pathogens.[4] As illustrated in Figure 41.1-2, the protein product is composed of 1,040 amino acids with several functional domains: two caspase recruitment domains (CARD1 and CARD2), a nucleotide binding domain (NBD), and a leucine-rich repeat (LRR) domain.[4] The LRR domain functions as a pattern recognition receptor for microbial components. The NBD mediates protein self-oligomerization, which is required for activation.[4] CARDs are domains involved in apoptosis pathways and in nuclear factor-κB (NF-κB) activation.

Sequencing of the *NOD2/CARD15* gene in Crohn disease patients revealed a cytosine insertion at position 3020 in exon 11 (Leu1007fsinsC) that gives rise to a stop codon and a truncated NOD2 protein.[1,2] This frameshift mutation and two other common missense mutations (Arg702Trp, Gly908Arg), all within or near the LRR domain of *NOD2/CARD15* (see Figure 41.1-2), have been highly associated with Crohn disease but not with ulcerative colitis.[1,2,5] This lack of an association with ulcerative colitis is consistent with previous negative linkage data at the IBD1 locus.[45] Allelic frequencies for Arg702Trp, Gly908Arg, and Leu1007fsinsC are consistently higher in white Crohn disease patients compared with controls.[41] In addition to the three major risk alleles, a number of extremely rare ("private") amino acid polymorphisms, particularly within or near the LRR domain, have been defined, each present in only a few families.[1,9] These rare ("private") mutations appear, nevertheless, to be similarly associated with disease

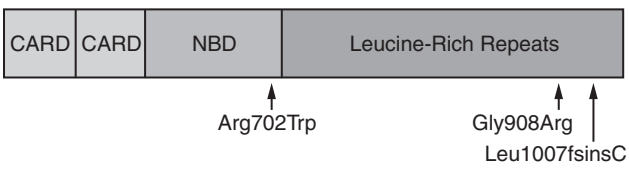

FIGURE 41.1-2 Structure of the NOD2/CARD15 protein. The three common Crohn disease–associated polymorphisms are located in the leucine-rich repeat domain. CARD = caspase recruitment domain; NBD = nucleotide binding domain.

pathogenesis. Little is known about the presence of *CARD15* mutations in ethnic groups other than whites. In studies of Japanese Crohn disease patients, none were carriers for any of the three major risk alleles.[46,47] A unique amino acid polymorphism has been identified in blacks.[41,48]

Having one copy of a risk allele confers a relatively small (two- to fourfold) increased risk for developing Crohn disease, whereas having two copies increases the risk 20- to 40-fold, indicating that *NOD2/CARD15* functions to a large extent in an autosomal recessive fashion.[1,5] Patients carrying two variant alleles are characterized by a younger age at onset.[9] These observations are, interestingly, in accordance with previous segregation analyses, which suggested that a major recessive gene might play a role in a subset of patients, particularly with early-onset Crohn disease.[40] Approximately 8 to 17% of patients carry two copies of the major *NOD2/CARD15* risk alleles (compared with less than 1% in white control populations).[41] Twenty-seven to 32% of Crohn disease patients carry one major risk allele, and roughly half carry one major or one rare ("private") variant allele (compared with about 20% of white controls).[9,41]

Several phenotypic correlations have emerged as large patient populations have been genotyped for the Arg702Trp, Gly908Arg, and Leu1007fsinsC *NOD2/CARD15* polymorphisms.[8,9,49–54] First, *NOD2/CARD15* polymorphisms are associated with both sporadic and familial Crohn disease.[1,2,5,9] Second, in multiple studies, a significant association has been found between ileal disease and the carriage of one or more *NOD2/CARD15* risk variant alleles.[8,9,49–54] Double-dose carriers are very uncommonly observed in colon-only Crohn disease.[8,9,50] Finally, some studies have demonstrated an association between *NOD2/CARD15* risk alleles and a stricturing phenotype.[50,51,53]

The mutational spectrum of *NOD2/CARD15* is favorable for genetic analyses because the three major polymorphisms represent more than 80% of the variant alleles and are easily determined in laboratories involved in molecular diagnosis.[1,9] As yet, however, knowledge of *NOD2/CARD15* status has little role in clinical practice. The lack of any association between *NOD2/CARD15* polymorphisms and ulcerative colitis initially suggested that *NOD2/CARD15* genotyping could help to classify indeterminate colitis, but this is precluded by the low frequency of *NOD2/CARD15* mutations in Crohn disease patients with an ulcerative colitis–like phenotype. To date, *NOD2/CARD15* status has not correlated with any treatment responsiveness, but only the response to infliximab therapy has hitherto been studied.[55,56] The usefulness of *NOD2/CARD15* genotyping in predicting outcomes needs to be studied in future clinical trials.

As expected for a complex genetic trait, *NOD2/CARD15* is neither necessary nor sufficient for disease development. The odds ratio of disease development is in the range of 20 to 40 for carriers of two Crohn disease–associated polymorphisms compared with carriers of two wild-type *NOD2/CARD15* alleles. Given a rough prevalence of 1 in 1,000 inhabitants in Western countries, it can be easily calculated that the probability of developing the disease (penetrance) is no more than 0.04 for the group at highest risk. With such a low risk, and in the absence of any preventive action, screening of family members is not currently recommended. It has been estimated, moreover, that the three main *NOD2/CARD15* polymorphisms explain no more than 20% of the genetic predisposition Crohn disease.[41] Such observations are expected for a complex genetic disorder, in which the disease results from the complex interplay between several genetic and environmental risk factors.

Search for Other Crohn Disease–Associated Genes

The number of individual genes that contribute to the overall susceptibility to Crohn disease is, as yet, unknown. Since the initial identification of IBD1 on chromosome 16q, several other genome-wide linkage studies have identified additional IBD susceptibility loci, indicating the complexity of IBD.[57–62] As recently reviewed,[41] significant linkages have been reported on chromosomes 1p, 5, 6, 12, 14, and 19 and suggestive linkages on chromosomes 1q, 3, 4, 7, 10, 16p, 22, and X.[41,57–62] One of the lessons learned from animal models of IBD is that entirely different genetic abnormalities can lead to similar clinical features of intestinal inflammation.[63] Genetic heterogeneity in Crohn disease (the ability of different genes to result in a similar disease phenotype) could well contribute to the discrepancies observed between linkage studies undertaken in different patient populations.[29,41,64] The evidence of two X chromosomal linkages in the huge number of affected sibling pair families studied by Hampe and colleagues[58] is interesting in light of the reported increased incidence of Crohn disease in girls with Turner syndrome.[65]

From localization to actual identification of other susceptibility genes is still a long walk. On chromosome 5q31-33 (IBD5),[60] significant associations have been reported between Crohn disease and several genetic haplotypes in a very narrowed region, which includes a cluster of cytokine genes.[66]

The list of genes studied via association (case control) studies in relation to IBD is growing rapidly. Genes considered and studied as candidates have mainly been those coding for factors involved in regulation of immune and inflammatory processes or maintenance of mucosal barrier function. Major histocompatibility complex (MHC) class II allelic associations are more robust for ulcerative colitis than for Crohn disease, although susceptibility associations have been found for human leukocyte antigen (HLA)-DRB1*0701 and HLA-DRB3*0301 and protective association for HLA-DRB1*1501.[8,67] Allelic variation in the promoter region of the tumor necrosis factor-α (TNF-α) gene within the MHC class III region has been associated with susceptibility to and progression of Crohn disease.[68,69] Other reported associations include polymorphisms in the intercellular adhesion molecule 1.[70] Most of the reported associations do not clearly indicate functional alterations in the gene.

ENVIRONMENTAL INFLUENCES

Environmental factors must also be important in the development of Crohn disease, as evidenced by the rapidly increasing incidence in recent decades[10] and by the lack of complete concordance for disease status among monozygotic twins.[30,31] Environmental influences, if shared by family members, may also contribute to familial aggregation, as is supported by a recent analysis of birth order of affected siblings in multiplex families.[71]

Search for a Specific Microbial Trigger

Over the past 30 years, there has been an intensive search for the antigens that trigger the immune response. The fundamental question regarding the pathogenesis of Crohn disease has been framed as follows: does the chronic, recurring inflammatory activity reflect an appropriate response to a persistently abnormal stimulus (eg, a persistent causative agent in the intestinal lumen) or an abnormally prolonged response to a ubiquitous stimulus?[6] Although the most widely held theory is that Crohn disease constitutes a dysregulated immune response to common bacterial antigens, the search for specific pathogens has not been abandoned. An infectious etiology for Crohn disease, with a direct cause-and-effect relationship between a single microorganism and inflammation in a genetically predisposed host, still remains plausible. The relationship between microbes and defined clinical entities is often ambiguous, but diseases of unknown etiology are unexpectedly proven to be infectious. The most striking example is peptic ulcer disease and *Helicobacter pylori*. A number of putative infectious agents have been proposed and discounted as etiologic factors since Crohn disease was first identified as an entity distinct from tuberculous ileitis.

Mycobacteria. *Mycobacterium paratuberculosis* causes Johne disease, an intestinal disorder in ruminants that clinically and histologically resembles Crohn disease. Mycobacteria (named *M. Linda* after the patient) isolated from a Crohn disease surgical specimen were administered orally to a young goat and caused a disease similar to Johne disease.[72] It is quite possible, however, that this organism, although pathogenic for goats, is merely a nonpathogenic secondary agent in humans. Despite intensive efforts, *Mycobacterium* species similar or identical to *M. paratuberculosis* can be cultured from relatively few patients with Crohn disease. Some, but not all, investigators have identified fragments of its deoxyribonucleic acid (DNA) by polymerase chain reaction more commonly in Crohn disease tissue than in tissue from patients with ulcerative colitis or controls.[73] Current evidence suggests that *M. paratuberculosis* is an environmental contaminant that preferentially invades the deeply ulcerated mucosa of Crohn disease patients to a greater extent than that of controls. The results, however, do not exclude pathologic infection of a subgroup of Crohn disease patients.

Viruses. Interest in a viral etiology of Crohn disease was rekindled by Wakefield and colleagues, who demonstrated evidence of persistent measles infection in foci of granulomatous vasculitis in the intestine of patients with Crohn disease.[74] It has been proposed that measles infection early in life leads to microvascular thrombosis, multifocal gastrointestinal infarction, and, eventually, gross pathologic sequelae such as inflammation, fibrosis, and strictures.[75] This has been tied in with a report of a cohort effect of a measles epidemic in childhood with subsequent Crohn disease and a striking report of three cases of serious Crohn disease in the children of three of four mothers affected with measles during pregnancy.[76] The same group of investigators reported that the first children participating in the measles vaccination program in the United Kingdom were more likely to subsequently develop Crohn disease than were nonvaccinated children.[77] Other investigators, however, have been unable to confirm either the epidemiologic observations or the identification of persistent measles virus infection by immunohistochemical staining of tissue from patients with Crohn disease.[78,79]

Role of Enteric Flora

The recent recognition that altered structure of the *NOD2/CARD15* receptor confers susceptibility to Crohn disease clearly links microbes to its pathogenesis.[2] Indeed, although Crohn disease is not likely usually due to a single mucosal pathogen, several lines of evidence indicate the crucial involvement of the intestinal microflora.[80–83] Increased numbers of bacteria have been reported in both inflamed and noninflamed colonic segments in patients with Crohn disease compared with noninflamed and inflammatory disease controls.[80]

Patients with ileal resections and a diverting ileostomy excluding the neoterminal ileum fail to develop recurrent disease until reanastomosis is performed.[81] Infusion of autologous luminal contents in excluded normal ileal loops of patients with Crohn disease rapidly induces new inflammation, indicating that fecal component(s) may promote disease flare-up.[82] Inflammation is prevented or lessened by a germ-free environment in several knockout and transgenic animal models of IBD.[83,84]

Role of Diet

It is logical to attempt to attribute the changing incidence of gastrointestinal disorders to different dietary exposures. However, no specific dietary toxin or antigen has been incriminated. The rarity of IBD has limited traditional epidemiologic methods of determining causation to case-control studies, which have failed to provide meaningful insight into disease pathogenesis. Persson and colleagues reviewed studies examining the reported preillness intake of refined sugar, cereals, fiber, and milk products of patients with Crohn disease or ulcerative colitis compared with that of controls.[85] An increased intake of refined sugar before the development of symptomatic Crohn disease has been fairly consistently reported, suggesting perhaps a modulating role, but the methodologic weaknesses of study design must be recognized.[85] The association may represent a behavioral adaptation to disease rather than a cause of disease.

Recently, nutritional factors modulating the risk of IBD development have been examined. In a correlation study from Japan, the increasing incidence of Crohn disease in this racially homogeneous population was shown to parallel increasing daily intake of animal protein, total fat, and animal fat, especially ω-6 polyunsaturated fatty acids (PUFAs) relative to ω-3 PUFAs.[86] These trends indicating "Westernization" of diet in Japan were determined from sequential population surveys of dietary habits. Omega-3 PUFAs found in marine oils have an anti-inflammatory effect through modulation of proinflammatory cytokine synthesis.[87]

Risk Factors: Early-Life Exposures

The finding of a birth cohort effect instead of a period effect in two independent Swedish studies indicates that events early in life influence the risk of developing IBD.[88,89] Maternal or neonatal infection has been investigated as the underlying cause of this clustering phenomenon.[90] Breastfeeding in infancy may reduce the risk of developing Crohn disease.[91]

Other Modulating Factors

Smoking. A clear dichotomy between ulcerative colitis and Crohn disease is indicated by the opposing effect of cigarette smoking on the two disorders.[92] Smoking decreases and cessation of smoking increases the risk of developing ulcerative colitis.[92] Smoking is a risk factor for Crohn disease, with a point estimate of between 2 and 5.[92] The risk for former smokers does not differ from the risk in people who have never smoked, indicating that smoking is not an initiator of Crohn disease but rather a promoter. Passive exposure to smoking and active smoking may be influential.[93] The components of tobacco responsible for these observations are uncertain, but most attention has been focused on nicotine. Cigarette smoking has been shown to cause morphologic injury to endothelial cells, leading to the formation of microthrombi. Hence, the increased risk of Crohn disease among smokers has been hypothesized to relate to potentiation of multifocal gastrointestinal infarction.

Oral Contraceptives. Oral contraceptive use has been implicated as a risk factor for Crohn disease, but the results are not as consistent as those for smoking, and the risk estimates are generally lower. In some studies, the increased risk has only been found in subgroups of smoking patients.[94] Oral contraceptive use may modestly increase the risk of Crohn disease 1.5 and 2.6 times in current smokers. It is biologically plausible that oral contraceptive use might further promote Crohn disease through similar effects on the mesenteric vasculature.

HOST-ENVIRONMENT INTERACTIONS

The intestinal inflammation of Crohn disease may be viewed as an exaggeration of the "physiologic" inflammatory response always present in the normal lamina propria of the intestine and colon. An enormous antigenic load is regularly presented through the lumen of the gastrointestinal tract, but an intact mucosal barrier and regulatory mechanisms normally prevent the immune and inflammatory responses important in defense against pathogenic agents from proceeding to cause tissue injury. A defect in mucosal barrier function, in antigen processing, or in immunoregulation could result in a chronic inflammatory state, lymphocyte proliferation, cytokine release, recruitment of auxiliary effectors such as neutrophils, and eventual tissue damage. Elucidation of the genetic basis of Crohn disease has the potential to uncover the primary mechanisms underlying its development. Identification of the *NOD2/CARD15* susceptibility gene has provided a tangible basis for investigating the interactions between host and environment that culminate in chronic inflammation.

Defective Mucosal Barrier

Mucosal permeability is increased in Crohn disease, but whether the defect is primary or secondary remains controversial. Approximately 10% of asymptomatic family members of Crohn disease patients exhibit increased permeability, and an additional subset have enhanced permeability after exposure to nonsteroidal anti-inflammatory drugs.[95,96] Whether this abnormal permeability is indicative of subclinical intestinal inflammation or represents a genetically determined predisposing factor is unclear. There is little doubt, however, that excessive permeability, allowing macromolecular uptake of bacterial and other luminal antigens, plays a role in perpetuating the inflammation in IBD.

Immunoregulatory Abnormalities

In general, immune-mediated inflammation can result from activation of innate or adaptive immune responses, which are tightly linked. Adaptive immune responses are initiated when antigen is presented to a T lymphocyte by an antigen-presenting cell. The innate immune system regulates the immediate response to microbial pathogens through "pattern recognition" receptors, which recognize bacterial products and constituents.

Defective Innate Immunity. *NOD2/CARD15* belongs to a family of such pattern recognition receptors located intracellularly, in contrast to Toll-like receptors located on the cell membrane.[97] As expected for a gene involved in innate immunity, *NOD2/CARD15* is mainly expressed in the monocyte-macrophage lineage (including dendritic cells) and granulocytes, cells present in Crohn disease lesions and involved in granuloma formation. Epithelial cells are able to express NOD2/CARD15 when activated, but the biologic relevance of this observation remains to be clarified.

Initially, lipopolysaccharide was thought to be the bacterial component recognized by the *NOD2/CARD15* receptor, but recent work has concluded instead that it is muramyl dipeptide, a component of peptidoglycan, that is associated with various bacteria and frequently contaminates preparations of lipopolysaccharide.[98] As illustrated in Figure 41.1-3, stimulation of *NOD2/CARD15* with bacterial proteins results in activation of the NF-κβ signaling cascade. Such activation occurs through receptor interacting protein 2 (RIP2, also

called RICK), a serine/threonine kinase that phosphorylates the inhibitor of NF-κB kinase and therefore allows the transport of NF-κβ to the nucleus.[99] Moreover, *NOD2/CARD15* may be up-regulated by proinflammatory cytokines, such as TNF-α, via a NF-κβ binding element in its promoter region, contributing to amplification of the inflammatory process.[100]

The recognition of *NOD2/CARD15* as a susceptibility gene identifies a primary abnormality in Crohn disease for the first time. Precisely how *NOD2/CARD15* polymorphisms confer susceptibility cannot as yet be definitively answered. The presence of caspase activation and recognition domains indicates that *NOD2/CARD15* plays a role in apoptosis. However, the Crohn disease–associated *NOD2/CARD15* polymorphisms are located in the LRR domain of the gene or in its vicinity, suggesting a defect in host-bacteria interaction, as had long been hypothesized. It is surprising, however, that the *NOD2/CARD15* Leu1007fsinsC frameshift mutation appears in functional experiments to be associated with a loss of NF-κβ activation in the presence of bacterial components.[2,101] In contrast, distinct polymorphisms in the *NOD2/CARD15* gene are associated with increased NF-κβ activation in Blau syndrome, another granulomatous condition.[102] Uveitis, arthritis, and skin rashes, but no digestive tract involvement, characterize this rare dominant mendelian trait.[102] It is postulated that Blau syndrome results from basal overactivation of the *NOD2/CARD15* pathway, not requiring interaction with bacterial components and by direct consequence without digestive lesion.[102]

The loss of function associated with Crohn disease–associated *NOD2/CARD15* polymorphisms is not as yet reconciled with the excess of NF-κβ activation observed in intestinal lesions. It is suggested that the defect in innate immunity may secondarily trigger an aberrant T-cell inflammatory response, leading to abnormal cytokine production, deregulated NF-κβ activation, and tissue damage.[63]

It remains to be seen whether other susceptibility genes, which undoubtedly exist, also code for proteins involved in the interpretation of the microbial environment by the innate immune system.

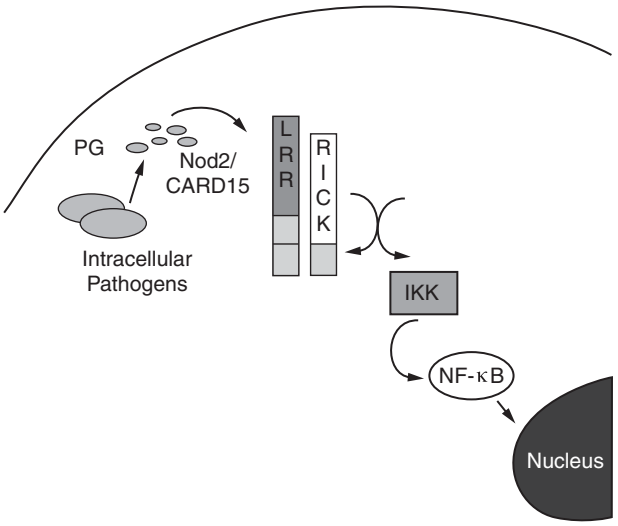

FIGURE 41.1-3 NOD2/CARD15 function in the innate immune response. Stimulation of NOD2/CARD15 by bacterial components, particularly peptidoglycan (PG), results in activation of the nuclear factor-κB (NF-κB) signaling cascade. Such activation occurs through RICK, a serine/threonine kinase that phosphorylates IKK (inhibitor of NF-κB kinase), thereby allowing transport of NF-κB into the nucleus. Crohn disease–associated polymorphisms in the *NOD2/CARD15* gene are associated with a loss of NF-κB activation in the presence of bacterial components. LRR = leucine-rich repeat; RICK = receptor interacting protein 2.

Adaptive Immune Response. Until the discovery of the *NOD2/CARD15* gene, most investigations of the pathogenic mucosal immune response in Crohn disease focused on the role of T cells, which are clearly important effectors. The pathophysiology of chronic intestinal inflammation from T-cell activation to tissue injury is summarized schematically in Figure 41.1-4. T-cell activation occurs on presentation of antigen by a macrophage or other antigen-presenting cell to a $CD4^+$ T cell. The antigen-specific activation of $CD4^+$ T cells occurs through binding of the CD4 molecule to MHC class II molecules bearing processed antigen on the surface of the activated macrophage. Following activation, $CD4^+$ T lymphocytes can differentiate into two subpopulations designated T helper 1 (Th1) and 2 (Th2) cells, which produce, respectively, predominantly interferon-β, interleukin (IL)-1, TNF-α, and IL-6 (Th1 cytokines) or IL-4, IL-5, and IL-10 (Th2 cytokines). Whether Th1 or Th2 responses predominate appears to depend on the specific costimulatory signal, the nature and concentration of the antigen, and the prevailing cytokine milieu.[63] Intracellular bacteria, which activate macrophages, promote Th1 cell differentiation by stimulating the synthesis of IL-12, the major Th1-inducing cytokine. Th1 responses predominate in Crohn disease. Amplification or suppression of the inflammatory response by T cells and macrophages depends on the relative balance of proinflammatory and anti-inflammatory immunoregulatory cells and mediators.[63] The unrestrained intestinal Th1 activation in Crohn disease appears to be due to defective regulation via regulatory T-cell populations and to T-cell resistance to normal apoptotic signals.[7,103] Tissue damage results from downstream effects of activated Th1 cells. Th1 cytokines, such as TNF-β, have many functions, including induction of the expression of adhesion molecules, thereby contributing to the recruitment of monocytes, lymphocytes, and granulocytes. Cells recruited to the mucosa release a vast number of substances with nonspecific inflammatory but directly injurious properties. These include arachidonic acid metabolites (prostaglandins, thromboxane, leukotrienes), free radicals (reactive oxygen metabolites and nitric oxide), platelet activating factor, and various proteases.

Control of Mucosal Immune Response. The mucosal immune system must tightly control the balance between responsiveness and nonresponsiveness (tolerance) to the millions of antigens continuously passing along the mucosal surface. The ability to distinguish between commensal and pathogenic organisms is vital to a normal mucosal immune system. Attempting to explain all of the epidemiologic and pathophysiologic observations, as dis-

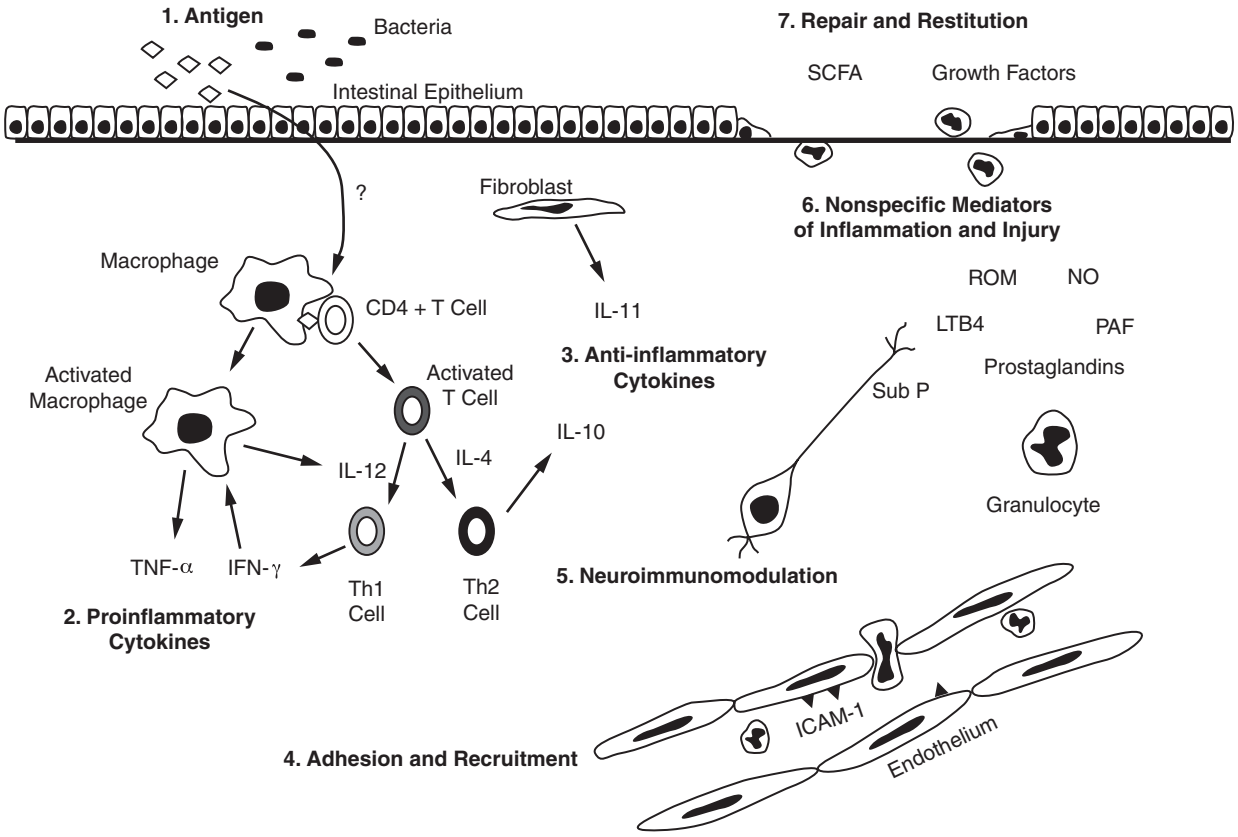

FIGURE 41.1-4 Proposed pathophysiology of chronic intestinal inflammation. Numbers in parentheses indicate sites for therapeutic interventions. The persistent mucosal inflammation in inflammatory bowel disease is triggered by antigen (1) believed to be bacterial in origin. Macrophages process antigen and present it in the context of human leukocyte antigen class II to CD4+ T cells. On activation, macrophages elaborate the proinflammatory cytokines (2) tumor necrosis factor-α (TNF–α) and interleukin (IL)-12, inducing T helper 1 (Th1) responses. Alternatively, elaboration of IL-4 may promote differentiation toward T helper 2 (Th2) responses and expression of the anti-inflammatory cytokine (3) IL-11. Cells are recruited from the periphery (4) via coordinated expression of integrins, chemokines, and adhesion molecules such as intercellular adhesion molecule 1 (ICAM-1). Elaboration of neutropeptides, such as substance P (Sub P), may modify the local responses (5). A variety of nonspecific mediators of inflammation and injury (6) may affect tissue destruction directly. Such mediators include reactive oxygen metabolites (ROM), nitric oxide (NO), leukotriene B_4 (LTB_4), platelet activating factor (PAF), and prostaglandins. Finally, host responses may induce nonspecific mechanisms of restitution of the wound and repair (7). Short-chain fatty acids (SCFA) and growth factors may contribute to this process. Adapted with permission from Sands BE. Novel therapies for inflammatory bowel disease. Gastroenterol Clin North Am 1999;28:324.

cussed above, it has been hypothesized that Crohn disease may be a disorder of mucosal immune interpretation of the microbial environment.[7] Genetic variants increasing susceptibility to Crohn disease and other chronic inflammatory disorders, such as allergies and asthma, may have persisted and expanded in human populations owing to a beneficial effect in mediating host-microbial interactions in an unsanitary world.[7] The survival advantage previously afforded by enhanced mucosal immune reactivity becomes a risk factor for immune-mediated disease as lifestyle and environmental conditions change.[7,104] Environmental factors, hence, may exert their influence and account for the changing incidence of Crohn disease at the level of immune regulation rather than via a transmissible infectious agent.[7]

PATHOLOGY

Anatomic Distribution

Crohn disease is a panenteric inflammatory process. Endoscopy with biopsies commonly identifies histologic abnormalities throughout the gastrointestinal tract. Nevertheless, classification according to the distribution of gross disease evident radiologically or macroscopically at endoscopy still guides treatment and may be useful prognostically. In comparison with the continuous distribution of ulcerative colitis, Crohn disease is characteristically segmental, with spared areas throughout the intestinal tract. The terminal ileum is the most commonly affected site.

During the most recent decade (1990–1999) at The Hospital for Sick Children, Toronto, 386 children and adolescents were evaluated at initial presentation by colonoscopy and small bowel radiography. Twenty-nine percent have had terminal ileal with or without cecal disease, 9% more isolated proximal (ileal or jejunal) disease, 42% ileocolonic inflammation, and 20% colon-only involvement. The extent of the disease was evaluated by colonoscopy and small bowel radiography. Data from 12 pediatric series, as pooled by Barton and Ferguson, were similar.[105] Crohn disease was localized to the small intestine (breakdown of terminal ileum versus diffuse or more

proximal small intestine not given) alone in 38%, in combination with the colon in another 38%, and in the colon only in 20%. Upper endoscopy with biopsy, if routinely performed, frequently reveals at least microscopic involvement of the esophagus, stomach, or duodenum, but gastroduodenal disease is only rarely the sole or predominant site of Crohn disease.[106]

Macroscopic Appearance

Gross inspection of the bowel in well-established Crohn disease reveals marked wall thickening. Mural thickening is the result of chronic inflammation and edema in all layers and is accompanied by luminal narrowing. The mesentery is thickened and contracted and may fix the intestine in one position. Mesenteric lymph nodes are frequently enlarged. Fat extends from the mesentery and "creeps" over the serosal surface of the bowel. The transmural inflammation may cause loops of intestine to be matted together. Fistulae are thought to arise when inflammation extends through the serosa into adjacent structures, such as another loop of bowel, the urinary bladder, or the vagina. Alternatively, a fistulous tract ends blindly in an inflammatory mass (phlegmon) adjacent to the bowel. Stricture formation may occur as a result of fibrous tissue proliferation involving first the submucosa and then the deeper layers of the bowel wall.

The earliest lesion of Crohn disease is the aphthous ulcer, which typically occurs over Peyer patches in the ileum and over lymphoid follicles in the colon. As the disease progresses, aphthoid ulcers enlarge and become stellate and, eventually, deeper longitudinal and transverse linear ulcers. Remaining islands of nonulcerated mucosa give a "cobblestone" appearance. Fissures develop from the base of ulcers and extend through the muscularis to the serosa. Free perforation is uncommon because serositis induces the adherence of other bowel loops into which the fissure extends. Bowel inflammation and ulceration are characteristically punctuated by "skip areas" of grossly and even microscopically normal mucosa.

Microscopic Findings

Mucosa that is thought to be normal grossly often reveals abnormalities such as edema and an increase in mononuclear cell density in the lamina propria. Microscopic inflammation in such relatively uninvolved sites is often strikingly focal.[107] Even within one histologic section, inflammation may be immediately adjacent to an uninflamed microscopic skip area. Mucosal changes may resemble ulcerative or infectious colitis with infiltration of the crypts by polymorphonuclear leukocytes (cryptitis or crypt abscesses) and distortion of crypt architecture. However, the presence of fibrosis and histiocytic proliferation in the submucosa suggests Crohn disease. The pathologic hallmark is transmural extension to all layers of the bowel wall and adventitia.[107]

Granulomas are not always found in pathologic specimens from patients with Crohn disease. The likelihood of finding granulomas in biopsy specimens is a function of the number of specimens taken, the number of sections examined, and the definition of a granuloma. Granulomas occur more commonly in the submucosa than in the mucosa. Hence, they are observed in 60% of surgical specimens but, less frequently, in 20 to 40% of mucosal biopsies (Figure 41.1-5).[107] Granulomas can also be found in lymph nodes, mesentery, and peritoneum. Intestinal granulomas can be found in a number of infectious diseases, including tuberculosis, fungal infections, chlamydial infections, and yersiniosis, as well as in sarcoidosis and foreign-body reactions. Granulomas in Crohn disease lack the caseating necrosis evident in tuberculosis.

CLINICAL PRESENTATION

The prevalence of individual symptoms among children and adolescents at the time of their diagnosis with Crohn disease is outlined in Table 41.1-3. As is evident in the table, the reported common symptoms during a year of prospective surveillance for pediatric Crohn disease in the United Kingdom and Ireland[22] were remarkably similar to

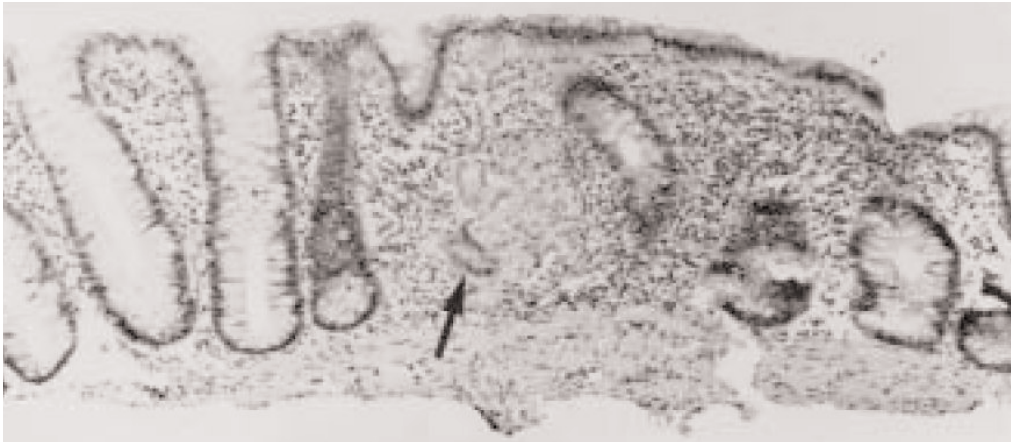

FIGURE 41.1-5 Colonic biopsy specimen from a child with active Crohn disease. Notice the distortion of the crypt architecture with a prominent noncaseating granuloma and giant cells (*arrow*) amid increased acute and chronic inflammation in the lamina propria (hematoxylin and eosin; × 40 original magnification).

TABLE 41.1-3 PREVALENCE OF INDIVIDUAL SYMPTOMS AT THE TIME OF DIAGNOSIS OF CROHN DISEASE*

	PERCENTAGE OF PATIENTS WITH EACH INDIVIDUAL SYMPTOM	
SYMPTOM	TORONTO PEDIATRIC IBD DATABASE (*N* = 386)	UNITED KINGDOM AND IRELAND SURVEILLANCE (*N* = 379)
Abdominal pain	86	72
Diarrhea	78	56
Blood in the stool	49	22
Weight loss	80	58
Fevers	38	Not stated
Perianal lesions	8 fistula or abscess, 19 tags, 22 fissures	7 fistula or abscess
Arthralgias/arthritis	17	8
Mouth ulcers	28	Not stated
Skin lesions	8	1

IBD = inflammatory bowel disease.
*Prospectively recorded single-center data from The Hospital for Sick Children, Toronto, during a 10-year period, 1990–1999, are compared with multicenter data reported by pediatric gastroenterologists as part of the United Kingdom/Ireland Inflammatory Bowel Disease Surveillance Project.[21]

those prospectively recorded during the past decade (1990–1999) among newly diagnosed children in Toronto. The mean interval between development of these presenting symptom(s) and diagnosis at The Hospital for Sick Children, Toronto, was 5.5 months. Several observations warrant emphasis.

In comparison with those of ulcerative colitis, the initial symptoms of Crohn disease are more subtle and varied, in part a reflection of its diffuse and diverse anatomic localization. A careful physical examination may detect clues to underlying Crohn disease when the presenting symptoms such as abdominal pain without other overt intestinal symptoms, isolated anemia, or weight loss do not immediately suggest IBD. A plateau in linear growth, delayed pubertal development, perianal lesions, and finger clubbing are tell-tale signs that may be easily overlooked unless specifically sought.

Classic Presentation

The constellation of abdominal pain, diarrhea, poor appetite, and weight loss constitutes the classic presentation of Crohn disease in any age group. As shown in Table 41.1-4, this symptom complex (with and without extraintestinal manifestations of IBD) comprises the mode of presentation in nearly 80% of children and adolescents. Abdominal pain is the most common single symptom at presentation. It is often periumbilical but may localize to the right lower quadrant or diffusely to the lower abdomen with colonic disease. Diarrhea, although common, need not be present, especially when disease is confined to the small intestine. Diarrhea, especially in the absence of significant left-sided colonic disease, is often not grossly bloody, unless there is bleeding from a perianal fissure. The less common modes of presentation in Table 41.1-4 deserve comment because they are more likely to be associated with diagnostic confusion and delay.

Growth and Pubertal Delay

Crohn disease may present as short stature. Impairment of linear growth and concomitant delay in sexual maturation may precede the development of intestinal symptoms and dominate the presentation.[108] Impaired growth in a young adolescent becomes strikingly obvious as healthy peers experience the rapid increase in height associated with normal puberty. Affected adolescents are often normal weight for height but low height for age, that is, stunted. As is discussed in detail later in this chapter, there is evidence that *both* chronic undernutrition secondary to anorexia *and* proinflammatory cytokines produced by the inflamed intestine contribute to the observed alterations in growth.[109,110]

Perianal Disease

Perianal lesions may be the isolated presenting feature of Crohn disease and an accompanying sign of other gastrointestinal symptoms. Perianal fistulae, large tags, or recurrent perianal abscesses in any child warrant investigation to exclude Crohn disease.

TABLE 41.1-4 MODES OF PRESENTATION OF CROHN DISEASE*

MODE	N (%)
Classic presentation (abdominal pain, diarrhea, weight loss ± extraintestinal manifestations)	235 (78.6)
Growth failure predominating	10 (3.3)
Extraintestinal manifestations predominating	25 (8.4)
Arthritis	13
Recurrent fevers	8
Recurrent oral ulcers	1
Oral cheilitis	1
Pyoderma gangrenosum	1
Recurrent acute pancreatitis	1
Anemia (as the major complaint)	8 (2.7)
Perianal disease predominating	11 (3.7)
Anorexia, weight loss predominating	6 (2.0)
Laparotomy for acute abdominal pain	4 (1.3)
Total	299

Adapted from Griffiths AM. Crohn disease. In: David TJ, editor. Recent advances in pediatrics. Edinburgh: Churchill Livingstone; 1992. p. 145–60.
*Data from The Hospital for Sick Children, Toronto, from 1980–1989.

Anemia

Iron deficiency anemia, unless explained by abnormal menstrual losses, reflects gastrointestinal blood loss until proven otherwise. Crohn disease is one condition that must be included in the differential diagnosis, even if other suggestive symptoms are not readily apparent.

Weight Loss

With similar subtlety, Crohn disease can masquerade as anorexia nervosa when weight loss predominates.

EXTRAINTESTINAL MANIFESTATIONS

Inflammatory extraintestinal manifestations of Crohn disease are more common with colonic disease than with isolated small bowel disease.[111,112] The most common target organs are the skin, joints, liver, eye, and bone.[111,112] At least one extraintestinal manifestation is seen in about 25 to 35% of adult patients with IBD.[111] The prevalence would likely be higher in children if fever was included. As previously reviewed, inflammatory extraintestinal manifestations in children most often parallel the activity of intestinal inflammation, but some follow an independent course.[112] Appreciation of this relationship determines whether specific therapy of the extraintestinal lesion is required. Other complications, although not inflammatory themselves, result directly from the presence of diseased bowel. These include nephrolithiasis and ureteral obstruction. Growth impairment is discussed separately and in detail because of its extreme significance and its multifactorial pathogenesis.

Joints

Arthritis is less common than arthralgias, but together they constitute the most common extraintestinal manifestations of Crohn disease (15%) in young patients.[112] As exemplified in Table 41.1-4, arthritis is the most likely of the extraintestinal manifestations to precede gastrointestinal manifestations of Crohn disease and dominate the clinical presentation. Typically, a few large joints are involved, especially the knees, hips, and ankles.[112,113] The appropriately named episodes of "colitic arthritis" tend to reflect inflammatory activity in the intestine and settle without deformity as the bowel disease is treated. An exception is the arthritis of juvenile ankylosing spondylitis, which occurs more frequently in IBD than in the general population and not only in those positive for HLA-B27.[112,113] Peripheral arthritis of the hips or sacroiliac joints precedes the spinal arthropathy, often requires specific anti-inflammatory treatment, and may progress independently of the bowel disease.[113]

Skin

The most commont cutaneous manifestations (with frequencies of occurrence based on a large adult series of Crohn disease patients) are erythema nodosum (8–15%) and pyoderma gangrenosum (1.3%).[111] Erythema nodosum tends to occur when the intestinal disease is active but does not necessarily indicate its severity. Pyoderma gangrenosum often runs its own course and requires specific medical treatment.[112]

Eye

Ocular lesions appear to be less common in young patients than in adults, but acute episcleritis. uveitis, and, rarely, orbital myositis do occur.[112] Asymptomatic uveitis has been described in Crohn disease predominantly when only the colon is involved, but there is no convincing evidence that it progresses to destructive ocular disease.[114]

Hepatobiliary

Primary sclerosing cholangitis (PSC) is associated most often with ulcerative colitis but may also occur with Crohn disease involving the colon. PSC preceded the clinical onset of the bowel disease in 50% of 17 predominantly adolescent patients (ulcerative colitis in 14, Crohn disease of the colon in 3) with the PSC-IBD combination identified in Toronto.[115]

Pancreas

Acute pancreatitis may occur, albeit rarely, as an extraintestinal manifestation of either ulcerative colitis or Crohn disease.[116] It can also be a direct complication of duodenal Crohn disease, PSC, or drug therapy (azathioprine, 6-mercaptopurine [6-MP], and 5-aminosalicylic acid [5-ASA]).

Renal

Renal complications of Crohn disease are not themselves inflammatory in nature but rather result from the presence of diseased bowel. These include ureteric obstruction and hydronephrosis from thickened, chronically inflamed bowel and an increased incidence of urinary stones.[117] Oxalate, urate, and phosphate stones may occur. In normal persons, oxalate in the lumen is bound to calcium, and the poorly absorbed calcium oxalate is excreted in the feces. However, when the ileum is diseased or has been resected, increased luminal concentrations of malabsorbed fatty acids compete for calcium. Oxalate absorption and subsequent renal excretion are therefore increased.

Vascular

Hypercoagulability from thrombocytosis, hyperfibrinogenemia, elevated factor V and factor VII, and depression of antithrombin III is seen in some patients with IBD.[118] Vascular complications have included deep vein thrombosis, pulmonary emboli, and cerebrovascular disease.[118]

Bone

Decreased bone density is increasingly recognized in Crohn disease.[119–122] The pathogenesis is multifactorial, but a major contributory factor appears to be inhibition of bone formation by cytokines.[123] As recently reported, bone density was reduced to osteoporotic levels in 25% of newly diagnosed children with Crohn disease prior to any corticosteroid therapy.[124] Rapid increments in bone mass are normally acquired during puberty, and peak bone mass attained is an important determinant of risk for subsequent osteoporosis in later life. Hence, childhood-onset Crohn disease may be a particular risk factor for long-term morbidity from clinical osteoporosis. Monitoring of bone densitometry and modification of other risk factors (exercise,

reduction in steroid use, hormonal treatment of chronic secondary amenorrhea in adolescent girls, calcium and vitamin D supplementation) are recommended. Treatment with bisphosphonates is now common among adult patients with Crohn disease and significantly decreased bone density, but experience with these agents in children has been very limited to date.

DISEASE COMPLICATIONS

Malnutrition

Weight loss and emaciation are the most prevalent nutritional disturbances in IBD. At the time of first diagnosis, approximately 85% of pediatric patients with Crohn disease have lost weight.[125] Multiple factors contribute to malnutrition. However, reduced intake, rather than excessive losses or increased needs, is the major cause of the caloric insufficiency. The child or adolescent may voluntarily refrain from eating to avoid aggravation of abdominal cramps and diarrhea. Moreover, disease-related anorexia may be profound. Cytokines produced by the inflamed bowel are likely responsible; TNF-α has been shown to produce anorexia.[110]

Intestinal malabsorption may factor in the equation leading to energy imbalance but is seldom a major cause. In Crohn disease involving the ileum, the processes of fat digestion and absorption may be altered either by loss of gut surface area owing to inflammation or by depletion of the circulating bile salt pool owing to bile acid malabsorption in the diseased ileum or deconjugation by bacteria. In a study years ago of adults prior to intestinal resection, Filipsson and colleagues found predominantly mild steatorrhea in 24% of patients with ileal disease, 26% of those with ileocolonic involvement, and 17% of those with Crohn colitis.[126]

Increased energy expenditure associated with active inflammation has been suggested as a further mechanism accounting for the frequency of malnutrition. In general, resting energy expenditure (REE) does not differ from normal in patients with inactive disease but can exceed predicted rates in the presence of fever and sepsis.[127] Furthermore, in comparison with comparably malnourished patients with anorexia nervosa, a lack of compensatory reduction in REE has recently been described in adolescents with Crohn disease.[128] A reduction in REE is a normal biologic response to conserve energy. Production of inflammatory mediators may explain the lack of REE adaptation in patients with Crohn disease and further augment the ongoing malnutrition.

Growth Impairment

Chronic malnutrition translates into impairment of linear growth and pubertal development in pediatric IBD. Inflammatory disease occurring during early adolescence is likely to have a major impact on nutritional status and growth because of the very rapid accumulation of lean body mass that normally occurs at this time. Further, boys are more vulnerable to disturbances in growth than girls are because their growth spurt comes at a later stage of normal pubertal development and is ultimately longer and greater.

Prevalence. Several recent studies have characterized the growth of children with Crohn disease as treated in the 1980s and into the 1990s.[129–133] These studies are important as a benchmark of outcomes with traditional therapy. It is to be hoped that the now better understanding of the pathogenesis of growth impairment, together with the greater efficacy of immunomodulatory and emerging biologic therapies in treating intestinal inflammation, may lead to enhanced growth of young patients diagnosed in the present decade.

As summarized in Table 41.1-5, the percentage of patients with Crohn disease whose growth is affected varies with the definition of growth impairment and with the nature of the population under study (tertiary referral center versus population based).[129–133] It has nevertheless been consistently observed that impairment of linear growth is common prior to recognition of Crohn disease and during the subsequent years and that height at maturity has often been compromised.[129–133] Height velocity is the most sensitive parameter by which to recognize impaired growth. It is important to obtain preillness heights so that the impact of the IBD can be fully appreciated. The greater the height deficit at diagnosis, the greater the demands for catch-up growth. This emphasizes the need for early recognition of Crohn disease in young patients and for new approaches to optimize the catch-up growth of those diagnosed late.

Pathophysiology. As summarized in Table 41.1-6, several interrelated factors contribute to growth impairment in children with Crohn disease. Chronic undernutrition has long been implicated and remains an important and

TABLE 41.1-5 PREVALENCE OF LINEAR GROWTH IMPAIRMENT IN PEDIATRIC CROHN DISEASE

STUDY	TIME OF ASSESSMENT	PATIENTS STUDIED	DEFINITION OF LINEAR GROWTH IMPAIRMENT	PERCENTAGE WITH GROWTH IMPAIRMENT
Kanof et al[108]	At diagnosis	Prepubertal (Tanner 1 or 2) patients (n = 50)	Decrease in height velocity	88
Kirschner[133]	At diagnosis		Decrease in height centile > 1 SD	36
Griffiths[129]	During follow-up	Prepubertal (Tanner 1 or 2) patients (n = 100)	Height velocity ≤ 2 SD for age for ≥ 2 yr	49
Hildebrand[130]	Before diagnosis or during follow-up	Population-based cohort of 46 children	Height velocity ≤ 2 SD for age for 1 yr	65
Markowitz[131]	At maturity	38 children in tertiary care setting	Failure to reach predicted adult height	37

TABLE 41.1-6 FACTORS CONTRIBUTING TO GROWTH ABNORMALITIES IN CHILDREN WITH CROHN DISEASE

FACTOR	REASON
Cytokines produced by chronically inflamed intestine	Direct role of inflammatory cytokines in linear growth inhibition (IGF-1 inhibition; interference with kinetics of bone growth)
Insufficient caloric intake	Food avoidance because of exacerbation of gastrointestinal symptoms by eating; cytokine-mediated anorexia
Stool losses	Mucosal inflammation leading to protein-losing enteropathy; steatorrhea if extensive
Increased nutritional needs	Fever, chronic deficits
Corticosteroid treatment	Inhibition of IGF-1

IGF-1 = insulin-like growth factor 1.

remediable cause of growth retardation.[109] A simple nutritional hypothesis fails, however, to explain all of the observations related to growth patterns among children with Crohn disease. Within the past decade, the direct growth-inhibiting effects of proinflammatory cytokines released from the inflamed intestine have been increasingly recognized and are now the focus of very intriguing research.[110,123,134] It is postulated that inflammatory mediators, such as TNF-α and IL-6 secreted from the diseased gut, may interfere with growth plate kinetics and thereby suppress linear growth.[110,123] Daily corticosteroid use may also inhibit growth, but it is often difficult to separate the relative contributions of disease activity from corticosteroid use in the pathogenesis of slow linear growth in pediatric Crohn disease.[129,132]

Insulin growth factor 1 (IGF-1), produced by the liver in response to growth hormone stimulation, normally mediates growth hormone's effects on the growth plate of bones. An association between impaired growth in Crohn disease and low IGF-1 levels is well recognized.[135] Malnutrition,[135] cytokines,[134,136] and chronic daily corticosteroid therapy[137] may all suppress IGF-1 production. Recently, transgenic mice with defective growth were found to overexpress IL-6.[134] Antibody to IL-6 partially corrected the growth defect, whereas administration of IL-6 led to a decrease in IGF-1 before food intake was affected. Further, IGF-1 levels were negatively correlated with IL-6 among children with juvenile rheumatoid arthritis. These findings suggest that an IL-6–mediated decrease in IGF-1 production may represent a major mechanism by which chronic inflammation leads to stunting of growth.

From the foregoing discussion, it is clear that enhancement of linear growth is best achieved through control of intestinal inflammation without chronic corticosteroid therapy and assurance of adequate nutrition.[138]

Perianal Disease

Perianal abscesses and fistulae occur in one-third of patients over time, more frequently in association with colorectal disease in comparison with small intestinal disease.[139] When skin tags and fissures are included, the prevalence of perianal disease among pediatric patients at tertiary care centers is 14 to 62%.[139] Tags and fissures are often asymptomatic, but large inflamed tags, deeper fissures, perianal fistulae, and abscesses may contribute significantly to the morbidity of Crohn disease. A recent analysis suggests that the IBD5 risk haplotype at the chromosome 5q31 locus is associated with perianal lesions, providing further molecular evidence of a genetic basis for the clinical heterogeneity of Crohn disease.[140] This putative association will need to be re-examined when an actual gene is identified at this locus.

INVESTIGATION AND DIAGNOSIS

There is no single test that can confidently confirm the suspicion of Crohn disease. The diagnosis is made based on a compatible clinical presentation, substantiated by radiologic assessment of the small bowel, endoscopy of the ileocolon with pathologic examination of mucosal biopsies, and exclusion of other causes of chronic intestinal inflammation. *Salmonella*, *Shigella*, *Campylobacter jejuni*, enteropathogenic *Escherichia coli*, and *Clostridium difficile* infections are excluded with stool cultures. Serologic techniques are important in the diagnosis of *Yersinia* infections and amebiasis. Intestinal tuberculosis and schistosomiasis should be excluded when risk factors exist.

Ultrasonography has been used as a noninvasive screening test for suspected Crohn disease. Radiology and ileocolonoscopy (with mucosal biopsies for histologic assessment) are used to define the nature and extent of intestinal inflammation and to distinguish ulcerative colitis from Crohn disease. Differentiation of isolated colonic Crohn disease from ulcerative colitis is made on the basis of macroscopic and microscopic criteria. When features of both types of IBD are present, the designation of indeterminate colitis is applied, pending clarification with time and subsequent evaluations.[141]

Some clinicians recommend upper gastrointestinal endoscopy with biopsies as an adjunctive tool in differentiating the type of colitis, but caution must be exercised in interpreting all gastric or duodenal inflammmation as indicative of Crohn disease.[142] Nonspecific histologic gastritis is common in ulcerative colitis and in Crohn disease; focal antral gastritis may be more specific for Crohn disease[142]; the finding of granulomata on gastric antral biopsy may clarify a diagnosis of Crohn disease in an otherwise indeterminate colitis.[142] Technologic advances in magnetic resonance imaging (MRI) or ultrasonography, which allow assessment of the depth of intramural inflammation, may provide adjunctive information facilitating differentiation of Crohn colitis from ulcerative colitis.[143,144] However, in a pilot study of MRI of the colon in children with IBD, there was poor interobserver reliability between radiologists and poor correlation with diagnosis based on conventional endoscopic and histologic criteria.[145]

Serologic Tests in the Diagnosis of IBD

Recently, antineutrophil cytoplasmic antibodies (ANCAs) and anti-*Saccharomyces cerevisiae* antibody (ASCAs) have been recommended as tools to facilitate screening for IBD among children with suggestive symptoms and to differentiate ulcerative colitis from Crohn disease.[146]

ASCAs are immunoglobulin (Ig)G and IgA antibodies that recognize mannose sequences in the cell wall of *S. cerevisiae* strain Su1. ASCA is detected in 55 to 60% of children and adults with Crohn disease and in 5 to 10% of controls with other gastrointestinal disorders, findings indicating good specificity but relatively poor sensitivity.[146–148] The specificity of the antibody response makes it unlikely that the elevated titers result merely from increased intestinal permeability. It is possible that *S. cerevisiae* shares antigenic determinants with another organism of true etiopathogenetic significance in Crohn disease. An increased prevalence in relatives is reported.[149] Whether such clustering is due to genetic or environmental factors is unknown.

ANCAs, originally described in Wegener granulomatosis and necrotizing vasculitis, are IgG antibodies directed against cytoplasmic components of neutrophils. The association with IBD of a subset of ANCA with a perinuclear staining pattern on immunofluorescence studies (pANCA) was first recognized for ulcerative colitis.[150] The specificity of perinuclear staining for IBD can be confirmed by its disappearance after desoxyribonuclease digestion of neutrophils. Studies in both adult and pediatric patients have consistently supported the specificity of pANCA for IBD versus infectious colitides and other gastrointestinal disorders.[148,151,152] However, sensitivity is poor because pANCA is positive in only 50 to 65% of pediatric patients with ulcerative colitis.[148,152] ANCAs are produced by B cells in the lamina propria of the colonic mucosa but do not appear to contribute directly to tissue injury in IBD. Rather, pANCA is considered a marker of the immunologic disturbance that underlies the development of chronic colonic inflammation. Further, there is evidence that HLA class II genes influence ANCA status.[153] Hence, ANCA may prove to be a marker of heterogeneity of genetic susceptibility to IBD.[153]

The relatively low sensitivities of serology for Crohn disease and ulcerative colitis argue against there being any additional value for ASCA or ANCA as routine or first-line screening tests for IBD compared with clinical acumen and the equally sensitive (albeit less specific) measurement of acute-phase reactants. Moreover, the need to perform definitive radiologic and endoscopic studies to guide therapy by defining the extent and nature of disease will not be averted by positive serologic tests.

Combined ANCA or ASCA testing has recently been recommended to help differentiate Crohn disease from ulcerative colitis.[146] However, differentiation of Crohn disease from ulcerative colitis is clinically problematic only when inflammation is largely confined to the colon. pANCA is positive in up to 35% of patients with colonic Crohn disease, and ASCA is less often detected in patients with Crohn disease confined to the colon.[147,148,154,155] Hence the utility of serology is less in the setting where it is needed most. In the one published study clearly reporting sensitivity, specificity, and predictive values of combined serologic testing, the sensitivity of ASCA+pANCA– serology for Crohn colitis versus ulcerative colitis was only 32%.[147] However, the specificities of ASCA+pANCA– serology for Crohn colitis and pANCA+ASCA– serology for ulcerative colitis are high, so the predictive values are also high in the presence of a positive test.

TREATMENT

Basic Principles Governing Treatment

The challenge in treating each child or adolescent with Crohn disease is to employ pharmacologic, nutritional, and, where appropriate, surgical interventions, not only to decrease mucosal inflammation and thereby alleviate symptoms but also to optimize growth, normalize pubertal development, facilitate normal social development, and avoid long-term disease-related complications.

Growth is a measure of the success of therapy. Based on the pathogenesis of growth impairment in Crohn disease, as discussed above and summarized in Table 41.1-7, optimization of treatment of intestinal inflammation and provision of adequate nutrition are of paramount importance in preventing or remedying growth impairment.[138] Control of gastrointestinal symptoms by long-term corticosteroid use, which impedes linear growth, does not constitute successful medical management. Optimal treatment of intestinal inflammation encompasses, as is discussed in detail in subsequent sections, immunomodulatory therapy, biologic therapies, nutritional therapies, and, if appropriate, resection of diseased bowel.

The natural history and severity of Crohn disease vary greatly among patients. Increasingly, gastroenterologists are acknowledging the heterogeneity of disease and the varying treatment responsiveness of individual patients. The identification of genetic or other markers predictive of disease behavior would constitute a significant advance, allowing early selection of the most appropriate treatment plan for individual patients.

Crohn disease has traditionally been thought of as a disease characterized by exacerbations and remissions. It must be acknowledged, however, that the term "remission," as generally applied, is a clinical concept. The persistence of endoscopic and histologic lesions, despite resolution of symptoms and biochemical abnormalities with corticosteroid therapy, has been well documented.[156,157] With emerging biologic agents and optimal use of immunomodulatory drugs, healing of intestinal lesions may, however, become a more achievable goal.

Nutritional Support

Nutritional support is a vital component of the management of patients with Crohn disease. Management goals must include correction and prevention of nutritional deficits and control of symptoms.

General Dietary Measures. For children and adolescents, the most important advice is to consume a diet liberal in protein with calories sufficient to maintain or

TABLE 41.1-7 SUMMARY OF EVIDENCE-BASED PHARMACOLOGIC TREATMENT OF CROHN DISEASE

TYPE OF CROHN DISEASE	ACTIVE DISEASE	MAINTENANCE OF REMISSION
ILEAL OR OTHER SMALL BOWEL DISEASE		
Mild	Oral 5-ASA (50–100 mg/kg/d up to 4 g/d) (Pentasa or Salofalk most suitable)	(Oral 5-ASA frequently used, but RCT data combined in meta-analysis do not support a benefit when compared with placebo)
Mild or moderate	Controlled ileal release budesonide (9 mg/daily) for ileal and/or right colonic inflammation	
Moderate or severe	Conventional corticosteroids (1 mg/kg/d up to 40–60 mg/d prednisone)	6-MP (1.5 mg/kg/d)* Azathioprine (2 mg/kg/d)* Methotrexate
Otherwise refractory and extensive	Infliximab	Infliximab
COLONIC DISEASE		
Mild to moderate	Oral 5-ASA (Asacol or Dipentum most suitable) Sulfasalazine† ?5-ASA enemas‡ Metronidazole (10–20 mg/kg/d up to 1 g daily) Ciprofloxacillin (20 mg/kg/d)	(Oral 5-ASA or sulfasalazine frequently used; RCT data combined in meta-analysis do not support a benefit when compared with placebo)
Moderate or severe	Conventional corticosteroids (1 mg/kg/d up to 40–60 mg/d prednisone) Methotrexate	6-MP (1.5 mg/kg/d)* Azathioprine (2 mg/kg/d)* Methotrexate
Otherwise refractory	Infliximab	Infliximab
PERIANAL		
	Metronidazole‡ (10–20 mg/kg/d) Ciprofloxacillin‡ (20 mg/kg/d) 6-MP/azathioprine* Methotrexate Infliximab	Metronidazole‡ (10–20 mg/kg/d) Ciprofloxacillin‡ (20 mg/kg/d) 6-MP/azathioprine* Methotrexate Infliximab

5-ASA = 5-aminosalicylic acid; 6MP = 6-mercaptopurine; RCT = randomized controlled trial.
*Delayed onset of action.
†May have an adjunctive role with corticosteroids.
‡Not subjected to controlled clinical trial.

restore weight and to support growth in children and adolescents. For children, recommendations for daily intakes of total calories and protein should be made according to their height for age and need for catch-up growth. Liquid dietary supplements may help motivated patients achieve these goals, although in young patients, these will often simply displace ingested calories from regular food without increasing total caloric intake.

Except in specific circumstances (eg, a low-residue diet to reduce obstructive symptoms in the setting of small intestinal stricture), a full diet for age is most appropriate.

Intensive Nutritional Support. Intensive nutritional support rather than simple dietary counseling to increase caloric intake is required when patients are significantly malnourished or when growth is retarded.[109,158,159] Adjunctive nutritional support in these contexts may be provided by enteral nutrition using formulated food or via parenteral nutrition using a centrally placed intravenous catheter. Nocturnal nasogastric infusion of formulated food has come to constitute the preferred and more frequent approach by virtue of its lower complication rates and easier and less costly administration.[158,159]

PHARMACOLOGIC TREATMENT

Drugs constitute the mainstay of treatment of children and adolescents with Crohn disease, although enteral nutrition using formulated food is commonly and effectively employed as an alternate primary therapy of active inflammation in some parts of the world, particularly in the United Kingdom. Table 41.1-7 summarizes validated pharmacologic options for the treatment of active inflammation and the maintenance of clinical remission based on the nature and localization of disease. Multicenter randomized controlled trials conducted predominantly among adults have helped establish the effectiveness of these therapies. For several of the commonly employed drugs, meta-analyses of individual controlled trial data have been performed. The efficacy of combination therapy (ie, more than one drug used in adjunctive fashion) has seldom been addressed in the clinical trial setting. Pharmacokinetic and dose-ranging studies have hitherto seldom been conducted in children; recommended dosages in Table 41.1-8 are based on extrapolation from adult studies.

The pharmacokinetics, mechanism of action, and potential adverse effects of individual drugs are discussed and the evidence of their therapeutic efficacy critically appraised in the forthcoming sections. The challenge at the present time is to wisely employ immunomodulatory and emerging biologic therapies, with the goal of improving outcomes, particularly for the subgroup of pediatric patients with otherwise chronically active, extensive, and disabling disease, formerly all too often associated with compromised growth.[129]

Systemic Corticosteroids. Corticosteroids have traditionally been the mainstay of treatment of active Crohn dis-

TABLE 41.1-8 SULFASALAZINE AND ORAL 5-AMINOSALICYLIC ACID ANALOGUES

GENERIC NAME	TRADE NAME	DOSAGE FORM	RELEASE MECHANISM	SITE OF RELEASE
Sulfasalazine	Azulfidine	500 mg tablets	Bacterial cleavage of diazo bond	Colon
Olsalazine	Dipentum (Pharmacia)	250 mg capsules	Bacterial cleavage of diazo bond	Colon
Mesalamine	Asacol (Proctor and Gamble, US and Canada; Tillots, UK)	400 mg tablets	pH-dependent breakdown	Distal ileum or right colon (pH > 7.0)
Mesalamine	Salofalk (Interfalk); Claversal (Smith, Kline & French, US); Rowasa (Reid-Rowell, US)	250 and 500 mg tablets	pH-dependent breakdown	From mid- to small bowel distally (pH > 5.6)
Mesalamine	Pentasa (Marion, US; Ferring, Canada; Ferring, Europe)	250 and 500 mg tablets	Timed release	Throughout small intestine and colon

ease. The various systemic glucocorticoids differ with respect to the duration of action, relative glucocorticoid potency, and relative mineralocorticoid activity. Oral prednisone in North America, the comparable prednisolone in Britain, and the slightly more potent methylprednisolone in Europe are favored. They offer the advantage of minimal mineralocorticoid effects, unlike parenteral hydrocortisone.

Pharmacology and Mechanism of Action. Prednisone is a synthetic glucocorticoid of intermediate potency. It is converted to its active form, prednisolone, in the liver. Both prednisone and prednisolone are promptly and completely absorbed in most individuals. The possibility of reduced absorption in patients with active Crohn disease of the small intestine must be borne in mind. The concept that the effects of corticosteroids at the tissue level outlast drug concentrations in serum is important to the derivation of treatment regimens. Intermittent rather than sustained high blood levels, as long as therapeutically efficacious, are preferable by virtue of causing fewer side effects.

The multifactorial mode of action of corticosteroids, although not completely understood, relates both to their inhibition of cell-mediated immunity and their anti-inflammatory effects. It has been recently demonstrated that steroids inhibit NF-κβ function, diminishing the release of inflammatory cytokines, IL-1 and IL-2.[160] Anti-inflammatory effects include decreased capillary permeability, impaired neutrophil and monocyte chemotaxis, and stabilization of lysosomal membranes. Eicosanoid production is decreased by inhibition of phospholipase, preventing arachidonic acid liberation from membranes. Additionally, steroids decrease diarrhea in inflammatory bowel disease by enhancing sodium and water absorption.

Potential Adverse Effects. The potential toxicity of systemic corticosteroids is well known and has been recently reviewed in the context of IBD.[161] Disfigurement by acne, moon facies, hirsutism, and cutaneous striae are the most commonly observed adverse effects with treatment of acute IBD and are particularly distressing to teenagers, despite assurances of reversibility following drug withdrawal. Pseudotumor cerebri, steroid psychosis, and proximal myopathy are other, fortunately rare, sequelae of steroid therapy. Corticosteroid use also may contribute to renal calculi formation via hypercalciuria. Aseptic necrosis of the femoral head is one of the most serious consequences of steroid therapy and may be mistaken for IBD arthropathy.

There are many reasons to avoid chronic corticosteroid use. Daily glucocorticoid administration suppresses IGF-1 and consequently inhibits linear growth.[137] Alternate-day corticosteroids allow IGF-1 secretion to normalize and hence do not have the same direct growth-inhibiting potential. The effects of corticosteroids on bone are of particular concern and include both enhanced bone resorption and diminished bone formation.[162] Corticosteroids decrease calcium absorption but increase urinary calcium excretion. The secondary hyperparathyroidism is associated with osteoclastic activity. Dietary supplementation with calcium and vitamin D may be of some prophylactic benefit in reducing the rate of corticosteroid-related bone calcium loss.[162] The incidence of posterior subcapsular cataracts correlates with the dose and duration of therapy. Glucocorticoids also increase intraocular pressure.

Evidence-Based Therapeutic Indications. Corticosteroids have been the most widely used pharmacologic agents for moderate or severe acute exacerbations of Crohn disease. Corticosteroid-induced clinical remission, however, is usually associated with persistence of endoscopic lesions.[156,157]

Active Disease. Steroid use in active Crohn disease of the small intestine alone or small intestine plus colon has been validated by the National Cooperative Crohn's Disease Study (NCCDS) and the European Cooperative Crohn's Disease Study (ECCDS).[163,164] Disease confined to the colon, which, interestingly, proved relatively refractory to corticosteroid therapy in the NCCDS, benefitted from combination therapy with sulfasalazine in the European study.[163,164] There are no studies directly comparing different corticosteroid dosing regimens in adults or in children. The NCCDS titrated the dose of prednisone to the level of disease activity with a daily dose range of 0.25 to 0.75 mg/kg (maximum 60 mg). The Canadian Pediatric Crohn Disease Study employed 1 mg/kg oral prednisone (maximum 40 mg) as a once-daily dose, with the option of increasing this dose briefly to 2 mg/kg/d (maximum 60 mg) if disease was refractory to the lower dose during the first 7 days of therapy. Overall, 92% of children and adolescents with active Crohn disease involving the small intestine alone or small intestine plus colon responded to this corti-

costeroid regimen.[165] In this trial, the daily prednisone dosage was tapered from 1 mg/kg/d by 5 mg each week beginning 4 weeks after attainment of clinical remission.

Maintenance of Remission. There is little justification from longitudinal placebo-controlled trials in adults for the use of low-dose corticosteroids (less than 20 mg prednisone daily in adults) to prevent relapse in patients with inactive Crohn disease.[163,164] The ECCDS did suggest a benefit to continuation of low-dose prednisolone after clinical remission of Crohn disease was induced by its use.[164] Such patients could be considered to have corticosteroid-dependent disease, as occurred in 45% of adult patients in a population-based study of outcomes in adult patients.[166] Such chronic daily corticosteroid administration is contraindicated in childhood because of the associated suppression of IGF-1 and the consequent inhibition of linear growth. Low-dose alternate-day prednisone therapy, formerly advocated for children as a strategy to maintain remission without impeding linear growth,[167] is not evidence based and has little place in current pediatric treatment alogorithms. In short, children with "corticosteroid-dependent" disease require alternative therapies.

Controlled Ileal Release Budesonide. *Pharmacology and Mechanism of Action.* Both therapeutic and adverse effects of glucocorticosteroids are mediated via the glucocorticosteroid receptor, which is uniform in all cells. To separate therapeutic from unwanted effects, glucocorticoids have been developed with a high affinity for the glucocorticosteroid receptor in the intestinal mucosa (and therefore high topical anti-inflammatory potency) but a rapid transformation to inactivated metabolites by the liver following absorption (and therefore low risk of systemic effects).[168] One such compound is budesonide, which has now been formulated into orally administered delayed-release capsule preparations to facilitate delivery to the terminal ileum and proximal colon. Microgranules of the nonhalogenated glucocorticoid bound to ethylcellulose are encapsulated by Eudragit L resin and released at a pH greater than 5.6 in one preparation.[169,170] Another delayed-release formulation more recently available in Europe releases budesonide at a pH greater than 6.[171] Budesonide possesses a high topical potency, with affinity for the glucocorticosteroid receptor 15 times that of prednisolone. Rapid metabolism in the liver to compounds with vastly lower affinity for the glucocorticosteroid receptor results in systemic bioavailability of only 10% compared with 80% for prednisolone.[168]

Adverse Effects. The major promise of oral budesonide formulated for intestinal release is decreased adverse systemic effects in comparison with classic corticosteroids. In clinical trials, no serious corticosteroid-related toxicity has been encountered, and the incidence of overall adverse effects has been less than with prednisolone.[169–178] A dose-related biochemical impairment of adrenal function as measured by basal cortisol levels and responses to adrenocorticotropic hormone stimulation has been observed, albeit also less than with conventional oral steroids.[169,170,173]

Evidence-Based Therapeutic Indications. *Active Crohn Disease.* The major clinical indication for the hitherto available oral budesonide formulations is in the treatment of Crohn disease involving the ileum and/or right colon. In one multicenter randomized placebo-controlled trial, 8 weeks of treatment of adults with 9 mg controlled ileal release (CIR) budesonide daily resulted in clinical remission in 51% of patients compared with 20% in the placebo group.[169] In two subsequent trials, the same CIR budesonide regimen induced remission in 60% and 53% of patients, respectively, compared with 60% and 66% of adult patients treated with prednisolone.[170,172] In a recently completed multicenter randomized controlled trial versus oral prednisone in children with active Crohn disease localized to the ileum and/or right colon, 55% achieved clinical remission with CIR budesonide compared with 71% with prednisolone.[173] Taken together, the extensive randomized controlled trial data and clinical experience in adults and children suggest that response rates with CIR budesonide are intuitively less than those achieved with optimal dosing of prednisone or prednisolone. Indeed, a treatment benefit for conventional corticosteroids has been confirmed in a meta-analysis.[174] Nevertheless, CIR budesonide may spare a proportion of young patients the adverse effects of short-term conventional steroids while successfully ameliorating their Crohn disease.[173]

Only the German Budesonide Study Group has studied the efficacy of an oral budesonide formulation in patients with more distal colonic disease. Comparable percentages of patients with distal colonic involvement and ileal with or without proximal colonic disease, 51% and 59%, respectively, attained clinical remission in this open-label study with the budesonide formulation designed for release at a pH greater than 6.[171]

Maintenance of Remission in Crohn Disease. Four randomized placebo-controlled studies have examined the efficacy of CIR budesonide in maintaining medically induced clinical remission.[175–178] CIR budesonide 6 mg daily in adults significantly prolonged the median duration of remission in comparison with placebo in two multicenter studies, but at 1 year following randomization, there was no difference in the percentage of patients remaining in continuous remission.[175,176] Two other placebo-controlled studies demonstrated a similar lack of efficacy at 1 year and no delay in the median time to relapse with daily doses of 6 mg or 3 mg CIR budesonide in comparison with placebo.[177,178] At present, long-term use to maintain remission in children must be considered experimental because of the marginal benefit observed among adults and the drug's hitherto unknown effect on linear growth.[179]

Sulfasalazine and Oral 5-ASA. Sulfasalazine and 5-ASA have a greater role in the management of ulcerative colitis than in Crohn disease. Indeed, the multiple oral 5-ASA formulations, designed in part to extend the scope of efficacy to include active and preventive treatment of Crohn disease, have, in this respect, proved disappointing.

Pharmacology and Mechanism of Action. Sulfasalazine consists of 5-ASA in azo-bond linkage with sulfapyridine. The sulfa moiety functions primarily as a carrier facilitating delivery of the therapeutically active 5-ASA to the colon. There it is released from the parent molecule

by bacterial cleavage of the diazo bond and acts locally in the colonic mucosa to impede the inflammatory process. The sulfapyridine component is 95% absorbed and undergoes acetylation, hydroxylation, and glucuronidation in the liver. 5-ASA is now recognized to modify neutrophil-mediated tissue damage through a multiplicity of actions, including inhibition of leukotriene biosynthesis via the lipoxygenase pathway of arachidonic acid metabolism, interference with myeloperoxidase activity, scavenging of reactive oxygen species, and inhibition of NF-κβ.[180] Even though the sulfapyridine component is therapeutically inert, there is new evidence that the complete parent molecule may act synergistically to enhance the anti-inflammatory effects of its component 5-ASA.[181]

5-ASA ingested orally in nonprotected form is rapidly absorbed in the proximal small intestine. Alternate delivery systems have been developed to facilitate transport and release of 5-ASA distally without the sulfa carrier. The plethora of available oral 5-ASA analogues differ importantly with respect to the mechanism and site of 5-ASA release.[182] The balance among release, local inactivation, and local absorption determines intraluminal drug levels at specific sites.[182,183] High 5-ASA concentrations within the intestinal wall are thought to optimize anti-inflammatory actions.

Table 41.1-8 lists the oral 5-ASA preparations currently in clinical use. These analogues are best understood in three groups. First, olsalazine, in which 5-ASA is attached to a second molecule of itself, depends, like sulfasalazine, on bacterial cleavage of the azo-bond. Other azo-bond derivatives are ipsalazide and balsalazide, which contain 5-ASA linked to an inert, unabsorbable carrier molecule. Second, the delayed-release preparations, known collectively as mesalazine in Europe and mesalamine in the United States, employ different acrylic-based resins, designed to break at a set pH, thereby making 5-ASA available to the intestinal mucosa. Finally, the timed-release formulation Pentasa contains 5-ASA in microgranules coated with a semipermeable membrane of ethylcellulose. Release occurs continually but at a rate affected by pH. The predicted sites of intestinal release are given in Table 41.1-8. However, 5-ASA preparations that require specific alterations in pH for release may not, for example, be distributed uniformly in patients with IBD, in whom the pH of luminal contents has been shown to differ from that of normal subjects.[184]

Potential Adverse Effects. Potential toxicity of sulfasalazine is considerable.[185] Overall, 20 to 25% of patients experience adverse reactions that either limit drug dosage or preclude use entirely. Undesirable effects fall into two categories, dose related and idiosyncratic or hypersensitivity, but the majority of both types seem attributable primarily to the therapeutically unimportant sulfapyridine component.[185] Dose-dependent adverse effects include nausea, vomiting, headaches, and mild hemolysis. The dose of sulfasalazine at which such reactions occur varies among individuals, partly reflecting acetylator status and its effect on sulfapyridine metabolism. Temporary interruption of therapy followed by a more gradual increase in dosage may avoid a recurrence of dose-dependent adverse effects. Glucose-6-phosphate dehydrogenase deficiency aggravates hemolysis and is therefore a contraindication to sulfasalazine administration. Idiosyncratic adverse reactions demand cessation of therapy rather than dose reduction. These, fortunately, are much less common than the dose-dependent effects and usually occur at the initiation of therapy. Fever, exanthems including Stevens-Johnson syndrome, pulmonary fibrosis, hepatotoxicity, and, rarely, agranulocytosis have all been reported. A known hypersensitivity to sulfonamides is a contraindication to sulfasalazine therapy. The infrequent occurrence of exacerbation of colitic symptoms is considered related to the 5-ASA constituent rather than to sulfapyridine.[186] Sulfasalazine also reversibly impairs male fertility.[187] Sperm morphology and motility revert to normal after discontinuation of the drug. Sulfasalazine reduces folate absorption, but supplementation to prevent anemia does not seem routinely necessary.

Eighty to 90% of patients intolerant of or allergic to sulfasalazine will tolerate oral 5-ASA.[188] Occasionally, the same adverse hypersensitivity reaction, such as fever and/or rash or exacerbation of colitic symptoms, is observed.[188] The most serious idiosyncratic reactions associated with sulfasalazine (ie, agranulocytosis, pulmonary complications) have not been reported with 5-ASA. Sulfasalazine-related impairment of male fertility resolves with a change to 5-ASA. However, several case reports of acute pancreatitis in association with mesalazine formulations have been published.[189] Olsalazine, which can act as a secretagogue in the distal ileum, causes diarrhea in 10 to 15% of patients.[190]

Evidence-Based Therapeutic Indications. *Active Disease.* In both the NCCDS and the ECCDS, sulfasalazine proved efficacious in treating inflammation involving the colon.[163,164] The European study suggested, furthermore, an adjunctive role in isolated colonic Crohn disease, which is often relatively resistant to corticosteroid therapy alone.[164] Isolated ileal Crohn disease was refractory to sulfasalazine therapy in both collaborative studies.

Timed-release and pH-dependent formulations designed to extend the scope of efficacy of 5-ASA have demonstrated, at most, modest benefit when employed at high dosages in adults with active disease.[191] For example, in one large multicenter study, 4 g of timed-release 5-ASA was superior to placebo and 1 g and 2 g of active drug, but the mean reduction in the Crohn Disease Activity Index (CDAI) even with the highest dosage was only 72 ± 13 points.[191] The best reported results have been in isolated ileitis, in which 4 g of a timed-release microgranular formulation achieved a mean change in CDAI of –123 at 12 weeks.[192] In keeping with these modest results, timed-release 5-ASA (4 g) induced clinical remission in significantly fewer patients (36%) than did CIR budesonide (69%).[193]

If children with mildly active Crohn disease are to be treated with 5-ASA or sulfasalazine, the choice of formulation should be made based on knowledge of the site(s) of intestinal inflammation to be targeted. Dosage of 5-ASA is extrapolated from adult studies; dose-ranging trials of efficacy, specifically in children, are lacking.

Maintenance of Remission. There is a paucity of data to support the efficacy of sulfasalazine in maintaining remission of quiescent Crohn disease. Neither the NCCDS

or the ECCDS demonstrated a statistically significant benefit in comparison with placebo.[163,164] The efficacy of oral 5-ASA in maintaining remission in Crohn disease has been observed in several individual randomized placebo-controlled trials, but initial enthusiasm has waned because additional data have been accrued. In an elegant meta-analysis, Camma and colleagues combined data from 2,097 patients with medically and surgically induced remission in 15 trials, including several large studies.[194] In the setting of medically induced remission, the pooled risk difference with oral 5-ASA versus placebo was not significant (–4.7%; 95% CI –9.6–2.8%). Hence, in keeping with earlier results achieved with sulfasalazine,[163,164] randomized controlled trial data in adults do not support the use of mesalamine therapy to maintain medically induced remission in patients with inactive Crohn disease.[194] Similarly, in a recently reported pediatric study, the 1-year remission rate with timed-release 5-ASA (50 mg/kg/d) initiated immediately following successful medical treatment of active disease was no different than with placebo (43% vs 37%).[195]

In the Camma and colleagues' meta-analysis, the therapeutic effectiveness of mesalamine appeared to be increased in the setting of a surgically induced remission, where the pooled risk difference was –13.1% (95% CI 0.05–0.21). As a measure of the efficacy of postoperative 5-ASA, the overall risk difference between the frequency of relapse in treated and control groups was calculated, allowing easy computation of the number of patients needing treatment to prevent one relapse.[194] Based on these data, one would need to treat eight patients following surgical resection to prevent one postoperative recurrence.[194] However, in the subsequently reported well-designed ECCDS of 318 adults, there was overall no significant difference in 18-month clinical relapse rates with timed-release 5-ASA (24.5 ± 3.6%) versus placebo (31.4 ± 3.7%) begun immediately postoperatively.[196] With these additional data in the trial literature, the number needed to treat rises to 25, and it becomes hard to defend an evidence-based recommendation for routine use of oral 5-ASA to maintain remission following intestinal resection of all grossly diseased bowel.[197] In a subgroup analysis of the ECCDS data, however, a delay in disease recrudescence was identified among patients who had undergone resection for isolated small bowel disease.[196]

Antibiotics and Probiotics. The clear implication of microbial flora in the pathophysiology of intestinal inflammation in Crohn disease has led to a resurgence of interest in the use of antibiotics and, more recently, probiotics. Supportive data are, however, relatively scarce. Metronidazole has been the most frequently studied drug, but, recently, ciprofloxacin has been employed alone or in combination.

Probiotics, defined as "living organisms, which upon ingestion, exert health benefits," belong generally to a large group of bacteria that make up the natural microflora and dwell as harmless commensals. Varieties of probiotics, which have been tested in limited fashion in inflammatory bowel disease, include lactobacilli, bifidobacteria, streptococci, nonpathogenic strains of *Escherichia coli*, and the yeast strain *Saccharomyces boulardii*.

Evidence-Based Therapeutic Indications. Metronidazole is well established, albeit not by randomized controlled trial data, in the control of perianal fistulae in Crohn disease.[198,199] In the treatment of intestinal inflammation, metronidazole was found to be at least as effective as sulfasalazine in a double-blind crossover Swedish multicenter study.[200] More recently, metronidazole was associated with a greater reduction in Crohn disease activity than was placebo, although not with a superior remission rate.[201] Two doses employed (10 mg/kg/d and 20 mg/kg/d) were comparable; the lower dose is preferred because of concerns about potential peripheral neuropathic effects with long-term use. In both studies, colonic inflammation responded better than disease confined to the small intestine.[200,201] In a small randomized study of metronidazole plus ciprofloxacin versus methylprednisolone for active Crohn disease, the clinical response rates were, respectively, 45% and 63%.[202] The combination of metronidazole plus ciprofloxacin did not enhance the efficacy of CIR budesonide in active Crohn disease involving the ileum and right colon.[203] There are supportive data for the use of metronidazole to delay recurrence after ileal resection.[204] Antimycobacterial regimens have not demonstrated benefit in comparison with placebo.[205]

The small number of IBD patients who have been treated in a randomized controlled fashion with probiotics have, to date, most often had ulcerative colitis or pouchitis. A recent combined antibiotic-probiotic regimen showed some promise in comparison with oral 5-ASA in the prevention of postoperative recurrence of Crohn disease.[206] As further trials of probiotic therapy are undertaken, it must be remembered that each strain exerts different and specialized functions. Viability and survival of organisms must be well documented; benefit of one preparation does not imply the efficacy of another.

Potential Adverse Effects. Metronidazole may cause a peripheral predominantly sensory neuropathy, which appears to be related to the dosage and duration of therapy.[207] Clinical experience suggests that paresthesiae always resolve, albeit at times very slowly, following discontinuation of metronidazole. Adolescents should be warned of metronidazole's disulfiram-like effect with alcohol ingestion. Another concern with long-term metronidazole therapy in young patients arises from its mutagenic and carcinogenic effects observed in laboratory animals. Ciprofloxacin has caused damage to growing bone in some laboratory animal species, but such adverse effects have not been observed in children despite substantial use in a variety of pediatric conditions.

Azathioprine and 6-MP. The immunomodulatory drugs azathioprine and 6-MP have become a mainstay of the management of pediatric Crohn disease, a reflection of the increased body of evidence in support of both their therapeutic efficacy and safety profile.[208] Their delayed onset of action precludes a role for azathioprine or 6-MP monotherapy in acute treatment.[208] Their importance as steroid-sparing agents in controlling intestinal inflamma-

tion and maintaining remission and thereby ameliorating growth in children is now well established.[208,209]

Pharmacology and Mechanism of Action. 6-MP is a purine analogue capable of interfering with endogenous purines, essential components of ribonucleic acid and DNA.[210] It has cytotoxic and immunosuppressive properties. Azathioprine was developed as a prodrug permitting liberation of 6-MP in tissues. The related structure of the two drugs leads one to anticipate similar clinical effects, but no directly comparative studies exist. There is some evidence from animal studies that azathioprine may have a better therapeutic index, that is, the ratio of therapeutic immunosuppressive to toxic dose.[211] The multiple mechanisms by which 6-MP and azathioprine modify immune responses are uncertain but include direct cytotoxicity, inhibition of cytokine synthesis, and, for azathioprine, additional immunologic effects of the nitroimidazoles, released from the prodrug at the same time as 6-MP.[210,211]

As shown in Figure 41.1-6, azathioprine undergoes a series of enzymatic reactions leading to the formation of 6-thioguanine nucleotide (6-TGN), considered the active but myelotoxic metabolite. Cytotoxicity results when these nucleotides are incorporated into DNA. The time to reach steady state for these cytotoxic intracellular metabolites may take up to several months, hence explaining the delayed median clinical response time of 3 to 4 months. In competing enzymatic pathways, thiopurine methyltransferase (TPMT) catalyzes the formation of 6-methylmercaptopurine ribonucleotides (6-MMPRs), metabolites that are therapeutically inactive and potentially hepatotoxic.[212]

Codominantly inherited polymorphic alleles confer high ($TPMT^H$) and low ($TPMT^L$) functional TMPT activity, which potentially impacts the therapeutic response to azathioprine and its toxicity. Approximately 89% of the population carry two wild-type $TPMT^H$ alleles ($TPMT^H/TPMT^H$) and have high TPMT activity (> 9.5 U/mL red blood cells [RBCs]); 11% are heterozygous ($TPMT^H/TPMT^L$) and have intermediate activity (5.0–9.5 U/mL RBCs); and 0.3% are homozygous for the variant $TPMT^L$ allele ($TPMT^L/TPMT^L$) and have low or undetectable activity (< 5.0 U/mL RBCs).

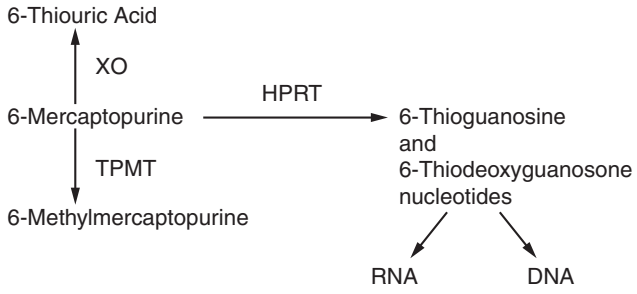

FIGURE 41.1-6 6-Mercaptopurine (6-MP) metabolism. The initial metabolism of 6-MP occurs along the competing routes catalyzed by thiopurine methyltransferase (TPMT), xanthine oxidase (XO), and hypoxanthine phosphoribosyltransferase (HPRT). Relative deficiency of TPMT or competition for XO leads to increased formation of 6-thioguanosine and 6-deoxythioguanosine nucleotides. The corporation of these nucleotides into ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) induces cytotoxicity.

Moreover, sulfasalazine and 5-ASA are recognized to inhibit TPMT activity.[212,213]

Potential Adverse Effects. Sandborn and colleagues categorized the adverse effects of azathioprine and 6-MP as allergic (dose independent) or nonallergic (dose and metabolism dependent).[214] The hypersensitivity reactions, which resolve with discontinuation of therapy, include fever, pancreatitis, rash, arthralgias, nausea, vomiting, and diarrhea. Nonallergic toxicities include severe leukopenia, thrombocytopenia, infection, hepatitis, and a theoretically increased malignancy risk.

Several groups have summarized the frequency of these acute and long-term unwanted complications of azathioprine or 6-MP administration.[214–218] Treatment was discontinued because of an adverse effect in 17 (18%) of 95 children and adolescents,[215] a higher overall percentage than in previous adult series.[214,216] The most common hypersensitivity reaction among adults and children is pancreatitis, occurring in 3 to 4% of patients and almost always within the first several weeks of starting therapy.[219] Fever and gastrointestinal intolerance manifest by vomiting necessitated cessation of treatment in 4% and 3%, respectively, of pediatric patients.[215] Bone marrow toxicity may be seen shortly after therapy is begun but also many years into therapy.[216,217] Accordingly, white blood cell counts should be monitored throughout the duration of therapy. Peripheral white blood cell counts below 2,500/mm^3 were reported in 2% of patients during more than 20 years of experience with 6-MP.[216] The British experience of 4% leukopenia with azathioprine includes two deaths, both related to bone marrow aplasia, among 714 patients with IBD.[217] Leukopenia is partly dose related, but individual susceptibility also varies, related in part to genetically determined differences in drug metabolism.[213]

It has been argued by some that TPMT activity and/or genotype be determined prior to commencing azathioprine or 6-MP as a means of avoiding serious myelosuppression. However, observed myelosuppression is, for the majority of patients, related to factors other than $TPMT^L$ activity.[213,219] Whatever the TPMT genotype, white cell counts will still need to be monitored throughout the duration of azathioprine or 6-MP therapy.[213,217,219] As an example, Colombel and colleagues reported TPMT genotype analysis in 41 Crohn disease patients, all of whom had experienced leukopenia (white cell count < 3.0×10^9/L) or thrombocytopenia (platelets < 10^9/L) leading to drug withdrawal in 83% and dosage reduction in the remainder.[219] Four patients (10%) were TPMT deficient (homozygous variant allele) and seven (17%) were heterozygous, but the remainder had wild-type activity (homozygous normal).[219] All 4 TPMT-deficient patients experienced bone marrow toxicity within 1.5 months, suggesting a role for TPMT in the prediction of early myelosuppression.[213,219] However, based on $TPMT^H$ and $TPMT^L$ allele frequencies, very low or undetectable TPMT activity would be encountered in only 3 patients of every 1,000 started on therapy. Three hundred patients would need to undergo TPMT assessment to detect one patient at short-term risk.[213]

Experience to date in IBD does not document an association of azathioprine or 6-MP use with increased risk of

subsequent malignancy, but continued observation is essential.[218] In a cohort of 775 adults treated with azathioprine for IBD, the frequency of lymphoma or other malignancy was not greater than expected among age-matched controls.[218] Infectious complications possibly attributable to or aggravated by azathioprine or 6-MP are described in 1 to 2% of patients overall.[216] One case of fatal varicella infection in a child with Crohn disease treated with 6-MP has been reported.[220] Susceptible children receiving azathioprine or 6-MP who are exposed to chickenpox should be given varicella-zoster Ig within 48 hours and have immunomodulatory agents discontinued.

Evidence-Based Therapeutic Indications. A meta-analysis of placebo-controlled trials employing azathioprine (2.0–2.5 mg/kg/d) or 6-MP (1.5 mg/kg/d) to treat active Crohn confirms the efficacy of these immunomodulatory drugs. The pooled odds ratio for response is 9.3 (95% CI 7.8–10.8).[208] Negative individual study results, which include the NCCDS,[163] are explained by withdrawal of corticosteroids immediately before commencement of azathioprine, premature outcome assessment, or employment of inadequate dosage. The meta-analysis demonstrated a requirement of 16 weeks to achieve therapeutic benefit, compatible with the hypothesis that the mode of action relates to gradual accumulation of intracellular cytotoxic metabolites. Pilot data suggesting that a large intravenous loading dose of azathioprine given at initiation of therapy might decrease the time to response were not supported in a subsequent randomized controlled trial.[221] Rather unexpectedly in light of its presumed mechanism of action, the addition of azathioprine to prednisolone therapy facilitated weaning of prednisolone dosage in a placebo-controlled 8-week trial in acute Crohn disease.[222]

Based on adult randomized controlled trial data and reported clinical experience among pediatric patients, it is reasonable to anticipate improved control of symptoms and reduced corticosteroid requirements in 60 to 70% of children and adolescents. Indicative of the now increased pediatric use of immunomodulatory drugs is the report of a placebo-controlled trial of concomitant 6-MP among newly diagnosed children treated with an initial course of prednisone.[209] In 1 year of follow-up, children treated with 6-MP experienced fewer relapses, received a lower cumulative prednisone dosage, and grew better.[209]

Direct measurement of intracellular metabolites in RBCs is feasible and is now often recommended in order that drug dosage be adjusted on an individual basis with greater safety.[223,224] There are some data from the treatment of other disorders to support the concept that individualized azathioprine or 6-MP dosing based on 6-TGN levels can improve response rates compared with conventional dosing regimens.[225,226] Data concerning 6-TGN levels and therapeutic response in IBD patients are, however, conflicting.[223,224,227,228] The earliest, preliminary, retrospective data were reported by Cuffari and colleagues in 1996.[227] Among 25 adolescent Crohn disease patients treated with 6-MP, a lack of clinical response judged by a high Harvey Bradshaw Index retrospectively calculated was associated with low RBC 6-TGN levels, but a satisfactory clinical response (low Harvey Bradshaw Index) was associated with a wide range of RBC 6-TGN levels.[227] Dubinsky and colleagues have published two observational studies concerning, respectively, 92 children and 62 adults who had been receiving 6-MP or azathioprine as treatment of their Crohn disease for at least 4 months.[223,224] RBC 6-TGN levels measured in these patients were found to correlate with a likelihood of clinical remission, as assessed by a modified Harvey Bradshaw Index less than 5 or fistula closure and steroid discontinuation. In the largest study to date conducted among 170 adults established on azathioprine or 6-MP therapy, no difference was found between whole-blood 6-TGN concentrations in patients in clinical remission compared with those with active disease.[228] Similarly, in a prospective randomized controlled trial comparing an intravenous loading dose of azathioprine with standard oral dosing, there was no correlation between RBC 6-TGN concentrations and either clinical response or occurrence of leukopenia.[221]

All studies to date in Crohn disease are observational and do not prospectively test the hypothesis that adjustment of azathioprine or 6-MP dosage to a target 6-TGN concentration will improve treatment outcomes. It would not be surprising, given the multiple possible mechanisms of action of azathioprine and its many metabolites, if a variable relationship exists between 6-TGN levels and efficacy. A double-blind multicenter trial comparing in randomized fashion azathioprine therapy at a standard 2.5 mg/kg/d dosage without subsequent metabolite monitoring versus therapy dosed according to TPMT enzyme activity and serial 6-TGN measurements is about to begin. At present, in a patient with an unsatisfactory clinical response, determination of 6-TGN and MMPR will at least detect noncompliance and may serve to influence the next therapeutic recommendation.

Methotrexate. Although methotrexate has a long history of substantial use in the treatment of rheumatoid arthritis, application to the treatment of Crohn disease has been very recent and is still limited. The onset of action appears to be more rapid than with azathioprine or 6-MP, but, otherwise, there are no comparative efficacy and safety data.

Mechanism of Action. Methotrexate inhibits the conversion of folic acid to its active form, tetrahydrofolate, which is necessary for thymidine synthesis. Thus, methotrexate impairs DNA synthesis. Additional anti-inflammatory properties may be related to a reduction in IL-1 production or induction of apoptosis of selected T-cell populations.

Potential Adverse Effects. In comparison with azathioprine and 6-MP, data establishing a safety prolife for use in IBD are sparse. Observed adverse effects in the one published multicenter trial in Crohn disease were minor: nausea and asymptomatic increases in liver enzymes.[229] Hypersensitivity pneumonitis is a rare but potentially serious complication but has not been observed in the limited experience in Crohn disease to date or reported in the much larger experience with pediatric rheumatic diseases.

Evidence-Based Therapeutic Indications. In a large placebo-controlled study that used intramuscular injections of methotrexate (25 mg weekly for 16 weeks), 39% of

patients whose Crohn disease was persistently active despite corticosteroid therapy achieved remission with methotrexate compared with 19% with placebo.[229] Clinical improvement was seen after 6 weeks of treatment. In a follow-up study, a benefit in maintaining remission in these adult patients was also observed.[230]

Eleven (78%) of 14 children and adolescents with Crohn disease previously intolerant or refractory to azathioprine or 6-MP therapy improved with methotrexate therapy.[231] These observations have now been confirmed in a larger, multicenter, but still retrospective analysis.[232]

Cyclosporine and Tacrolimus. The results from randomized controlled trials of cyclosporine in Crohn disease have been disappointing,[233–236] as is briefly summarized. In the first study conducted among adults with active corticosteroid-resistant Crohn disease, the percentage "improving" (59%) with high-dose oral cyclosporine (5–7.5 mg/kg/d) was greater than with placebo (32%), but the threshold level for judging clinical response was set very low.[233] A subsequent multicenter Canadian placebo-controlled study involving approximately 300 patients demonstrated no benefit from a mean of 4.8 mg/kg/d cyclosporine administered for 18 months.[234] One-third of patients had active disease, and two-thirds were in remission at initial randomization. Two other multicenter trials in adults yielded essentially negative results.[235,236] Oral cyclosporine was not as effective as prednisolone in newly diagnosed children with Crohn disease.[237] Intravenous cyclosporine was helpful in closing severe fistula in one study in adults.[238]

Biologic Agents: Infliximab. As the pathways involved in the pathogenesis of intestinal inflammation have been elucidated, a new class of therapeutic agents, referred to as biologics and having specific molecular targets, has been developed. These biologic therapies include cytokines, antibodies, and adhesion molecules. Infliximab, a chimeric (murine-human) monoclonal antibody directed against TNF-α, is the only biologic currently approved for treatment of Crohn disease. Infliximab has ushered in a new era in the management of pediatric Crohn disease; thus far, data from huge clinical trials in adults have influenced treatment algorithms.

Pharmacology and Mechanism of Action. Infliximab is an antibody of the IgG1 isotype that binds specifically to circulating and membrane-bound TNF-α.[239] TNF-α is a key proinflammatory cytokine possessing many properties relevant to intestinal inflammation.[240] The product of activated macrophages, it is capable of activating other macrophages and priming neutrophils, inducing proteases critical to tissue destruction, enhancing chloride secretion from intestinal epithelial cells, and inducing acute-phase reactants. It may also increase the expression of adhesion molecules, thereby contributing to the recruitment of monocytes, lymphocytes, and granulocytes. The expression of TNF-α is markedly increased in Crohn disease.[240] The efficacy of its blockade with infliximab attests to its important pathogenic role. Induction of apoptosis following infliximab binding to membrane-bound TNF-α appears to be particularly important to efficacy in Crohn disease.[241]

Evidence-Based Therapeutic Indications. In a multicenter placebo-controlled trial of three doses of infliximab in 108 adult patients with medically resistant, moderate to severe Crohn disease, 81% of patients treated with a single infusion of 5 mg/kg were significantly improved and 48% achieved remission by the end of 4 weeks.[239] In another placebo-controlled study of three infusions of infliximab (at 0, 2, and 6 weeks), 68% of patients with fistulous disease in the 5 mg/kg arm had at least a 50% reduction from baseline in the number of fistulae draining.[242] After a median 6 to 12 weeks, however, there is a predictable loss of infliximab-induced benefit in the treatment of inflammatory and fistulizing disease.[239,242] The ability of repeated infusions given at 8-weekly intervals to sustain clinical response has been demonstrated in large maintenance placebo-controlled and dose-ranging studies in adults with both inflammatory and fistulizing disease.[243,244] These studies were not designed to compare regularly scheduled infliximab infusions with episodic administration at the time of exacerbation. Mucosal healing of intestinal inflammation was observed in 30% of the adult patients with otherwise refractory inflammatory Crohn disease who underwent repeated colonoscopic examination during the ACCENT 1 trial.[243] In comparison with 5 mg/kg, doses of 10 mg/kg were associated with a longer duration of clinical response.[243]

A major efficacy-limiting problem is the development of antibodies to infliximab (ATI), previously called human-antichimeric antibodies. These are directed against the murine-TNF-α binding regions of infliximab. Their development, therefore, is associated with a shorter duration or loss of responsiveness, as well as with an increased frequency of infusion reactions.[245] Among rheumatoid arthritis patients, concomitant use of methotrexate reduced the frequency of antibody formation and had an adjunctive therapeutic effect.[246] In a retrospective analysis among adults with Crohn disease, concomitant administration of azathioprine or 6-MP was associated with a lower incidence of ATI formation and of infusion reactions, as well as with prolongation of clinical response following each infusion.[245] These observations constitute the basis for recommendations that patients treated with infliximab should also receive an immunomodulatory drug.[245] Use of an intravenous corticosteroid drug at the time of infliximab infusion is another strategy to reduce ATI formation.[247]

Potential Adverse Effects. The adverse effects of infliximab therapy have been monitored in randomized controlled trials among Crohn disease and rheumatoid arthritis and as part of postmarketing surveillance. Delayed hypersensitivity reactions are reported particularly after a long interval of no treatment. These are associated with rapid induction of high-titer ATI and are characterized by malaise, fever, and muscle ache 1 to 12 days following infusion. No end-organ damage results. Infliximab may induce anti–double-stranded DNA antibodies, which are usually low titer and infrequently associated with the clinical signs and symptoms of systemic lupus erythematosus.[248]

TNF-α is an integral component of innate and adaptive immune responses and is absolutely necessary for host defense against certain intracellular bacteria, in particular

Mycobacterium tuberculosis. It is not surprising, therefore, that infliximab treatment is associated with an increased incidence of tuberculosis, usually a consequence of reactivation of latent disease and often presenting in a disseminated form.[249] Pretreatment screening for latent tuberculosis, which would constitute a contraindication to infliximab therapy, is essential. Other infections complicating infliximab therapy are much rarer but include histoplasmosis and a case of *Listeria* meningitis in a teenager treated for Crohn disease.[250]

Clinical trial experience in adults with rheumatoid arthritis and Crohn disease raised an initial concern about an excess of lymphoma development, but reported occurrences of malignancy in postmarketing surveillance have not exceeded expected rates. However, the very recent introduction of infliximab precludes any data concerning long-term safety.

Clinical Use. Evidence-based guidelines for infliximab use among adult patients with inflammatory Crohn disease have endorsed its use among patients refractory to or intolerant of corticosteroids and conventional immunomodulatory agents.[251] Such "step-up" recommendations constitute a balance between the often dramatic short-term efficacy and the possibility of mucosal healing and the known short-term and unknown long-term adverse effects. Infliximab has a similar role in the treatment of children and adolescents with severe disease refractory to optimally employed conventional medical therapies and not amenable to localized resection.[252] Pediatric gastroenterologists must stay aware of evolving recommendations concerning treatment regimens and concomitant medications in order that efficacy be maximized and problems, including ATI formation, be minimized.

Other Biologics. Other biologic agents that have been less efficacious in Crohn disease include TNF-α receptor and CDP571, a humanized anti–TNF-α antibody of IgG4 type.[253,254] The greater efficacy achieved with infliximab is felt to be due to its ability to induce apoptosis.[241] The results with IL-10 in Crohn disease have been disappointing.[255] Recently, some diminution of active inflammation was observed with natulizimab ($\alpha_4\beta$ integrin) in a randomized controlled trial among adults.[256]

Primary Nutritional Therapy

Liquid diet therapy is an effective alternative to corticosteroids among children and adolescents with active Crohn disease. Indeed, enteral nutrition is regarded as first-line therapy for pediatric Crohn disease in centers in the United Kingdom[138] and is commonly employed in other parts of Europe and Canada.

Enteral Nutrition in Crohn Disease. *Mechanism of Action.* The mode of action of enteral nutrition as primary treatment of active Crohn disease remains conjectural. Hypotheses have included alteration in intestinal microbial flora, elimination of dietary antigen uptake, diminution of intestinal synthesis of inflammatory mediators via reduction of dietary fat, and overall nutritional repletion or provision of important micronutrients to the diseased intestine.[257] The effects of enteral nutrition on the gut microbial flora deserve further exploration in light of the now well-established role of microbes in disease pathogenesis.

Evidence-Based Therapeutic Indications. *Active Disease.* The potential role of exclusive enteral nutrition as primary therapy of active Crohn disease was discovered fortuitously. Patients given elemental formulae preoperatively experienced an improvement not only in their nutritional status as intended but also in the inflammatory activity of their disease. These observations of primary therapeutic efficacy were first confirmed in the small controlled trial conducted by O'Morain and colleagues.[258] However, in the subsequent much larger ECCDS randomized trials of enteral nutrition versus corticosteroid and sulfasalazine therapy, the drug therapy proved superior to semielemental formulae containing oligopeptides as the protein source.[259,260]

The controversy surrounding seemingly divergent outcomes has fueled several meta-analyses, each of which has concluded that there is a treatment benefit to corticosteroids in comparison with enteral nutrition.[261] Analyzing results of eight trials on an intention-to-treat basis, the pooled odds ratio for the likelihood of clinical remission with liquid diet therapy versus corticosteroids is 0.35 (95% CI 0.23–0.53).[261] Furthermore, poor compliance, although contributory, does not constitute the major explanation for the lower response rates to enteral nutrition, as is evident from secondary meta-analysis excluding dropouts for apparent intolerance.[261]

No controlled trials of enteral nutrition versus placebo or less effective drugs in active Crohn disease have been conducted. However, comparison of observed response rates to exclusive liquid diet therapy (53–82%) with usual placebo response rates (18–42%) in the controlled clinical trial setting suggests that enteral nutrition is of therapeutic benefit, when tolerated, even if efficacy does not equal that of corticosteroid treatment.[261] Moreover, a reduction in gastrointestinal protein loss, a decrease in intestinal permeability, and a reduction in fecal excretion of indium-labeled leukocytes have each been demonstrated, suggesting a direct effect on intestinal inflammation.[262,263] Open trials in children have documented endoscopic healing and decreased mucosal cytokine production following exclusive enteral nutrition.[264,265]

Factors Influencing Efficacy. *Anatomic Localization of Inflammation.* Anecdotal evidence suggests that Crohn colitis responds less well to enteral feeding than ileocolitis or isolated small bowel disease. Children with Crohn disease confined to the colon were excluded from the Canadian pediatric multicenter study.[165] The results of the ECCDS did not confirm a relationship between site(s) of intestinal inflammation and outcome, but the numbers of patients with isolated colonic disease were small even in these trials.[259,260] In one trial comparing two types of enteral nutrition, two-thirds of patients had disease confined to the colon, but excellent clinical response rates of 67% and 73% to elemental and polymeric formulae, respectively, were less observed.[266]

Formula Composition. Several clinical trials have compared the efficacy of elemental versus nonelemental formulae of varying protein and fat composition. Existing data combined in a meta-analysis do not support an advantage to elemental (amino acid based) feedings compared with more palatable polymeric formulations. The importance of fat composition to efficacy is less clear, but there may be a small treatment benefit achieved by a reduction in the content of total fat or ω-6 PUFAs.[267]

"Bowel Rest." When employed in the treatment of active Crohn disease, enteral nutrition is generally combined with "bowel rest." The necessity of complete avoidance of other food is challenged by the results of a randomized controlled trial of adjunctive nutritional support.[268] Partial parenteral nutrition plus an ad libitum oral diet proved as effective in inducing clinical remission as either elemental liquid diets administered by nasogastric tube or total parenteral nutrition and complete bowel rest among patients hospitalized because of continuing activity of their disease despite high-dose steroid therapy.[268]

Age and Disease Duration. Several small trials of enteral nutrition in children with extremely high success rates have been reported,[269] but caution is always required in drawing firm conclusions from very small studies.[270] The Canadian Pediatric Collaborative Trial of enteral nutrition versus corticosteroids[165] employed an oligopeptide-containing liquid diet very similar in composition to that used among adults in the ECCDS.[260] These two trials included, respectively, 78 children and 107 adults. The differences in patient populations and outcomes are summarized in Table 41.1-9. It seems likely that the higher remission rate overall with enteral nutrition in the pediatric study (75%) versus the adult study (53%) reflects differences in the nature of the patients randomized rather than an inherent difference in the responsiveness of childhood Crohn disease. Indeed, the subgroup of children with disease in relapse and of longer duration had a rate of clinical response to enteral nutrition very similar to that of adult patients. Alternatively, it could be argued that new-onset disease is more responsive to enteral nutrition, an observation that has also been made with infliximab therapy.[271]

Enteral nutrition does seem to be more feasible, if not inherently more efficacious, in pediatric populations. The formula can be infused nocturnally via a nasogastric tube in the home setting and will not interfere with normal activities. With the support of experienced nurses and physicians, enteral nutrition is, in general, well accepted by young patients. There is enough evidence of its short-term efficacy in active disease to support presentation as an alternate primary treatment to all young patients, particularly with predominantly small intestinal disease, for whom corticosteroids are being considered.

Maintenance of Remission. One of the limitations of liquid diet therapy has been the observed tendency for symptoms to recur promptly following its cessation. In most studies, 60 to 70% of patients experience a relapse within 12 months of stopping enteral nutrition and resuming a normal diet.[266] Chronic intermittent bowel rest with nocturnal infusion of an elemental diet 1 month of 4 has been recommended as a means of sustaining remission.[272] The beneficial effects on disease activity and growth of such cyclic enteral nutrition were confirmed in a recent randomized controlled trial versus alternate-day prednisone.[273] Phase 2 of the Canadian Pediatric Crohn Disease Study examined the effects of alternate-day administration of 0.3 mg/kg oral prednisone on the rates of clinical relapse and growth in comparison with cycles of exclusive enteral nutrition. During an 18-month period of follow-up, the difference between the percentage of patients remaining in continuous remission with alternate-day prednisone (47%) and with cyclic enteral nutrition (67%) was not statistically significant, but linear growth was significantly better among patients randomized to receive the nutritional therapy.[273] Continuation of nocturnal nasogastric feeding four to five times weekly as a supplement to an unrestricted ad libitum daytime diet was also associated with prolonged disease quiescence and improved growth in a historical cohort study.[159] In the long term, allowing normal food at times when family and friends are eating is particularly important in achieving compliance.[159]

Another "nutritional" strategy for maintaining remission has involved the use of fish oil supplements. A placebo-controlled study employing enteric-coated fish-oil capsules designed for ileal release demonstrated a substantial reduction in clinical relapse rate among patients with Crohn disease in clinical but not biochemical remission at baseline.[274] Eicosapentaenoic acid (fish oil) competes with arachidonic acid, thereby reducing its metabolism to leukotriene B_4, an amplifier of intestinal inflammation, by the 5-lipoxygenase pathway.

TABLE 41.1-9 ENTERAL NUTRITION AS PRIMARY TREATMENT OF ACTIVE CROHN DISEASE: COMPARISON OF TWO MULTICENTER ADULT AND PEDIATRIC CONTROLLED TRIALS

STUDY	RESPONSE	BASELINE CDAI	COLON ONLY INVOLVED
European Cooperative Crohn Disease Study IV[261] (*n* = 107)	53% to EN 85% to CS	323 (± 12 SEM) 316 (± 11 SEM)	22%
Canadian Pediatric Crohn Disease Study[165] (*n* = 78)	75% to EN 89% to CS	260 (242–278)† 309 (282–335)†	None*
Canadian Pediatric Crohn Disease Study subgroup with disease in relapse[165] (n = 21)	50% to EN 85% to CS	269 (233–304)† 292 (251–333)†	None*

CDAI = Crohn Disease Activity Index; CS = corticosteroids; EN = enteral nutrition; SEM = standard error of mean.
*Crohn disease confined to the colon was an exclusion criterion.
†Values in parentheses represent 95% confidence intervals.

Total Parenteral Nutrition

Greenberg has summarized studies of total parenteral nutrition (TPN) as primary treatment of active Crohn disease.[275] In contrast to studies of enteral nutrition in active disease, there are numerous retrospective reports but few prospective randomized controlled trials of TPN. From retrospective series among Crohn disease patients, one can expect an in-hospital remission rate of 64% after 14 to 21 days of treatment, with patients with isolated colonic Crohn disease responding less often than those with disease involving the small intestine alone or small intestine plus colon.[275] Two small prospective controlled trials suggest that TPN combined with bowel rest is of no primary therapeutic efficacy in the management of patients with acute Crohn colitis, although it will improve their nutritional status.[275] Only two randomized studies have compared TPN with exclusive enteral nutrition in the treatment of active disease; remission rates with either modality were comparable.[276,277] There is no evidence to suggest that TPN is superior to enteral nutrition in the treatment of acute inflammation.

Surgical Treatment

Optimal management of young patients with IBD often includes appropriate and timely referral for intestinal resection, which is increasingly performed laparoscopically.[278]

Indications for Intestinal Resection. The most common reasons for surgery include intractable symptoms despite medical therapy, intestinal complications including obstruction, intra-abdominal abscess, enterovesicular fistula, and, less frequently, free perforation or intractable hemorrhage. Many children have experienced growth impairment by the time resection is considered, an indication of the failure of medical treatment to adequately control intestinal inflammation.

Outcome. "To cut is not to cure."[279] Crohn disease is a chronic panenteric inflammatory process that cannot be eradicated by current medical therapy or by resection of gross disease. Patients who undergo ileal resection typically develop recurrent disease just proximal to the ileocolonic anastomosis, whereas the site of recurrence can be on either or both sides of the anastomosis following segmental colonic resection. Macroscopic lesions have been found in the neoterminal ileum of 72% of patients routinely colonoscoped 1 year following intestinal resection and ileocolic anastomosis.[279] However, the possibility of a significant asymptomatic interval, during which normal growth and pubertal development can resume, makes such surgery an attractive therapeutic option for young patients, despite the likelihood of eventual disease recrudescence. A substantial improvement in height velocity can be anticipated postoperatively in previously growth-impaired adolescents provided that the surgery is performed prior to or during early puberty.[280,281] The amount of catch-up growth achieved will depend on the duration of the clinical remission.

Several studies among adult patients have documented the cumulative rates of recurrence of symptomatic disease. The incidence of clinical recurrence averages about 10% per year postoperatively. Similarly, based on the return of symptoms with radiologic evidence of disease, the median postoperative recurrence-free interval was 5.1 years in a series of 82 children and adolescents undergoing a first resection.[280]

Factors Influencing Outcome. Risk factors for postoperative symptomatic recurrence have been examined in many studies of large cohorts of adult patients, but few reliable "prognostic factors" have been consistently identified.[280,282,283] The length of recurrent ileal disease appears comparable to the length of ileum inflamed preoperatively.[284] The rates of recurrence are lower after surgical resection with ileostomy than after resection with ileocolonic anastomosis.[285] The outcome after colectomy and end-ileostomy for isolated Crohn colitis is particularly good: 15% cumulative probability of symptomatic recurrence after 20 years versus 64% in patients with ileocolitis as their initial presentation in one typical study.[286] In two pediatric studies, anatomic distribution of disease was the most important factor influencing outcome.[280,281] Patients with extensive ileocolonic involvement experienced an excess of early recurrences (50% by 1 year) in comparison with children with preoperative disease in the terminal ileum with or without the right colon or in the more proximal small intestine (50% by 5 years).[280] Fistulizing disease and stenosing disease have been considered, respectively, poor and good prognostic factors for the length of postoperative remission in adult studies.[282,287,288] However, children undergoing resection because of stenosing or fistulizing complications (eg, bowel obstruction or intra-abdominal abscess) had delayed recrudescence of disease in comparison with those operated on simply for inflammatory symptoms refractory to medical therapy.[280] An early operative approach to localized disease and for complications of chronic inflammation is supported by these data.[138,280,281] The rapid return of symptoms after major resection in patients with extensive ileocolitis suggests that medical strategies should be maintained in this group.

Maintenance of disease quiescence via adjuvant pharmacologic treatment following surgery has proved as difficult as maintenance of medically induced remission. As discussed earlier in the chapter, meta-analysis of the now very large number of patients treated in a randomized fashion with oral 5-ASA in placebo-controlled trials no longer supports its routine use following resection of all gross disease.[194,195,197] Patients undergoing resection of isolated ileal disease may benefit.[196] Two studies have suggested a benefit to postoperative antibiotic administration in delaying endoscopic and clinical remission, but the number of patients on which this conclusion is currently based is small.[204,206] CIR budesonide administered postoperatively has not been helpful.[289] Data concerning 6-MP in the maintenance specifically of surgically induced clinical remission are very limited.[290] Smoking is consistently associated with higher recurrence rates, particularly in female patients, in all studies in which this variable has been examined.[291]

Management of Perianal Disease

At least until the advent of infliximab, the first line of medical therapy for perianal fistulae has been metronidazole. Eighty-three percent of adult patients with a variety of chronic perianal and rectovaginal fistulae and unhealed perineal wounds responded to 20 mg/kg/d over 2 to 4 months.[198] Subsequent follow-up for as long as 36 months indicated that although perianal disease seldom relapsed on full-dose therapy, a reduction in dose or cessation of therapy was often associated with exacerbation. Metronidazole could be successfully discontinued in only 28% of patients.[199] The place of other antibiotics in the management of perianal disease is less substantiated, but ciprofloxacin appears to offer benefit as sole or adjunctive therapy. Benefits from azathioprine or 6-MP, methotrexate, and cyclosporine have also been observed. Incision and drainage are indicated for abscesses. Other surgical treatments include fistulotomy for simple or low fistulae, fistulotomy with seton insertion for complex or high fistulae, and intestinal diversion for highly destructive lesions. As previously discussed, infliximab infused at baseline, 2 weeks, and 6 weeks proved highly successful in achieving a reduction in drainage and clinical closure of perianal fistulae.[242] Prior to infliximab, examination under anesthesia or MRI is required in order that any abscesses be identified and properly drained, usually with a seton.[292] As for intestinal inflammatory disease, regularly repeated infusions may be required to sustain clinical response.[244] MRI examinations can be used to detect persistence of fistulae despite the apparent clinical closure.

CLINICAL COURSE AND PROGNOSIS

Disease Severity

Crohn disease is heterogeneous in nature and varying in severity. Its protean nature precludes universally applicable statements about clinical course or prognosis. As depicted in Figure 41.1-7, the spectra of disease patterns among consecutively diagnosed prepubertal patients in Toronto in the past two decades were similar. In the 5-year follow-up, roughly one-third had mild symptoms only and another third more troublesome exacerbations but clear-cut remissions. The remaining third experience chronically active steroid-dependent or steroid-refractory disease, but half of these benefit significantly from resection and then experience a sustained remission. Thirty-six percent of the children diagnosed during the 1980s and the 1990s underwent intestinal resection by 5 years of follow-up.[116]

Disease Behavior

Recently, there have been attempts to characterize and categorize Crohn disease as "penetrating/fistulizing," "stenosing," or simply "inflammatory."[293] The fistulizing subgroup includes any patient with enteroenteric, enterovesicular, enterocutaneous, or perianal fistula or intra-abdominal abscess. The stenosing category includes patients with persistent abdominal pain and radiologic documentation of marked stenosis of a segment of small or large intestine. Although it has been argued that such behavior may be genetically determined, an analysis of the prevalence of each phenotype according to the duration of disease suggests that stricturing and fistulizing behaviors become progressively more common over time.[294] This is in contrast to major localization of disease, which is consistent over time[295] and appears to be genetically influenced.[8,41,54] Nevertheless, consideration of the disease behavior at a given time may guide therapy.

Overall Mortality and Cancer Risk

Death from Crohn disease is extremely rare in the pediatric population. The two disease-related deaths since 1980 among roughly 700 children and adolescents in Toronto occurred as a result of postoperative septic complications. In adulthood, there appears to be an increased mortality risk compared with age-matched controls (standardized mortality ratio 1.51).[295]

Persons with Crohn colitis may be at similar risk of developing carcinoma of the colon as those with ulcerative colitis. The absolute cumulative frequency of risk for development of colorectal cancer in extensive Crohn colitis was reported to be 8% after two decades of disease.[296] Accompanying severe perianal disease has been suggested as an additional risk factor. Colorectal carcinoma has been reported in a 21 year old diagnosed with Crohn disease at the age of 8 years.[297]

CARING FOR THE WHOLE CHILD

The need to address the psychosocial impact of Crohn disease on children and adolescents as well as on their fami-

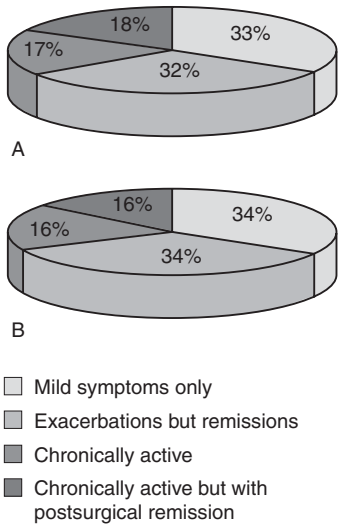

FIGURE 41.1-7 Clinical course of Crohn disease among consecutively diagnosed children at The Hospital for Sick Children, Toronto, Canada, during two decades. All children were prepubertal or in early puberty (Tanner stage 1 or 2) at the time of presentation. Patients were classified via retrospective chart review as having mild symptoms only, at least moderate excerbations but remissions, or chronically active disease (a portion of these achieving sustained remission following resection). *A*, 1980–1988 cohort (n = 100); follow-up 4.9 ± 1.8 years. *B*, 1990–1999 cohort (n = 163); follow-up: 4.7 ± 1.7 years.

lies is increasingly recognized.[298] The concept of health-related quality of life (HRQOL) has been defined as the functional effect of an illness and its treatment on a patient, as perceived by the patient.[298] HRQOL is determined not only by physical well-being but also by psychological state, degree of social support, effects of treatment, and complications.[298] Assessment of HRQOL provides a global measure of the patient's perceptions, illness experience, and functional status.[298] Increasingly, appraisal of the impact of treatments on HRQOL is being expected in clinical trials and in health policy making.

Several recent studies have explored the concerns of young patients with inflammatory bowel disease with quite consistent findings.[299,300] Although, in focus groups, children initially denied that Crohn disease interfered with their lives in any way, it became apparent that many were frustrated and angry about the physical symptoms and a lack of understanding of their illness by others.[300] North American and English children shared similar concerns about body image, having to miss out on things, uncertainty about exacerbations and future health, and unpleasant treatments.[299,301] A disease-specific instrument incorporating these patient-generated concerns has been developed to facilitate assessment of the overall impact of pediatric IBD.[299,302] Activity indices such as the Pediatric Crohn's Disease Activity Index[303] are designed to allow determination of the effects of treatment on the basic disease process, but they do not tell us how our patients are functioning day to day and how they feel about their chronic illness. Two adolescents may respond to the same physical symptoms with different outlooks and subsequent endeavors and achievements. Recognition of the disparity between the activity and severity of Crohn disease and emotional or functional disability may be facilitated by assessment of HRQOL.

When it is recognized that a child or adolescent needs help coping with his or her illness, it is equally important that there be effective interventions to restore emotional health and improve function. Although there are opinions and guidelines, strategies involving a multidisciplinary approach have not been subjected to scrutiny and appraisal, as have other therapies. Adolescents have a reluctance to participate in peer support groups, but whether "dyadic peer support," that is, matching with another patient of similar or somewhat older age, may be beneficial is beginning to be explored.

From the time of recognition of Crohn disease, it is important to create an atmosphere that permits both the child and parents to express their concerns about the physical state and the psychological aspects of the disease. As time goes by, an empathetic relationship between the medical team and the patients may enhance the likelihood of emotional expression. Support in coping with a chronic illness must be provided, but it is also important to understand and alleviate other stresses in the family environment, which may hinder coping with the illness. The overall impact of Crohn disease on a child is the result of the complex interplay among physical, psychological, and social factors. If depression is identified, this must be dealt with psychotherapeutically by a psychiatrist or psychologist. It is important to see the child alone to make psychological communication easier. Education is vital because well-informed patients and families may be more likely to develop active coping strategies.

FINAL COMMENTS: CROHN DISEASE IN 2004

The past several years have witnessed exciting progress in unraveling the etiopathogenesis of Crohn disease. The identification of the first gene conferring susceptibility is a landmark achievement in a complex disorder. The recognition of other genes determining susceptibility and influencing phenotypic expression is eagerly awaited.[304,305] Novel techniques allowing characterization of microbes inhabiting the gut hold promise that the microbial trigger(s) of unrestrained inflammation in susceptible hosts will ultimately be defined. The interactions between intestinal microbial flora and the signaling pathways involved in the innate and adaptive immune response are being continually elucidated. With better understanding of these pathways have come biologic agents, which already have had a major therapeutic impact on this often disabling disease.

Although "step-up" versus "top-down" therapy can be debated, optimal management entails identification of genetic or clinical predictors of outcomes and responsiveness to treatments so that recommendations can be individualized. Pediatric gastroenterologists have become aware of the need to consistently characterize the phenotype and, as it becomes possible, the genotype, thereby establishing a framework for multicenter evaluation of emerging therapies in what is clearly a heterogeneous chronic inflammatory disorder if not a spectrum of related but distinct "Crohn diseases."

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2. Ulcerative Colitis

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Ulcerative colitis (UC) is a chronic inflammatory disease of the colonic and rectal mucosa of unknown etiology affecting children and adults. Although much of the available knowledge about this disease has been derived from studies of adult patients, in recent years, the number of investigations performed on pediatric patients has increased dramatically. The following discussion summarizes the key aspects of UC in children, emphasizing the unique aspects of the disease in childhood.

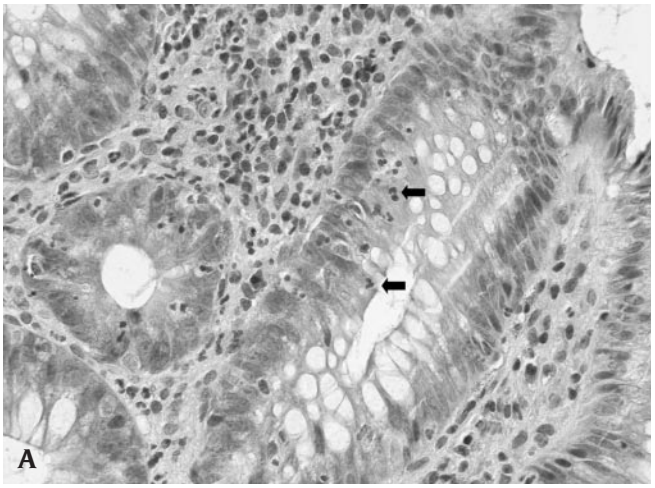
PATHOLOGY

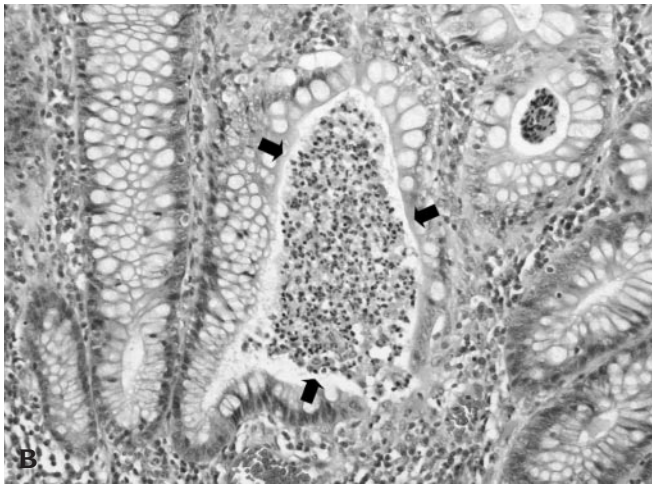
The first description of UC was in 1875 by Wilks and Moxon, who differentiated UC from infectious colitis.[1] In 1932, Crohn described regional enteritis, a transmural disease of the small intestine, as well as a type of transmural colitis, which was distinct from UC.[2,3] In 1960, formal criteria differentiating UC from Crohn colitis were established.[4] According to the classic definition, the inflammation in UC is limited to the colon and rectum. In untreated UC, the distal colon is most severely affected, and the rectum is virtually always involved. Inflammation is limited primarily to the mucosa, and involvement is continuous, extending from the rectum proximally and with varying degrees of ulceration, hemorrhage, edema, and regenerating epithelium.[5] In contrast to adult patients, who most commonly have disease confined to the left colon, only 15% of children have isolated rectal involvement or ulcerative proctitis, 20% have left-sided disease, and pancolitis is the most common pattern.[6] Although UC is limited to the colon, "backwash ileitis" owing to nonspecific inflammation of the terminal ileum may occur but is generally not associated with clinical small bowel disease. Grossly, the colonic mucosa appears erythematous, hemorrhagic, and roughened in active disease, whereas the inactive or quiescent phase is characterized by flattening of the mucosa and effacement of the normal haustral markings. In severely active colitis in which the mucosa has been destroyed, inflammation may extend into the submucosa and occasionally down to the muscularis. Such ulcers are typically broad and shallow rather than fissuring or knife-like, as would be more typical of Crohn disease (CD). Intervening areas of regenerating granulation tissue and residual mucosa may form islands of tissue called pseudopolyps. Thickening of the bowel wall and fibrosis are rare, although shortening of the colon and focal colonic strictures may occur in long-standing disease.

The histopathologic appearance of the mucosa in UC is characterized by continuous acute and chronic inflammation with diffuse mucosal infiltration by polymorphonuclear leukocytes and mononuclear cells.[5] The colonic epithelium shows the most characteristic changes. Cryptitis, crypt abscesses, mucin depletion, and surface erosion or ulceration typify the active inflammation, whereas chronic changes involve crypt architectural distortion with branching and atrophy, diminished numbers of goblet cells, and Paneth cell metaplasia in the left colon (Figure 41.2-1). Additional features of chronicity include villiform change of the mucosa and the appearance of basal plasma cells and lymphoid aggregates. In contrast to Crohn colitis, no granulomas or transmural inflammation and little fibrosis are present.

Recently, the classic pathologic description of UC has been subject to revision. It has become apparent that pharmacologic therapy may significantly alter the gross and histopathologic appearance of UC. Therapy may completely normalize the colonic mucosa histologically[7] or induce patchiness or discontinuity.[8] Given that these changes may mimic the appearance of CD in the colon and potentially lead to misdiagnosis, complete colonoscopy should be performed before treatment to maximize the ability to make a definitive diagnosis. Furthermore, children with new-onset disease may demonstrate unusual histologic features, such as absence or patchiness of features of chronicity and relative or absolute rectal sparing, even prior to the initiation of therapy.[9,10] Discontinuous disease has also been noted in adult patients who may demonstrate involvement of the appendix without surrounding cecal involvement.[11]

In contrast to the classic features of UC discussed above, CD tends to be characterized by focal involvement with skip lesions, transmural inflammation, and granulomas (Table 41.2-1). In an individual case, however, the histologic features may not clearly fall into either category, and the patient is said to have indeterminate colitis. Although there is no established formal definition of indeterminate colitis, in most cases, the diagnosis of indeterminate colitis is given when chronic colitis is present, but the clinician cannot reliably distinguish between CD and UC based on the available clinical, radiographic, endoscopic, and histologic data.[12–14] The prevalence of indeterminate colitis in adults and children with inflammatory bowel disease (IBD) is estimated to be 10 to 20%.[15,16] Approximately one-third to half of these patients will later be classified as UC or CD.[14,17]





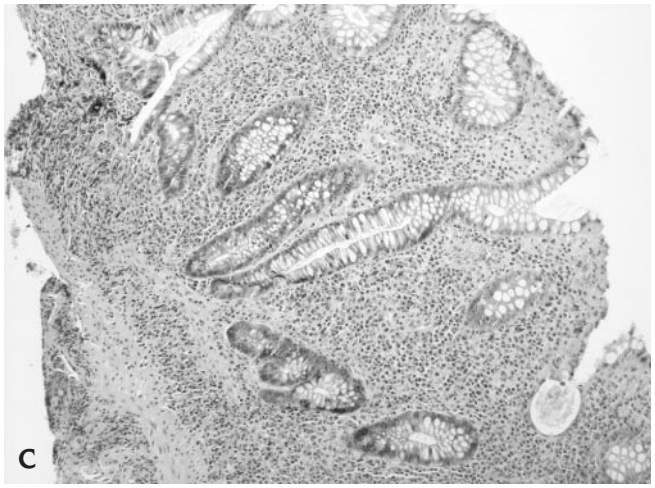


FIGURE 41.2-1 *A*, Active colitis with polymorphonuclear leukocytes invading the crypt epithelium resulting in cryptitis (hematoxylin and eosin; ×400 original magnification); *B*, Active colitis with a crypt abscess (hematoxylin and eosin; ×200 original magnification); *C,* Chronic colitis characterized by irregular glandular architecture with atrophic and branching crypts (hematoxylin and eosin; ×100 original magnification).

EPIDEMIOLOGY

The distribution of age at onset of UC is bimodal, with peaks occurring in the second and third decades and again in the fifth and sixth decades.[18] Between 15 and 40% of all patients with UC present before age 20 years.[19] The highest incidence in children is noted between the ages of 10 and 18 years. Although infants with UC have been reported, the disease is quite rare in individuals under 2 years of age.

The incidence and prevalence of UC vary considerably across different geographic areas. UC is common in North America and Northern Europe, particularly in the United Kingdom and Scandinavia, but is less prevalent in Southern and Central Europe and is seen quite infrequently in Asia, Africa, and South America.[19] Determination of meaningful estimates of incidence and prevalence is difficult because of variation in the definition of age limits for the pediatric population, inexact and nonstandardized criteria for diagnosis, and incomplete ascertainment of cases.[20] In the general population in Northern Europe and the United States, the incidence of UC has ranged from 3.9 to 7.3 cases per 100,000 people/year, with the prevalence ranging from 41.1 to 79.9 cases per 100,000 people.[21,22] The incidence of UC increased gradually in the pediatric population until 1978 but then became relatively stable worldwide. Current estimates for Northern Europe and the United States range between 1.5 and 4.0 cases per 100,000 children/year (Table 41.2-2).[16,22–26] The most recent surveys from Scotland and Sweden again suggest an increase in incidence in childhood UC over the last decade.[26,27]

Classically, the incidence of UC has been described as being highest in the white population. However, recent studies have demonstrated that blacks in the United States and the British Isles may have a comparable incidence and that the disease may actually occur more frequently in the Asian population in the British Isles.[27] UC is more common among Jewish than non-Jewish peoples, although disease rates in people of Jewish background also vary by geographic area.[28] Males and females are affected equally.

ETIOLOGY AND PATHOGENESIS

As with CD, the cause of UC remains unknown; however, a growing body of evidence suggests that both genetic and environmental factors play a role in pathogenesis (Table 41.2-3).[29] The most current theory proposes that disease results from an inappropriate and exaggerated immune response to normal constituents of the mucosal microflora.[30]

Evidence for an inherited predisposition to UC exists, although genetic factors may be less important than in

TABLE 41.2-1 COMPARISON OF PATHOLOGIC FEATURES OF ULCERATIVE COLITIS AND CROHN DISEASE

FEATURE	ULCERATIVE COLITIS	CROHN DISEASE
Gross/endoscopic		
Colonic involvement	Typically diffuse, continuous, extending proximally from rectum	Focal disease characterized by skip lesions
Rectal involvement	Almost always involved	Frequently spared
Ileal involvement	Nonspecific "backwash ileitis"	Typically involved with ulceration and nodularity
Ulceration	Broad and shallow	Early aphthous lesions, ulcers knife-like and fissuring, intervening areas of edema may give "cobblestone" appearance
Microscopic		
Depth of inflammation	Mucosal, except in severe disease	Typically transmural
Granulomas	Absent, except for occasional giant cell reaction to damaged crypts	Noncaseating granulomas commonly seen
Fibrosis	Unusual	Typical

patients with CD. Fifteen to 25% of patients with UC have other family members with IBD. Furthermore, the incidence is approximately 10 times greater when there is a positive family history.[31] The highest risk for a child developing UC occurs when an affected parent is Jewish, when both parents are affected, or when a sibling is affected. There appears to be a high concordance for the diagnosis of UC or CD among family members with IBD. In one study of families with two or more affected family members with IBD, among 83 families in which the proband had UC, 72 relatives had UC (81%; 72 of 89), and only 17 had CD (19%; 17 of 89).[32] Incidence studies of UC in twins reveal a pair concordance rate of 14 to 19% among monozygotic twins and a very low percent concordance rate of 0 to 5% among dizygotic twins.[33,34] This difference between monozygotic and dizygotic twins is much more striking in CD, with a pair concordance rate of 50% among monozygotic twins and 0 to 4% among dizygotic twins.[33,34] Although the concordance rates of disease are higher in monozygotic twins, the incomplete concordance suggests that nongenetic factors also contribute to the development of IBD.

Candidate gene studies suggest an association between human leukocyte antigen (HLA) class II genes and IBD, and this association is stronger in UC than in CD.[29] Two of the reported HLA associations with UC include the serologic subtype HLA-DR2 (particularly the deoxyribonucleic acid [DNA] genotype HLA-DRB1*1502 in the Japanese population) and the DNA genotype HLA-DRB1*0103 (especially with extensive UC).[29,35] Systematic genomic studies using linkage analysis report the presence of non-HLA susceptibility genes for UC located on chromosomes 3, 7, and 12.[36] The association of UC with Turner syndrome, a disorder with a clear genetic basis, also lends support for the importance of inherited factors in its pathogenesis.[37]

The incomplete concordance among monozygotic twins for UC suggests that nongenetic factors also contribute to the development of IBD. Multiple environmental factors also have been postulated to be important in the etiology of UC. Evidence of a birth cohort phenomenon suggests that early life exposure to environmental risk factors may influence the development of UC.[38,39] Koletzko and colleagues found that children with UC were three times more likely to experience a diarrheal illness during infancy than were unaffected siblings.[40] Glassman and colleagues reported an association between early cow's milk sensitivity and UC.[41] As is true for CD, the impact of breastfeeding on the development of UC remains controversial; some published studies suggest a protective effect,[42] whereas other studies show no effect.[40,43] Multiple studies report a lower risk of UC with appendectomy at a young age[44–46]; however, not all studies demonstrate this relationship.[47] In a population-based, case-control study, UC was inversely related to appendectomy only in those individuals who had surgery before 20 years of age.[45] Furthermore, the risk of UC was reduced only in patients who underwent appendectomy for inflammatory conditions (eg, appendicitis or mesenteric lymphadenitis) but not in patients who had the procedure for nonspecific abdominal

TABLE 41.2-2 STUDIES OF THE INCIDENCE OF ULCERATIVE COLITIS IN CHILDREN AND ADOLESCENTS

LEAD AUTHOR AND STUDY LOCATION	AGE RANGE	TIME PERIOD STUDIED	INCIDENCE (CASES/10^5 PERSONS/YR)
Calkins (1984),[22] Baltimore, Maryland	< 10 yr	1977–1979	M 0.34; F 0.36
	≥10 yr and ≤ 19 yr	1977–1979	M 2.25; F 1.32
Barton (1989),[23] Scotland	> 5 yr and ≤ 16 yr for UC	1968–1983	
		1968	1.9
		1983	1.6
Olafsdottir (1989),[24] Western Norway	≤ 15 yr	1984–1985	4.3
Langholz (1997),[25] Copenhagen, Denmark	< 15 yr	1962–1987	2.0
Lindberg (2000),[26] Stockholm, Sweden	≤ 15 yr	1984–1986	1.4
		1993–1995	3.2
Bentsen (2002),[16] Southeastern Norway	< 16 yr	1990–1993	2.1

UC = ulcerative colitis.

TABLE 41.2-3 ETIOLOGIC FACTORS IN THE PATHOGENESIS OF ULCERATIVE COLITIS

EVIDENCE FOR GENETIC PREDISPOSITION
Frequent positive family history (15–25%)
Higher rate of concordance in monozygotic twins than in dizygotic twins
Association with specific HLA class II genes
Association with other genetic disorders, eg, Turner syndrome
ENVIRONMENTAL FACTORS
Early childhood events, eg, diarrheal illness; may increase risk
Appendectomy at an early age; may decrease risk
Psychological stress; may cause exacerbations
Smoking tobacco; decreases risk
Drugs
Nonsteroidal anti-inflammatory drugs; may cause exacerbations
Oral contraceptives; conflicting data
Microbial factors; important in pathogenesis

pain.[45] These findings may suggest that the inflammatory condition that preceded the appendectomy, rather than the appendectomy itself, may be inversely related to the development of UC later in life.

Although the early theories that UC was a psychological disorder have been discounted, up to 40% of patients report psychological stress as a potential trigger of their disease.[48] Furthermore, stress has been demonstrated to be associated with relapse in animal models of colitis.[49] More research is required to assess the effect of stress on the immune system and the potential impact of stress on patients with UC.

The lower risk of UC among current smokers is one of the most consistent findings in multiple studies, although the potential mechanism of this association is still unknown. Current smokers have approximately half the risk of developing UC compared with nonsmokers.[50,51] Little information is available regarding the possible association of childhood exposure to passive smoking and the development of UC. One study demonstrated that passive smoke decreased the risk of developing UC in adulthood,[52] whereas another study demonstrated an association between passive smoke and the development of both childhood UC and CD.[53]

A number of published studies, mostly case reports and series, suggest that nonsteroidal anti-inflammatory drug (NSAID) use may precede the onset of IBD, lead to a reactivation of quiescent IBD, or exacerbate already active IBD in humans.[54–57] Cytoprotective prostaglandins exert predominantly anti-inflammatory effects in the setting of colonic inflammation, and it has been postulated that NSAIDs may worsen colitis by inhibiting the synthesis of these prostaglandins in the colon.[58] However, further study is needed to clarify any true association of NSAID therapy with the onset or exacerbation of UC, to identify those individuals at most risk, and to determine the safety profile of cyclooxygenase 2 inhibitors in comparison with standard NSAIDs in regard to potential colonic mucosa injury. Physicians may choose to advise their IBD patients to avoid NSAID medications until more information is available. However, some may argue that there is insufficient evidence to eliminate its use completely, especially among UC patients who may benefit from NSAID therapy, such as for arthritis.

The association between oral contraceptive use and UC remains controversial, with some studies suggesting an increased risk of UC and other studies showing no increased risk.[50,59]

The intestine is exposed to numerous luminal dietary antigens; thus, a potential relationship between dietary intake and the development of UC seems plausible. Ecologic and case-control studies have examined the association between certain foods and UC,[60,61] but, presently, there is no definitive link between diet and the development of UC. The collection of preillness dietary information and other methodologic issues of these retrospectively designed dietary studies hinder the interpretation of findings. For example, the ascertainment of recalled dietary intake after the onset of disease (retrospective design) may reflect changes in the diet secondary to the effects of the disease itself rather than the individual's dietary habits preceding the onset of disease.

Although no specific causative infectious agent has been identified, bacterial factors are likely to play a role in the initiation or perpetuation of intestinal inflammation by serving as antigens or costimulatory factors.[62] Several lines of evidence support the hypothesis that intestinal bacteria are involved in the development of UC in the genetically predisposed individual. Colitis in animals occurs in a number of different genetically altered rodents, including interleukin (IL)-2, IL-10, and T-cell receptor knockout mice and HLA-B27 transgenic rats.[63] Interestingly, most of these animal models do not develop colitis in germ-free environments, emphasizing the importance of bacteria in the development of intestinal inflammation in these colitis models. These findings suggest that multiple genes may contribute to the pathogenesis of IBD and that the interaction between genes and the environment is essential. Furthermore, modification of the intestinal flora with probiotic preparations or nonpathogenic *Escherichia coli* may help maintain remission of UC.[64–66] Increasing evidence suggests that probiotic preparations may be effective for preventive and maintenance therapy for pouchitis.[67,68] Of note, patients with UC who undergo colectomy and an ileal pouch-anal canal anastomosis (IPAA) experience pouchitis more commonly than patients with familial polyposis who undergo the same procedure.[69] The potential benefit of probiotic therapy and the higher incidence of pouchitis among patients with UC may further support the theory of an abnormal immune response to luminal bacteria in the genetically susceptible host, leading to the development of UC.

Ultimately, the damage to the large intestine that characterizes UC is the result of genetically determined dysfunction of the mucosal immune system. Inflammation occurs as a result of either excessive effector T-cell function or deficient regulatory T-cell function.[30] In UC, evidence suggests that an excessive T helper (Th)2-cell response occurs rather than the Th1-cell pattern typical of CD (Table 41.2-4). Th2 cells provide more efficient help in the activation of B cells and thus support a humoral immune response. The more frequent production of autoantibodies, such as antineutrophil cytoplasmic antibodies[70] and antitropomyosin antibodies,[71] in UC is thus consistent with an excessive Th2-cell

TABLE 41.2-4 CONTRASTING EFFECTOR T-CELL PATTERNS IN ULCERATIVE COLITIS AND CROHN DISEASE

	ULCERATIVE COLITIS TH2 RESPONSE	CROHN DISEASE TH1 RESPONSE
Cytokines	IL-5, IL-13	IFN-γ, IL-12, TNF-α
Autoantibodies	Frequent, eg, p-ANCA, antitropomyosin	Less frequent
Mucosal IgG subclass predominance	IgG1, IgG4	IgG2
Macrophage activation	Absent	Present

IFN = interferon; Ig = immunoglobulin; IL = interleukin; p-ANCA = perinuclear antineutrophil cytoplasmic antibodies; TNF = tumor necrosis factor.

response. Although IL-4 secretion is not increased in UC, the secretion of other cytokines characteristic of a Th2-cell response, namely IL-5 and IL-13, is elevated.[30] Furthermore, the immunoglobulin subclasses typically associated with a Th2 cell response, IgG1 and IgG4, are found in higher concentration in the serum of individuals with UC.[72]

Although there is mounting evidence that excessive effector T-cell function occurs in IBD, abnormal regulatory T-cell function may also be of critical importance in the pathogenesis of UC. Such abnormal regulation could lead to loss of normal oral tolerance to antigens of the mucosal microflora and result in persistent inflammation.[73] Abnormalities of antigen-presenting cells may also play a role in this abnormal response to the intestinal microflora.[30]

The epithelial cell barrier that limits or modifies exposure of the mucosal immune system to the microflora is a result of both passive or mechanical and active functions. Recent evidence suggests that epithelial cells can actively recognize luminal antigens through the expression of Toll-like receptors or other mechanisms. Thus, defects in either the passive or active epithelial barrier function might result in initiation or potentiation of mucosal inflammation by microbial antigens.[30]

Once the mucosal immune system is activated, cytokines and chemokines play important roles in the promotion and amplification of the inflammatory cascade. Levels of IL-8 are elevated in biopsy specimens and in rectal dialysates from patients with active UC.[74–76] IL-8 and other cytokines facilitate the recruitment and transmigration of neutrophils across mucosal surfaces.[77,78] In turn, these neutrophils produce leukotrienes and other mediators, which promote inflammation and contribute to tissue damage.

CLINICAL FEATURES

PRESENTATION

The cardinal symptoms of UC are diarrhea, rectal bleeding, and abdominal pain. However, several different patterns of presentation of UC are recognized, differing by the extent and severity of mucosal inflammation and the degree of systemic disturbance. Most commonly, UC presents with the insidious onset of diarrhea, later associated with hematochezia, but usually without systemic signs of fever, weight loss, or hypoalbuminemia.[79,80] In these patients, the physical examination is usually normal, with no abdominal tenderness.

Approximately 30% of cases have moderate signs of systemic illness and present with bloody diarrhea, cramps often associated with urgency to defecate, malaise, low-grade or intermittent fever, anorexia with weight loss, and mild anemia and hypoalbuminemia. Physical examination may reveal abdominal tenderness, and stool analysis shows varying amounts of blood and leukocytes.[79,80]

Severe colitis occurs in approximately 10% of patients and is characterized by five or more bloody stools per day, significant anemia, hypoalbuminemia, fever, tachycardia, and weight loss. The abdomen may be diffusely tender or distended. Children with this presentation are less likely to have a lasting response to medical therapy and may require early colectomy.[81] Adult and pediatric criteria for the diagnosis of severe or fulminant colitis are compared in Table 41.2-5.[81,82]

Isolated rectal involvement is unusual in children but can be recognized by the symptom complex of tenesmus, urgency, and the passage of formed or semiformed stool with blood and mucus.[83] Limited distal proctitis or proctosigmoiditis may extend to involve the more proximal colon with time in approximately one-third to half of cases.[84–87]

TABLE 41.2-5 CRITERIA FOR THE DIAGNOSIS OF SEVERE ULCERATIVE COLITIS

FEATURE	TRUELOVE AND WITTS[82]	WERLIN AND GRAND[81]*
Bloody stools	≥ 6 per day	≥ 5 per day
Fever	Mean evening temperature > 37.5°C or temperature ≥ 37.8°C at least 2 of 4 d	> 100°F during the first hospital day
Tachycardia	> 90 bpm	≥ 90 bpm
Anemia	Hgb ≤ 75% of normal value	Hct ≤ 30%
Hypoalbuminemia		Serum albumin ≤ 3.0 g/dL
Erythrocyte sedimentation rate	> 30 mm/h	

Hct = hematocrit; Hgb = hemoglobulin.
*Patients must fulfill four of the five listed criteria or have toxic megacolon to be categorized as having severe colitis.

Although, by definition, UC is confined to the colon, children with UC may develop symptoms of gastroesophageal reflux or dyspepsia, with associated inflammation of the upper gastrointestinal tract.[88,89] In a controlled, blinded study of children with UC who underwent upper endoscopy, esophagitis was noted in 50%, gastritis in 69%, and duodenitis in 23% of patients.[88] In the same study, patients with CD were noted to have a higher incidence of inflammation in each of these areas. Moreover, granulomas of the upper gastrointestinal tract were seen in 40% of the patients with CD and duodenal cryptitis was noted in 26% of patients with CD but in none of the patients with UC. Focally enhanced gastritis, thought to be a specific feature of CD, has also been recognized in UC.[89] Despite the accumulating evidence of frequent upper gastrointestinal involvement in UC, more research is needed to determine whether this is a primary manifestation of UC or secondary to concomitant peptic disease.

Extraintestinal manifestations of disease may rarely be the presenting features and precede the gastrointestinal manifestations of UC. Although less commonly seen than in CD, the first sign of UC may be growth failure characterized by decreased linear growth velocity.[90,91] Children with UC may develop axial or perpheral arthritis, erythema nodosum, pyoderma gangrenosum, or primary sclerosing cholangitis prior to the recognition of colitis.[79]

Extraintestinal Manifestations

Approximately 25 to 35% of patients with IBD develop extraintestinal symptoms (Table 41.2-6).[92,93] Extraintestinal manifestations of IBD may occur before, during, or after the development of gastrointestinal symptoms and may even appear after surgical removal of diseased bowel.[93–97] Furthermore, the clinical activity of the extraintestinal manifestations may or may not correlate with the activity of intestinal inflammation.

Arthropathy is a frequent extraintestinal manifestation of UC. It occurs in approximately 20 to 25% of patients and occasionally may be the presenting manifestation of UC. Two major forms have been recognized: a peripheral arthropathy and an axial arthropathy.

The peripheral arthritis of IBD usually presents as an asymmetric, nondeforming, migratory polyarthritis of one or more large joints (knees, ankles, hips, wrists, or elbows). Exacerbations of peripheral joint disease seem to parallel increased activity of bowel disease in UC or CD.[98] Orchard and colleagues, in a study of 976 adults with UC, suggested that there may be two subtypes of peripheral arthropathy: a pauciarticular form with fewer than five swollen joints and a polyarticular form with more than five joints. Of these two subtypes, the pauciarticular arthropathy is more likely to correlate with exacerbations of bowel disease.[99] Treatment of the mucosal inflammation associated with UC generally results in improvement of the peripheral arthropathy; however, judicious use of NSAIDs or other anti-inflammatory agents may be required. In addition to its value in the treatment of colonic inflammation, sulfasalazine may have a direct effect on the arthritis.

Axial arthropathies, ankylosing spondylitis or sacroiliitis, occur in 1 to 4% of patients and are associated with HLA-B27 positivity in the majority of cases.[92–94,100] Ankylosing spondylitis associated with IBD runs a course independent of the activity of bowel disease and may progress to permanent deformity, despite colectomy.[93,94,100] Patients with UC also frequently develop enthesopathy characterized by inflammation at the site of attachment of tendons and ligaments to bone.

Pyoderma gangrenosum and erythema nodosum are the two major skin manifestations associated with UC and CD. Pyoderma gangrenosum occurs in less than 1 to 5% of patients with UC (although it is more commonly seen in UC than in CD)[92,95] and often is associated with active disease and extensive colonic involvement.[95,101] The classic pyoderma gangrenosum lesion often begins as a discrete pustule with surrounding erythema and then extends peripherally to develop into an ulceration with a well-defined border and a deep erythematous to violaceous

TABLE 41.2-6 PRINCIPAL EXTRAINTESTINAL MANIFESTATIONS OF ULCERATIVE COLITIS

Musculoskeletal
Peripheral arthopathy
Ankylosing spondylitis/sacroiliitis
Enthesopathy
Hypertrophic osteoarthropathy
Decreased bone density*
Skin
Pyoderma gangrenosum
Erythema nodosum
Acne*
Alopecia*
Ophthalmologic
Episcleritis
Uveitis
Cataracts*
Increased intraocular pressure*
Hepatobiliary
Fatty liver disease*
Sclerosing cholangitis
Autoimmune hepatitis
Cholelithiasis
Hematologic
Coagulation abnormalities
Iron deficiency anemia
Autoimmune hemolytic anemia*
Neutropenia*
Thrombocytosis
Immune thrombocytopenic purpura
Renal
Nephrolithiasis
Pancreas
Pancreatitis*
Cardiorespiratory
Pericariditis*
Pneumonitis*
Growth and development
Delayed growth*
Delayed puberty

*These complications may be the result of drug side effects.

color.[102] The lesions of pyoderma gangrenosum tend to be multiple and localize below the knees and often develop at sites of trauma and previous surgical sites, including scars and ileostomy stomas.[101–103] Approximately 40% of patients with UC and pyoderma gangrenosum also develop joint symptoms.[95] Treatment of pyoderma gangrenosum involves intralesional or systemic therapy, and remission is difficult to achieve. Erythema nodosum occurs more frequently with CD (27%) in comparison with UC (4%) and usually coincides with increased bowel disease activity.[92,95,100] Erythema nodosum lesions appear as tender, warm, red nodules or raised plaques and usually localize to the extensor surfaces of the lower extremities, particularly over the shins.[104] Both pyoderma gangrenosum and erythema nodosum can precede the development of bowel symptoms, and pyoderma gangrenosum can occur even after bowel resection.[95] Other skin manifestations are unusual and include Sweet syndrome (acute febrile neutrophilic dermatosis) and, rarely, oral lesions.[105]

Ophthalmologic abnormalities are described in approximately 1 to 3% of children with IBD.[93] Episcleritis and uveitis are the more common ocular disorders reported.[106] Episcleritis usually presents with hyperemia of the sclera and conjunctiva, with or without pain and without loss of vision, and is treated with topical corticosteroids.[93,105] Uveitis is typically characterized by acute or subacute pain of the eye with visual blurring, photophobia, and headache and is diagnosed with a slit-lamp examination.[105] However, uveitis associated with IBD in children may be asymptomatic; thus, the incidence of associated eye findings may be underestimated in the literature.[107] Because of the potential risk of developing blindness, uveitis should be treated promptly with topical or systemic steroids.[105] Ocular inflammation appears to develop more commonly in patients with other extraintestinal manifestations, including arthritis, and may be associated with genes in the HLA region.[97,107] In addition, corticosteroid use increases the risk of increased intraocular pressure and the development of posterior subcapsular cataracts.[108,109] Given the potential eye complications, children with IBD should be monitored carefully at regular intervals, especially when receiving systemic corticosteroid therapy.

Hepatic abnormalities in children with UC have been well described. Although these are typically identified after the UC diagnosis, they may also precede the gastrointestinal symptoms.[110] Transient elevations of alanine aminotransferase occur in 12% of children with UC and appear to be related to medications or disease activity.[110] Persistent alanine aminotransferase elevations suggest the presence of primary sclerosing cholangitis or autoimmune chronic active hepatitis. Among children with UC, approximately 3.5% develop sclerosing cholangitis and less than 1% develop chronic active hepatitis.[110] The diagnosis of primary sclerosing cholangitis may be suspected based on symptoms of chronic fatigue, anorexia, pruritus, or jaundice, although many children may be asymptomatic.[111] γ-Glutamyl transpeptidase and alkaline phosphatase levels are typically elevated.[96] The diagnosis of primary sclerosing cholangitis is best established through a combination of cholangiography and liver biopsy.[111] Medical treatment has little impact on disease course, although ursodeoxycholic acid may be of some benefit.[112] There is a paucity of literature addressing the long-term outcome of children with primary sclerosing cholangitis specifically associated with UC.[110] In children with primary sclerosing cholangitis (with or without IBD), later age at presentation, splenomegaly, and prolonged prothrombin time at presentation were associated with poor outcome, defined as death or required listing for transplant.[96] Fatty changes of the liver observed on liver biopsies of patients with UC or CD may be secondary to malnutrition, protein losses, anemia, and corticosteroid use.[113] An increased risk of cholelithiasis has been reported in individuals with UC but is more commonly observed in CD.[114]

Thromboembolic disease, including cerebrovascular involvement, deep vein thrombosis, and pulmonary emboli, can lead to significant morbidity for individuals with IBD.[115–117] Multiple coagulation abnormalities are observed in IBD, including thrombocytosis; elevated levels of fibrinogen, factor V, and factor VII; and depressed levels of antithrombin III and factor V Leiden.[105,116] Controversy still remains as to whether the thromboembolic complications are directly related to the altered hemostatic states observed in UC and CD and may involve a more complex multifactorial cascade involving gene-gene and gene-environment interactions.[118] Rarely, other hematologic abnormalities associated with UC occur, including immune thrombocytopenic purpura[119] and autoimmune hemolytic anemia.[120]

Low bone mineral density occurs in children with UC but less often than in children with CD.[121] As with CD, corticosteroid use increases the risk of osteopenia. Chronic recurrent multifocal osteomyelitis, a rare disorder characterized by aseptic inflammation of the long bones and clavicles, was recently found to be associated with both UC and CD.[122] Other extraintestinal manifestations of UC include nephrolithiasis,[92,123] pancreatitis (related or unrelated to medications),[124,125] and pulmonary and cardiac involvement.[93]

Complications

A number of serious complications may occur in the course of UC. Whereas some of these, such as toxic megacolon, perforation of the colon, and massive hemorrhage, may occur with a severe exacerbation at any point of time, strictures and colon cancer typically happen in the setting of long-standing disease.

Toxic megacolon is a life-threatening complication of UC characterized by total or segmental nonobstructive dilatation of the colon of at least 6 cm in transverse diameter (as defined in adults) associated with systemic toxicity.[126–128] Although reported to occur in up to 5% of UC patients over their lifetime, it is relatively rarely encountered in our pediatric experience, and the overall incidence seems to be decreasing secondary to earlier recognition and improved management of severe colitis.[128] In contrast to typical UC, in which the inflammatory changes are limited to the mucosa, in toxic megacolon, the severe inflammation extends into the deeper layers of the colonic wall.[129]

The pathogenesis of toxic megacolon is probably multifactorial.[128] The extension of inflammation to the muscularis mucosa and myenteric plexus leads to paralysis of colonic smooth muscle, hypokalemia and hypomagnesemia further impair neuromuscular function, and low oncotic pressure owing to hypoproteinemia results in edema. Nitric oxide, an inhibitor of smooth muscle tone, may also play a role.[130] Disrupted mucosal integrity may allow entry of bacteria to submucosal tissues, leading to necrosis and peritonitis. Absorptive function of the mucosa is also impaired, resulting in increased luminal fluid volume and electrolyte losses.[131]

Risk factors for toxic megacolon include drugs that impair motility, such as anticholinergic agents, narcotic agents prescribed for analgesia or antidiarrheal effects, and antidepressants with anticholinergic effects.[129,132,133] Diagnostic procedures involving distention of the colon, such as colonoscopy and barium enema, may impair blood flow and increase mucosal uptake of bacterial products.[128] Metabolic abnormalities, such as hypokalemia, hypomagnesemia, or hypoproteinemia, may compromise colonic epithelial integrity and motor function. The early discontinuation or rapid tapering of steroids or 5-aminosalicylic acid (5-ASA) agents has been associated with the subsequent development of toxic megacolon.[128,129]

Toxic dilatation of the colon is accompanied by fever, abdominal distention, and tenderness, with or without peritoneal signs. In addition, tachycardia, hypokalemia, hypomagnesemia, hypoalbuminemia, dehydration, hypotension, and mental status changes can develop with progressive disease.[126,128] Leukocytosis with a predominance of immature neutrophils or anemia may be present. It is important to consider that clinical signs, particularly fever and tenderness, may be masked by high-dose steroid treatment. Therefore, a high index of suspicion is prudent regarding any change in clinical appearance, including attention to reduction in stool frequency, which may reflect colonic dysmotility rather than clinical improvement. Abdominal radiography reveals colonic dilatation, most frequently involving the transverse colon, sometimes accompanied by inflammatory changes including absent or markedly edematous haustral pattern (Figure 41.2-2).[134] Because the transverse colon is the most anterior portion of the colon, air will tend to accumulate in this segment of the colon in the supine position; however, with repositioning of the patient, the colonic air will redistribute, filling other segments of the bowel.[135]

The patient with toxic megacolon is at risk of colonic perforation, gram-negative sepsis, and massive hemorrhage.[126] Once the disorder is recognized, optimal management mandates prompt surgical consultation and should include stool bacterial culture with assay for *Clostridium difficile* toxin and treatment with broad-spectrum antibiotics and high-dose steroids. Effective monitoring requires serial physical examination and periodic (every 8 to 12 hours) supine and upright radiographs to assess colonic caliber and exclude the presence of intra-abdominal free air indicative of perforation. A nasogastric tube or, if necessary, passage of a long tube into the distal intestine may decompress the colon and minimize further fluid accumulation. Fluid and electrolyte homeostasis should be restored with intravenous normal saline, albumin, and blood. If not previously indicated, parenteral alimentation should begin in anticipation of a prolonged period of restricted enteral intake. Because most distention occurs in the anteriorly located transverse colon, positioning the patient prone, with or without a rectal tube, may facilitate colonic decompression. Patients who fail to respond to these aggressive medical measures and have persistence of toxic dilatation for longer than 48 hours, perforation, or ongoing hemorrhage may require emergency colectomy.

Massive hemorrhage in patients with UC may occur with or without toxic megacolon. Such hemorrhage is managed with blood transfusions and aggressive treatment for the underlying UC and may require urgent colectomy.[136] Colonic perforation may also occur with or without accompanying toxic colonic dilatation and requires urgent colectomy.[137] Intra-abdominal and hepatic abscesses occur less commonly than in CD, except after perforation or colectomy.

In long-standing UC, a colonic stricture may occur. Although in adults this may be due to carcinoma, in the pediatric age group, benign postinflammatory fibrotic strictures are most likely. These strictures usually occur in the rectum and sigmoid, are due to smooth muscle hypertrophy, and are thought to be potentially reversible.[138–140]

Individuals with long-standing UC are at a markedly increased risk of developing colorectal cancer.[141–143] This

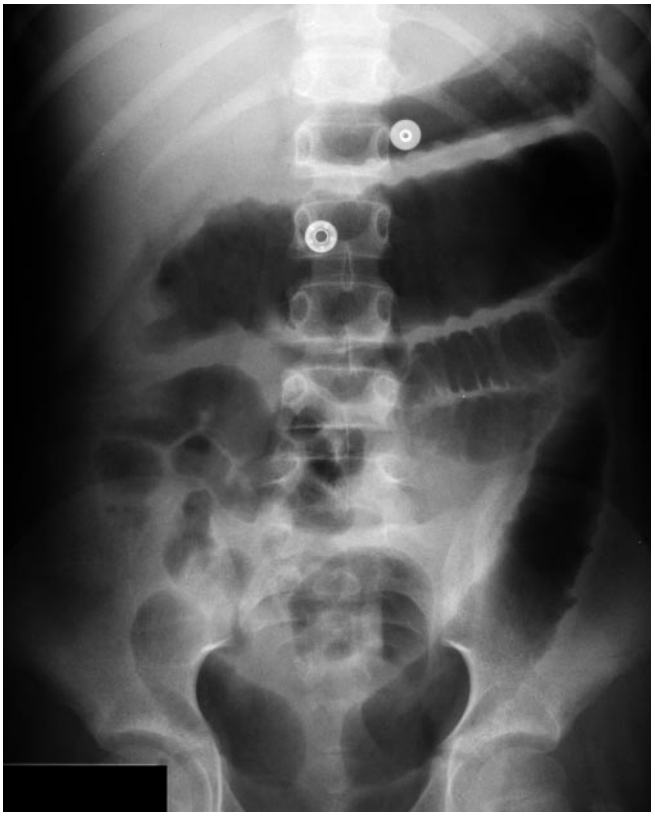


FIGURE 41.2-2 Massive dilatation of the transverse colon in a patient with fulminant ulcerative colitis developing abdominal distention. Courtesy of Carlo Buonomo and the Radiology Teaching Collection, Children's Hospital, Boston.

risk increases with longer duration of disease and more extensive colonic involvement.[144,145] In patients with less than 8 to 10 years of disease, the risk is minimal but thereafter increases by 0.5 to 1.0% per year.[146] Early age at onset has not been consistently demonstrated to be an independent risk factor.[147] The risk of colorectal cancer is minimal in patients with proctitis or proctosigmoiditis, intermediate in patients with left-sided colitis, and highest in patients with pancolitis.[147] In a population-based cohort study in Sweden, Ekbom and colleagues reported standardized incidence ratios for colorectal cancer of 1.7 for ulcerative proctitis, 2.8 for left-sided colitis, and 14.8 for pancolitis.[142] Other risk factors for colorectal cancer in patients with UC include concurrent primary sclerosing cholangitis,[148–150] a family history of colorectal cancer,[151] and the occurrence of backwash ileitis.[152] In contrast, some retrospective studies suggest that 5-ASA agents may provide a protective effect in the development of colorectal cancer in patients with UC,[153,154] but other studies do not support these findings.[155] Recently, ursodeoxycholic acid has been demonstrated to be effective as a chemopreventive agent in patients with UC and primary sclerosing cholangitis.[156,157] The role of pharmacologic therapy and vitamin supplements, such as folate, in the development of colorectal cancer in patients with UC remains controversial, and further prospective studies are needed.[158]

Colonoscopic screening programs are designed to detect patients with low- or high-grade dysplasia prior to the development of cancer and to detect cancer as early as possible. However, there are no randomized controlled trials examining the effectiveness of surveillance colonoscopy for dysplasia and colorectal cancer in patients with UC, and screening may even miss cancer.[159,160] Furthermore, there is significant interobserver variation among pathologists in the determination of the presence and grade of dysplasia.[161,162] Current practice includes surveillance colonoscopy every 1 to 2 years beginning at 8 years after the diagnosis of disease for pancolitis and 15 years in those with left-sided colitis.[160] However, it may be prudent to perform the initial surveillance colonoscopy beginning at 8 to 10 years after diagnosis on all patients with UC to reassess the true extent of disease.[160] Biopsies are performed in four quadrants at 10 cm intervals from the cecum to the descending colon and then at 5 cm intervals beginning in the sigmoid colon. The American College of Gastroenterology recommends colectomy for cancer and high-grade dysplasia and dysplasia-associated lesion or mass, both of which have a high risk of associated malignancy, and low-grade dysplasia, despite the fact that some patients with low-grade dysplasia may not progress.[163] It is not clear whether surveillance practice should differ for patients with younger-onset UC, and there are no published formal recommendations to guide surveillance colonoscopy in children.[159]

DIAGNOSIS

Differential Diagnosis

The diagnosis of UC is established by the information gathered from a detailed history, physical examination, and a combination of laboratory, radiologic, and endoscopic studies. The goal of the evaluation of the child with suspected colitis is to exclude other etiologies such as an infectious process and to distinguish UC from CD. Physical examination should include assessment of height, weight, and body mass index; abdominal distention, tenderness, or mass; extraintestinal manifestations (eg, aphthous stomatitis, pyoderma gangrenosum, uveitis, or arthritis); fecal blood on rectal examination; and perianal abnormalities (eg, fistulae, fissures, or tags). Colonic inflammation is typically accompanied by bloody diarrhea with abdominal cramping. Rectal bleeding may be caused by a number of other disorders in children, including polyps, vascular abnormalities, Meckel diverticulae, intestinal duplications, surgical disorders such as intussusception and other causes of bowel ischemia, or anal disease, such as hemorrhoids or anal fissures. The bleeding from Meckel diverticulum is usually painless, copious, and without fecal leukocytes. Polyps also usually cause painless bleeding, which is often low grade and chronic but may be massive. Fissures may be secondary to constipation or trauma or may be among the perianal manifestations of CD, particularly if inflammation is prominent.

The differential diagnosis of colitis depends on the age of the child at the time of evaluation. In infancy, necrotizing enterocolitis, Hirschsprung enterocolitis, and allergic colitis must be considered. In contrast, in the older child and adolescent, enteric infection and IBD are the most common diagnoses. Infection with *Salmonella*, *Shigella*, *Campylobacter*, *Yersinia*, *Aeromonas*, certain strains of *E. coli*, and *Entamoeba histolytica* may resemble UC and should be excluded. Although *C. difficile* usually causes diarrhea without accompanying rectal bleeding, pseudomembranous colitis may develop. Vascular injury and consequent ischemic colitis may occur in children with Henoch-Schönlein purpura and hemolytic uremic syndrome. In the latter disorder, the characteristic intravascular hemolysis and renal injury characteristic of hemolytic uremic syndrome may not be present initially. A complete list of the causes of colitis is provided in Table 41.2-7.

In addition to details of the clinical presentation, the history should include a family history, recent antibiotic therapy, infectious exposures, growth and sexual development, and the presence of extraintestinal manifestations of UC. Findings on physical examination may help to distinguish UC from CD; for example, pronounced growth failure or a perianal abscess strongly suggests the diagnosis of CD. A severely ill child with UC may have tachycardia, orthostatic hypotension, fever, or dehydration. Such findings in the presence of abdominal distention and a concerning abdominal examination may herald a fulminant presentation of UC with increased risk of developing toxic megacolon.

Laboratory Assessment

Initial laboratory evaluation should include blood tests, stool for occult blood and stool cultures, and a urinalysis. A complete blood cell count with differential may reveal a leukocytosis with or without left shift, microcytic anemia, and thrombocytosis. Thrombocytosis, hypoalbuminemia, and

TABLE 41.2-7 DIFFERENTIAL DIAGNOSIS OF COLITIS

INFECTIOUS ETIOLOGIES
Bacterial: *Campylobacter*, *Salmonella*, *Shigella*, *Escherichia coli* (enterohemorrhagic strains) *Yersinia*, *Aeromonas*, *Plesiomonas*, *Clostridium difficile*, *Gonococcus*, tuberculosis
Parasitic: *Entamoeba histolytica*
Viral: cytomegalovirus, HIV
CHRONIC IDIOPATHIC
Ulcerative colitis
Crohn disease
Behçet disease
Lymphocytic colitis
Collagenous colitis
Eosinophilic colitis
VASCULITIS
Henoch-Schönlein purpura
Hemolytic uremic syndrome
OTHER
Ischemic colitis
Allergic colitis
Enterocolitis associated with Hirschsprung disease
Diversion colitis
Chemotherapy-induced colitis
Radiation-induced colitis
Graft-versus-host disease
Necrotizing enterocolitis (in neonates)

elevated erythrocyte sedimentation rate or C-reactive protein may indicate increased disease activity.[164–166] Unlike the erythocyte sedimentation rate, the assay for C-reactive protein is not affected by the hematocrit or other serum proteins and thus may be a superior marker for intestinal inflammation. The presence of anemia with low mean corpuscular volume, wide red blood cell distribution width, and low iron levels may indicate an iron-deficient anemia secondary to ongoing fecal blood losses or malabsorption of iron. However, even children with significant mucosal inflammation may have normal laboratory test results. In a study of children with UC or Crohn colitis, 13 of 36 patients with UC (36%) had normal blood test results, including 7 of 28 UC patients with macroscopic findings on colonoscopy and 12 of 31 UC patients with histologic moderate or severe chronic inflammation.[166] Although insensitive, a positive assay for fecal leukocytes by Wright stain or methylene blue preparation may suggest colitis. More recently, assays of white blood cell proteins, such as calprotectin or lactoferrin, have been proposed as more reliable indicators of colonic inflammation.[167–169] Stool cultures should be performed to detect enteric infections. It is important to note, however, that a significant percentage of patients with bacterial colitis will have negative cultures. In sexually active patients, rectal cultures for gonorrhea should be considered. *C. difficile* is detected more frequently in patients with UC than in the general population. Assay for both *C. difficile* toxins should be obtained in all patients, regardless of prior antibiotic treatment.[170–172]

Several unique serum antibodies have been proposed as tools for use in screening symptomatic children for IBD and discriminating UC from CD.[173,174] Perinuclear antineutrophil cytoplasmic antibodies (p-ANCAs) are seen in 60 to 80% of subjects with UC compared with 10 to 27% of those with CD.[173,175,176] In contrast, anti–*Saccharomyces cerevisiae* antibodies (ASCAs) are commonly found in individuals with CD but are rarely seen in UC. In a study of 173 children, assay of ASCAs yielded a sensitivity of 55% and a specificity of 95% for CD, and assay of ANCAs had a sensitivity of 57% and a specificity of 92% for UC.[173] Using modified cutoff values to optimize the sensitivity of the ASCA and ANCA assays in a study of 128 pediatric patients undergoing evaluation for IBD, Dubinsky and colleagues noted an increase in sensitivity for detecting IBD from 69 to 81%, but this was accompanied by an increase in false-positive rates among the children without IBD.[174] Therefore, these tests should not be considered a substitute for the traditional evaluation of a child for UC. Furthermore, because a significant fraction of children with CD may have positive assays for p-ANCAs, serologic studies may not reliably discriminate between CD and UC. In particular, p-ANCAs tend to test positive in the serum of patients with CD who exhibit UC features.[173,177] Additional evidence will be required before the routine use of serologic testing can be endorsed in the child with suspected UC.

RADIOLOGY

Plain abdominal radiographs, both upright and supine, are typically used to detect complications of UC, such as colonic dilatation, obstruction owing to stricture, and pneumoperitoneum from perforation. The extent of disease may occasionally be ascertained by noting the distribution of abnormal haustral patterns as outlined by air.[178–180] These films form a baseline for later comparisons. With the increasing application of endoscopic procedures, the barium enema has been assigned a very limited role in the evaluation of children with UC. A barium enema should never be performed in patients with acute, active colitis because of the risk of precipitating toxic dilatation. Single-contrast barium enema is rarely performed except in young children but may help in assessing the character and extent of colonic disease. In mild to moderate colitis without dilatation, an air-contrast barium enema may reveal enough detail to detect mucosal granularity, ulcerations, and nodular haustral folds. Even without air contrast, a barium enema may reveal the chronic changes of foreshortening, loss of haustra, pseudopolyps, and strictures. An upper gastrointestinal series with small bowel follow-through should be performed to assess for any evidence of abnormality of the terminal ileum or a more proximal gastrointestinal tract and the presence of fistulae, which would suggest CD. The only exception is the finding of UC-associated "backwash ileitis," which should be distinguished from terminal ileal disease of CD. With backwash ileitis, the ileum appears patulous and inflamed, involvement is limited to the distal ileal segment, and pancolitis is present; there should be no evidence of extensive ulcerations or stricturing, as seen with CD.[140] A late film during the upper gastrointestinal series to study the right colon (anterograde colonogram) may uncover colonic involvement.[181] This technique is safer than a barium enema, although lack of colonic distention may make interpretation difficult. Computed tomography is not typically performed in the initial assessment for UC but may demonstrate diffuse bowel wall thickening, marked rectal wall thickening, and perirectal fibrofatty pro-

liferation.[140] In moderately to severely ill patients with bowel dilatation, extensive bleeding, persistent fever, or abdominal mass, computed tomography may identify an intra-abdominal abscess. Recently, Doppler ultrasonography[182,183] and leukocyte-labeled scintigraphy[184] have been proposed as accurate means of assessing the extent and activity of UC; however, more study is needed before routine application of these studies can be recommended.

Endoscopic Assessment

Flexible sigmoidoscopic or colonoscopic inspection of the colon and ileum, in conjunction with mucosal biopsies, provides the most sensitive and specific evaluation of intestinal inflammation. Although in older children and adults, sigmoidoscopy can be performed with more limited sedation and preparation of the colon, these advantages do not apply to young children; therefore, full colonoscopy with examination of the terminal ileum at diagnosis may be preferable. To establish the extent of disease involvement by colonoscopy, we recommend biopsies from the terminal ileum and each segment of the colon even if there are no macroscopic findings at a particular level of the colon. However, colonoscopy is contraindicated in severe colitis because of the risks of perforation and hemorrhage and the induction of toxic megacolon. In patients with severe disease, a limited proctosigmoidoscopy often suffices to establish the diagnosis. Excessive bowel distention with air insufflation should be avoided, and the examination should be halted on encountering areas of severe inflammation.

In UC, typical findings seen by the endoscopist include a diffuse, continuous involvement of the mucosa starting at the rectum and extending more proximally into the colon. In children with UC, however, rectal sparing has occasionally been noted.[9,10] Also, previous topical therapy may also result in the appearance of distal sparing. The colonic mucosa often appears edematous, erythematous, and friable, with loss of vascular pattern, minute surface erosions, and ulcerations. Larger, deeper ulcerations with associated exudate may develop in more severe disease. With long-standing UC, pseudopolyps may be present. In contrast, in CD, colonoscopy may reveal focal ulcerations (aphthous lesions) with intervening areas of normal-appearing mucosa (skip lesions). In severe or chronic CD, linear ulcerations, nodularity (cobblestoning), and strictures or stenoses may be present. In general, the ulcerations in CD are deeper and focal versus the diffuse, superficial ulcerations typical of UC.[185–187]

Although inflammation of the upper gastrointestinal tract has been reported primarily in children with CD, such inflammation can also be seen in UC.[88,89,188,189] Nevertheless, the pattern of inflammation (eg, presence of granulomas characteristic of CD) may differentiate the two entities. Therefore, some centers recommend initial upper endoscopy in addition to colonoscopy in all patients with suspected IBD.

THERAPY

Overview

UC is a chronic disease requiring careful monitoring, patient education, and expert management by a team consisting of the primary clinician, gastroenterologist, nurse, nutritionist, social worker, and psychiatrist or psychologist. An alliance with a pediatric surgeon familiar with IBD is essential for management of potential complications. Because the success of management depends on the degree to which the patient and family understand and participate in the treatment, they must be educated about the disease and incorporated as members of the team, often a major challenge for the physician. Support groups and organizations such as the Crohn's and Colitis Foundation of America are indispensable not only in disseminating information but also in providing a community to counter the isolation that often attends the diagnosis of a chronic disease.

The effective management of UC in children demands special considerations. Poor compliance with recommendations for medical therapy is a major reason for treatment failure, especially in the pediatric age group. Younger children may not be able to swallow tablets or capsules or refuse medications on the basis of taste. Extemporaneous suspensions and the administration of capsule contents or crushed tablets in applesauce or foods of similar consistency are possible options for these patients. Older children and adolescents are unlikely to be compliant with medication programs that require multiple daily doses of medication; accordingly, regimens should be streamlined. In the choice of medication, the pediatric gastroenterologist must also consider the unique aspects of the pediatric patient, such as growth, pubertal development, and psychosocial adjustment to disease, and the potential adverse effects of certain drugs. For example, corticosteroids, which may inhibit growth and cause cosmetic problems, can cause devastating problems for the teenage child. Monitoring children during therapy presents challenges as well. The physician must balance the need to perform follow-up blood tests and radiologic and endoscopic procedures against the trauma that these tests may cause for certain children.

The goals of medical therapy of UC in children are the induction of remission with control of symptoms, the prevention of relapse, the avoidance of complications, and the provision of optimal quality of life. With the advent of more effective agents, mucosal healing has been proposed as a goal of therapy, but this approach remains controversial and is often impractical. The choice of therapy depends on the severity of the inflammation and the distribution of inflammation in the colon. The following discussion reviews the utility of the various individual agents used in the management of UC (Table 41.2-8) and offers an approach to therapy based on disease severity and distribution.

Pharmacologic Agents

Aminosalicylates are the principal therapeutic agents for treatment of mild to moderate UC in childhood. Both the initially discovered agent sulfasalazine and newer formulations containing 5-aminosalicylate have proven efficacious in the induction of remission in mild to moderate disease and in the subsequent maintenance of remission.

A combination of 5-aminosalicylate and sulfapyridine linked by an azo bond, sulfasalazine is split by colonic flora into its two constituents. The 5-aminosalicylate is poorly

TABLE 41.2-8 COMMONLY PRESCRIBED MEDICATIONS FOR ULCERATIVE COLITIS

MEDICATION	DOSAGE	MAJOR SIDE EFFECTS
SULFASALAZINE	40–75 mg/kg/d PO, divided bid or tid; adult dose: 4–6 g/d, divided bid or tid	Nausea, headaches, diarrhea, photosensitivity, hypersensitivity reaction, pancreatitis, azoospermia, hemolytic anemia, neutropenia
MESALAMINE		
Oral formulation	30–75 mg/kg/d PO divided bid to qid (depending on preparation); adult dose: 2.4–4.8 g/d divided bid to qid	Nausea, headaches, diarrhea, pancreatitis, nephritis, pericarditis, pleuritis
Enema formulation	2–4 g PR q 12–24 h	
Suppository formulation	500 mg PR q 12–24 h	
CORTICOSTEROIDS		
Intravenous or oral formulation	1–2 mg/kg/d of prednisone or equivalent, IV or PO, divided q 12–24 h (maximum 60 mg/d)	Cushing syndrome, growth suppression, immunosuppression, hypertension, hyperglycemia, increased appetite, osteoporosis, aseptic necrosis (hip), cataracts
Enema formulation	50–100 mg of hydrocortisone PR qhs	
Suppository formulation	25 mg of hydrocortisone acetate PR qhs	
Foam formulation	One application PR qhs	
6-MERCAPTOPURINE	1.0–2.0 mg/kg/d PO qd	Nausea, emesis, immunosuppression, hepatotoxicity, pancreatitis, myelosuppresion
AZATHIOPRINE	1.5–2.5 mg/kg/d PO qd	Nausea, emesis, immunosuppression, hepatotoxicity, pancreatitis, myelosuppression
CYCLOSPORINE	Induction regimen for fulminant colitis: initial dose: 4–6 mg/kg/d IV continuous or divided bid; maintenance oral dose varies according to oral preparation	Nephrotoxicity, hypertension, headache, hirsutism, immunosuppression, nausea, emesis, diarrhea, tremor, hypomagnesemia, hyperkalemia, hepatotoxicity, seizures, gingival hyperplasia

absorbed from the colon and is considered to be the active anti-inflammatory moiety.[190] The proposed mechanisms of action of 5-aminosalicylate include inhibition of the synthesis of the leukotriene B_4, a potent chemotactic agent, from arachidonic acid and the scavenging of reactive oxygen metabolites.[191,192] The sulfapyridine is absorbed and excreted in the urine and is responsible for many of the common dose-related and transient side effects of headache, malaise, nausea, vomiting, anorexia, heartburn, epigastric distress, and diarrhea.[193] In addition, a number of rare and potentially severe hypersensitivity reactions, including skin eruptions, fever, leukopenia, agranulocytosis, aplastic anemia, pancreatitis, systemic lupus erythematosis, and pulmonary dysfunction, may occur. Sulfasalazine interferes with folic acid absorption and may cause megaloblastic anemia if a folate supplement is not administered. About 80% of adult men develop reversible abnormalities of sperm, which can compromise fertility.[191] Parodoxically, cases of exacerbation of colitis have been reported and probably represent salicylate hypersensitivity.[194] Children at risk for glucose-6-phosphatase deficiency should be screened prior to sulfasalazine treatment. Given the potential for intolerance, the dosage of sulfasalazine is gradually increased over 1 week from 10 mg/kg daily to 40 to 50 mg/kg, up to a maximum of 75 mg/kg in two to four divided doses. Symptoms and blood counts should be monitored closely. Because sulfasalazine impairs folate absorption, 1 mg daily folate supplements are usually provided.

Newer agents that use alternative systems have been created to deliver 5-aminosalicylate (mesalamine) to the colon without sulfapyridine.[195] Asacol and Pentasa contain 5-aminosalicylate in a delayed-release coating to prevent proximal absorption of the drug and ensure delivery to the small intestine and colon. Two azo-bonded compounds, which, like sulfasalazine, are cleaved in the colon, are available: olsalazine, which contains two 5-aminosalicylate moieties, and balsalazide, which contains a 5-aminosalicylate molecule bonded to 4-aminobenzolyl-β-alanine. All of these agents have been demonstrated to be effective in the treatment of acute UC and in the maintenance of remission in adults. Although some studies suggest an advantage of azo-bond ASA agents (ie, balsalazide) in comparison with delayed-release ASA agents (ie, mesalamine), the overall data suggest equivalence between the different oral aminosalicylates when comparable amounts of the ASA are released at the site of disease activity.[196] Anecdotal evidence for efficacy of oral aminosalicylates in children with UC exists, but the only controlled trial failed to demonstrate a therapeutic effect comparable to that of sulfasalazine.[197] In this multicenter, randomized, double-blind study, the efficacy and safety of olsalazine (30 mg/kg/d; maximum 2 g/d) were compared with those of sulfasalazine (60 mg/kg/d; maximum 6 g/d) in the treatment of mild to moderate UC. The findings demonstrated clinical remission after 3 months in 79% of the sulfasalazine-treated children in comparison with 39% of the olsalazine-treated children. The authors suggested the low dosage of olsalazine as a possible explanation for the difference in efficacy between sulfasalazine and olsalazine. Only 10 to 15% of patients receiving 5-aminosalicylate compounds in clinical trials experienced side effects, including nausea, vomiting, headache, abdominal pain, diarrhea, rash, and hair loss.[198] Occasionally, severe side effects, such as pancreatitis, pericarditis, nephritis, pneumonitis, and an exacerbation of colitis, may occur.

Olsalazine use is associated with a significantly higher risk of diarrhea. Oligospermia and folate deficiency associated with sulfasalazine use do not occur with the newer 5-aminosalicylate compounds.

The choice of an appropriate oral aminosalicylate for use in children must consider ease of administration. Children who cannot swallow tablets may be able to accept extemporaneous suspensions of sulfasalazine or the content of capsules, in the form of powder or beads, when mixed in applesauce or other foods of similar consistency. Cost should also be considered.

Topical aminosalicylates are effective in the treatment of proctitis, proctosigmoiditis, or left-sided UC.[199–201] To be effective, topical therapy must reach the most proximal extent of the disease activity. Mesalamine suppositories spread to the upper rectum, and enemas and foams can reach the splenic flexure or into the distal transverse colon.[191,202] Mesalamine enemas or suppositories are effective as first-line therapy and maintenance therapy for mild to moderately active left-sided UC or proctitis, respectively.[196] Rectal mesalamine may be superior to oral mesalamine in the treatment of active ulcerative proctitis.[203] Mesalamine enemas may be superior to rectal corticosteroids[204] and are also effective in treating distal colitis that is unresponsive to oral aminosalicylates or corticosteroids.[191,202] Combination therapy with oral and topical mesalamine is more effective than one agent alone in the treatment of mild to moderate distal colitis.[205] Topical agents may rarely be associated with side effects.

Corticosteroids are effective in the induction of remission in children with moderate to severe UC. In this context, steroids do not seem to adversely affect surgical outcome if surgery becomes necessary. Treatment is begun with a relatively high dosage of prednisone or methylprednisolone (1 to 2 mg/kg/d, up to a maximum of 40 to 60 mg) in two doses and sustained until disease is controlled, usually within 2 weeks, and then maintained in a single daily dose for another 2 to 4 weeks. Subsequently, with the adjunctive use of a 5-aminosalicylate, the dose is tapered gradually by 5 mg decrements weekly to a dose approximating the child's endogenous production of corticosteroid (approximately 5 mg of prednisone/m^2 surface area) and is then gradually withdrawn over an additional 2 to 4 weeks. If exacerbation of disease activity prevents complete withdrawal of steroids, chronic alternate-day steroid therapy may be necessary. In an uncontrolled study of 20 children with active UC, 85% of the children achieved clinical remission with combination therapy of corticosteroids (oral prednisolone for pancolitis or topical prednisolone for distal colitis) and mesalamine (20–40 mg/kg/d).[206] Reassessment with colonoscopy at 8 weeks demonstrated complete endoscopic remission in 40% and full histologic remission in only 15%, suggesting that clinical remission may not correlate with endoscopic or histologic remission. Potential short-term complications of steroid therapy in patients with UC include increased appetite, weight gain, fluid retention, mood swings, hyperglycemia, hypertension, insomnia, acne, and facial swelling (moon facies). Complications of long-term steroid therapy (usually of greater than 3 months) include growth retardation, osteopenia with compression fractures, aseptic necrosis of the hip, and cataracts.[207] Effects of steroid therapy on growth may be complex because disease activity itself may cause growth retardation. Some patients resume normal growth velocity only after steroid suppression. Other patients whose disease remains in remission may show catch-up growth velocity only after steroid withdrawal.[80,208] Currently available oral preparations of rapidly metabolized steroids, such as budesonide, which may have decreased systemic toxicity, have not yet been proven to effectively treat patients with pancolitis.[209]

Topical corticosteroid therapy may be sensible when disease is limited to the distal colon and rectum. Corticosteroid suppositories, foam, or enemas can be used as first-line induction therapy in patients with mild or moderately active ulcerative proctitis or left-sided UC[196] or may be used in an attempt to reduce the dose of systemic steroids necessary to control disease. Rectal administration of hydrocortisone or prednisolone permits more direct delivery of steroids to distal UC sites; however, as with oral steroid therapy, prolonged treatment with topical steroids may induce systemic steroid side effects, including adrenal suppression.[199,202] Budesonide in an enema preparation may induce remission in distal colitis with fewer systemic steroid side effects.[210] In a randomized, double-blind, placebo-controlled trial, budesonide enema therapy (2.0 mg or 8.0 mg/enema, once per evening) effectively induced remission, with significant improvement in sigmoidoscopy and histopathology scores, compared with placebo in adults with active distal UC or proctitis.[210]

Immunosuppressive agents are being administered increasingly as adjuncts to steroid therapy. 6-Mercaptopurine (6-MP) and azathioprine, which is converted to 6-MP in the liver, are appropriate agents in cases of UC refractory to or chronically dependent on steroid therapy. These immunomodulatory agents can reduce disease activity and allow the withdrawal of steroid therapy in children with steroid-dependent UC; however, the time to clinical response is usually 4 to 8 weeks.[211,212] In a small open-label trial of children with CD or UC, six of nine patients with UC reduced their steroid therapy by at least 75% with azathioprine therapy.[211] In another series of 16 children with severe, steroid-dependent or steroid-refractory UC, 6-MP or azathioprine therapy allowed the discontinuation of steroid therapy in 75%, and 67% remained without steroid therapy at 3 to 65 months.[212] Given their steroid-sparing effects and reasonable tolerance by children with IBD, 6-MP and azathioprine offer an alternative maintenance treatment of IBD in children. In a study of 95 children with either CD or UC, only 18% required discontinuation of the medication; the majority of side effects responded to dose reduction or improved spontaneously.[213] Side effects reported include aminotransferase elevations and hepatitis, pancreatitis, bone marrow depression, hypersensitivity reactions, and recurrent infections.[213,214] Therefore, frequent monitoring for complications with complete blood counts and liver function tests is advised, especially at the initiation of therapy. Furthermore, because evaluation for thiopurine

methyltransferase genetic polymorphism can identify those children at higher risk for drug toxicity,[215] genetic analysis or a functional measure of enzyme activity should be performed on all patients prior to beginning treatment. For a child with normal drug metabolism, the starting dose of 6-MP is 1 to 1.5 mg/kg/d as a single dose and of azathioprine is 1.5 to 2 mg/kg/d. Children with intermediate levels of enzyme should be given lower doses, and homozygotes with very low levels should not receive the medication. Some clinicians have used 6-MP metabolite levels to monitor for compliance and potential toxicity and to permit safe dose adjustment in nonresponding patients.[216]

Given the high relapse rate with withdrawal of 6-MP in adults with UC,[217] the majority of children requiring azathioprine or 6-MP to suppress disease activity most likely will require long-term maintenance therapy with these agents. There are no studies in children addressing this issue to date. At the present time, evidence does not suggest any definitive increased risk of malignancy secondary to long-term use of azathioprine in adults.[218] However, one article has identified a slightly increased risk of Epstein-Barr virus–associated lymphoma in a large cohort of patients treated with long-term 6-MP or azathioprine.[219]

Methotrexate has been recommended as an alternative for patients intolerant of 6-MP but appears to be less effective for the treatment of UC than for CD.[220] Although open-label trials in adults with UC suggested a benefit of methotrexate in the induction of remission,[221,222] a double-blind, randomized trial failed to show any advantage of methotrexate in the induction or maintenance of remission in adults with chronic active UC in comparison with placebo.[223] Although methotrexate can be useful in treating children with CD intolerant to 6-MP, there are currently no published studies describing its efficacy in children with UC.[224] Studies evaluating the effectiveness of mycophenolate mofetil, another immunodulatory agent, for UC show mixed results and may suggest increased side effects compared with other immunomodulatory agents, such as azathioprine.[225–227]

For adult patients with severe colitis unresponsive to intravenous corticosteroid therapy, cyclosporine has been demonstrated to be very effective, and, in contrast to most other immunosuppressive agents, a response occurs within a mean of 7 days.[228] However, such intensive immunosuppression should not be started if surgery is believed to be imminent, such as in a septic patient, a patient with toxic megacolon, or a patient with a suspected perforation. In children with steroid-refractory UC, intravenous or oral cyclosporine therapy has also successfully induced remission.[229–231] In a study of 14 children treated with oral cyclosporine therapy for severe active colitis unresponsive to high-dose intravenous steroids, 80% achieved clinical remission within 2 to 9 days; however, the majority of children who initially responded to cyclosporine eventually required colectomy within 1 year.[231] The exact dosage of cyclosporine varies in different protocols, depending on whether intravenous or oral medication is used.[228,230,231] In addition to the potential complications of immunosuppression, cyclosporine commonly produces renal toxicity, hypertension, tremors, paresthesias, and hypertrichosis. Children on cyclosporine or tacrolimus should receive prophylaxis for *Pneumocystis carinii* and have careful monitoring of electrolytes, blood glucose, renal function, blood pressure, and neurologic status. A recent review outlines specific recommendations on the initiation and monitoring of cyclosporine therapy in children with IBD.[232] A related agent, tacrolimus can also induce remission when administered orally to children with severe active colitis.[233] However, the majority of the children who responded to either agent relapsed or underwent colectomy within 1 year.[231,233] Therefore, cyclosporine and tacrolimus may be most useful in delaying emergent colectomy at a time when the child's health status is most compromised and may allow time to improve the child's health and emotional status in preparation for future "elective" colectomy.[231] If either cyclosporine or tacrolimus is used as induction therapy, the aim should be to transition patients to a less toxic maintenance medication, such as 6-MP, over a 3- to 6-month period.[234]

In open, uncontrolled trials, therapy with infliximab (chimeric monoclonal anti–tumor necrosis factor-α antibody) has resulted in clinical improvement in adults and children with UC.[235–237] In an open-label study of 9 children and adolescents with moderate to severe UC, 7 children (77%) responded based on a decrease in disease activity as measured by the Physician Global Assessment and corticosteroid therapy was discontinued in six children (66%).[237] A small, double-blind, placebo-controlled clinical trial of infliximab in 11 adults with severe, active, steroid-refractory UC suggested a clinical benefit for patients treated with one dose of infliximab (5 mg, 10 mg, or 20 mg/kg/dose) compared with those who received placebo.[238] In this study, 4 of 8 UC patients receiving infliximab improved compared with 0 of 3 receiving placebo. In a larger, randomized, double-blind, placebo-controlled study of 43 adults with moderately active, glucocorticoid-resistant UC, there was no significant difference in remission rate or sigmoidoscopic score between the group treated with infliximab (5 mg/kg/dose at week 0 and 2) and the placebo group.[239] Given the risk of infusion reactions, anti–nuclear antibody formation, and opportunistic infections and the unclear therapeutic benefit, the role of infliximab in the treatment of UC, especially in children, cannot be supported without further study.[240–242]

Despite the potential role of infectious agents in the pathogenesis of UC,[63] the use of antibiotic therapy in the treatment of UC remains controversial. There is a lack of consistent evidence of the effectiveness of antibiotics in the induction and maintenance of remission in UC.[243–246] Nevertheless, empiric broad-spectrum antibiotics often are administered in the setting of severe active UC,[81,247] especially if there is concern for toxic megacolon or other surgical complications.[128] Probiotics have shown some promise for the maintenance of remission in adults with UC.[64–66] In an open-label trial of the probiotic VSL #3, containing a mixture of bifidobacteria, lactobacilli, and *Streptococcus salivarius*, administered to adults with inactive UC, 75% of patients remained in remission during the

12-month study.[65] In two randomized, controlled, comparison trials, nonpathogenic *E. coli* strains and mesalamine maintained similar rates of remission in adults with quiescent UC.[64,66] There have been no formal studies on the effectiveness of probiotics in children with UC.

A number of other agents have been proposed for the treatment of UC, but insufficient evidence of efficacy or troublesome side effects have prevented them from becoming part of the standard therapeutic armamentarium. Given the decreased risk of UC in smokers, nicotine has been studied as a potential treatment. Despite limited evidence that transdermal or topical nicotine therapy may be beneficial in adults with active UC, the potential side effects make this an inappropriate therapy for children.[248,249] Several placebo-controlled studies suggest a benefit of adjunctive therapy with fish oil supplementation containing eicosapentaenoic acid, a potent inhibitor of leukotriene B_4 synthesis, in the treatment of active UC.[250–252] However, fish oil supplementation does not appear to show any benefit in maintenance therapy.[251,253] Furthermore, therapeutic dosing requires that a large number of capsules be taken daily, and the fishy odor and diarrhea associated with therapy limit tolerance. Randomized, controlled trials in adults with active distal UC suggest that therapy with topical short-chain fatty acid preparations results in clinical improvement, but there is no statistically significant advantage in comparison with placebo.[254,255] In several open-label trials, adults with steroid-resistant UC showed a clinical response to heparin therapy[256–258]; however, a controlled trial did not show any advantage of heparin treatment for moderate to severe UC compared with corticosteroid therapy.[259] In an open-label pilot study, daclizumab (humanized anti-IL-2R antibody [CD 25]) resulted in clinical and endoscopic improvement in adults with refractory UC[260]; further study is needed to determine the effectiveness of this novel therapy.

Approach to Medical Therapy

The first step in the medical therapy of UC is the induction of remission (Table 41.2-9). In the child with mild to moderate pancolitis or left-sided UC, with no or only minimal systemic signs, oral aminosalicylates are usually the first line of therapy. Moderate to severe disease usually requires oral corticosteroids for the induction of remission. Restriction of residue in the diet has been anecdotally reported to reduce symptoms during a flare of disease.[261] Severe or fulminant colitis, as defined by more than five to six bloody bowel movements per day, fever, tachycardia, anemia, and hypoalbuminemia, requires hospitalization and intravenous corticosteroids. Intravenous fluids for rehydration and correction of electrolyte imbalances should be administered, with transfusion of packed red blood cells and albumin if indicated. Central venous access for parenteral alimentation is advisable for patients who are unable to tolerate enteral nutrition. Empiric broad-spectrum antibiotic coverage should be considered, especially if there is a suspicion of a toxic megacolon or another surgical complication. Early surgical consultation is recommended. Failure of the patient with fulminant colitis to respond to 7 to 14 days of intravenous steroid therapy should prompt consideration of cyclosporine or tacrolimus therapy or colectomy.

For the cooperative child with mild to moderate proctitis or proctosigmoiditis, topical aminosalicylate suppositories or enemas are appropriate first-line therapy. Topical steroids in the form of suppositories, foam, or enemas are an alternative for nonresponsive disease. Children who fail to respond to topical therapy may need systemic therapy as outlined above for more extensive disease.

The second phase of therapy is the maintenance of remission. Generally, aminosalicylates are the first choice for maintenance therapy. For children with frequent relapses requiring corticosteroid therapy or dependence on steroids, the addition of maintenance treatment with 6-MP or azathioprine should be considered. Remission of proctitis or proctosigmoiditis may be maintained by alternate-day or twice-weekly topical therapy, although oral aminosalicylates are generally preferred by children.

Nutritional Therapy

The provision of adequate nutrients is essential for optimal healing of UC.[262,263] Children with UC usually develop nutritional issues as a result of poor oral intake secondary to symptoms rather than malabsorption or increased metabolic demands; thus, promotion of good nutritional intake is an essential part of therapy.[264] The goals of nutritional therapy are to establish metabolic homeostasis by correcting nutrient deficits and replacing ongoing losses, to provide sufficient

TABLE 41.2-9 RECOMMENDED APPROACH TO MEDICAL THERAPY OF ULCERATIVE COLITIS

DISEASE EXTENT AND SEVERITY	INDUCTION PHASE*	MAINTENANCE PHASE*
Extensive colitis		
Mild to moderate	Oral aminosalicylates	Oral aminosalicylates
Moderate to severe	Oral corticosteroids	1. Oral aminosalicylates 2. 6-MP or azathioprine
Distal colitis	1. Topical aminosalicylates 2. Topical corticosteroids 3. Oral aminosalicylates or corticosteroids	1. Intermittent topical aminosalicylates or corticosteroids 2. Oral aminosalicylates
Fulminant colitis	1. Intravenous corticosteroids 2. Cyclosporine or tacrolimus or colectomy	Oral aminosalicylates and 6-MP or azathioprine

6-MP = 6-mercaptopurine.
*When multiple alternatives are listed, these should be prescribed in the order presented.

energy and protein for positive nitrogen balance or net protein synthesis, and to promote catch-up growth toward premorbid percentiles. Enteral nutrition is preferred to total parenteral nutrition when possible. Continuous nocturnal nasogastric infusions of enteral formula may be necessary in those who cannot voluntarily increase their intake. In contrast to CD, enteral nutrition is not an effective primary therapy for active UC.[196,232] Studies suggest no advantage of total nutritional support and bowel rest in addition to conventional medical therapy in the treatment of UC.[265,266] However, bowel rest and total parenteral nutrition are clearly indicated in patients with severe colitis and possible surgical complications. Furthermore, correction of nutrient deficiencies and maintenance of adequate nutritional status are critical in preparing the patient for surgery.[262]

Surgery

Although medical therapy remains the first-line treatment for UC, colectomy may be required for patients with severe or medically refractory disease or to prevent colon cancer. Because the inflammation in UC is limited to the colon, colonic resection will most often result in resolution of symptoms. However, because colectomy is not without potential long-term complications, such as the development of pouchitis in patients who undergo ileoanal anastomosis,[267] resection of the colon should not be promoted as a "cure" of UC.

Timely surgical intervention in the appropriate setting may avoid complications of UC. Standard indications for colectomy in a patient with UC include fulminant colitis or a complication of colitis, such as massive hemorrhage, perforation, stricture, or toxic megacolon; failure of medical therapy; steroid dependency; and the presence of colonic dysplasia.[268] Retardation of growth and sexual maturation, despite optimal medical therapy and nutritional supplementation, may also prompt consideration of surgery because prepubertal children may experience catch-up growth after colectomy. In one series, 11 of 18 children increased their median height velocity from 3.85 cm/yr preoperatively to 7.35 cm/yr postoperatively.[269] Improvement in medical therapy, however, has led to a decline in the frequency of colectomy. At one center, a retrospective review of children and adolescents with UC revealed a decrease in the frequency of colectomy from 48.9% from 1955 through 1964 to 26.2% from 1965 through 1974.[6]

There are no early predictors to help determine who will proceed to colectomy; however, children with more severe disease at presentation are more likely to undergo colectomy. Hyams and colleagues, in a retrospective review, reported that the 5-year colectomy rate in patients with mild disease at presentation was 8% compared with 26% in patients with moderate to severe disease at presentation.[270] In another retrospective review of 73 children with UC between the ages of 1 and 18 years, the combination of steroid dependency and pancolitis was associated with an increased need for colectomy.[271]

Except in the setting of emergent colectomy, a complete evaluation should be performed to ensure that there is no evidence of CD prior to colectomy. Prior endoscopies and pathology reports should be carefully reviewed, and if not done recently, an upper gastrointestinal series with small bowel follow-through, colonoscopy, and endoscopy should be performed. If there is evidence suggesting the possibility of CD, the patient and family need to be informed of the potential for postoperative recurrence and the relative contraindications of ileoanal pull-through procedures in patients with CD.

The current standard surgical option for UC is colectomy with IPAA.[268] This procedure removes the entire colon and the rectal mucosa, avoids permanent ileostomy, and preserves anorectal function. If the rectal mucosa is in good condition, many centers use the two-stage operative approach. During the first stage, a subtotal colectomy of the cecum to proximal rectum, the removal of the distal rectal and proximal anal mucosa, and the formation of the ileal pouch are performed. In this initial stage, a diverting loop ileostomy is performed to allow the pouch to heal. The second stage involves closure of the loop ileostomy with restoration of fecal flow to the pouch. The creation of a distal ileal reservoir, usually in a "J" or "S" configuration, helps improve continence postoperatively, and elimination of a complete rectal dissection preserves the anorectal sphincter apparatus and parasympathetic innervation to the bladder and genitalia. Surgeons at some centers also complete the IPAA in one stage, without the loop ileostomy.[272] Some authors believe that the omission of the diverting ileostomy may increase the risk of anastomotic leaks and prolong recovery,[273] and the need to retain the anal transition zone may result in persistent mucosal inflammation and the potential for malignant transformation. Thus, candidate patients for the one-stage IPAA should be selected carefully.[274]

If the patient presents for emergent surgical intervention, such as with fulminant colitis, a three-stage operative approach may be used. At the time of acute presentation, a subtotal colectomy is performed with formation of a rectal stump with the remaining unresected rectum and ileostomy. After the first operation, the rectal mucosa is treated with topical therapy to induce mucosal healing. At the second operation, the distal rectal and proximal anal mucosa is removed, and the ileal pouch is created. The ileostomy is reversed at the third operation.

The potential complications of IPAA include small bowel obstruction, pelvic sepsis, anastomotic leak, fecal incontinence, pouchitis, strictures, or fistulae.[268,274,275] The development of fistulae raises the suspicion of CD. In one series of children aged 9 to 16 years who underwent proctocolectomy with IPAA, late complications (bowel obstruction, pouch fistula) occurred in 11 of 29 (38%) and pouchitis developed in 9 of 29 (31%) of the children with UC.[276] Median follow-up was 4 years (range 6 months to 9 years). In this same study, daytime continence was noted in 100% and nighttime continence in 93%. The median frequency of bowel movements was 4 per 24 hours, and 7% of patients had nighttime bowel movements.

Pouchitis, or inflammation of the newly created ileal reservoir, is the most significant chronic complication in UC patients undergoing IPAA. In adult series, approxi-

mately 40% of patients will have a single episode of pouchitis, 15% will have recurrent pouchitis, and 5% will develop chronic pouchitis.[277] Symptoms of pouchitis include diarrhea, rectal bleeding, abdominal cramping, urgency and incontinence of stool, malaise, and fever.[277] Patients with UC who undergo IPAA have pouchitis more commonly than patients with familial polyposis who undergo the same procedure,[69] suggesting that this complication is related to an underlying defect characterizing UC. Pouchitis may occur more frequently in children and adults with extraintestinal manifestations of UC,[278] especially primary sclerosing cholangitis.[279,280] Laboratory studies may demonstrate anemia and an elevated erythrocyte sedimentation rate. The definitive diagnosis is established by flexible sigmoidoscopy of the pouch with biopsies.

Broad-spectrum antibiotics are usually the first-line treatment for pouchitis.[278,281] Metronidazole is the most commonly used antibiotic, but alternative therapies include ciprofloxacin, amoxicillin-clavulanic acid, erythromycin, and tetracycline.[277,278] If there is no improvement with antibiotics, other options include mesalamine enemas and steroid enemas or oral therapy with mesalamine, sulfasalazine, or steroids.[277,282,283] Other therapies that may occasionally be effective include cyclosporine enemas, short-chain fatty acid enemas, butyrate suppositories, and glutamine suppositories.[277,284–286] Probiotic therapy may prevent the onset of acute pouchitis after ileostomy closure and effectively maintain remission of chronic pouchitis.[67,68] A double-blind, placebo-controlled study evaluated the efficacy of a probiotic preparation, VSL#3 (containing a mixture of bifidobacteria, lactobacilli, and *S. salivarius*), compared with placebo in maintenance of remission of chronic pouchitis in 40 patients in clinical and endoscopic remission. Three patients (15%) in the VSL#3 group had relapses within the 9-month follow-up period compared with 20 (100%) in the placebo group.[67] In another double-blind, placebo-controlled study performed by the same authors in 40 patients, VSL#3, administered immediately after ileostomy closure for 1 year, effectively reduced the onset of acute pouchitis in the VSL#3 group (10%) in comparison with the placebo group (40%).[68]

Several studies suggest that there is a low risk of dysplasia in the ileal pouch,[287,288] which may be associated with chronic pouchitis.[289] Furthermore, the development of adenocarcinoma has been reported in the ileal pouch.[290] At present, the long-term risk of development of dysplasia is not yet known, and definite recommendations for endoscopic screening of the pouch are not available.

PROGNOSIS

Most children with UC have the potential for a full, active life with good general health. Ten percent of patients experience only their presenting episode of colitis. Twenty percent of patients have intermittent symptoms, 50% have chronic disease, and the remaining 20% have chronically active, incapacitating disease.[291] Limited distal UC diagnosed in adults and children can extend further to involve more proximal colon with time.[84–87] Disease involvement eventually will extend beyond the rectosigmoid in approximately 29% of children and adolescents with ulcerative proctitis and 58% with proctosigmoiditis.[84,86] The extension of disease may necessitate a change in medical therapy to control disease activity and may have implications for an increased risk of colorectal cancer in the future.[143] In addition, the physician should always be alert for signs of the upper gastrointestinal, perianal, or ileal pouch involvement that may unmask CD.

Advances in medical therapy have dramatically altered the prognosis for medical management of UC, with fewer patients requiring surgery to control the disease and more patients avoiding the long-term complications of steroids. The majority of patients are able to resume full activities, including school and athletics. In general, UC has no specific effect on fertility and poses no risk to the fetus, although an increased risk of preterm births has been reported in some studies.[292–294] In contrast, women with UC who undergo proctocolectomy with IPAA may experience reduced fertility.[294] If UC is active at the time of conception, the course of disease activity may be worse during the pregnancy.[295]

In the treatment of children and adolescents with UC, it is essential to care for not only the physical components of the disease but also the psychosocial aspects, including quality of life and coping abilities of the child and family. Previous studies report an increased risk of psychiatric and behavioral issues in children with IBD, including depression, anxiety, and low self-esteem.[296–299] Burke and colleagues reported that children are at increased risk for depression as early as the time of diagnosis.[298] Adolescents with IBD may experience a delay in autonomy, which may affect their ability to gain independence from caregivers.[300,301] MacPhee and colleagues reported that in contrast to the typical adolescent, adolescents with IBD tend to seek emotional support from family members rather than from peers and maintain extended family in their social support networks.[300] They also found that these affected adolescents often depend on their parents' coping skills rather than their own coping strategies to deal with stressors. In fact, the adolescents' quality of life health scores correlated with the parental coping styles rather than adolescent coping styles. Studies have reported a lack of correlation between severity of illness and psychiatric and behavioral issues in children and adolescents with IBD.[298,302] Interestingly, stressful life events and a lack of family cohesion may be more predictive of adolescent depression in new-onset IBD,[298] and affected adolescents' health-related quality of life scores appear to correlate more with satisfaction and degree of closeness with their social supports, such as parents, than with disease severity.[300]

In contrast to previous studies, Gold and colleagues reported normal psychosocial adjustment among their population of children and adolescents with IBD and speculated that the professional supports provided by their clinic may have been instrumental in these findings.[303] The findings of these studies emphasize the need for further study of the risks of psychiatric and behavioral issues and affected quality of life among children with IBD. More recently, two

patient-generated, disease-specific, health-related quality of life questionnaires for children with IBD have been validated, the IMPACT and IMPACT-II questionnaires.[299,304] Hopefully, these instruments, along with more generalized health-related quality of life questionnaires, will provide vital information to improve the care of children with IBD.

Despite the successes of medical management, currently there is no true "cure." The medications used to control the disease have potential morbidity, and the risk of colonic carcinoma is significant and cumulative, warranting careful surveillance even in those children without relapses of disease activity. Confronting a chronic disease, with frequent medical visits and frustrating relapses, the prospects of surgery in childhood and adolescence, and the need to cope with such issues, is a tremendous emotional burden for the patient and family, requiring ongoing psychosocial support. Physicians need to be able to recognize the warning signs of depression and other psychiatric and behavioral problems and offer appropriate psychiatric and psychological support if necessary. Colectomy may result in resolution of symptoms and almost eliminate the risk of colorectal carcinoma but presents its own risks of potential morbidity, discomfort, and mortality. All of these medical, surgical, and psychosocial factors must be considered and reconciled by the patient, family, and medical team in the long-term management of children with UC.

CONCLUSION

UC in children results from the complex interplay of genetic and environmental factors. Diagnosis and management of this chronic disease remain challenging, particularly in view of the unique requirements of pediatric patients who must complete their physical and emotional development. Colectomy with ileoanal anastomosis provides long-term relief of symptoms for most patients failing medical therapy, but others develop pouchitis, which may itself become a chronic IBD. For these patients, the true "cure" must await further study of the genetic basis of UC and its pathogenesis.

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3. Undetermined Colitis and Other Inflammatory Diseases

Barbara S. Kirschner, MD

This chapter describes four inflammatory disorders that are distinguishable from the chronic inflammatory bowel diseases, ulcerative colitis, Crohn disease, and indeterminate colitis (see Chapter 41.1, "Crohn Disease," and Chapter 41.2, "Ulcerative Colitis"): eosinophilic gastroenteropathy (EG), hemolytic uremic syndrome (HUS), Henoch-Schönlein purpura (HSP), and Behçet syndrome (BS).

EOSINOPHILIC GASTROENTEROPATHY

EG is a chronic, relapsing disorder in which eosinophils constitute the predominant cell type in the inflammatory infiltrate of the gastrointestinal (GI) tract. The entity was described first by Kaijser in 1937.[1] Subsequently, patterns of clinical presentation were correlated with the extent of the eosinophilic infiltration (diffuse vs circumscribed) by Ureles and colleagues[2] and the depth of the eosinophilic reaction (mucosal, muscular, and serosal) by Klein and colleagues.[3]

In some cases in which specific foods clearly precipitate symptoms, this condition may be difficult to distinguish from the allergic gastroenteropathy described by Waldman and colleagues.[4] However, at least 50% of patients with EG have no personal or family history of atopy, and often dietary restriction does not cause resolution of symptoms.[5–7] Furthermore, allergic gastroenteropathy is characterized by mucosal injury in infants (partial or subtotal villous atrophy) rather than tissue eosinophilia.[8–11] In older children, those with symptoms of recurrent abdominal pain and positive elimination-challenge tests to foods were more likely to show endoscopic findings of esophagitis, gastric erosions, and lymphonodular or erosive duodenitis than villous atrophy or tissue eosinophilia.[12]

Isolated eosinophilic infiltration involving only the esophageal mucosa may occur, often mimicking the symptoms of reflux esophagitis. Multiple concentric esophageal furrows or rings may been seen on endoscopy.[13] Esophageal pH probe studies are normal, and eosinophils remain in the esophageal mucosa despite treatment with antireflux medications.[14–17]

Epidemiology

EG is rare, with a peak incidence in the second and third decades. Katz and colleagues described 12 children with GI symptoms and blood and tissue eosinophilia.[5] These patients could be divided into two groups: those with milk-sensitive enteropathy and those with EG. The first group, infants with allergic histories, who developed GI symptoms at about 5 months of age and improved with milk elimination, had immunoglobulin (Ig)G milk antibodies but not raised total IgE or IgE milk antibodies. The second group was older, averaging 4 years of age, with chronic symptoms, including growth failure, that did not respond to dietary manipulation. These children had signs of systemic allergy (asthma, rhinitis, urticaria) and elevated serum total IgE and IgE milk-specific antibodies. Intermittent use of corticosteroids was necessary to control the disease in the latter group.

Eosinophilic infiltration of the mucosa is associated with abdominal pain, vomiting, peripheral edema resulting from protein-losing enteropathy in severe cases, and hematochezia when the colon is diseased.[3–6] Several reports of EG in children have observed that the small bowel, especially the jejunum, is the most commonly involved site.[6,18] In children, inflammation of the submucosa and muscularis may be prominent, particularly in the antral region, and frequently causes obstructive symptoms. Serosal infiltration is the least common form and may be accompanied by eosinophilic ascites.[18,19] Formula or food intolerance, especially to cow's milk protein, soy protein, or beef, may be associated with eosinophilic infiltration of the mucosa. Even breast milk colitis may be associated with increased eosinophils in rectal biopsies.[20,21] Infectious agents such as *Giardia lamblia*, the roundworm *Eustoma rotundaturn*, cytomegalovirus, dog hookworm, and *Anisakis* (acquired following the ingestion of some species of raw fish) have been implicated as possible agents that trigger an eosinophilic response. Oral medications, such as gold, have been observed to cause EG.[22,23] Two cases of EG following liver transplant were reported by Dhawan and colleagues.[24] In each case, protracted intestinal symptoms developed several weeks post-transplant, and mucosal biopsies showed intense eosinophilic infiltration. Clinical improvement was achieved with corticosteroid therapy.[24]

Pathogenesis

The etiologic basis of EG is unknown. The prevalence of allergic histories varies widely in different series, from nil to greater than 70%.[4,6,7,14] Total IgE is elevated in some patients and normal in others. Serum IgE levels demonstrate variable responses to food challenge, suggesting that both IgE-mediated and IgE-independent mechanisms may be important in different patients with EG.

Although food sensitivity may play a role in some patients, the inciting factor is undetermined in most. Instillation into the jejunum of foods thought to provoke symptoms in specific patients has caused tissue eosinophilia in some[3,25] but not in others.[3,5] Spergel and colleagues identified 26 patients with eosinophilic esophagitis who underwent skin-prick testing and patch testing to identify potential food antigens.[26] Milk and egg were the most common positive foods with skin-prick testing and wheat with patch testing. In these patients, symptoms resolved or improved, as did esophageal eosinophil counts, when these foods were avoided. In contrast, dietary restriction of specific foods identified by history and skin tests did not alleviate symptoms in the majority of children with eosinophilic esophagitis reported by Teitelbaum and colleagues.[7]

Eosinophil recruitment and activation are induced mainly by three cytokines: interleukin (IL)-3, granulocyte-macrophage colony-stimulating factor (GM-CSF), and IL-5. Desreumaux and colleagues detected IL-3, GM-CSF, and IL-5 in the duodenal and colonic biopsies of 9 of 10 patients with EG but 0 of 10 controls.[27] They suggested that the presence of these cytokines in the mucosa may perpetuate eosinophilic infiltration via autocrine or paracrine actions.

Teitelbaum and colleagues studied the immunopathologic features of eosinophilic esophagitis in 11 children before and after treatment with fluticasone proprionate.[7] Greater numbers of mucosal CD3 and CD8 lymphocytes and CD1a dendritic antigen-presenting cells were seen in children with eosinophilic esophagitis versus normal controls. They postulated that the latter cells may stimulate T-cell proliferation and the production of eosinophil chemokines, such as eotaxin. Once activated, the eosinophils may release cytokines, leukotrienes, and granule proteins (such as major basic protein), which are not only cytotoxic but also may stimulate other cells, leading to smooth muscle contraction and dysphagia, as well as the production of tumor necrosis factor (TNF)-α and IL-8.

Clinical Manifestations

Gastrointestinal Symptoms. The most common GI symptoms of EG are vomiting (50%), abdominal pain (40%), and growth failure (35–100%).[6,7,14] Gastroduodenal or small bowel edema and cellular infiltration of the submucosa and muscularis may cause obstructive symptoms. Infiltration in the muscle layer of the esophagus may cause dysphagia when a mass effect is present. When the appendiceal wall is diffusely infiltrated with eosinophils, symptoms resemble those of acute appendicitis. Rare GI complications include ileal perforation and colonic stricture.[17] Diarrhea may be associated with rectal bleeding (23%), especially in infants.[5,6,19,28] Friesen and colleagues described 11 children with "dyspepsia" or upper abdominal pain that occurred at night or with eating.[29] Systemic symptoms included fatigue, headache, dizziness, and joint pain in two-thirds of the patients. Gastric and duodenal mucosal histology was compatible with EG. Ascites containing large numbers of eosinophils accompanies serosal inflammation. Circumscribed polypoid lesions with heavy eosinophilic inflammation in the antrum or small intestine can cause obstructive symptoms (which may mimic pyloric stenosis in infants) or intussusception.[18,30]

Eosinophilic esophagitis usually presents with vomiting, pain, and dysphagia, symptoms that mimic reflux esophagitis. Recent studies have suggested that a personal history of atopy (food allergy, reactive airway disease, allergic rhinitis or conjunctivitis, or atopic dermatitis) can be identified in the majority of patients with eosinophilic esophagitis. Eosinophilic esophagitis can be differentiated from reflux esophagitis by the presence of a normal barium esophagram, 24-hour pH probe study, density of eosinophils within the esophageal mucoal biopsy, and lack of response to acid-reducing therapies.[7,14–17]

Peripheral Edema. Edema of the extremities and periorbital regions may develop in children with EG and hypoproteinemia. Hypoalbuminemia, present in 33 to 100% of children, results from protein-losing enteropathy.[4–6]

Laboratory Findings. Peripheral blood eosinophilia occurs in 70 to 100% of children with EG.[5,6,19] Increased bone marrow precursors may be observed in the absence of increased circulating eosinophils. Elevated serum food-specific IgE antibodies occur in some patients, although their specificity and meaning are uncertain. Iron deficiency is common, especially in children with enteric blood loss.[4,6,8] Intestinal absorptive function as assessed by D-xylose excretion and fecal fat determinations in a few pediatric patients have been normal.[4] As mentioned above, esophageal pH probe studies are normal in eosinophilic esophagitis but abnormal in patients with reflux esophagitis.

Diagnosis

The diagnosis of EG is strongly suggested when the characteristic symptoms are accompanied by blood eosinophilia. Further supportive evidence may come from a careful dietary history that elicits a temporal relationship between ingestion of specific foods and the onset of symptoms.

Total serum protein, albumin and circulating IgE levels, and specific food-related IgE antibodies may be abnormal in some patients but are often not found, even in cases of documented reactions to specific foods, especially cow's milk protein.

Radiographic studies may demonstrate narrowing and nodularity of the antrum and/or duodenum or thickened mucosal folds in the small bowel. Intestinal dilatation and flocculation of barium occur in some cases.

Endoscopic examination of the esophagus may show granularity with furrows or rings involving varying extents of mucosa, including that proximal to the distal esophagus.[13,14] Biopsies from children with eosinophilic esophagitis show increased eosinophilic density compared with healthy controls and those with reflux esophagitis, although the criteria differ among published studies from more than 5 versus less than 5 eosinophils per high-power field (HPF) to more than 15 versus less than 15 eosinophils per HPF.[7,14,16] The stomach and duodenum may show nodular-

ity, erythema, friability, erosions, and ulceration in affected areas. Kokkonen and colleagues observed that children with recurrent abdominal pain who demonstrate lymphonodular hyperplasia involving at least 50% of the duodenal bulb are more likely to have tissue eosinophilia and food-induced symptoms than children without duodenal lymphonodular hyperplasia.[31] Gastric biopsies may demonstrate EG more consistently than intestinal biopsies because the intensity of the latter infiltrate varies even within the same individual.[5] Decreased vascular pattern, friability, and erosions have also been observed in EG involving the colon.[6,28]

Proof of diagnosis depends on finding excessive eosinophils in biopsy or resected tissue specimens, assuming that other causes of eosinophilic infiltrates, such as lymphoma, Crohn disease, parasitism, vasculitis (especially polyarteritis nodosa), chronic granulomatous disease, and the hypereosinophilic disorder Job syndrome, have been excluded. Hoefer and colleagues noted that lymphadenopathy and inflammation of the omentum and mesentery may be present in EG, but isolated ileal involvement and "fat wrapping" (characteristic of Crohn disease) are absent.[18] Most patients with EG have more than 10 eosinophils per HPF in the antral or duodenal mucosa.[32] Allergic proctocolitis in infants usually shows focal eosinophilic infiltration ranging from 15 to 60 eosinophils per HPF.[28,33] In the study of 35 infants with allergic colitis described by Machida and colleagues, tissue eosinophil concentrations were more than 20 per HPF compared with 8.6 per HPF in control infants.[34] Macroscopic changes in the infants with colitis varied from patchy erythema with loss of vascular pattern to frank ulceration; prominent lymphonodular hyperplasia was seen in 10 of these infants.[34]

Management

When specific foods provoke the characteristic symptoms, dietary elimination and use of an elemental diet may resolve GI symptoms, associated blood, and tissue eosinophilia and improve growth in children with EG and eosinophilic esophagitis.[4,5,15,17,26,35,36] The elemental formulas Neocate or Neocate-1-Plus (Scientific Hospital Supplies Ltd, Liverpool, UK) were given to 10 patients with eosinophilic esophagitis, aged 10 months to 12 years, for a minimum of 6 weeks. [35,36] Complete resolution of symptoms occurred in 8 of 10 patients, along with a significant reduction in intraepithelial eosinophils. In a second larger study of 51 children with eosinophilic esophagitis, an elemental diet for 4 weeks led to clinical improvement within a mean of 8.5 days and a reduction in esophageal eosinophils from 33.7 to 1.0 per HPF.[15] When symptoms persist, corticosteroids (1.0 to 2.0 mg/kg/d)[7,16,37] and possibly oral disodium cromoglycate (50 to 200 mg 4 times daily)[6,38] control symptoms in most children. Low-dose (5 to 10 mg) or alternate-day corticosteroids are needed in some cases for long-term control.

More recently, the use of swallowed fluticasone propionate, administered via an inhaler without a spacer, has been used in children with eosinophilic esophagitis.[7,39] Initially, doses of 220 µg/puff 4 puffs twice daily for 6 weeks were studied.[38] Subsequently, lower doses (2 to 4 years old, 44 µg/puff; 5 to 10 years, 110 µg/puff; 11 years and older, 220 µg/puff) twice daily for 8 weeks resolved symptoms and significantly reduced eosinophil density in mucosal biopsies.[7] A rapid clinical response was observed within 1 week. Two patients of 13 fluticasone-treated children developed *Candida* esophagitis, which responded to fluconazole.[7]

Ketotifen, an H_1 antihistamine that stabilizes mast cells, may also benefit children with EG.[40] Melamed and others described six children, with a mean age 16.9 years, whose prolonged abdominal pain, elevated circulating IgE levels, and mucosal eosinophilic infiltrates normalized after receiving 2 to 4 mg daily for 4 to 6 months.[40]

Limited results have been described using montelukast (Singulair), a leukotriene antagonist, in eight pediatric patients "unresponsive to standard therapies" with eosinophilic gastroenteropathy involving the esophagus (four patients), duodenum (two patients), or colon (two patients).[41] The patients ranged in age from 2 to 17 years and received 5 to 10 mg daily. Symptoms resolved in all within 2 to 4 weeks.

Occasionally, bowel resection or gastroenterostomy has been performed for gastric outlet or small bowel obstruction caused by EG,[3,6,18,38] but most of these complications were reported prior to the use of corticosteroid therapy.

HEMOLYTIC UREMIC SYNDROME

HUS, first described by Gasser and colleagues in 1955,[42] is defined as the development of microangiopathic hemolytic anemia, thrombocytopenia, and renal insufficiency in a previously healthy person. Now several forms of this syndrome, varying in their etiology, age at diagnosis, and prognosis, are recognized.[43–46] A prodrome of GI symptoms averaging 3 to 16 days precedes the development of the HUS in 90 to 100% of children.[47–49] Atypical or incomplete forms of HUS include *Escherichia coli* O157:H7 diarrhea with hemolytic anemia and thrombocytopenia but without renal injury, as well as HUS associated with invasive pneumococcal disease rather than diarrhea[50,51]

Epidemiology

Characteristically, HUS occurs in children less than 5 years of age.[47–49] It is endemic in Argentina, southern Africa, and the western United States, and epidemics have occurred in many other parts of the world. Between 1971 and 1980, Tarr and Hickman observed a fourfold increase in incidence in King County, Washington, from 0.63 to 2.81 cases per 100,000 children younger than 15 years.[49]

An epidemic form occurring in the summer, associated with the abrupt onset of diarrhea,[47,49] has a good prognosis, with an expected mortality under 6%.[49] A sporadic form in older children has neither seasonal influence nor obvious prodrome and has a higher mortality.[46] Siblings who develop HUS during infancy more than 1 year apart are thought to have an autosomal recessive trait that predisposes them to HUS and a mortality rate of 68%.[43–45]

Most cases of HUS in the Pacific Northwest (58%) are preceded by enteric infection with Shiga toxin–producing *E. coli* (STEC) O157:H7.[52] This *E. coli* serotype has a dis-

tinct cell membrane adhesion molecule, intimin, which attaches to follicle-associated epithelium of ileal Peyer patches.[53] Over a 2-year period, the organism was isolated from the stools of nine patients with HUS in British Columbia's Children's Hospital, an incidence far in excess of the incidence in all stools submitted for routine diagnosis (1.9%).[54] In a national prospective study of postdiarrheal HUS in the United States, 80% of patients had serum samples positive for *E. coli* O157 lipopolysaccharide antibody titers.[55] This O157:H7 serotype was originally described as a cause of hemorrhagic colitis.[56] Subsequently, isolated cases of HUS were reported following outbreaks of gastroenteritis (both bloody and nonbloody) with *E. coli* O157:H7.[52,57] Among children presenting with STEC diarrhea, HUS developed in 18% of children with *E. coli* O157:H7 but none of those with the non-O157:H7 serotype.[58] Isolates of this serotype produce a toxin cytotoxic for Vero and HeLa cells in tissue culture (verocytotoxin).[54] Dissemination of *E. coli* O157:H7 has been reported to occur through contaminated food (such as undercooked hamburger, unpasteurized milk and yogurt, unpasteurized apple cider, and vegetables, such as raw alfalfa and radish sprouts, grown in a garden fertilized with cow manure), contaminated municipal water supplies, and person-to-person spread in a day-care center.[47,52,59] More recently, reports of HUS have followed trips to dairy farms (where 13% of the calves were colonized with *E. coli* O157:H7) and petting zoos (where O157 was isolated from goats and sheep).[60,61] Thus, *E. coli* O157:H7 appears to cause a spectrum of disease, including diarrhea, hemorrhagic colitis, and HUS. The factors that determine which disease entity develops in an individual child are unknown, but geographic and genetic influences have been suggested.

Non-O157:H7 STEC strains appear to account for a larger number of cases of HUS in children in Europe than in the United States. Of 394 children studied in Germany and Austria, O26 was detected in 15% and O111 in 43%.[62] These authors emphasized the rising importance of non-O157:H7 serotypes in HUS. Similar findings were noted in an Australian population of 98 children with HUS.[63] Serotype O111:H- was the most common isolate, and none had O157:H7. Enteric infections with various non–*E. coli* organisms, including *Shigella*, *Salmonella*, *Campylobacter*, *Yersinia*, and enteroviruses, have been associated with the development of HUS.[47,64]

HUS has been associated with invasive *Streptococcus pneumoniae* infections in the absence of diarrheal disease.[65] Seven children, mean age 16 months, developed HUS after *S. pneumoniae* pneumonia (five patients) or meningitis (two patients). These children represented 23% of all HUS cases seen in Atlanta, Georgia, between 1994 and 1996. In contrast to diarrhea-associated HUS, which occurred between June and August, the *S. pneumoniae* HUS cases were seen year round. All seven patients required dialysis, compared with 48% of the diarrhea-associated HUS. The findings were not consistent with pneumococcal septic shock and disseminated intravascular coagulation because D-dimers and prothrombin time were not elevated and fibrinogen was not decreased. The authors reiterated the position of the Academy of Pediatrics that vancomycin be included in the therapy of suspected or proven invasive *S. pneumoniae* infections. A more recent study comparing invasive pneumococcal HUS with diarrhea-associated HUS noted that the former patients were younger (22.1 vs 49 months), were more likely to require dialysis (75% vs 59%), had longer duration of hospitalization (32.2 vs 16.1 days), and needed more platelet transfusions (83% vs 47%) and red blood cell transfusions (7.8 vs 2.0).[66]

HUS has also been reported following renal transplant. Although most cases were associated with cyclosporine therapy, it has been suggested that tacrolimus may cause exacerbations of HUS in patients previously treated with cyclosporine.[67]

Pathogenesis

The central lesion in HUS is vascular endothelial damage. Endothelial cells show swelling and separate from the basement membrane with widening of the subendothelial space.[68] Glomerular involvement occurs in the classic form in young children, whereas arteriolar lesions with intimal and subintimal edema, proliferation, and necrosis are more common in older children.[46,68]

The precipitating cause of this vascular injury is probably the release of Shiga-like toxins (SLT-1, SLT-2, and SLT-2 variants), also called verotoxins, which damage the microvasculature of the intestinal wall, leading to hemorrhagic and ulcerative lesions.[47,56] The B subunits of the SLTs bind to high-affinity glycolipid (GB_3) cell-surface receptors on target organs and are internalized by endocytosis.[47] The verotoxins produced by *E. coli* O157:H7 thus cause endothelial damage and gain entrance to the circulation, leading to severe cell injury or death.[52,54,56,57] Lipopolysaccharide can also damage endothelial cells and promote thrombosis. Shiga-like toxin also induces TNF production in the kidney but not in other tissues. Alternatively, perturbations in endothelial prostacyclin metabolism are postulated to be important in sporadic HUS.[68] Prostacyclin (PGI_2), a potent inhibitor of platelet aggregation, is normally produced in endothelial cells, and studies have suggested that PGI_2 deficiency, the presence of an inhibitor of PGI_2 production, or rapid degradation of PGI_2 may be important in patients with HUS.[68] Proulx and colleagues observed increased circulating pro- and anti-inflammatory cytokines (IL-6, IL-8, IL-10, and IL-1Ra) in children who developed HUS in contrast to normal controls and children with non–verotoxin-associated hemorrhagic colitis.[69] IL-8, an activator of white blood cells, may explain the leukocytosis seen in HUS.[47]

The vascular injury induces microangiopathic hemolytic anemia, thrombocytopenia, and local deposits of fibrin microthrombi. Ischemic changes result in focal or generalized renal cortical necrosis and damage to other organs (colon, liver, myocardium, brain, pancreas, and adrenal glands).[47,68,70]

Genetic Aspects

Recent studies have identified a subset of patients with atypical HUS who have mutations in the gene coding for the sol-

uble complement regulator factor H gene (*FH1*).[71] In a German series of 111 patients with atypical HUS, 14% had *FH1* mutations.[72] The mutant proteins cause reduced binding of the central component C3b/C3d to heparin and endothelial cells, leading to progression of endothelial cell and microvascular damage.[73] Other mutations of the human complement regulator membrane cofactor protein were found in three families with multiple affected individuals with HUS.[74] The mutations were also associated with reduced C3b binding and diminished ability to prevent complement activation.

Clinical Manifestations

Gastrointestinal. Gastrointestinal lesions occur in 90 to 100% of children with HUS. In 70 to 80%, bloody diarrhea precedes the recognition of HUS by 3 to 16 days[47–49]; other children have a prodrome of abdominal pain, nonbloody diarrhea, and/or vomiting. Resolution of the GI symptoms usually begins before the onset of renal insufficiency, with hematochezia clearing first, followed by improvement in the diarrhea and abdominal pain. However, the colitis may persist for as long as 2 months.[47] Rectal prolapse has been described in up to 10% of patients with colitis.[47] Occasionally, patients have thrombosis of vessels in the muscularis and serosa, resulting in necrosis and perforation of the colon.[75]

The endoscopic appearance of the bowel is characterized by hyperemia, edema, and petechiae, sometimes in association with ulceration.[75,76] Berman reported that the gross appearance was indistinguishable from that of chronic nonspecific ulcerative colitis, but rectal biopsies in these children showed only edema and submucosal hemorrhage with scant inflammation.[76] In a series of eight patients who underwent colectomy for HUS, the findings were limited to the transverse and left colon in seven, with only one patient showing involvement of the right colon.[77]

Barium enema examinations may demonstrate focal or diffuse bowel wall edema, thumbprinting, filling defects, mucosal irregularity, fine marginal spiculations, colonic spasm, or colonic dilatation (Figure 41.3-1).[75,76] Initially, the findings may mimic chronic inflammatory bowel disease until the full syndrome becomes apparent.

Hemolytic Anemia. Microangiopathic hemolytic anemia with fragmented erythrocytes occurs in all children with HUS. It usually develops suddenly within 1 to 2 days, resulting in a mean hemoglobin concentration of 6.1 g/d.[48] Several mechanisms are responsible for the anemia, including mechanical stress owing to microvascular disease, oxidative injury to red blood cells, and direct Shiga-like toxin injury.[47] Transfusions are required in 64% of children.[48]

Thrombocytopenia. Thrombocytopenia (platelet count under 150,000/mm^3) occurs in 92% of children with HUS owing to platelet trapping within organs. As mentioned above, platelet aggregation defects may occur in the presence of normal platelet counts. Other tests of coagulation status, including prothrombin time and partial thromboplastin time, are normal.[47,48,68]

Renal Insufficiency. Although all children with HUS are azotemic (mean peak blood urea nitrogen of 95 mg/dL), the severity of the renal impairment is variable. Oliguria (less than 15 mL urine per kilogram body weight per day) and anuria (less than 25 mL urine per day) each occur in 32% of affected children.[48] In approximately 50% of cases, the anuria lasts for up to 3 days, but oliguria may persist for weeks. One-third of children have no documented oliguria. Hypertension, which is mild, begins early in the course of the illness in most patients,[47] is usually labile, and is easily controlled. However, some children may require peritoneal dialysis. The time for creatinine clearance to return to normal averages 3.7 months and corresponds to the duration of the preceding oliguria. Chronic renal insufficiency persists in approximately 9.5% of children.[48]

Neurologic Disease. Central nervous system (CNS) manifestations of HUS (changes in consciousness and abnormal movements, tone, and posture) are found in one-third of children with HUS.[47] The frequency of seizures has decreased with careful attention to fluid and electrolyte balance and implementation of dialysis. Serious CNS complications, such as cerebral edema or stroke, which are reported in up to 5% of children, may be fatal.[47]

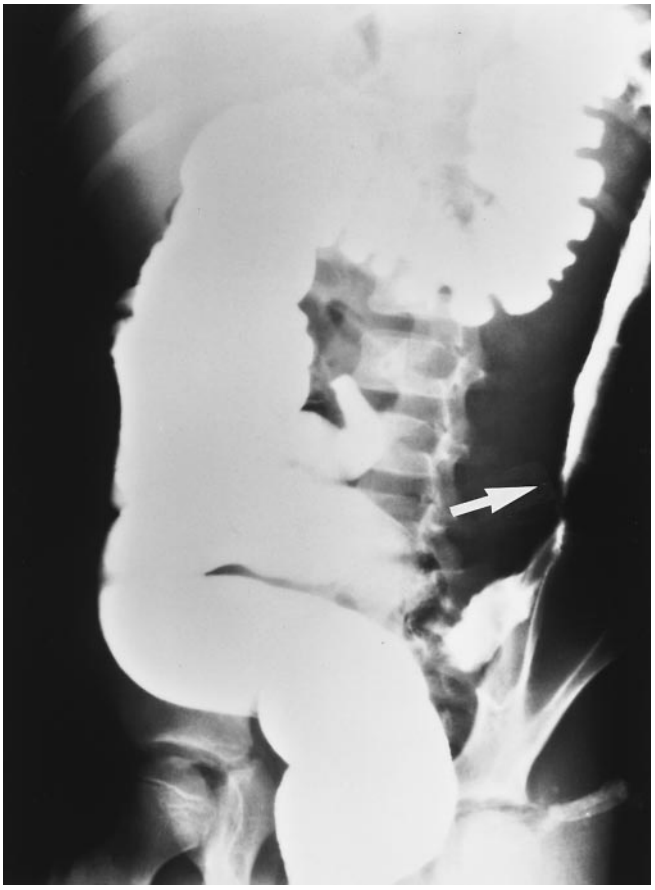

FIGURE 41.3-1 Barium enema in a 6-year-old child with hemolytic uremic syndrome. Extensive spasm and narrowing of the descending colon (*arrow*) are illustrated during the period of bloody diarrhea. Gastrointestinal symptoms resolved 1 week later but were followed by the onset of acute renal failure.

Liver Disease. Elevations of hepatic enzymes (2- to 20-fold above normal) for serum glutamic-oxaloacetic transaminase, serum glutamic-pyruvic transaminase, γ-glutamyl transpeptidase, alkaline phosphatase, and 5′ nucleotidase have been observed in most patients in whom these tests were done.[75,78] This transient hepatocellular injury may be caused by focal hepatic hypoxia. Similarly, elevated serum amylase and lipase levels are observed in about 20% of patients, as well as hyperglycemia in 4 to 15%.[47]

Diagnosis

HUS in childhood usually occurs in a previously healthy child who develops diarrhea (generally bloody) and abdominal pain followed by the acute onset of hemolytic anemia, thrombocytopenia, and renal insufficiency. Initially, the GI manifestations may be mimicked by intussusception, enteric infection, or inflammatory bowel disease (especially ulcerative colitis), which can be excluded by appropriate radiologic, microbiologic, and histologic studies. Stool specimens should be obtained for enteric pathogens, including *E. coli* O157:H7. This serotype metabolizes sorbitol slowly; therefore, *E. coli* strains with this characteristic can be sent to specialized laboratories for confirmation. However, the yield is low if specimens are sent after the first week of diarrhrea.[47] The deoxyribonucleic acid (DNA) probes for genes associated with toxin production and polymerase chain reaction methods are being evaluated.[47] Typical hematologic and renal findings are discussed above.

Treatment

Severe GI symptoms require hospitalization and intravenous fluids during the prodromal period prior to the development of HUS. Because *E. coli* O157:H7 is sensitive to ampicillin and amoxicillin, some authors suggest treating all cases with one of these antibiotics.[54] Butler and colleagues observed that the administration of ampicillin to children with ampicillin-resistant strains of *Shigella dysenteriae* 1 was associated with a greater incidence of HUS than occurred in non–antibiotic-treated children infected with the same organism.[79] They also postulated that the risk of HUS might be reduced if children were treated early with appropriate antibiotics. Tarr and colleagues cautioned against the empiric use of antibiotics for *E. coli* O157:H7 colitis until the risks and benefits have been analyzed by controlled clinical trials.[80] These authors expressed concern that the incidence of HUS might be increased through proliferation of *E. coli* O157:H7 or the release of cytotoxins through bacterial lysis or "sublethal damage." In a prospective study of 71 children under 10 years of age with *E. coli* O157:H7 diarrhea, Wong and colleagues observed that antibiotic treatment conferred a relative risk of 14.3 when compared with children who had not received antibiotic therapy.[81] It should be noted that a meta-analysis of nine studies of whether antibiotic use increased the risk of HUS did not detect greater risk.[82] Rarely, surgical intervention may be necessitated by bowel necrosis.[75]

Primary attention should be directed toward management of fluid and electrolyte balance, renal insufficiency, hypertension, seizures, and hemolytic anemia. Siegler recommends that after correcting for necessary previous losses, fluids should be limited to ongoing loss (insensible water loss plus urine and GI output).[47] Sodium should be withheld from children with edema and hyponatremia. Similarly, potassium should not be given unless levels fall into the low normal range. Nutrition is important in these catabolic, hypoalbuminemic patients. For children with ongoing diarrhea or vomiting, transpyloric feeds or total parenteral nutrition (TPN) may be required. Peritoneal dialysis is employed as necessary in infants and preschool-age children except if there is severe colitis or abdominal tenderness.[5,47,48,83] There is no direct evidence that anticoagulant therapy is beneficial.[45,84]

For children with severe anemia (hematocrit < 15%) or those symptomatic from anemia, packed red blood cell transfusions (10 mL/kg) can be given. Those with *Pneumococcus*-related HUS should receive blood products free of T-antigen antibody.[47] Siegler cautions against the use of platelet infusions, except for severe bleeding or those requiring invasive vascular procedures (such as TPN line placement) or surgery because exogenous platelets may provide further substrate for aggregation and microthrombus formation.[47] Plasma transfusions, plasmapheresis, or exchange transfusion has been reported to improve the anemia, thrombocytopenia, and renal insufficiency in some children with HUS and may be considered in patients demonstrating a poor response to supportive therapy.[83–85] Intravenous gammaglobulin administered to nine children did not demonstrate benefit on the duration of hemorrhagic colitis, anuria, or hospitalization when compared with nine children with HUS who did not receive this form of therapy.[86] Prostacyclin levels that were low prior to plasma therapy become normal following the plasmapheresis.[87] The possible future role of antitoxin directed against the verotoxin remains to be investigated.[46]

Hypertension usually responds to short-acting calcium channel blockers (such as nifedipine or nicardipine). Siegler recommends using either nifedipine at 0.25 to 0.5 mg/kg/dose as needed every 2 to 6 hours or nicardipine 0.5 to 1.0 mg/kg/dose every 6 to 8 hours as needed. Nicardipine can also be administered as a constant intravenous infusion of 1.0 μg/kg/min.[47] The use of dialysis has been responsible for the reduced mortality in HUS. Siegler recommends that dialysis be instituted for severe uncontrollable hyperkalemia, fluid overload associated with pulmonary edema, or severe uremic symptoms such as encephalopathy.[47] Other indications are a blood urea nitrogen over 150 mg/dL, a need for TPN when adequate fluid intake cannot be tolerated, and severe CNS dysfunction.

Future potential therapeutic approaches include oral administration of the GB_3 receptor, intravenous antibodies to Shiga-like toxins or lipopolysaccharide, and inhibitors of TNF production and platelet aggregation.[47]

HENOCH-SCHÖNLEIN PURPURA

HSP, a multisystem vasculitic disorder, primarily affects children under 7 years of age. Schönlein described an asso-

ciation of arthritis with purpura in 1837.[88] The clinical syndrome, subsequently extended to include abdominal pain and GI bleeding by Henoch in 1874,[89] is characterized by urticarial or purpuric skin lesions, colicky abdominal pain, sometimes with hematochezia, and arthralgias or arthritis and hematuria, which may be accompanied by proteinuria. Symptoms persist for an average of 3.9 weeks, although recurrences are common, occurring in 40% of patients.[90]

Epidemiology

In a review of 131 children with HSP, more than 75% were less than 7 years of age.[90] Seasonal variation was noted, with one-third of cases occurring during the spring. An upper respiratory infection or fever occurred 1 to 3 weeks before symptoms of HUS in 90% of children. A cluster of 20 cases was identified in Connecticut, of whom 10 were from Hartford County,[91] but no etiologic agent was identified. Although the disorder has been known as anaphylactoid purpura, family and personal allergic histories are not more frequent than expected for a comparable age group.[90] Genetic susceptibility is suggested by an analysis of *DRB1* polymorphisms; increased frequencies of *DRB1**01 and *DRB1**11 with decreased *DRB1**07 are observed in patients with HSP compared with controls.[92,93]

Pathogenesis

Factors that contribute to the development of HSP have been further elucidated since the last publication of this chapter. Clinical observations suggest that infectious agents (including β-hemolytic streptococci, *Bartonella henselae*, varicella and other viruses, and *Mycoplasma*) or medications (including penicillins, ciprofloxacin, acetylsalicylic acid, vancomycin, levodopa, cocaine, acetylcholinesterase inhibitors, carbamazepine, and streptokinase) may trigger an immune response, leading to IgA1 immune complex (IgA1C) deposition in blood vessel walls in susceptible hosts.[90,91,94–96] IgA1C is normally cleared by the asialoglycoprotein receptor of hepatocytes, which binds the oligosaccharide chains of the IgA1 Fc fragment.[96] High levels of IgA1C are found in the circulation of patients with HSP nephritis. Abnormally glycosylated IgA1 aggregates, which may be less well cleared by hepatocytes, have been identified in patients with HSP nephritis but not those without renal involvement.[96,97] Activation of the alternate complement pathway may generate chemotactic factors and polymorphonuclear infiltration. Antineutrophilic cytoplasmic antibodies are detected in 10% of patients.[98]

Serum TNF levels are higher during the acute phase of HSP than during remission and in those with renal involvement in contrast to those with normal renal function. Immunohistochemical staining of skin lesions reveals intracellular TNF in the nucleated epidermal layer, with lesser amounts of IL-1 and IL-6 staining, suggesting that these cytokines may contribute to the inflammation in HSP.[99]

Microscopic findings show perivascular infiltrates of polymorphonuclear leukocytes, lymphocytes, and occasional histiocytes around small blood vessels in the skin and GI tract, as well as IgA deposits in capillary walls and the dermal-epidermal junction. Mucosal IgA deposition can be seen in the biopsies of the duodenum in the absence of vasculitis.[100] Intimal proliferation and thrombosis have been described in cerebral vessels in children with seizures. Vasculitis results in edema and hemorrhage in various organs, including the intestine, pancreas, gallbladder, lung, myocardium, testis, and cerebral cortex.[90,101–103] In glomeruli, there are diffuse polymorphonuclear infiltrates or hyalinization with thickening of the basement membrane of Bowman capsule.

Clinical Manifestations

Skin Involvement. Skin lesions occur in 97 to 100% of children with HSP.[90] Usually, there is an urticarial eruption on the extensor surfaces of the legs, buttocks, and arms, which changes to red, nonblanching macules. Children under 2 years of age may present with scalp, facial, or extremity edema, whereas older children often show petechiae, especially on the lower extremities. Edema of the lower extremities, which is more common in children less than 3 years of age, is often seen in combination with facial or scalp swelling. Prominent purpuric lesions involving the face and ears were recently described in 6 infants less than 1 year of age.[104]

Gastrointestinal Involvement. GI symptoms occur in 65 to 90% of children.[90,105,106] Colicky abdominal pain, often associated with vomiting, results from submucosal edema and hemorrhage. Abdominal pain develops within 8 days of the rash in 75% of pediatric patients, although intervals as long as 150 days between GI symptoms and skin findings have been observed.[105] Hypoproteinemia may develop secondary to protein-losing enteropathy. Obvious GI bleeding (melena or hematochezia) occurs in 25% of children and hematemesis in approximately 10%. Children with abdominal pain frequently show tenderness to direct palpation (75%), but rebound tenderness is uncommon (9%).[105] Stools are guaiac positive in 50% of children. Rare intestinal complications include intussusception (2–3%), perforation, pancreatitis, and cholecystitis.[90,101–103] When intussusception develops, it is seen in children 5 to 7 years of age; early surgical intervention markedly reduces mortality.[90,106,107] Intussusception in HSP usually originates in the ileum (90%) or jejunium (7%), and approximately 58% of cases are confined to the small bowel.[108] In contrast, most idiopathic intussusceptions are ileocolonic. In one series of children who underwent exploratory laparotomy, excessive amounts of peritoneal fluid were observed. Rarely, small bowel obstruction may develop from entero-entero fistulae or late-onset stricture formation.[109,110]

Endoscopic examination may demonstrate coalescing purpuric lesions, especially in the descending duodenum, gastritis, or punctate erythematous and ulcerative changes in the colon.[100,109,111] Tomomasa and colleagues described endoscopic findings in nine children with HSP.[111] No esophageal abnormalities were observed. Gastric changes in two children consisted of diffuse mucosal edema, patchy erythema, and multiple erosions. Diffuse severe erythema with erosions was noted in the duodenal bulb (two patients) and

the second portion of the duodenum (three children). Rectosigmoid examination in six children demonstrated shallow ulcers in two, but studies were normal in the other four. Endoscopic biopsies revealed polymorphonuclear infiltrates in the lamina propria, predominantly around blood vessels.

Radiologic studies of the small bowel and colon show thumbprinting (Figure 41.3-2), representing submucosal edema and hemorrhage, spasm, ulceration, and pseudotumor,[101] usually in the jejunum and ileum (Figure 41.3-3), but the colon may be affected. Similar findings may be seen in lymphoproliferative disorders, other hemorrhagic conditions (hemophilia, leukemia), scleroderma, and Crohn disease.[101]

In a series of 139 pediatric patients, remission occurred within 4 weeks in 84 patients, 5 to 8 weeks in 19 children, and 9 to 53 weeks in 18 children.[112] Thus, symptoms of extrarenal HSP often last more than 1 month. The authors also noted that renal involvement was more common in children whose extrarenal symptoms lasted for 6 weeks versus 3 weeks for those not developing renal disease. Relapses of GI symptoms have been reported 7 years after the initial episode.[102]

Joint Involvement. Arthritis and arthralgias, usually oligoarticular and nonmigratory, occur in 65% of children with HSP. Ankles and knees are involved more than the upper extremities.

Renal Manifestations. Hematuria (gross or microscopic) occurring in approximately 40% of all children with HSP[90] is reported in 70% of children with GI disease.[106] Two-thirds of children with hematuria also develop proteinuria. Muller and colleagues reported that measuring urinary tubular marker proteins (*N*-acetyl-β-D-glucosaminidase and α_1-microglobulin) correlated with the extent of early and late renal involvement and suggested that their use may be helpful in identifying those who will develop HSP nephritis.[113] Renal lesions are more frequent in older children than in those less than 2 years of age and usually follow skin and GI involvement.[90] Serious complications include hypertension (sometimes leading to hypertensive encephalopathy) and renal failure. Glomerular lesions include glomerular proliferation, hyalinization, and IgA deposits in the mesangium. Autopsy studies of the kidneys demonstrate endothelial thickening, thrombosis, and medial necrosis of small renal arteries. In Europe, HSP accounts for 15% of children receiving dialysis for renal failure.[114]

Hepatobiliary Involvement. Chao and colleagues described hepatobiliary involvement in 20 of 225 children with HSP.[115] Symptoms included right upper qudrant pain (80%), nausea (45%), lethargy (20%), and vomiting (15%). Laboratory tests revealed elevated alanine transaminase in 15 of 20 patients (75%) and elevated γ-glutamyl transpeptidase in 6 of 20 patients (30%). Abdominal ultra-

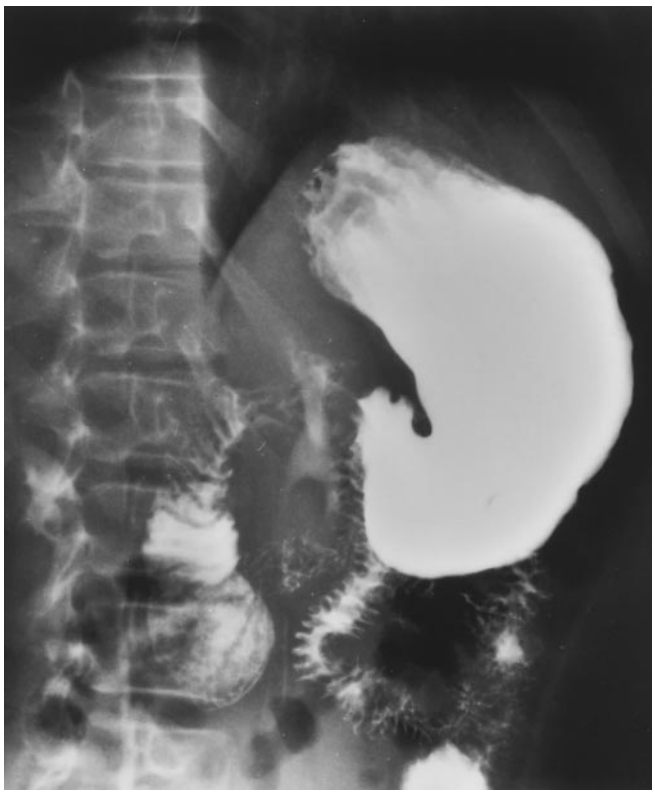

FIGURE 41.3-2 Upper gastrointestinal series demonstrating focally thickened folds and thumbprinting due to submucosal edema in the proximal jejunum in a 14-year-old girl with recurrent episodes of colicky abdominal pain. Four weeks later she developed typical skin lesions of Henoch-Schönlein purpura on the lower extremities.

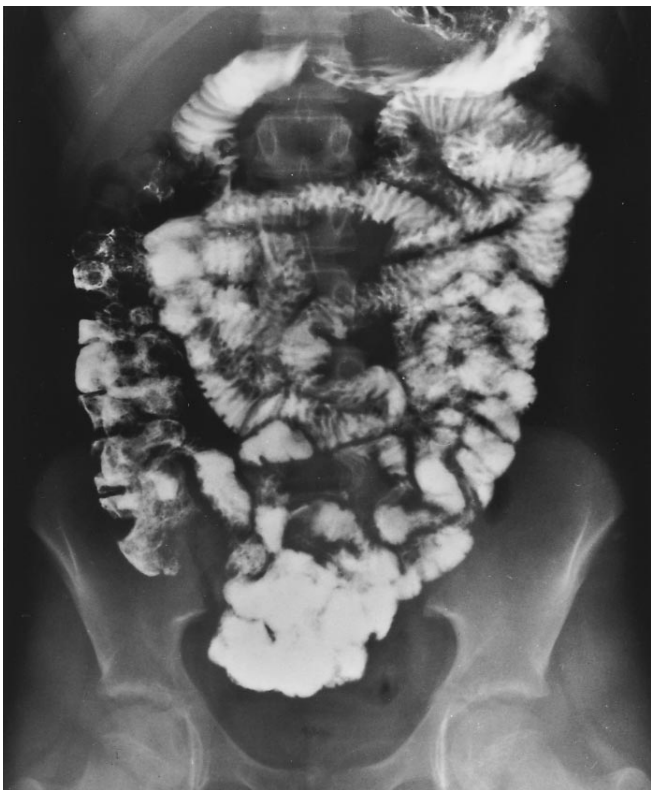

FIGURE 41.3-3 Upper gastrointestinal and small bowel follow-through demonstrating extensive thickening of mucosal folds in the descending duodenum and entire jejunum in a 10-year-old boy with recurrent vomiting and scattered macular erythematous lesions on the buttocks and lower extremities due to Henoch-Schönlein purpura.

sonography demonstrated hepatomegaly in 75% and gallbladder wall thickening in 25% of this group. The findings subsided within 3 to 7 days after steroid therapy. Viola and colleagues described a child with ischemic necrosis of the bile ducts (which they attributed to HSP vasculitis of the peribiliary vessels), which resulted in biliary cirrhosis and liver transplant.[116]

Scrotal Involvement. Vasculitis of the scrotum may result in findings and symptoms that must be differentiated from torson of the spermatic cord.[117] In one study, 22 of 93 children (24%) with HSP showed signs of scrotal involvement. Of the 22 patients, 8 had surgical exploration and none had testicular torsion. The authors recommended the use of high-resolution Doppler ultrasonography to identify those patients in whom surgical intervention could be avoided.

Other Presentations of HSP Vasculitis. Case reports describing children with HSP have included rare presentations, including ataxia owing to brainstem vasculitis, loss of vision from retinal artery occlusion, pulmonary hemorrhage, and hypertension without other signs of renal disease.

Diagnosis

The diagnosis of HSP rests on the presence of characteristic clinical features with supporting laboratory, endoscopic, and radiologic studies as indicated. Peripheral blood counts show leukocytosis (10,000 to 20,000/mm^3) with left shift in half of the children. Erythrocyte sedimentation is elevated (greater than 20 mm/h) in 75% of children.[118] Urinalyses may not demonstrate hematuria or proteinuria until several weeks after the initial presentation. The presence of microscopic blood should be checked in stool specimens. High-frequency ultrasonography may be helpful in equivocal cases, as when abdominal pain develops prior to skin or renal involvement.[119,120] Findings of HSP include thickened bowel wall (3–11 mm), which may be diffuse or focal, free peritoneal fluid, impaired peristalsis of affected loops, and bowel dilatation. Serial examinations during the course of the disease can determine whether the lesions are extending or resolving and whether there is re-expansion of the small bowel lumen and reappearance of peristalsis. Complications such as intussusception and perforation can be detected by this method.[119,120]

Endoscopic evaluation may be indicated to exclude other conditions if the diagnosis is unclear.

Histologic examination of skin biopsies from recent lesions demonstrate leukoclastic vasculitis, often with IgA1 (but not IgA2) deposits. IgA1 deposits may not be present in necrotic or old lesions.[121]

Physicians should be aware that activation of coagulation secondary to endothelial damage may result in D-dimer concentrations more than 10 times the upper limit of normal.[122] However, in none of the 15 children were the platelet count, prothrombin time, thrombin time, protein S and C, or antithrombin 3 levels abnormal. The authors caution against interpreting the abnormal D-dimers as indicating disseminated intravascular coagulation in patients with HSP.

Management

Therapeutic intervention for HSP is usually directed at specific complications, such as severe abdominal pain, hypertension, renal insufficiency, and GI or cerebral hemorrhage. The efficacy of other recommended medical approaches has not been studied in controlled clinical trials.

Gastrointestinal Disease. Children with severe abdominal pain should be admitted to the hospital because of the potential risk of intussusception, hemorrhage, or perforation. Supportive care with intravenous fluids and nasogastric suction is helpful in comforting severely symptomatic children. Corticosteroids used for GI manifestations of HSP are controversial. Allen and colleagues suggest that abdominal pain resolves in the majority of children after steroid therapy and that tapering this medication in some patients leads to clinical relapse, which improves with reinstitution of steroids.[90] Rosenblum and Winter retrospectively compared the hospital course of 48 children with HSP, 58% of whom were treated with prednisone (1–2 mg/kg/d) and 42% of whom did not receive corticosteroids.[105] Abdominal pain resolved within 24 hours in 44% of the steroid-treated group but in only 14% of the nonsteroid-treated group. At 48 hours, resolution was 65% in the steroid group and 45% in the other group, although this difference was not statistically significant. By 72 hours after admission, resolution of pain was similar in both groups (75%). The report concluded that corticosteroid administration may hasten resolution of abdominal pain but that controlled trials should be undertaken.

Whether intravenous gammaglobulin is beneficial in persistent steroid-dependent HSP has not been reported. Jordan suggested that glomerulonephritis may have been precipitated in one patient by this therapy.[123] Relapse is frequent in HSP, with 40% of children developing symptoms as late as 7 years after the initial episode.[90,102] In one child with severe cerebral and retinal vasculitis, intravenous pulse methylprednisolone was not effective, but plasmaphoresis led to prompt resolution of symptoms.[124]

Surgical treatment is required rarely for massive gastric hemorrhage, intussusception, obstruction, intestinal perforation, or cholecystitis.[90,102,125] Careful and repeated physical examinations are necessary to identify early signs of these complications.

Renal Disease. Management of the renal complications of HSP will not be discussed, but, clearly, blood pressure and renal output must be carefully monitored. Urine samples should be analyzed regularly for hematuria and proteinuria. Antihypertensive medications, fluid restriction, and dialysis may be necessary.[126] Recently, the combined use of corticosteroids and azathioprine was reported to reduce the progression to chronic HSP nephritis when compared with historical controls.[127] Intravenous pulse methylprednisolone or oral prednisone followed by oral

cyclophosphamide reduces proteinuria and may lessen the development of renal insufficiency.[128]

BEHÇET SYNDROME

BS is a multisystem vasculitic disorder reported first in 1937.[129] The original description included aphthous stomatitis, genital ulcers, and uveitis. Because there are no pathologic or laboratory findings that definitively establish the diagnosis, several clinical criteria have been proposed.[130,131] Although the disease affects predominantly young adults in Japan, the Mediterranean region, and the Middle East, pediatric cases have been described.[132–143] Genetic predisposition is suggested by the observation that the class I human leukocyte antigen (HLA) HLA-B51 is positive in 62.8% of patients with BS compared with 24.6% of healthy controls.[144] A positive family history is also higher in HLA-B51–positive patients (83%) versus HLA-B51–negative patients (58%). Immunologic processes are thought to be causally related to the vasculitis; thus, current forms of therapy use immunomodulatory medications. The clinical presentation of BS may closely resemble that of Crohn disease, but the presence of genital ulcers, very severe oral ulceration, and neurologic complications aids in differentiating the two conditions.[134,145]

Epidemiologic Aspects

The prevalence of BS varies greatly among different countries. It is most common in Japan (10 per 100,000) compared with England (0.6 per 100,000) and the United States (0.3 per 100,000).[131] The incidence appears to have increased between 1958 and 1977 in Japan. Presentation prior to the onset of puberty is unusual.[146] In one series of 297 patients, only 34 had symptoms prior to 19 years of age, with the youngest being 13 years old.[146] Ammann and colleagues described six children whose onset of symptoms of BS began at 2 months to 11 years of age.[133] Although the majority of adult patients are male (70%), half of the pediatric patients are female.[133,134]

Familial cases are uncommon, but isolated instances involving parents with affected children have been described.[147] A transient form of BS may occur in neonates born to mothers with the disease.[135]

Pathogenesis

The cause of BS is unknown, but genetic and immunologic factors probably contribute to the development of this disorder. Peripheral blood mononuclear cells show a significantly higher number of IL-2–producing $CD4^+$ cells (T helper 1 response) than do controls.[148]

The primary histopathologic lesion consists of vasculitis that affects predominantly small vessels. Initially, there is endothelial proliferation and infiltration with mononuclear cells, which is followed by a polymorphonuclear response. The same vascular lesion may occur in large veins and cause thrombophlebitis, as well as in large arteries, leading to gangrene or aneurysm formation. Studies have shown deposition of C3 and C9 in blood vessel walls and circulating immune complexes in patients with BS. In the newborn form, transient circulating IgG immune complexes and reduced total hemolytic complement were detected, suggesting transplacental passage of immune complexes or autoantibodies.[135] Some authors reported that serum IL-8 more reliably reflects disease activity than the erythrocyte sedimentation rate or C-reactive protein level.[149] However, this was not confirmed in a different center.[150]

Lehner and colleagues observed that specific HLA haplotypes are associated with some forms of BS.[151] When compared with controls, HLA-B5 occurs more frequently in patients with ocular involvement, HLA-B27 is increased in those with arthritic symptoms, and HLA-B12 correlates with mucocutaneous signs. The allele *B**5101 is found in 80% of patients with BS compared with 26% in controls.[152] Additional associations with *B**5101 were the development of BS at a younger age and the presence of erythema nodosum. However, there is no association between homozygosity for HLA-B51 and the severity of the course of BS.[144]

Recently, genetic linkage analysis in a Turkish population has suggested that chromosome 6p may be a second susceptibility locus for BS.[153] In addition, six patients with trisomy 8 have been described with BS and myelodysplastic syndrome.[154]

Clinical Manifestations

Several classifications have been proposed to define the diagnostic criteria for BS.[129,130] These consider the major manifestations (buccal ulceration, genital ulceration, uveitis, and skin lesions) and its less frequent signs (GI lesions, thrombophlebitis, arthritis, CNS lesions, and family history).

Major Manifestations. ***Oral Ulcers.*** Painful recurrent oral ulcers are the most common feature of this disease. They persist for 7 to 14 days, subside spontaneously, and recur several days to months later. Because this finding may occur in up to 10% of the normal population, additional manifestations are necessary to establish the diagnosis of the disease.

Genital Ulcers. Ulcerations on the genitalia are reported in 93 to 98% of patients with BS.[134,155] However, the rates are lower in children at first presentation (32%).[139] Their gross appearance and clinical course are similar to those of the oral ulcers and occur equally in male and female children.

Skin Lesions. The cutaneous manifestations of the disease are varied and include folliculitis, erythema nodosum, acne, vesicles, pustules, and other nonspecific lesions.[155] The formation of a sterile pustule at the site of needle trauma (behçetian reaction) occurs in approximately 40% of pediatric patients.[139]

Ocular Involvement. The signs of ocular disease are iritis with hypopyon and posterior uveitis. Visual impairment is usually bilateral and may result in optic nerve atrophy, glaucoma, and cataracts. This manifestation is much less common in Western countries than in Japan and Turkey, where it is a major cause of blindness. Between 21 and 47% of pediatric patients have ocular involvement.[134,139]

Minor Manifestations. ***Gastrointestinal Disease.*** The frequency of GI involvement in patients with BS varies widely in different series and perhaps in different regions. Yazici and colleagues reported that no patient had GI signs of BS in their review of 297 patients from Turkey.[146] Japanese authors have observed intestinal symptoms in at least 15% of patients with BS.[156] The frequency is higher in Japanese children with BS, with 58% having GI disease.[140] The most frequent complaints are colicky abdominal pain and nonbloody diarrhea, but vomiting, flatulence, and constipation may occur. Perianal ulceration may occur with or without concurrent genital ulceration.[142] Rare cases of esophageal involvement have been reported. GI involvement has been demonstrated by technetium 99m leukocyte scintigraphy and follow-up by ileocolonoscopy in children with BS without GI symptoms.[143] Radiologic examinations demonstrate thickened mucosal folds, pseudopolyps, deformity of bowel loops, ulcerations, and fistulae.[112] Ulcerations are localized or diffuse, with the majority (76%) occurring in the ileocecal region.[124] Extension of the ulcers to the serosal surface may result in perforation. Recurrence is often at the site of anastomosis, and 44% of surgically treated cases require reoperation.[124] Postoperative azathioprine results in a lower reoperation rate: 7% versus 25% at 2 years and 25% versus 47% at 5 years.[157]

The typical colonoscopic findings in BS are ileocecal location (96%), solitary ulcer (67%), size greater than 1.0 cm (76%) with a mean size of 2.9 cm, and deep ulcers (80%; 62% with discreet margins).[158] The differentiation of BS from chronic nonspecific ulcerative colitis and especially Crohn disease depends on the character of the intestinal endoscopic and radiologic findings and the associated extraintestinal manifestations. The correct extraintestinal diagnosis may be obscured by the presence of BS and ulcerative colitis or Crohn disease in the same family.[145] Baba and colleagues noted that the ileocecal location and the depth of the ulcers in BS distinguish this disease from chronic ulcerative colitis.[156] In comparison with Crohn disease, there was less inflammation in the area surrounding the ulcer, and granulomas were not seen.[156,159] Fistula formation has been reported in BS.[134,155] The recurrent genital ulcers and CNS signs seen in BS are rarely found in ulcerative colitis or Crohn disease.[145]

In contrast to children with Crohn disease, growth based on the mean height standard deviation score at the time of diagnosis is usually normal in BS: 0.38 ± 1.08 at 1 year and 0.35 at 2 years after diagnosis.[142] Body mass index is reported to be normal in children with BS. Kim and colleagues analyzed the response to medical therapy and need for surgical intervention based on the colonoscopic appearance of ulcers in 50 patients with BS.[160] The most common were volcano-type ulcers (50%), followed by aphthous ulcers (28%) and geographic ulcers (22%). Complete remission following medical therapy was better for geographic ulcers (73%) and aphthous ulcers (64%) than for the volcano type (24%). Remissions were achieved after surgical intervention in 52% of patients with volcano-type ulcers. However, recurrence rates were also higher for the volcano-type ulcers (47%) than the geographic and aphthous ulcer types (11% and 9%, respectively).

Vascular Disease. The vasculitic process described above can affect both arterial and venous systems. Small vessel disease accounts for many systemic signs, but large vessel involvement may result in severe complications. Recurrent superficial thrombophlebitis, vena cava thrombosis, and arterial occlusions leading to infarction and hemorrhage from rupture of aneurysmal dilatations have been reported. Increases in the Q–T interval, ventricular arrhythmias, and sudden death were described in 1997.[161]

Arthritis. Chronic nonmigratory seronegative pauciarticular arthritis affecting the knees, ankles, hips, elbows, and wrists occurs in up to 50% of patients with BS. The course tends to be nondestructive, with rare radiologic evidence of bone erosion, although synovial thickening and effusion may occur.[155]

Neurologic Manifestations. Headaches are reported in 37% of children with BS.[139] More severe neurologic involvement is reported in 1 to 20% of patients with BS. Episodes may be transient or progressive and include pyramidal signs, organic confusional states leading to dementia, meningoencephalitis, cranial nerve palsies, dural thrombosis, pseudotumor cerebri, seizures, and quadriparesis.[119] There may be mild pleocytosis with or without an elevation of protein in the cerebrospinal fluid. Low-dose methotrexate (7.5–12.5 mg per week) appears to have a beneficial effect in reducing cerebrospinal fluid IL-6 levels and dementia.[162]

Diagnosis

The diagnosis depends on the presence of characteristic clinical findings.[131] The criteria of Mason and Barnes, described in 1969, include three major and two minor criteria (described under "Clinical Manifestations").[130] Subsequently, an International Study Group (ISG) recommended that the diagnosis should be based on the presence of three or more episodes of oral aphthous ulcers plus two of the following lesions: recurrent aphthous genital ulcers, uveitis or retinal vasculitis, cutaneous vasculitis, or cutaneous hyperactivity to needle prick (positive pathergy test).[131] The technique of performing the pathergy test affects the rate of positive response.[163] The positivity rate was statistically higher with nondisposable blunt needles than with disposable sharp needles. Recently, the specificity of the ISG classification compared 302 patients with BS and a control group of 438 patients with other conditions, including ulcerative colitis, Crohn disease, and familial Mediterranean fever.[164] Of those diagnosed with BS, 98% fulfilled the ISG criteria compared with 1% of the control group.

Fujikawa and Suemitsu described 31 pediatric patients with BS and observed that the diagnosis in children may be difficult because of the long interval between disease onset and the presence of sufficient symptoms to satisfy the diagnostic criteria of BS.[137] The prevalence of oral ulcers increased from 77% in the first 6 months to 100% during the course of the illness. Similarly, genital ulcers increased from 45 to 58%, uveitis from 10 to 29%, and skin lesions from 39 to 55%. Krause and colleagues noted that the interval between the first disease sign and the full disease

complex in children was 3.9 ± 3.5 years, thus emphasizing the difficulty in diagnosing BS in pediatric patients.[139]

Management

Therapeutic intervention for active disease must take into account the range and seriousness of complications as described by Yazici and colleagues.[146] Corticosteroid medications form the mainstay of treatment for BS.[165] Relief of symptoms occurs initially in most patients, but recurrences are common. Topical preparations are usually effective for genital ulcers, whereas oral or intravenous administration is required for uveitis, intestinal lesions, and neurologic manifestations. A prospective randomized 2-year trial of colchicine (1–2 mg/kg/d) versus placebo was conducted in adults with BS.[166] There was a reduction in genital ulcers, erythema nodosum, and arthritis in the colchicine group relative to the placebo group. Colchicine use in children was described in five patients (dose: 13.5 ± 8.1 μg/kg/d increasing to 26 ± 9.2 μg/kg/d), with a response in two patients but only a partial response in three others.[142]

Immunosuppressive agents such as colchicines, azathioprine, chlorambucil (combined with prednisone 1.0 mg/kg/d), thalidomide (1 mg/kg dose at varying intervals of once daily to once weekly), and cyclosporine (10 mg/kg/d) have been effective in patients with severe disease who did not respond to steroids or who could not tolerate a reduction in steroid dosage.[142,165,167–171]

Yazici and colleagues and Greenwood and colleagues observed that azathioprine (2.5 mg/kg/d) was beneficial in controlling the progression of eye disease, recurrent uveitis, arthritis, the frequency of genital ulceration, reducing steroid requirement, and preventing relapse.[167,168] This finding was confirmed by Hamuryudan and colleagues, who observed significantly less deterioration in visual acuity in patients receiving azathioprine versus placebo.[169] Two children with oral, genital, and intestinal ulcerations that did not improve with steroid therapy achieved long-term control following treatment with chlorambucil.[133] Short-term improvement in GI symptoms was observed in a child treated with colchicine.[134]

Thalidomide may induce remission in refractory patients, including one infant who initially responded to 10 mg/kg per day for 4 weeks and subsequently relapsed but responded to 5 mg/kg daily.[141,170] Kari and colleagues reported thalidomide use for a mean duration of 2.2 years (1.3–4.3 years) in 5 children with BS who had been unresponsive to corticosteroids.[142] The dose range was very broad, varying from 0.6 to 1.1 mg/kg/d to 2.4 to 3.7 mg/kg two to three times weekly. Three achieved complete remission and two partial remission. Neuropathy developed in two children, and in one, it was irreversible after 1.3 years of treatment with 1 mg/kg/d. In some cases beginning in childhood, symptoms resolve; however, a mortality of 3% has been reported and was related to large vessel involvement.[138]

There is evidence that a subpopulation of T cells (CD45RA+Vγ Vδ2+) in patients with BS produces high levels of TNF-α when stimulated with phorbol myristate and anti-CD3.[172] Thus, it is not surprising that infliximab has been used in a few patients who were either refractory to corticosteroids, colchicines, thalidomide or cyclosporine,[173] chronically active and steroid dependent,[174] or sight-threatening panuveitis.[175] In most cases, infliximab was administered in the usual dose of 5 mg/kg. Response may occur as early as 24 hours followed by corticosteroid tapering and remission by 2 weeks.[173,174] In the five patients reported with panuveitis, response was noted within 24 hours and complete remission within 7 days in all.[175]

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4. *Surgical Aspects*

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Most children with inflammatory bowel disease (IBD) can be managed using a combination of nutritional and pharmacologic approaches. Initially, the goal of therapy is to bring about a remission of the disease, and subsequent therapy is designed to prevent recurrence. Although, historically, these measures have been successful in many patients, and the continual development of newer pharmacologic agents has improved the armamentarium of the pediatric gastroenterologist, there continue to be a number of situations in which surgery may be the most appropriate course of action. The surgeon may participate in the care of these children for a number of reasons, including resection of diseased tissue, repair of narrowed or perforated intestine, bypass or defunctioning of involved bowel, drainage of abscesses, and provision of access for enteral or parenteral nutrition.

Indications for surgical management of pediatric IBD are summarized in Table 41.4-1. Although infrequent, complications such as free perforation, toxic megacolon, and massive bleeding are indications for emergent surgical intervention. In the majority of cases, surgery is indicated only after nonoperative management has failed or when complications of medical management have occurred. This is particularly true in children with small bowel Crohn disease, who are at higher risk for recurrence and further loss of bowel length.

This chapter reviews the surgical management of Crohn disease and ulcerative colitis and highlights some of the special issues that these diseases pose in childhood.

TABLE 41.4-1 INDICATIONS FOR SURGICAL INTERVENTION IN THE MANAGEMENT OF INFLAMMATORY BOWEL DISEASE IN CHILDREN

FAILURE OF MEDICAL MANAGEMENT
Complications of steroid or other drug therapy
Persistent symptoms despite maximal medical therapy
COMPLICATIONS
Crohn disease
Perforation
Fistula formation
Excessive or uncontrolled bleeding
Fibrous stricture with intestinal obstruction
Ongoing sepsis
Growth failure
Ulcerative colitis
Excessive or uncontrolled bleeding
Toxic megacolon
Mucosal dysplasia or fear of colon cancer

PRINCIPLES OF PREOPERATIVE ASSESSMENT AND PREPARATION

Prior to considering surgery for a child with IBD, a number of steps must be taken, including confirmation of the diagnosis, staging of the disease, complete assessment of any complication that might lead to consideration of surgery, evaluation of the surgical risk, and optimization of the patient's condition to minimize that risk. It is also important to have a series of full and complete discussions with the child and family so that they understand the risks and potential benefits of surgery.

The extent of disease should be assessed prospectively using a combination of barium studies and endoscopy. In children with colitis, it is important to differentiate between ulcerative colitis and Crohn colitis because the surgical options differ between these two conditions. This determination is made on the basis of colonoscopic biopsies, the distribution of the disease in the colon, and the presence or absence of disease elsewhere in the gastrointestinal tract. Computed tomography, ultrasonography, and magnetic resonance imaging may also be very useful in evaluating the presence of inflammation or thickening and in identifying the presence and location of fistulae or abscesses.

The goal of preoperative preparation is to achieve normal nutritional status and to minimize the dose of steroids. These steps are very important because the risk of serious complications such as anastomotic leak and sepsis is increased in patients who are malnourished or on chronic high-dose corticosteroids.[1] This is usually accomplished by using bowel rest, nutritional support, and "steroid-sparing" medical therapy. Nutritional therapy with concomitant bowel rest can be accomplished using either parenteral or enteral routes. Parenteral nutrition, although effective, is associated with significant risks from metabolic derangements, trace element deficiencies, central line complications, and a much higher cost. For enteral nutrition, most physicians use elemental feeding through a nasogastric or gastrostomy tube. There is, however, no convincing evidence that elemental diets are better than the use of standard enteral formulas.[2] Preoperative nutritional therapy over a 4- to 6-week period is extremely effective, safe, and well tolerated.[3] The addition of immunosuppressive agents such as azathioprine, 6-mercaptopurine, cyclosporine, methotrexate, tacrolimus, or infliximab may also help to decrease or eliminate the use of steroids.

CROHN DISEASE

The surgical approach to the child with Crohn disease depends on the location of the disease and the indication for surgery.

Ileocecal Disease

Children with ileocecal disease may develop fixed fibrotic changes, local perforation and abscess formation, or fistulization to the colon, bladder, vagina, proximal small bowel, or skin. Children may also be considered for surgery if they experience recurrent symptoms whenever the steroids are tapered, and the disease is therefore controlled only on unacceptably high doses of steroids. The decision to recommend surgery in these children may be difficult and should be made collaboratively between the family, the gastroenterologist, and the surgeon. The decision must be based on the balance of the risk of long-term high-dose steroids and the surgical risks (including infection, anastomotic leak, adhesions, and recurrent disease). Once a decision has been made, the extent of disease must be confirmed, and other studies may be done as necessary. Examples may include computed tomography or magnetic resonance imaging to assess the possibility of local abscess or fistula formation and renal ultrasonography to look for hydronephrosis owing to ureteral compression.

The surgical goal is removal of the grossly involved bowel. Although, previously, many surgeons did frozen sections of the resection margins to ensure that all microscopic disease had been removed, this is no longer recommended because it does not result in a lower recurrence or complication rate[4] and increases the risk of short bowel syndrome. The anastomosis can be hand-sewn or stapled, and the factors known to increase the rate of anastomotic leak include malnutrition, previous perforation or contamination, and chronic high-dose steroid administration.[1] In patients who have intra-abdominal sepsis or in whom anastomotic healing is felt to be compromised, a temporary Brooke ileostomy or a proximal defunctioning loop ileostomy should be done.

Ileocolic resection has traditionally been performed using a midline or transverse lower abdominal incision. Recently, a number of authors have described a laparoscopy-assisted approach to this procedure that results in less pain, shorter hospitalization, and an improved cosmetic appearance (Figure 41.4-1).[5] The laparoscopic approach can be used successfully even in patients with previous intra-abdominal abscesses or fistulae. Laparoscopy may also be useful in some cases in which the diagnosis of Crohn disease may be in doubt.

A small group of patients may present with multiple areas of symptomatic small bowel disease, with or without ileocecal involvement. Indications for surgery are similar to those mentioned above, but multiple resections would result in excessive loss of bowel length. In patients with multiple, short areas of involvement, strictureplasty has been advocated (Figure 41.4-2). The results of this procedure in both adults and children have been encouraging, with no evidence of a higher rate of leak, infection, or recurrence.[6] Patients with ileocecal disease combined with obstructive proximal disease may require ileocolic resection and a combination of proximal resections and/or strictureplasties, and the specific operation must be individualized.

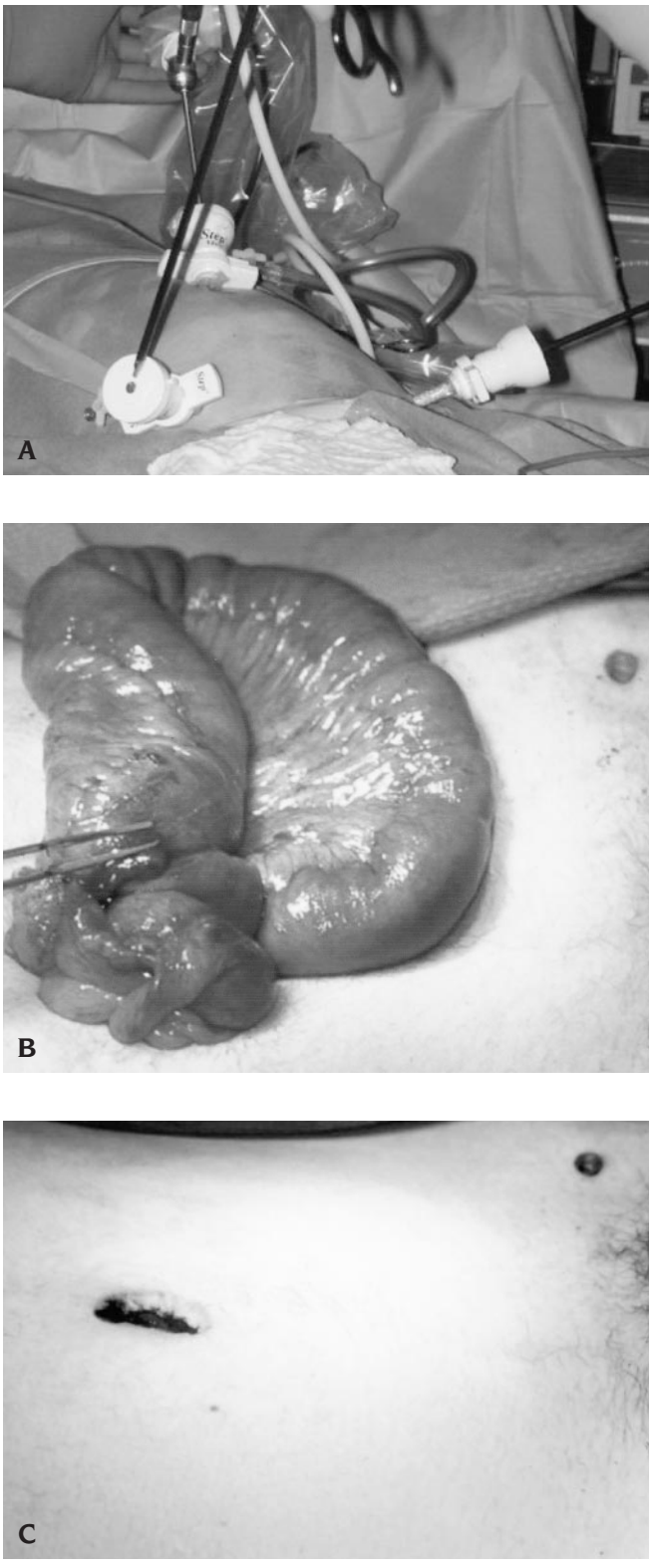

FIGURE 41.4-1 Laparoscopy-assisted ileocolic resection. The right colon and ileum are mobilized laparoscopically using several 3 to 5 mm ports (*A*), and the final resection and anastomosis are done through a small umbilical incision (*B*). The final result is shown (*C*).

FIGURE 41.4-2 Strictureplasty for multiple strictures in small bowel Crohn disease. The bowel is incised longitudinally across the stricture and then closed transversely. Strictureplasty should be performed on all strictures that cannot permit passage of a 10 cc Foley balloon.

The principles are to surgically treat only bowel that is thought to be causing symptoms, to do strictureplasties on short narrow areas, and to conserve small bowel length as much as possible. Like ileocolic resection, these procedures can usually be done using a laparoscopy-assisted approach.

Perianal Disease

Perianal disease can present a difficult management problem in children because anal fistulae are often deep and complex and surgical treatment may lead to permanent sphincter damage. The first-order treatment should consist of metronidazole and immunosuppressive agents, which may permit healing of the fistulae without the need for surgery. True perirectal abscesses require surgical drainage, but as conservative an approach as possible should be taken. The use of a defunctioning ileostomy for perianal disease is controversial, and it is likely that the same efficacy can be achieved through the use of elemental feeding or total parenteral nutrition with bowel rest. However, a stoma may provide better control of pain and perineal excoriation because it completely diverts the fecal stream. In extremely severe cases of perianal Crohn disease in which there has been extensive destruction of the anal sphincter, a total proctocolectomy with permanent ileostomy may be necessary.

Fistulizing Crohn Disease

Fistulae between the small bowel or colon and the skin, intestine, bladder, or vagina often can be treated medically using bowel rest, steroids, metronidazole, and immunosuppressive agents, although at least 50% of these will recur after therapy is discontinued. In cases in which medical management fails and the fistula is symptomatic, excision of the fistula with local resection of the involved bowel should be done.[7] In cases of fistulae to bladder or vagina, the defect in the normal structure can often be repaired without the need for resection. Some authors believe that fistulizing Crohn disease is an absolute indication for surgical intervention, although improving results with newer medical approaches, such as infliximab, may permit some patients to avoid surgery in this setting.[8]

Gastroduodenal Disease

Although relatively common, this form of Crohn disease can almost always be successfully managed using acid-reducing agents such as H_2 blockers or omeprazole, as well as standard medical therapy for Crohn disease. Surgery may be necessary for rare cases involving fistulae, strictures, or severe symptoms.[9]

Crohn Colitis

Crohn colitis often presents a complicated picture. Isolated colonic disease can usually be differentiated from ulcerative colitis by the presence of skip lesions or evidence of transmural involvement. Often both endoscopy and a barium enema are necessary because endoscopy provides mucosal detail and biopsy tissue, and the barium study permits assessment of distensibility (ie, fibrosis) and fistula formation. In cases in which only a small segment of colon is involved, local resection with primary anastomosis can be done. However, this approach is associated with a high incidence of recurrence or anastomotic complications (Figure 41.4-3).

Most patients have multiple areas of involvement, and at least a subtotal colectomy is necessary. The options for these children are a Brooke ileostomy, an ileorectal anastomosis, or a total proctocolectomy. Sphincter-saving pouch procedures and continent ileostomies are associated with a

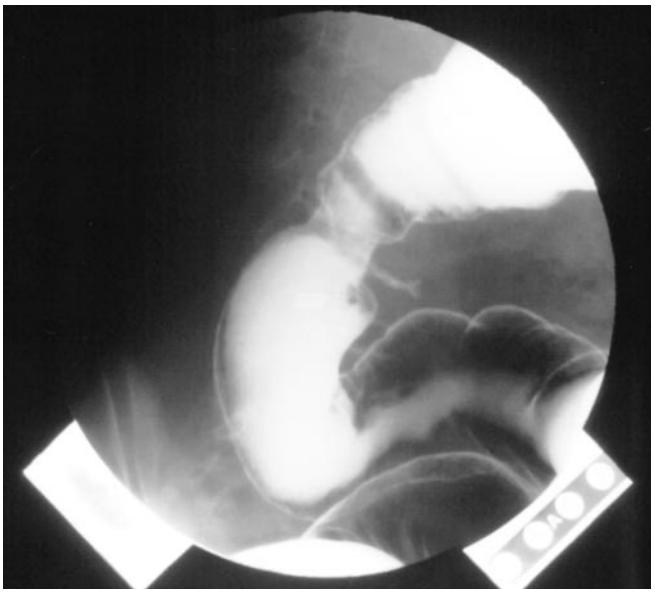

FIGURE 41.4-3 Child with a short segment of colonic disease, which was managed by local resection and anastomosis. The child then developed recurrent disease at the anastomosis with development of a recurrent stricture and fistula, requiring a more extensive resection.

prohibitive complication rate and are contraindicated in the presence of Crohn disease.[10] Although functional results in children are extremely good following subtotal colectomy with ileorectal anastomosis, at least 50% of these children will ultimately require a permanent ileostomy because of recurrent rectal disease.[11]

ULCERATIVE COLITIS

Emergency Management

For children with ulcerative colitis, emergent surgical intervention is carried out for life-threatening bleeding or toxic megacolon. The primary goal of surgery should be to remove the area that is bleeding or that is pathologically distended. Although a definitive procedure may be appealing, in the vast majority of emergency cases, a subtotal colectomy should be performed with ileostomy and either mucous fistula or Hartmann pouch. Some authors have recommended ileostomy with "blow hole" decompression for patients with toxic megacolon as an alternative to colectomy.[12]

Nonemergency Management

For most children with ulcerative colitis, surgery is considered because of symptoms that are resistant to medical management or because of complications (or the fear of complications) from pharmacologic therapy. Because ulcerative colitis is a mucosal disease, surgical removal of all colonic mucosa results in cure. Options for definitive surgery include total proctocolectomy with either Brooke or continent (Kock) ileostomy or colectomy with ileoanal anastomosis ("restorative" proctocolectomy) (Figure 41.4-4). Although the former alternatives were commonly used in the past, the

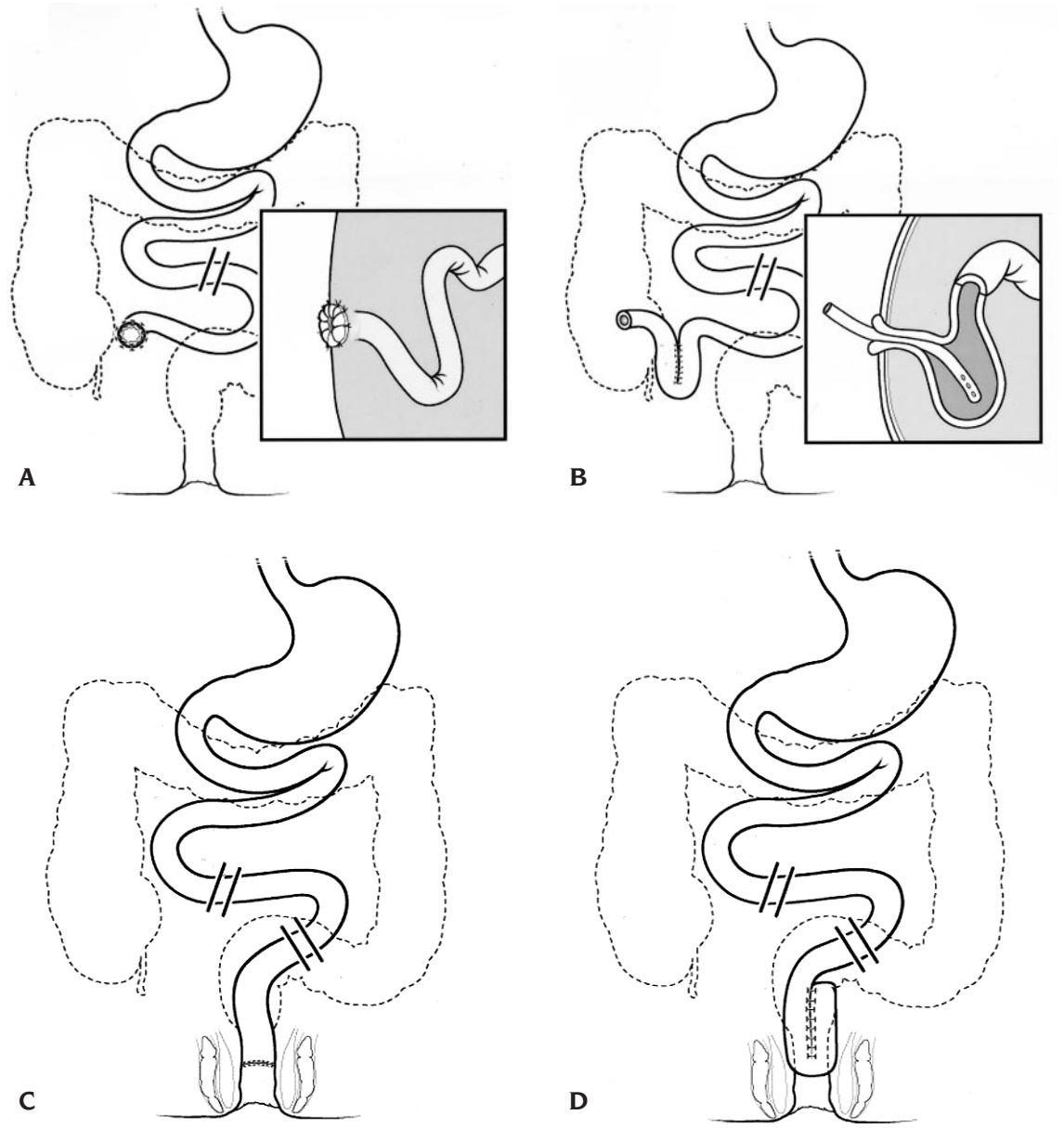

FIGURE 41.4-4 Options for reconstruction after colectomy for ulcerative colitis. A, Proctocolectomy and Brooke ileostomy; *B*, proctocolectomy with continent ileostomy (Kock pouch); *C*, subtotal colectomy with straight ileoanal anastomosis; *D*, subtotal colectomy with J pouch.

need for a permanent stoma and the high complication rate of the continent ileostomy[13] have made total proctocolectomy unappealing for most children, and by far the most common approach currently is subtotal (or "total abdominal") colectomy with restorative proctocolectomy.

A number of controversies exist as to the best approach to restorative proctocolectomy in children. In some cases, a subtotal colectomy may be performed as the primary procedure, with a reconstructive operation done in a second stage. Indications for this approach include an emergency situation, high preoperative steroid dose, poor nutrition, or concern that the diagnosis may be granulomatous colitis (Crohn disease) rather than ulcerative colitis. If the colectomy and reconstruction are to be done simultaneously, a proximal defunctioning ileostomy is usually, but not always, placed.[14,15] Controversy exists regarding a number of technical issues in restorative proctocolectomy, including straight ileoanal pull-through versus pouch formation, shape of the pouch (J, S, or W), or stapled versus handsewn anastomosis. Potential advantages of the straight pull-through include a simpler procedure, lower incidence of complications, absence of postoperative pouchitis (see below), and avoidance of a defunctioning ileostomy.[16] Potential advantages of a pouch include lower stool frequency and a decreased incidence of incontinence and perianal excoriation.[17] The use of a stapled anastomosis permits retention of a short length of transitional epithelium. This, theoretically, results in improved sensation with less incontinence, an advantage that is counterbalanced by the long-term risk of cancer and the need for periodic lifelong surveillance using proctoscopy. Thus far, comparative studies in children have failed to document a clear advantage to either approach.[18,19]

There is controversy around the appropriateness of restorative proctocolecomy in patients with "indeterminate" colitis. In general, it is best to start with a subtotal colectomy and ileostomy in these patients and wait for permanent sections on the resected colon. If, at that point, the diagnosis of ulcerative colitis is still not certain, a pouch procedure can be safely done,[19] but the patient and family must understand that the risks of pouchitits or the ultimate development of Crohn disease may be higher than are seen with clearly defined ulcerative colitis.

Both colectomy and formation of a J pouch have been described as laparoscopic or laparoscopy-assisted procedures (Figure 41.4-5). The advantages of this approach include less pain, shorter time to a full diet, and a better cosmetic result.[20] In addition, because laparoscopic surgery is thought to result in a lower incidence of adhesions,[21] it is hoped that laparoscopic surgery will decrease the relatively high incidence of postoperative small bowel obstruction seen in patients undergoing colectomy.

COMPLICATIONS AND POSTOPERATIVE MANAGEMENT

As with any abdominal procedure, patients undergoing surgery for IBD may develop wound infection, adhesions, stoma dysfunction, and ventral hernias. However, the more disturbing complications include anastomotic leak, recurrent Crohn disease, and pouchitis.

The major risk factors for the development of an anastomotic leak are administration of steroids and nutritional status. A leak can be diagnosed clinically by the presence of fever, local pain and tenderness, ileus, and fluid drainage. Radiographs may demonstrate increasing free intraperitoneal air. It is rarely necessary to do contrast studies soon after an anastomosis or pouch procedure to document a leak; in fact, such a study could potentially make matters worse. Abdominal computed tomography or ultrasonography may show free air or fluid accumulation. Treatment of documented leak usually consists of laparotomy or laparoscopy with defunctioning ileostomy. In selected cases in which there is a small leak that is not accompanied by systemic sepsis and that is well localized, management with bowel rest and antibiotics may be effective. Primary revision of the anastomosis or pouch, without proximal diversion, should never be attempted.

Crohn disease usually recurs in the region of an anastomosis and may occur weeks to many years following surgery. Early recurrence is highest in patients with active inflammation at the resection margins and patients with multiple anastomoses.[4] The risk of late recurrence is very high, especially in children, and approaches 90 to 100% in series with long-term follow-up.[22] A number of recent randomized trials in adults have demonstrated that chronic postoperative administration of 5-acetylsalicylic acid, metronidazole, or azathioprine results in decreased recurrence rates in patients with both Crohn colitis and ileitis.[23] Overall, patients can be given the "rule of thirds" with respect to outcome from surgery: roughly one-third will have a relatively prompt recurrence, one-third will have 5 to 10 years before their next recurrence, and one-third will go more than 10 years or indefinitely without recurrent disease.

Inflammation in the ileal pouch ("pouchitis") is a common complication following ileoanal pull-through for ulcerative colitis. It occurs in approximately 40% of patients, and the incidence is not influenced by the type of

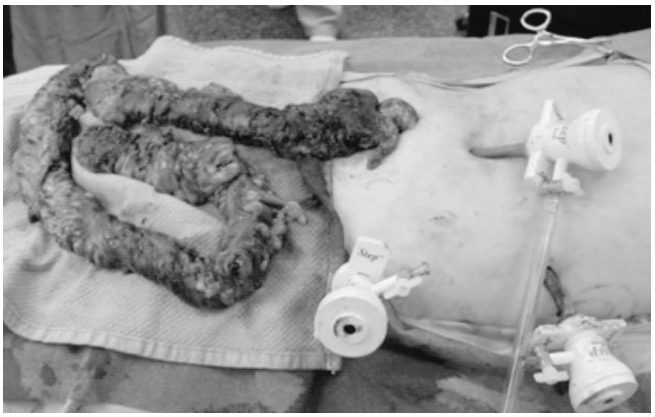

FIGURE 41.4-5 Laparoscopic approach to subtotal colectomy and ileostomy. The dissection is done using four port sites (one of which will become the ileostomy site), and the colon is ultimately removed through the ileostomy site.

pouch or method of anastomosis.[24] The etiology of pouchitis is unknown. Interestingly, it is never seen in patients who have had restorative proctocolectomy for familial polyposis, suggesting that the same mechanism for inflammation in ulcerative colitis may cause pouchitis.[25] Inflammation is only rarely seen in patients undergoing straight ileoanal pull-through without a pouch, suggesting that stasis within the pouch may play a role. Pouchitis may be the first sign of unsuspected small bowel Crohn disease in patients who have been initially diagnosed as having ulcerative colitis or indeterminant colitis. Most patients have mild pouchitis, which can be successfully treated with metronidazole or ciprofloxacin. In approximately 15% of patients, more aggressive therapy with local steroids or 5-acetylsalicyclic acid may be necessary, and in approximately 5%, the pouchitis is severe enough that the pouch must be removed. Other complications, such as anastomotic stricture, perforation with pelvic sepsis or fistula formation, or poor pouch emptying, may also result in loss of the pouch.[26] Successful conversion to a continent ileostomy has been reported in some patients with a failed pelvic pouch.[27]

PSYCHOSOCIAL CONSIDERATIONS

IBD presents some unique psychosocial issues in children when compared with the same disease processes in adults. As with any chronic illness in childhood, the disease impacts not only on the child but on the entire family as well. It is crucial for the attending physician and surgeon to be aware of this and to involve the family in discussions and the decision-making process.

Most children with IBD are adolescents and are going through a normal process of developing independence from their parents and struggling with their self-image.[28] The disease may interfere with this process by imposing dependency on parents; creating self-image problems owing to cushingoid features, delay in onset of puberty; and stomas; and socially isolating the child because of frequent hospitalizations, chronic pain, and diarrhea. Withdrawal from steroid medication, which often occurs during the postoperative period, commonly causes depression, which must be expected and explained to the child and family so that the feelings are not misinterpreted. It is imperative for the health care team to be aware of and sensitive to these issues because the mental health of the child impacts so strongly on physical recuperation.

Finally, quality of life is an important factor in the decision to perform surgery and in the assessment of postoperative results. Several quality of life indexes have been described for use in adults with Crohn disease and ulcerative colitis, which may be useful for surgeons in clinical practice.[29,30] Quality of life issues have also been assessed in great detail for adults undergoing restorative proctocolectomy for ulcerative colitis.[31,32] However, as yet, there have been no specific attempts to measure quality of life in children and adolescents with IBD. Despite this, for many children with IBD, the decision to have an operation may revolve more around a quality of life issue than a medical one, and the surgeon must be patient and respectful while the child and family struggle to make these difficult choices.

TEAM APPROACH

The decision to operate on a child with IBD should be made by the child and family in collaboration with both the surgeon and the pediatric gastroenterologist and with the help and support of social workers, nurse clinicians, psychologists, dietitians, and other allied health professionals. It is helpful if the surgeon becomes involved in discussions with the family early in the process. This gives the surgeon the opportunity to educate the family with respect to the risks and benefits of surgery and permits a rapport to be established between the family and the surgeon. Having a surgeon consult with the family should not be considered as a "failure" on the part of the gastroenterologist. Ideally, the family should meet periodically with the team as a whole to ensure that the information being transmitted to the child and family is consistent and clear.

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CHAPTER 42

NECROTIZING ENTEROCOLITIS

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Michael Caplan, MD

Neonatal necrotizing enterocolitis (NEC) is an inflammatory bowel necrosis that primarily afflicts premature neonates.[1,2] Despite the significant morbidity associated with this disease, the pathophysiology is poorly understood. NEC differs from other conditions in that the inherent immaturity of the premature intestine appears to be the greatest risk factor rather than any particular insult. Thus, this chapter reviews the clinical features and pathophysiology of this disease with particular attention to what is understood about the unique susceptibility of the preterm intestine. Although full-term infants may develop this disease, these infants generally have specific underlying risk factors for gut compromise such as birth asphyxia, polycythemia, exchange transfusion, intrauterine growth restriction, cyanotic congenital heart disease, gastroschisis, or myelomeningocele (Table 42-1).[3–7] In these patients, NEC often presents within the first days of life. Thus, the pathophysiology in full-term infants may be quite different from that in premature infants and is not considered further in this chapter.

CLINICAL FEATURES

NEC affects approximately 1 to 5% of all neonatal intensive care unit (NICU) admissions and 12% of premature infants < 1,500 g, characteristically between 7 and 14 days of life.[8–10] Increasingly, NEC has been documented several weeks after birth, particularly in very low birth weight infants.[11] Susceptibility to NEC appears inversely related to gestational age, with the primary risk being gut immaturity itself.[12–15] NEC presents with variable symptoms, which may include evidence of gastrointestinal dysfunction such as abdominal distention, feeding intolerance, gastric aspirates, bilious vomiting, and hematochezia, with progression to pneumoperitoneum and/or systemic signs of shock and rapid death in severe cases.[16,17] Laboratory values are significant for leukocytosis, thrombocytopenia, electrolyte imbalance, and acidosis. The pathognomonic feature is pneumatosis intestinalis, which is detected on abdominal radiograph. The disease progression has classically been defined by the Bell staging system (Table 42-2). Stage I or suspected NEC is characterized by abdominal distention, increased gastric residuals, hematochezia, vomiting, lethargy, apnea, bradycardia, and hemeoccult positive stools. Stage II or definite NEC is stage I plus pneumatosis intestinalis (Figure 42-1) or portal venous air. Stage III or advanced NEC is progression to shock, disseminated intravascular coagulation, acidosis, thrombocytopenia, neutropenia, peritonitis, or pneumoperitoneum. There does not appear to be a sex or race predilection. Mortality is reported at 10 to 40% of infants with NEC. Other sequelae include stricture, short gut syndrome, abscess formation, and recurrence of disease

TABLE 42-1 RISK FACTORS FOR NECROTIZING ENTEROCOLITIS

PRETERM INFANT	FULL-TERM INFANT
Prematurity—immature intestine	Birth asphyxia
Ischemia	Polycythemia
Enteral feeds	Exchange transfusion
Bacterial colonization	Intrauterine growth restriction
Cyanotic congenital heart disease	
Gastroschisis	
Myelomeningocele	

PATHOLOGY AND HISTOLOGY

NEC primarily affects the terminal ileum and proximal colon, a watershed area for the superior and inferior mesenteric arteries. Pathologic and histologic specimens have shown a combination of ischemic necrosis, acute and chronic inflammation, bacterial overgrowth, and tissue repair, suggesting that NEC is an evolving process rather than an acute event.[18,19] Specifically, surgical specimens reveal evidence of apoptosis of enterocytes in apical villi.[20] Inflammation can be limited to the mucosa and submucosa of the intestine or progress to transmural involvement in the most severe cases. In addition, lesions may include submucosal or subserosal collections of gas, which may represent bacterial fermentation of intraluminal substrates.[19]

PATHOGENESIS

Multiple theories have been proposed for the pathogenesis of this disease; however, despite multiple epidemiologic and animal studies, a single explanation has eluded investigators. The primary risk factors appear to be prematurity, ischemia, enteral feeding, and bacterial colonization. The classic theory of the pathogenesis of NEC, as described by Santulli and colleagues in 1975, suggests that the disease is

TABLE 42-2 BELL STAGING CRITERIA

STAGE	SYSTEMIC SIGNS	INTESTINAL SIGNS	RADIOLOGIC SIGNS
Stage I: suspected NEC	Temperature instability; apnea; bradycardia; lethargy	Abdominal distention; gastric residuals; vomiting; hematochezia	Normal or mild ileus
Stage II			
Definite NEC	Same as I	Same as I plus abdominal tenderness	Pneumatosis intestinalis or portal air
Advanced NEC	Progression to shock; DIC; metabolic acidosis; thrombocytopenia; neutropenia	Peritonitis	Pneumoperitoneum

DIC = disseminated intravascular coagulation; NEC = neonatal necrotizing enterocolitis.

caused by the triad of injury to the intestinal mucosa, bacteria, and a substrate such as formula feedings in the bowel wall.[19,21] Although the pathogenesis for NEC appears to be multifactorial, the major risk factor appears to be prematurity because more than 90% of NEC occurs in premature infants, and gestational age and birth weight correlate inversely with a higher incidence of disease.

Several factors potentially make the preterm infant more susceptible to NEC, including immature intestinal host defense,[22,23] blood flow regulation,[24,25] bacterial colonization,[26–30] and inflammatory response.[31–33] Furthermore, it is known that the fetal intestine is exposed to amniotic fluid containing hormones and peptides that may have a role in intestinal maturation. Preterm infants may not have completed this maturation process when colonized by bacteria and initially fed, potentially placing them at higher risk for NEC. At this stage, the fetal intestine is normally protected in its sterile environment and may not be prepared to respond to bacterial interaction.

HOST DEFENSE

Several aspects of the gastrointestinal host defense are developmentally regulated, putting the premature infant at a disadvantage (Figure 42-2). First, there are physical barriers such as mucous membranes, intestinal epithelia and microvilli, epithelial tight junctions, and mucin. Animal studies have shown that pathogenic organisms adhere and translocate across the intestine to a greater extent in immature versus mature animals. Abnormal peristaltic activity in these infants may increase bacterial adherence, allowing for bacterial overgrowth that could increase endotoxin exposure and predispose the infant to NEC.[34–36] Cell surface glycoconjugates serve as adhesion sites for a variety of microbes, and the immature intestine has a different pattern of carbohydrate residues than the adult intestine, which may result in increased pathogenic colonization in preterm infants.[37,38] Furthermore, it is known that intestinal mucus, which protects against bacterial and toxin invasion, is different in developing animals and perhaps in premature infants.[39]

Next, there is immaturity of the functional barrier that limits growth of bacteria that breach the physical barrier. This functional barrier is composed of the immunologic host defense and various biochemical factors. It is known that numbers of intestinal B and T lymphocytes are decreased in neonates and do not approach adult levels until 3 to 4 weeks of life. Newborns also have reduced levels of secretory immunoglobulin (Ig)A in salivary samples, presumably reflecting decreased activity in the intestine.[40–42]

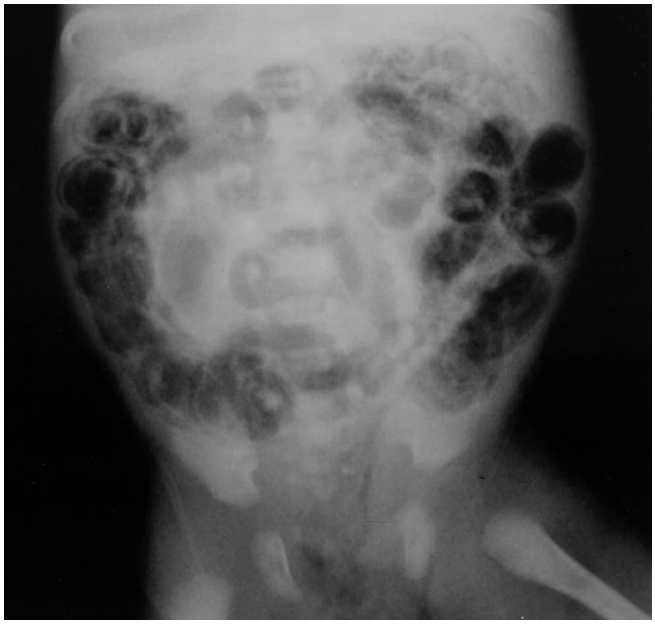

FIGURE 42-1 Abdominal radiograph depicting pneumatosis intestinalis in a premature infant with necrotizing enterocolitis.

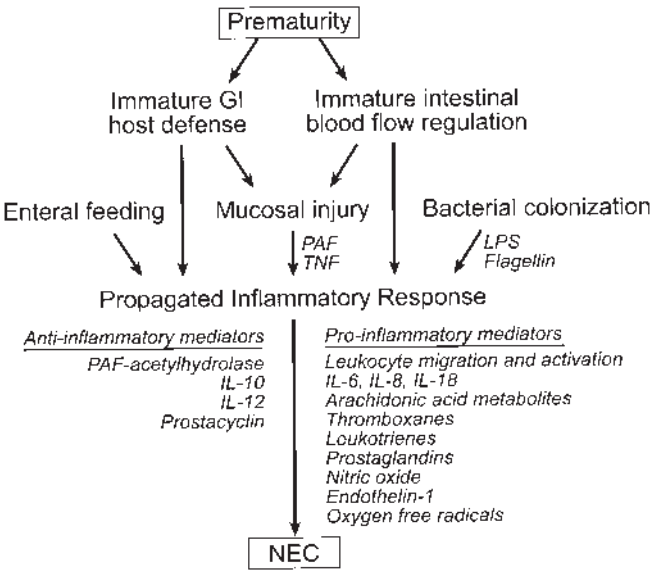

FIGURE 42-2 Aspects of immature intestinal host defense in the premature infant that may contribute to susceptibility to necrotizing enterocolitis. GI = gastrointestinal; IL = interleukin; LPS = lipoplysaccharide; NEC = neonatal necrotizing enterocolitis; PAF = platelet activating factor; TNF = tumor necrosis factor.

Additionally, the premature infant has lower gastric acid production than do older children, and immature proteolytic enzyme activity may lead to incomplete breakdown of toxins.[40] Finally, key bacteriostatic proteins are secreted from the intestinal epithelium. Intestinal trefoil factor is one such molecule that appears to be developmentally regulated and deficient in premature infants.[43–45] Human defensins (or cryptidins) are bacteriostatic proteins synthesized and secreted from Paneth cells that protect against bacterial translocation and are also altered in premature infants and those with NEC.[46,47]

INTESTINAL BLOOD FLOW REGULATION

Early observations suggested that profound intestinal ischemia was a critical predisposing factor for NEC.[48,49] It was hypothesized that similar to the diving reflex observed in aquatic animals, in periods of stress, blood flow was diverted away from the splanchnic circulation, resulting in intestinal necrosis. Although early epidemiologic observations identified asphyxia as an important risk factor, subsequent studies have shown that the majority of NEC cases are not associated with profound impairment of intestinal perfusion.[16]

Neonatal animals have been shown to have differences in intestinal circulation that may predispose them to NEC. Studies have shown that the newborn has compromised intestinal flow in response to circulatory stress. In response to hypotension, newborn animals appear to have defective pressure/flow autoregulation, resulting in decreased intestinal oxygen delivery and tissue oxygenation.[23,50,51] In addition, in the face of arterial hypoxemia, the newborn intestinal circulatory response differs from that of older animals. Although following modest hypoxemia, intestinal vasodilation and increased intestinal perfusion occur; severe hypoxemia causes vasoconstriction, intestinal ischemia, and/or hypoxia, mediated in part by loss of nitric oxide production. Multiple chemical mediators (nitric oxide, endothelin, substance P, norepinephrine, and angiotensin) impact on intestinal vasomotor tone, and in the stressed newborn, abnormal regulation of these may result in compromised circulatory autoregulation, leading to perpetuation of intestinal ischemia and tissue necrosis.[52–54]

ENTERAL FEEDING

Enteral alimentation has long been considered an important risk factor for the initiation of NEC. Feeds are thought to play a major role because 90 to 95% of all infants with NEC have been enterally fed, although the majority of fed premature infants do not develop the disease.[13] Although the precise relationship between enteral feedings and NEC remains poorly understood, studies have identified volume and rate of feeding advancement, osmolality, and substrate fermentation as important factors.[55,56] Preterm infants are unable to fully digest carbohydrates and proteins, leading to the production of organic acids, which may be harmful to the developing intestine.[57] Furthermore, human studies and carefully controlled animal models have shown that formula-fed infants have a higher incidence of NEC than breastfed infants.[13,30] Breast milk contains multiple bioactive factors that influence host immunity, inflammation, and mucosal protection, including secretory IgA, leukocytes, lactoferrin, lysozyme, cytokines, growth factors, enzymes, oligosaccharides, and polyunsaturated fatty acids. Specific host defense factors acquired from breast milk, such as epidermal growth factor, polyunsaturated fatty acids, acetylhydrolase, IgA, and macrophages, are effective in reducing the incidence of disease in animals, and some have been effective in human trials.[31,41,58,59]

BACTERIAL COLONIZATION

Although clusters of cases of NEC have been reported, no specific organism has been linked to this disease. It is unclear whether bacteria are a primary effector of NEC or are passive participants, entering the bowel wall through a breech in the intestinal mucosal barrier. Only about 30% of infants with this disease have positive blood cultures, and, in general, organisms present in the usual intestinal flora (eg, *Escherichia coli*; *Klebsiella*, *Enterococcus*, *Clostridium* species; and coagulase-negative *Staphylococcus*) have been found in the stool samples of affected infants.[18,27,60]

Colonization of the newborn intestine is affected by the environment, variation in pH, intestinal peristalsis, bacterial opposition, and type of feeding. Before birth, the infant gut is sterile, and no cases of NEC have been described in utero, supporting the importance of bacterial colonization in the pathophysiology. The intestine is initially colonized with a complex flora that reflects maternal flora.[61,62] The preterm infant is next exposed to bacteria in the NICU, with colonization frequently affected by the use of broad-spectrum antibiotics.[40,60] Although a wide range of aerobic and anaerobic flora colonizes normal infants by 10 days of age, infants in the NICU undergo delayed colonization with a limited number of bacterial species that tend to be virulent.[9,63]

Feeding is another variable in the acquisition of intestinal flora. In breastfed infants, bifidobacterium is a primary organism, with lactobacillus and streptococcus as minor components. In formula-fed infants, similar amounts of bacteroides and bifidobacterium are found with minor components of the more pathogenic species, *Staphylococcus*, *E. coli*, and *Clostridium*.[62,64–67] Animal models have shown that certain bacteria, such as adherent *E. coli*, produce disease in a rabbit model of NEC, whereas nonpathogenic strains of gram-positive organisms prevent disease.[68] Furthermore, preliminary work has suggested that early colonization by probiotics (facultative anaerobes such as bifidobacteria and lactobacilli) reduces the risk of NEC in animal and human studies.[29,69] In preterm infants, colonization with *Bifidobacterium* is delayed for several weeks. Because binding of pathogenic organisms can be influenced by the underlying microbial ecology through competition for binding sites or nutrients, production of inhibiting agents, alteration in pH, and synthesis of growth factors, promotion of the growth of competitive nonpathogenic strains of bacteria may protect the infant.[63,68,70]

INFLAMMATORY CASCADE

Evidence from human and animal studies suggests that various perinatal insults to the immature intestinal mucosal barrier lead to an inflammatory cascade, resulting in NEC in some patients.[30,58] Inflammation can be initiated by exogenous stimuli such as bacteria and bacterial products such as lipopolysaccharide and flagellin, as well as by endogenous inflammatory mediators such as tumor necrosis factor-α (TNF-α) and interleukin (IL)-1β.[71–75] In response to stimuli, enterocytes can release cytokines, such as IL-6, TNF-α, and IL-8.[76] These stimuli have distinct interactions with the intestinal epithelium and collectively result in a propagated inflammatory response (Figure 42-3). Asphyxia and/or ischemia-reperfusion also activate the early mediators of inflammation in many tissues, including in the intestine. Neonatal animal studies have shown that the stress of formula feeding stimulates phospholipase A_2 gene expression, intestinal platelet activating factor (PAF) production, and stimulation of apoptosis and the inflammatory response, with resulting NEC.[58,59] If counterregulatory responses to these inflammatory events are insufficient, pathologic changes to gut mucosa occur.

Intestinal specimens from patients with acute NEC demonstrate increased IL-1β, IL-8, IL-11, and TNF-α messenger ribonucleic acid compared with controls.[77–79] Other studies have documented increased IL-6 levels in patients with NEC, with some studies suggesting that IL-6 levels may correlate to severity of disease.[27,80,81] Furthermore, patients with NEC have elevated serum PAF and TNF-α levels but decreased acetylhydrolase levels compared with age-matched controls (Figure 42-4).[32] Acetylhydrolase is a PAF breakdown enzyme; thus, the higher PAF levels may result from decreased PAF catabolism and from increased PAF production. PAF is a potent phospholipid inflammatory mediator associated with NEC.[32,82] In rat models, PAF has been shown to produce bowel necrosis similar to NEC, an effect that can be inhibited by PAF receptor antagonists.[83,84]

The balance of pro- and anti-inflammatory influences appears to be a fundamental issue. Evidence suggests that the premature neonate may be predisposed to intestinal inflammation. Neonates are markedly deficient in their ability to degrade PAF owing to decreased activity of the specific PAF degrading enzyme PAF acetylhydrolase.[32,82] PAF acetylhydrolase is present in breast milk but absent in commercial formula, and this may, in part, explain the beneficial effects of breast milk feeding. In neonatal rats, maternal milk feedings increased amounts of the anti-inflammatory cytokine IL-10 and reduced the incidence of NEC, whereas in human milk specimens, a significant percentage of NEC patient-pairs was deficient in this important cytokine.[85,86] Studies have compared the proinflammatory response to lipopolysaccharide and IL-1β in intestinal cell lines and have found that IL-8 secretion is significantly higher in fetal intestinal epithelium compared with mature, adult intestine.[33] These results suggest that the neonatal balance of the inflammatory response may be weighted toward the proinflammatory side and is more likely to result in the pathologic outcome of NEC.

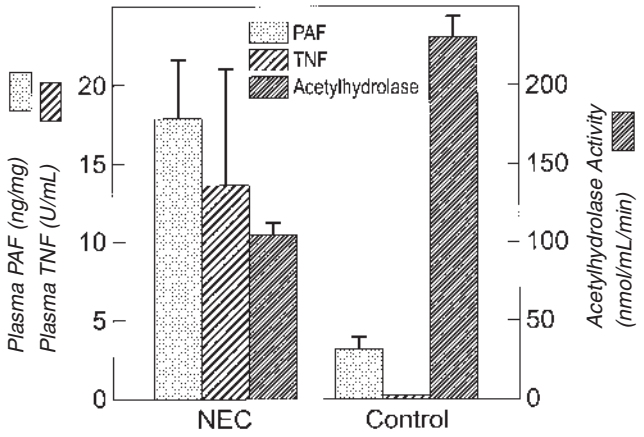

FIGURE 42-3 Potential mechanism by which risk factors for necrotizing enterocolitis (NEC) in preterm infants lead to propagation of an inflammatory response, resulting in NEC. PAF = platelet activating factor; TNF = tumor necrosis factor.

TREATMENT

Treatment remains supportive care only, and specific preventive steps have not been conclusively identified (Table 42-3). Treatment depends on prompt recognition of the disease. Therapy includes cessation of enteral feeds, nasogastric decompression, vigorous fluid resuscitation, broad-spectrum antibiotics, correction of anemia and thrombocytopenia, and respiratory support as needed. Patients need to be closely monitored by clinical examination and abdominal radiography for evidence of perforation. Radiographs may show nonspecific signs, such as intestinal distention and ileus, as well as pathognomonic pneumatosis intestinalis. Surgery is indicated for extraintestinal air (intestinal perforation or portal venous gas), evidence of ongoing necrosis (persistent metabolic acidosis indicating necrotic tissue, progressive shock, persistent thrombocytopenia), abdominal wall cellulites, or a fixed loop on serial abdominal radiographs.

PREVENTION

Several studies have explored measurement of cytokine levels to determine which infants will progress to severe NEC. IL-8, IL-10, and IL-1 receptor antagonist levels have been found to correlate to disease severity but are currently not used clinically.[86] Studies have indicated that early, hypocaloric, or trophic feeds are safe and improve gastrointestinal function in very low birth weight infants.[10] In addition, mother's milk has been found to be more protective than formula in humans and in animal models of the disease.[13,31,87–89] The specific protective factor(s) in human milk has yet to be conclusively identified, and studies are ongoing. Animal and human studies have documented decreased NEC in response to orally administered polyunsaturated fatty acids.[58,90] Epidermal growth factor given with feeds decreased the incidence of NEC in an animal model and has been used intravenously to treat an older child with intestinal necrosis in one clinical case report.[31,91]

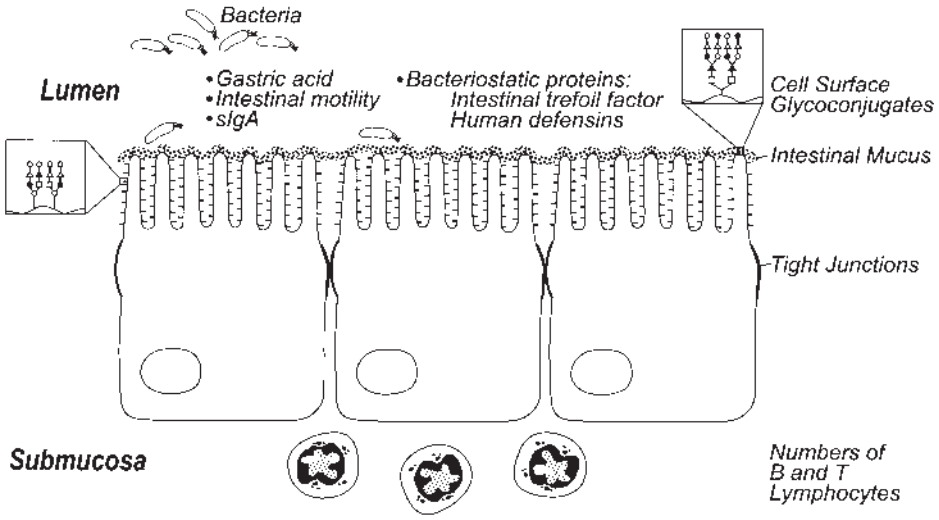

FIGURE 42-4 Plasma platelet activating factor (PAF) levels (*open bars*), plasma tumor necrosis factor (TNF) levels (*horizontally hatched bars*), and plasma acetylhydrolase activity (*vertically hatched bars*) in patients with necrotizing enterocolitis (NEC) and control subjects. Plasma PAF and TNF levels were higher in NEC patients than in control patients. Plasma acetylhydrolase activity was lower in NEC patients than in age-matched control subjects. Reproduced with permission from Caplan MS et al.[32] sIgA = secretory immunoglobulin A.

Halac and colleagues have shown that prenatal steroids reduce the incidence of NEC.[92] Cortisone acetate is known to accelerate maturation of the immature intestine, and studies in a rat model of NEC have shown that prenatal cortisone decreased the morbidity and mortality secondary to NEC.[93] Other studies have suggested prevention of NEC by IgA-IgG feeding of low birth weight infants.[41] Lastly, supplementation with *Bifidobacterium* has been shown to reduce NEC in a rat model of the disease.[29]

CONCLUSION

NEC is a devastating condition without a precise etiology. It specifically affects preterm infants and appears to be the result of an inflammatory response to risk factors, including bacterial colonization, intestinal ischemia/hypoxia, and formula feeding. The balance of pro- and anti-inflammatory influences appears to be a fundamental factor. The premature infant differs from term infants and older patients in multiple ways, including the complex system of intestinal host defense, intestinal motility, bacterial colonization patterns, autoregulation of splanchnic blood flow, and regulation of the inflammatory cascade. Preterm infants are uniquely susceptible because of an immature immune system that is unable to sufficiently protect against pathogenic organisms; in fact, the immature enterocyte may itself increase injury by excessive cytokine production in response to gram-negative organisms. Although preventing prematurity would be the most successful approach to prevent NEC, strategies to modulate inflammatory reactions may decrease intestinal injury.

TABLE 42-3 TREATMENT

Cessation of enteral feeds
Nasogastric decompression
Fluid resuscitation
Broad-spectrum antibiotics
Correction of anemia and thrombocytopenia
Respiratory support as needed
Careful monitoring of clinical examination
Serial abdominal radiographs
Surgical indications: extraintestinal air, ongoing necrosis, abdominal wall cellulitus, fixed loop on serial abdominal radiographs

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CHAPTER 43

CONGENITAL DISEASE OF DYSFUNCTION AND ABSORPTION

1. *Genetically Determined Disaccharidase Deficiency*

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Klaus-Peter Zimmer, MD

The hydrolysis of carbohydrates, essential constituents of mammalian diet, in the intestinal lumen is achieved by a family of microvillar enzymes, the disaccharidases. Prominent members of this family are the enzymes sucrase-isomaltase (SI), maltase-glucoamylase (MGA), and lactase-phlorizin hydrolase (LPH).[1–5] Starch, glycogen, sucrose, and maltose are examples of well-known diet carbohydrates that comprise monosaccharides associated with each other through α-glycosidic linkages and are hydrolyzed by SI and MGA to monosaccharides, which are eventually transported across the brush border membrane of epithelial cells into the cell interior. Only a few examples of α-glycosidically linked monosaccharides are known, such as that present in one of the major and most essential carbohydrates in mammalian milk, lactose, which constitutes the primary diet source for the newborn.[6]

The digestive enzymes are membrane-bound glycoproteins that are efficiently expressed at the apical or microvillus membrane of the enterocytes.[2–4] The absence of these enzymes in the intestinal lumen is associated with carbohydrate malabsorption. Malabsorption following maldigestion causes diarrhea with soft to watery, often acid, but rarely fatty stools. Osmotic diarrhea arises when the intestine cannot digest and/or absorb one or more nutrients. Molecules (especially those of smaller size) cause an osmotic force, which drives water into the gut lumen. Stool volumes are smaller than in secretory diarrhea and depend on the amount (and size) of undigested and unabsorbed molecules (mainly carbohydrates) concentrated in the bowel. Osmotic diarrhea regresses with discontinuation of oral feeding. Ion gap and osmolarity are increased in the stool while the pH is below 6.0.

Bacterial flora of the colon salvage carbohydrates, which were not digested and absorbed in the small intestine, by fermentation to gases (hydrogen, methane, and carbon dioxide) and to lactic acid as well as to volatile short-chain fatty acids (acetic, propionic, butyric, isobutyric, valeric, and isovaleric), the last being absorbed by the colonic mucosa.[7] Indeed, fermentative diarrhea develops when the salvaging capacity of the colonic flora to an undigested carbohydrate is exhausted.

Diarrhea attributable to carbohydrate malabsorption is usually more severe in children (especially infants) than in adults because the intestinal passage of nutrients is more rapid in children. Further impact of unabsorbed carbohydrates on intestinal motility consists of inhibition of gastric emptying and acceleration of small intestinal transit.[8] These effects are more tremendous when malabsorption is associated with conditions such as irritable bowel syndrome[9,10] that are known to accelerate bowel transit.

Diagnosing a disaccharidase deficiency starts with a precise nutritional history, which often points the way to the diagnostic and therapeutic approach. The onset of clinical features often coincides with the introduction of specific disaccharides, such as sucrose and lactose. The parents may already have started a dietary restriction based on empiric observations. In contrast, the causal needs of some patients are suitable to overestimate some unspecific complaints. The assessment of symptoms associated with disaccharidase deficiencies, such as vomiting, abdominal pain, meteorism, and diarrhea, can be difficult because the clinical features show a broad heterogeneity and change with age. Therefore, it is important to evaluate noninvasive tests such as stool examinations (pH and reducing substances), oral tolerance, and, preferentially, hydrogen breath test in the context of specific loads with suspected disaccharides. Breath testing uses the bacterial carbohydrate metabolism in the colon with the production of hydrogen, which is absorbed

by the mucosa of the colon and excreted in breath. The (invasive) determination of enzyme activities from homogenates of proximal small intestinal biopsies is required independent of the clinical (therapeutic) relevance. Finally, the therapeutic (long term) effect of disaccharide restriction must be considered in the conclusive consultation of the patient. Taken together, the diagnosis of a disaccharide deficiency should not be founded only on the result of one of the mentioned diagnostic approaches but in the context of the patients' complaints and sensitive and specific laboratory evaluations.

SUCRASE-ISOMALTASE

Structure, Biosynthesis, and Polarized Sorting

The SI enzyme complex (EC 3.2.1.48-10), the most abundant glycoprotein in the intestinal brush border membrane, is an integral membrane protein that is composed of two subunits, sucrase and isomaltase (isomaltase ~ 145 kDa and sucrase ~ 130 kDa).[11] Sucrase and isomaltase are enzymatically active toward α-glycosidically linked carbohydrates, the most abundant sugars in mammalian diet. Sucrase digests primarily 1,2-α- and 1,4-α-glucopyranosidic bonds and is responsible for the terminal digestion of dietary sucrose and starch. Isomaltase hydrolyzes mainly α-1,6 linkages. By virtue of these hydrolytic properties, the SI enzyme complex belongs to a superfamily of α-glucosidases in higher organisms, including, for example, brush border MGA (see later), mammalian lysosomal α-glucosidase, invertase and glucoamylase of *Saccharomyces cerevisiae*, and glucoamylase of *Schwanniomyces occidentalis*.[12]

SI is a type II membrane glycoprotein in which the N-terminal end exhibits a cytosolic orientation and the C-terminal extrudes into the lumen, that is, SI has an N_{in}/C_{out} orientation. The transmembrane domain in this type of membrane protein also comprises the signal sequence required for translocation of the newly synthesized protein into the endoplasmic reticulum (ER) (Figure 43.1-1 depicts a schematic presentation of the structure and biosynthesis of SI).[13] An interesting structural feature of SI is a heavily *O*-glycosylated serine and threonine-rich stalk domain in the immediate vicinity of the membrane.[2,13] The extensive *O*-glycosylation of this short domain imposes rigidity and inflexibility, resembling that of a stalk. These structural features may play a role in protecting SI from being digested by pancreatic secretions at the membrane interface (thus decreasing the turnover rate), thus prolonging the life cycle of this molecule in the intestinal lumen. Another important region is the cytoplasmic tail, which contains 12–amino acid residues and has been proposed to undergo phosphorylation in vivo at the conserved Ser_6, raising the possibility that this post-translational event may be implicated in essential regulatory processes of the protein.[14] Cloning of full-length complementary deoxyribonucleic acids (cDNAs) encoding the rabbit and human species of SI have shown that 38% of the amino acid sequences of sucrase and isomaltase are identical, and an additional 34% show conservative changes.[13] The striking similarity between the two subunits has led to the concept that SI has emerged from one cycle of duplication of a single gene. Furthermore, sequence comparison of SI with the human lysosomal α-glucosidase and the glucoamylase from the yeast *S. occidentalis* suggests that these proteins have evolved from the same ancestral gene.[12] Although no three-dimensional structure of this large protein is so far available, the striking sequence similarities between sucrase and isomaltase and algorithmic predictions suggest the existence of subdomains within the two species that reveal similar folding patterns, and the native folded state of the SI enzyme complex can be referred to as a pseudodimer.[13] This is further favored by the behavior of sucrase and isomaltase toward proteases. With the exception of the major extracellular cleavage step of the mature precursor form, pro-SI, by luminal trypsin (see later), both species are resistant toward pancreatic proteases and do not reveal altered proteolytic maps, as would be expected for differently folded proteins.[2] It is likely that both subunits can function as autonomous and independent units within the

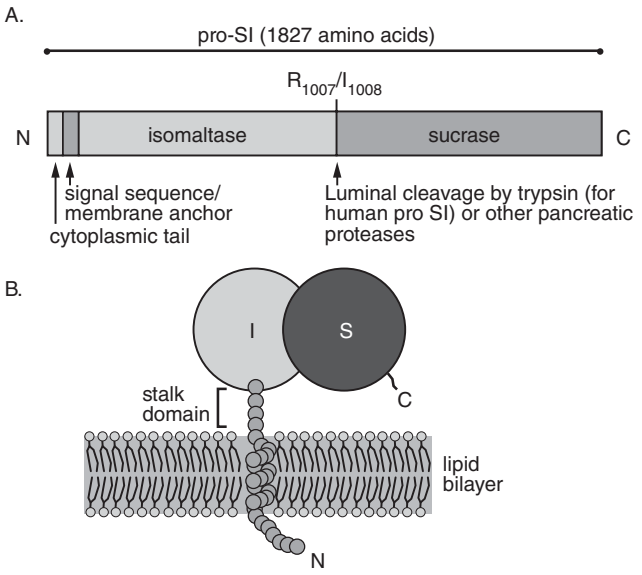

FIGURE 43.1-1 Structure and biosynthesis of pro–sucrase-isomaltase (pro-SI) in human small intestinal cells. *A*, Pro-SI is a type II integral membrane glycoprotein that is synthesized with an uncleavable signal sequence for translocation into the endoplasmic reticulum and consists of 1,827 amino acids. Pro-SI therefore has an N_{in}/C_{out} orientation. The N-terminal starts with a cytoplasmic tail (Met_1-Ser_{12}) followed by a membrane anchor that also contains the signal sequence Leu_{13}-Ala_{32}. A serine/threonine-rich stalk region encompasses the sequences Thr_{33}-Ser_{61} and is the site of extensive *O*-glycosylation of pro-SI. Isomaltase ends with amino acid residue Arg_{1007}, and sucrase starts immediately thereafter with Ile_{1008}. The Arg/Ile peptide sequence between isomaltase and sucrase is a trypsin site where the mature large precursor pro-SI is cleaved in the intestinal lumen by pancreatic secretions. The human enzyme is cleaved by pancreatic secretions (trypsin in the case of the human enzyme and elastase for the rat enzyme). Sucrase and isomaltase remain strongly associated with each other through ionic interactions. The membrane orientation of pro-SI is depicted in *B* with the cytosolic N-terminal, a luminal C-terminal, and the stalk domain next to the membrane. I = isomaltase; S = sucrase.

same polypeptide precursor. Nevertheless, individual expression of the subunits in heterologous transfection systems revealed the necessity of isomaltase for the presence of sucrase, which functions as a C-terminally located intramolecular chaperone in the folding of isomaltase.[15] In contrast, it is independent of the presence of isomaltase and acquires correct folding, enzymatic activity, and competent intracellular transport. The enzyme complex is expressed and synthesized exclusively by differentiated intestinal epithelial cells initially as a mannose-rich polypeptide precursor of an apparent molecular mass of 210 kD. The transport of this form from the ER to the Golgi cisternae occurs at a relatively slow rate (half-time of ~ 105–110 minutes) (for comparison, the intestinal proteins dipeptidyl peptidase IV and aminopeptidase N are transported at a half-time of ~ 15 minutes and 20 minutes, respectively).[2,4] Major post-translational processing of pro-SI occurs in the Golgi apparatus, leading to the generation of a complex *N*- and *O*-glycosylated mature pro-SI.[2] In particular, *O*-glycosylation is an essential modification that has implications for the further transport of pro-SI to the brush border membrane. Initial evaluation of the role of *O*-glycans on the sorting of pro-SI employed the inhibitor of *O*-glycosylation, benzyl-*N*-acetyl-β-D-galactoseaminide (benzyl-*N*-acetylgalactosamine), in the intestinal cell line Caco-2 cells.[16] This reagent leads to a drastic reduction in the size of pro-SI owing to impaired *O*-glycosylation. Concomitantly, the sorting behavior is dramatically altered from an efficient exclusive apical targeting to a random delivery of the modified pro-SI glycoform to both membranes, the apical and basolateral.[16] Deletion analyses have enabled the identification of the apical sorting signal within the stalk region of pro-SI.[17] The mechanism by which the sorting of pro-SI occurs is through association of SI with detergent-insoluble membrane microdomains enriched in glycosphingolipids and cholesterol, known as lipid rafts. In fact, inhibition of sphingolipid synthesis by fumonisin results in a random delivery of pro-SI to the apical and the basolateral membranes. A direct involvement of *O*-linked glycans in the association of pro-SI with lipid rafts is likely to be the driving mechanism for apical sorting of pro-SI.[16,17]

The isomaltase and the sucrase subunits are *O*-glycosylated. Moreover, *O*-glycosylation of the sucrase subunit is heterogeneous, as revealed by the presence of at least four glycoforms of differently *O*-glycosylated sucrase species, whereas the *O*-glycosylation pattern of the isomaltase subunit is more unique, suggesting a more efficient and consistent glycosylation of isomaltase compared with that of sucrase.[2] The variations in the *O*-glycosylation pattern of the subunits are presumably the consequence of spatial constraints, whereby isomaltase is readily *O*-glycosylated because it contains a serine/threonine-rich stalk region that is located in immediate proximity of the membrane, whereas the sucrase is more distal.[13] *O*-Glycosylation of pro-SI is not only required for correct targeting of pro-SI to the apical membrane, it also modulates the activity of isomaltase, which increases concomitantly with *O*-glycosylation.[2]

The final step along the biosynthetic pathway of pro-SI is the proteolytic cleavage in the intestinal lumen of the fully glycosylated precursor (pro-SI_C; c stands for complex glycosylated, 245 kD) by trypsin[11] or other pancreatic proteases into its two mature, enzymatically active subunits, sucrase (S_C, 130 kD) and isomaltase (I_C, 145 kD). These two subunits remain associated with each other through noncovalent strong ionic interactions.

The expression of pro-SI depends exclusively on the differentiated state of the intestine. In many tissues, cell differentiation is associated with altered gene expression owing to activation or repression of gene transcription. Gene activation and subsequent expression by tissue-specific transcription factors generate novel cellular phenotypic markers that are associated with significant morphologic and functional alterations of the differentiated cell. Differentiation of intestinal crypt cells to columnar epithelial cells, for example, results in the generation of two structurally and functionally different domains, the apical and basolateral domains,[18] and is characterized by the expression of genes encoding cell surface proteins that are implicated in the final steps of digestion of micromolecular nutrients, transports, and receptors. Among these genes are those encoding the disaccharidases, SI, LPH, and MGA.[19] Functional, immunohistochemical, and in situ hybridization studies have demonstrated that the pattern of expression of these disaccharidases, which are barely detectable in the crypts, reaches maximum levels between the lower and midvillus and decreases at the villus tip.[4,20]

Meanwhile, the structure and sequence of the SI gene are known.[21] Much of the information about the structure of the SI gene has described 5′-flanking regions and their role in transcription and regulation.[22] In addition to this, the sequences of the full-length cDNAs of the chicken, rabbit, rat, and human species are known.[22–25] One of the intriguing features of the gene structure of SI, which is perhaps unique for this enzyme, is that its first exon is untranslated, and the second exon starts with the initiation ATG codon.

Molecular Basis of Congenital Sucrase-Isomaltase Deficiency

The expression of SI and its activity increase during weaning and persist at high levels in adulthood.[26] The regulation of the SI activity is a transcriptional event that implicates SI- and intestine-specific nuclear factors. Meanwhile, several studies have identified several of these factors, as well as the structure of the SI gene. The SI gene consists of 48 exons, many of which reveal a predicted β-sheet or βα-barrel secondary structures. A very similar exon-intron junction organization reveals the gene of MGA, suggesting a close evolutionary structural and functional relation between the two genes.[21] A critical factor in regulating the SI promoter is *Cdx2*, an intestine-specific caudal-related homeobox gene that may also have a broader role in intestinal development and morphogenesis.[18]

Expression studies of promoter regions of the SI gene have assigned the intestine-specific promoter elements to a region between –183 and +54. On the other hand, several stretches within the sequence –3424 to +54 have an inhibitory effect on the level of transcription of SI. Assessment of the interaction of nuclear proteins with the pro-

moter by footprint analyses has identified three specific footprints for SI (denoted SIF1, SIF2, and SIF3 for SI footprints 1, 2, and 3, respectively).[27,28] Only SIF1 was found to be intestine specific because it was not identified in the nonintestinal cell line HepG2, in contrast to SIF2 and SIF3. Nevertheless, all three elements are implicated in positive regulation of SI gene transcription.[27]

Negative regulation of SI expression is potentially possible. Modulatory effects of certain additives, such as glucose, on the expression levels of SI in the intestinal cell line Caco-2 cells proposed negative regulatory mechanisms that operate at the messenger ribonucleic acid (mRNA) level of SI.[29] Fructose has also been reported to suppress post-translationally the expression of brush border enzymes.[30]

Post-translational regulation is the most common mechanism that significantly affects the physiology and cellular expression of SI. These effects are elicited by altered structural features: impaired, defective, or abnormal post-translational processing of an otherwise normally expressed SI protein and aberrant distribution of SI on the apical and basolateral domains of the enterocytes. These abnormalities in the cell biology and protein chemistry of SI constitute the basis for the congenital sucrase-isomaltase deficiency (CSID) in humans.[27,31–33] In addition to its clinical relevance, CSID has been used as a promising approach to characterize various steps in the biosynthesis, transport, and sorting of SI as a general model for cell surface membrane proteins and highly polarized proteins in epithelial cells.

Substantial progress has been made to elucidate the molecular basis of CSID. Different molecular defects or mutations in the SI gene have been proposed to be responsible for CSID. A number of studies with duodenal biopsy specimens from patients with this disorder at the subcellular and molecular levels have led to the identification of several phenotypes of SI in CSID that could be discriminated on the basis of subcellular localization, type, and nature of the mutation or residual enzymatic activities (Table 43.1-1 and Figure 43.1-2).[31–33] The emerging concept from all of these studies is that these phenotypes are generated by point mutations in the coding region of the SI protein. Our group was the first to successfully clone and characterize a cDNA encoding SI from a biopsy sample of a patient with CSID and to identify a single point mutation that is responsible for the impaired transport behavior of SI in phenotype II.[34] In the meantime, mutations correlating with other CSID phenotypes have been identified, and in what follows, a survey of the features of the individual phenotypes is presented (see also Table 43.1-1 and Figures 43.1-2 and 43.1-3).

Phenotype I Is Blocked in the ER. Phenotype I is characterized by a misfolded immature form of SI that is not capable of passing through the quality control machinery and is blocked in the ER as a mannose-rich glycosylated protein that is ultimately degraded.[33] Immunoprecipitation with several epitope-specific monoclonal anti-SI antibodies followed by enzymatic measurements demonstrated that the activity of this phenotype is below the detection limit. This phenotype is the predominant one among the CSID phenotypes. Most of the mutations that affect the trafficking of SI generate a misfolded form that is blocked in the ER. Recently, a CSID case with properties similar to those of phenotype I has been analyzed at the molecular and cellular levels.[35] Whereas biosynthetic labelings of an intestinal biopsy specimen and immunoelectron microscopy reveal predominant localization of SI in the ER and hence are similar to phenotype I, a partial conversion of the SI protein to a complex glycosylated mature form suggests a classification of this case as a subtype of phenotype I. The SI cDNA in this phenotype revealed a point mutation that results in an exchange of a leucine by a proline at position 620 (L620P) of the isomaltase subunit. The expression of this mutation in a heterologous cell line generates a protein with similar characteristics and intracellular localization to the intestinal phenotype. Thus, it was partially complex glycosylated and localized predominantly in the ER and, to

TABLE 43.2-1 NATURALLY OCCURRING PHENOTYPES OF CONGENITAL SUCRASE-ISOMALTASE DEFICIENCY

PHENOTYPE	CELLULAR LOCALIZATION OF SI	ENZYMATIC ACTIVITY	MOLECULAR FORMS	MUTATION	REFERENCE
I	ER	Completely inactive	Mannose-rich 210 kDa pro-SI	L620P	31–33,35
II	ER, ER/*cis*-Golgi intermediate compartment and *cis*-Golgi	Completely inactive	Predominant mannose-rich 210 kDa pro-SI and partial complex 245 kDa pro-SI	Q1098P	31, 33, 34
III	Brush border membrane	Completely inactive	Mannose-rich 210 kDa pro-SI and complex 245 kDa pro-SI	Not identified	31
IV	Random on apical and basolateral membranes	Active sucrase and isomaltase	Mannose-rich 210 kDa pro-SI and complex 245 kDa pro-SI	Q117R	33, 39
V	Intracellular cleavage, degradation of sucrase, isomaltase is correctly located at the apical membrane	Active isomaltase and absent sucrase activity	Mannose-rich 210 kDa pro-SI, complex 245 kDa pro-SI, and 150 kDa isomaltase	Not identified	33
VI	Intracellular cleavage, enzyme secreted	Active sucrase and isomaltase	Mannose-rich 210 kDa pro-SI and mannose-rich 207 kDa cleaved pro-SI and complex glycosylated 240 kDa cleaved pro-SI	L340P	40

ER = endoplasmic reticulum; SI = sucrase-isomaltase.

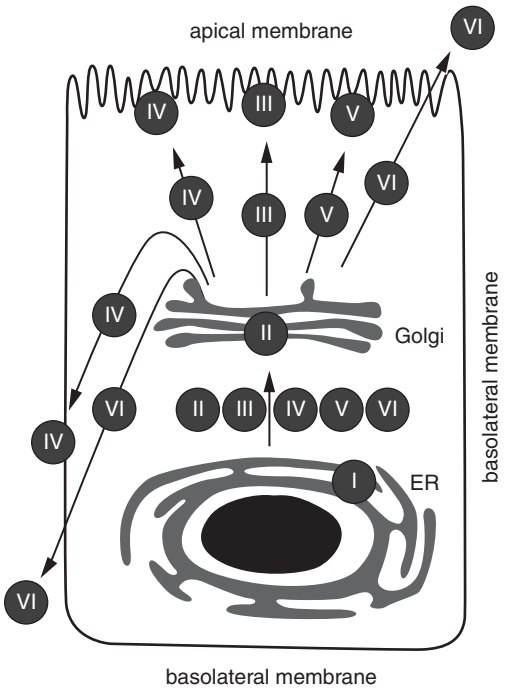

FIGURE 43.1-2 Schematic representation of the phenotypes of congenital sucrase-isomaltase deficiency. Phenotype I is blocked in the endoplasmic reticulum (ER) and phenotype II in the ER/*cis*-Golgi intermediate compartment and *cis*-Golgi.[31,34,37] Phenotype III is transported normally to the cell surface, but the sucrase subunit is enzymatically inactive.[31] Phenotype IV is randomly distributed on the apical and basolateral membranes.[33,39] In phenotype V, only the isomaltase subunit is transported to the apical membrane, whereas sucrase undergoes intracellular degradation.[33] Phenotype VI is characterized by an intracellular cleavage of pro-SI in the ER, generating a pro–sucrase-isomaltase that lacks its membrane-anchoring domain and stalk region and is secreted from both sides of the membranes of the enterocytes as an active protein.[40]

a lesser extent, at the cell surface. In addition, examination of the folding pattern in a proteolytic sensitivity assay results in an entire degradation of this mutant with the protease trypsin owing to additionally exposed cleavage sites in the malfolded protein.

Phenotype II Is Blocked in the *cis*-Golgi and ER Golgi Intermediate Compartments. The second most common phenotype of CSID is phenotype II. One of the most significant cellular features of phenotype II is an intracellular accumulation in the Golgi apparatus.[33] Some cases of CSID reveal the SI protein in the *cis*-Golgi in addition to the ER and the ER/*cis*-Golgi intermediate compartment (ERGIC), and in other cases, an accumulation in the medial and *trans*-Golgi has been observed.[32] In all of these cases, an enzymatically inactive mannose-rich polypeptide could be immunoisolated. Work done in the laboratory has succeeded in the identification of a mutation in this phenotype that results in a substitution of a glutamine by a proline in the sucrase subunit of SI at amino acid 1098 (Q1098P).[34] One of the consequences of this mutation is the synthesis of a temperature-sensitive form of SI that accumulates in the *cis*-Golgi compartment and ERGIC.[36] This observation and type of localization are surprising for membrane proteins because the consensus has emerged that proteins fulfilling the requirements of the quality control machinery in the ER and exiting this organelle acquire transport competence up to their final destination. For SI, this destination should be the cell surface; in phenotype II, however, the SI protein is blocked in the *cis*-Golgi. As such, this observation has important implications for current concepts of membrane and protein transport because this suggests that the Q1098P mutation generates a recognition site for a protein involved in a hypothetical quality control mechanism that operates beyond the ER, and such an event has not yet been described. The Q1098P substitution has therefore been investigated in more detail, and we could demonstrate that it is not only functional in intestinal epithelial cells but also produces a similar phenotype when expressed in nonpolarized COS-1 cells.[37] It is apparent therefore that (1) the Q1098P mutation is responsible for the generation of the SI phenotype II and (2) the onset of this CSID phenotype does not involve cellular factors specifically expressed in the small intestine. One important observation is that the folding state of the mutant SI protein is not as stable as its wild-type counterpart and is degraded within a relatively short period of time. The interesting aspect of these folding experiments is that the sucrase and the isomaltase subunits in the mutant phenotype II are degraded. Although malfolding of

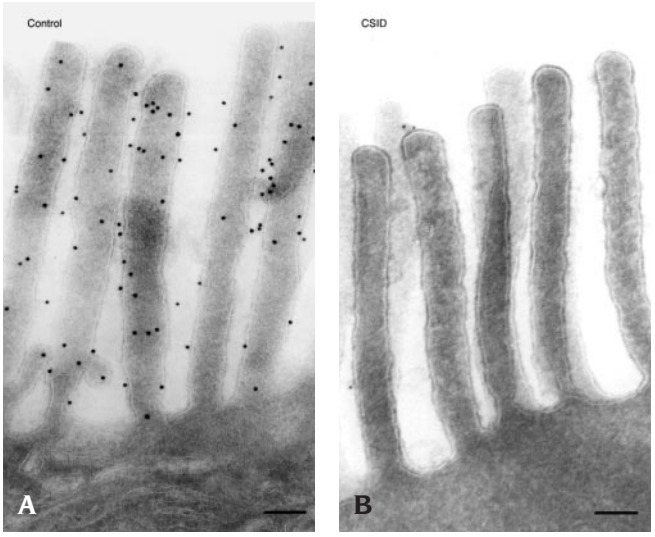

FIGURE 43.1-3 Immunogold labeling of sucrase-isomaltase (SI) within a thin frozen section of a duodenal biopsy of a control and a patient with congenital sucrase-isomaltase deficiency (CSID). Strong immunogold labeling corresponding to SI could be detected at the microvillar membrane of enterocytes from normal controls. In one case of CSID, the microvillar membrane was devoid of immunogold labeling owing to a defective intracellular transport behavior of SI. The absence of SI at the apical membrane could be observed in CSID phenotypes I, II, and VI.[31,34,37,40] Phenotype III is characterized by normal levels of SI in the brush border membrane,[31] whereas phenotype IV reveals marked reduction in the appearance of SI in the apical membrane, with the concomitant labeling of the basolateral membrane.[33,39] In phenotype V, only isomaltase could be detected in the brush border membrane.[33]

the sucrase subunit as a result of the mutation Q1098P is plausible, the altered structure of the isomaltase in the context of SI suggests that the association between sucrase and isomaltase takes place very early in the ER, whereby the sucrase subunit plays a decisive role in the folding of isomaltase and could be designated an intramolecular chaperone. In fact, more recent evidence has proven this hypothesis and provided further data in which an alternating association between the ER chaperones immunoglobulin binding protein (BiP) and calnexin, together with sucrase, occurs during the folding process of isomaltase.[15] Therefore, it is clear that sucrase is an important and crucial player in the acquisition of isomaltase to its physiologic function and transport competence within the cell on its way to the cell surface.

One of the interesting features of the Q1098P mutation is its presence in a region that shares striking homologies among human, rat, and rabbit sucrase and isomaltase variants, as well as human lysosomal α-glucosidase and *S. occidentalis* glucoamylase.[12] The fact that all of these proteins, which have been suggested to have evolved from a common ancestral gene, are transported along the secretory pathway to their final destinations proposes a key role for the region containing the Q1098P mutation in the sorting of these proteins from the ER and proposes a common function of this or structurally similar regions in other proteins along the secretory pathway. Indeed, we could show that the Q1098P mutation of sucrase elicits a similar effect when introduced at the corresponding amino acid position 244 in lysosomal α-glucosidase.[37] The mutated lysosomal α-glucosidase precursor does not undergo maturation in the Golgi, is not cleaved into mature enzyme, and is not transported through the Golgi cisternae. It is therefore conceivable that the Q1098P mutation has introduced a retention signal for the *cis*-Golgi or the mutation has led to a structural alteration in lysosomal α-glucosidase that functions as a recognition site for a quality control machinery operating in the intermediate compartment or *cis*-Golgi. The components of this hypothetical machinery must be unraveled.

Phenotype II Is a Temperature-Sensitive Mutant. One striking feature of phenotype II, in addition to the unusual block of SI in the *cis*-Golgi and ERGIC, is that the mutation Q1098P generates a temperature-sensitive mutant SI protein. In fact, biochemical and confocal microscopy analyses could unequivocally demonstrate that correct folding, intracellular transport, and full enzymatic activity can be restored by expression of the mutant SI (indicated $SI_{Q/P}$) at the permissive temperature of 20°C instead of 37°C.[36] Moreover, the acquisition of normal protein trafficking and function appears to use several cycles of anterograde and retrograde steps between the ER and the Golgi, implicating the molecular chaperones calnexin and BiP. A similar temperature-sensitive feature has also been proposed to be generated by the main genetic alteration in cystic fibrosis, the ΔF508 deletion in the cystic fibrosis transmembrane regulator.[38] However, the Q1098P mutation in phenotype II of SI is the first of its kind to generate a temperature-sensitive mutant implicated in an intestinal enzyme deficiency or an intestinal disorder.

Identification of New Cases of Phenotype II. Screening a cohort of patients with CSID has led to the identification of the Q1098P mutation in three more cases (H. Y. Naim and M. Keiser, unpublished data, 2003). All of these cases revealed a pattern of biosynthesis and processing similar to that obtained in the original case. The new cases were from East European patients, whereas the original one was from a West European individual. Despite the different ethnic origins of the patients, a similar mutation was identified, strongly suggesting that the genetic basis for phenotype II is exclusively caused by Q1098P mutation and is phenotypically characterized by a temperature-sensitive protein that is blocked in an early Golgi compartment. These new cases therefore provide strong support for the existence of a hypothetical quality control mechanism beyond the ER.

Normal Intracellular Protein Transport and Absent Enzymatic Activity in Phenotype III. In sharp contrast to the phenotypes I and II is phenotype III. Here SI is transported to the cell surface with kinetics similar to that of the wild-type protein. It is correctly folded because it reacted efficiently with different epitope-specific monoclonal antibodies and is correctly sorted to the apical membrane.[31] These criteria are adequate to propose that gross structural alterations did not occur in this specific phenotype of SI. Although further information about the location of the putative mutation in this phenotype is lacking, it is reasonable to assume that the mutation has immediately affected the catalytic site of sucrase, Asp^{1349}, or is located in immediate vicinity of the catalytic domain of sucrase, which is the Asp Gly Leu Trp Ile Asp Met Asn Glu (DGLWIDMNE) stretch.[13] The isomaltase subunit, on the other hand, expresses normal activity and is therefore not affected in this phenotype.

Random Delivery of SI to the Apical and the Basolateral Membranes Is the Characteristic of Phenotype IV. SI is almost exclusively (95%) located at the apical membrane of intestinal epithelial cells. This striking polarity and high sorting fidelity are required for an efficient function of the protein in the intestinal lumen. Deviation from this polarity pattern and an impaired sorting profile are associated with reduced function of SI and subsequent malabsorption. Analysis of two cases of CSID using immunoelectron microscopy demonstrated an altered distribution of SI from an exclusive apical to a random localization at the apical membrane and basolateral membranes.[33,39] Molecular characterization of this novel phenotype revealed a point mutation in the coding region of the SI gene that results in an amino acid substitution of a glutamine by arginine at residue 117 (Q117R) of the isomaltase subunit.[39] This amino acid exchange is located in a domain revealing features of a trefoil motif or a P domain in the immediate vicinity of the heavily *O*-glycosylated stalk domain. In wild-type SI, the stalk domain itself is directly involved in the targeting of the SI molecule to the apical membrane through an interaction of its *O*-glycosylated carbohydrate content with a putative lectin receptor that recruits SI to lipid rafts.[16] Although the location of the muta-

tion in the P domain of SI did not affect *O*-glycosylation of the mutant SI protein per se, random targeting of SI to the apical and basolateral membranes occurred. Unlike wild-type SI, the mutant protein is completely extractable with Triton X-100, despite the presence of *O*-glycans, which are required for the association of SI with detergent-insoluble lipid microdomains. This finding indicated that the *O*-glycan units were not adequately recognized in the context of the mutant SI by a putative lectin-like sorting receptor owing to a sterical hindrance or altered folding of the P domain generated by the mutation Q117R. Importantly, this naturally occurring mutant phenotype was the first of its kind of an apically sorted protein; moreover, it provided strong support to the notion of an active apical sorting mediated by sorting signals and a corresponding receptor.

Intracellular Proteolytic Cleavage at Two Different Sites in Phenotypes V and VI. Human SI is transported to the brush border membrane as a single-chain polypeptide, pro-SI, which is cleaved in the intestinal lumen by pancreatic trypsin to isomaltase and sucrase.[2] In phenotypes V and VI, the pro-SI precursor is intracellularly cleaved.[33,40] The cleavage sites are, however, different. In phenotype V, the cleavage occurs relatively late in the biosynthesis, in the *trans*-Golgi or *trans*-Golgi network.[33] In fact, biosynthetic labeling of an intestinal biopsy specimen and immunoisolation of SI revealed normal levels of the ER-located, mannose-rich polypeptide at an early labeling time point of 30 minutes. Within 4 hours of labeling, the complex glycosylated mature SI appears to be concomitant with the detection of a polypeptide of an apparent molecular weight similar to that of isomaltase.[33] The sucrase subunit was not detected by immunoprecipitation with monoclonal antisucrase antibodies. This observation was supported by immunolabeling of intestinal biopsy specimens. Here only the isomaltase subunit was detected at the apical membrane with an anti-isomaltase antibody. Obviously, the sucrase subunit in this phenotype undergoes an intracellular degradation immediately after being proteolytically processed from the precursor protein, whereas the isomaltase is transported correctly to the apical membrane. This phenotype provided the first indication that the isomaltase contains all of the necessary information required for apical transport of SI. Later, this hypothesis was experimentally verified, and the signals for apical sorting were identified in the *O*-glycosylated stalk region, and the membrane-anchoring domain and both domains are located in the isomaltase subunit.[16,17] The putative mutation in this phenotype has not yet been identified. Another mode of cleavage characterizes phenotype VI.[40] Biosynthesis of SI in intestinal explants revealed, in addition to the mannose-rich pro-SI polypeptide precursor, another band of smaller apparent molecular weight. The glycosylation pattern of this species was also of the mannose-rich type, suggesting that this band is a truncated form of the mannose-rich polypeptide and that the cleavage occurs within the ER. This view was supported by cDNA cloning of the SI from this phenotype and identification of the point mutation, as well as expression of the mutant cDNA in COS cells. The molecular basis of this phenotype is a point mutation isomaltase subunit that converts a leucine to proline at residue 340 of isomaltase. Expression of the mutant in COS cells and biosynthetic pulse-chase experiments revealed that pro-SI is cleaved early in the biosynthesis, in the ER, and the cleaved protein is transported efficiently along the secretory pathway, processed in the Golgi apparatus, and ultimately secreted into the exterior milieu as an active enzyme. This is a novel pathogenetic mechanism of a disorder that is elicited by the conversion of an integral membrane glycoprotein into a secreted species that is lost from the cell surface.

Epidemiology

The prevalence of CSID is 0.2% in individuals of European descent[41] and appears to be much higher in Greenland, Alaskan, and Canadian native people. It has been estimated to be 5% in indigenous Greenlanders.[42] Heterozygotes, which are defined as sucrase activity level below the lower limit for the normal population and with normal small bowel morphology, represent about 2 to 9% of European Americans.[43,44] CSID is transmitted by autosomal recessive inheritance. It was first described by Weijers and colleagues in 1960.[45]

Pathophysiology

Several factors, some of them less favorably present in infants, contribute to the development and extent of symptoms in CSID patients:

- Residual enzyme activity
- Amount of fed carbohydrate (in association with other foods)
- Gastric emptying
- Small bowel transit
- Degree of fermentation of unabsorbed carbohydrates by colonic bacteria
- Absorption of the colon

Sucrase hydrolyzes α-1,2- and α-1,4-glucosidic bonds, whereas isomaltase cleaves α-1,6-linkages. Sucrase-isomaltase overlaps with MGA, which digests the end and intermediary products of α-amylolysis of starch, such as maltose, maltotriose, and low- and high-molecular-weight branched dextrins, by hydrolyzing α-1,4-glucosidic linkages. However, only 20% of maltase activity is covered by MGA and 80% by SI.[5] Several factors led to the observation that patients with CSID tolerate starch better than sucrose:

1. MGA activity is preserved.
2. Starch fed to infants and toddlers has a low amount of α-1,6-glucosidic bonds, which may by sufficiently hydrolyzed by the residual isomaltase activity.
3. Colonic bacteria ferment starch in infants by 6 months of age.

Clinical Features

SI-deficient infants receiving human milk and lactose-containing formulas do not develop symptoms. Some parents introduced empirically a low-sucrose diet. Populations such as the Greenland Eskimos use traditionally low-carbohydrate

diets with a high amount of protein and fat. Later, introduction of sucrose and its reduction in quantity led to a delay in the onset of diarrhea.[46] Characteristic symptoms of patients with CSID are crying spells, vomiting, meteorism, watery diarrhea, mild steatorrhea, and chronic diarrhea.[47] Occasionally, dehydration, malnutrition, failure to thrive and to grow, developmental retardation, and muscular hypotonia were observed, indicating broad clinical heterogeneity.[41,48]

Presenting symptoms depend more on age and the amount of ingested sucrose than on the intestinal levels of SI activity. Symptoms and especially starch tolerance spontaneously improve with age. Chronic watery diarrhea and failure to thrive are common in infants and toddlers, chronic diarrhea with normal growth is usually found in preschool children, and irritable bowel syndrome and recurrent abdominal pain are typical symptoms of schoolchildren.[41] Adult patients present with refractory diarrhea or unspecific complaints.[49–51] Symptoms may present at the time of puberty, possibly unmasked by gastroenteritis. Onset of symptoms in adulthood with diagnosis up to the age of 59 years has been reported.[52]

Clinical heterogeneity includes (adult) patients with CSID who do not develop diarrhea following ingestion of large amounts of sucrose. In a few cases, CSID was associated with nephrocalcinosis, renal calculi,[50,51] metabolic acidosis, and hypercalcemia.[50,53]

The diagnosis of CSID is often missed or delayed because diseases such as food allergy, cystic fibrosis, and celiac disease or other causes of recurrent diarrhea are suggested. Transient hypoglycemia, acidosis, and lethargy may even lead one to consider inborn errors of metabolism.

Diagnostic Evaluation

A major step in diagnosing CSID is to recognize the complaints and clinical features of patients in relation to age-dependent alterations and composition of nutrition; sometimes, the parents started an empiric low-sucrose diet.

Stool and urine examinations serve as screening methods with limited sensitivity and specificity. A stool pH between 5.0 and 6.0 is an indicative but, finally, unreliable screening test for the diagnosis of sugar malabsorption.[54] The detection of reducing substances estimated with Clinitest tablets (normal < 0.5%) in stool, whose sucrose is hydrolyzed by boiling with 0.1 N HCl, has been proposed as a screening test for the diagnosis of CSID.[55] However, this method is not specific for sucrose.[54] Stool pH and reducing substances are falsely negative as soon as nonabsorbed carbohydrates are totally fermented in the colon. In contrast, reducing substances tend to be falsely positive in neonates and patients who have undergone a colectomy. The differential urinary excretion of sucrose in relation to lactulose can be used to diagnose SI deficiency[56]; however, this noninvasive sugar absorption test has not been approved in a larger series of patients.

A rise of blood glucose of less than 20 mg/dL after a 2.0 g/kg sucrose load indicates sucrose intolerance. Capillary blood samples show higher peak rises up to 25 mg/dL in blood glucose compared with venous samples.[57] Oral tolerance tests produce false-positive results between 24 and 33% owing to delayed gastric emptying.[58] False-positive results are also due to increased intestinal transit time. Monitoring of symptoms and determination of reducing substances in the stools within 6 hours improve the diagnostic values of this method.

Patients with CSID show a rise of breath hydrogen of more than 20 ppm over baseline between 90 and 180 (240) minutes after an oral load of 2.0 g/kg sucrose.[59,60] False-negative results are obtained by a delay in gastric emptying, the acid milieu of the colon (unrestricted diet prior to the test),[61] and nonhydrogen producers (following antibiotic therapy).[62,63] Up to 18% of patients who are referred for the evaluation of lactose intolerance are hydrogen nonexcretors.[64] Fast intestinal transit produces false-positive results on the breath test. The sucrose tolerance test, the hydrogen breath test, and sucrase activity did not strictly correlate in children who suffered from chronic diarrhea.[65]

The diagnosis of CSID requires the determination of SI activity with normal histology of the mucosa and the level of other disaccharidases (lactase, MGA, and alkaline phosphatase) in the normal range (differential diagnosis: secondary deficiency owing to general mucosa damage). Enterocytes of patients with CSID lack the sucrase activity of the enzyme SI, whereas the isomaltase activity can vary from absent to practically normal. Disaccharidase activities show a 40% reduction in the duodenum compared with the proximal jejunum.[66,67] In some SI-deficient patients, MGA activity is reduced in addition to isomaltase activity.[68] Combined deficiency of sucrase, lactase, and MGA, which seems to be caused by pleiotropic regulatory factors, has recently been described.[69] The differential diagnosis of CSID is broad, including the various causes of chronic diarrhea in childhood.

Therapy

Lifelong sucrose restriction is an effective therapeutic option for patients with CSID; it works well and is cheaper (as supplementation with sucrase). Because patients with CSID tolerate smaller or larger amounts of sucrose, the degree of restriction finally depends on the individual complaints of a patient. Foods with nutrients with high sucrose concentrations are beetroot (6.0), pea (4.5), honey (3.0), soybean flour (4.5), and onion (2.9 g/100 g edible parts). Care has to be taken with sucrose included in glucose polymer formulas[70] and medications. Because isomaltase function is also affected, the diet also excludes starch and glucose polymers, that is, foods with a high amylopectin content such as wheat (cereals, bread, pasta) and potatoes, especially in the first years of life. Tolerance to starch improves during the first 3 to 4 years. Rice starch and maize starch are the best digested. Arguing with compliance problems of a sucrose-restricted diet, it has to be considered that no short- or long-term complications of noncompliance are known, with the exception of severe (missed) cases.[50]

Lyophilized baker's yeast (*S. cerevisiae*), which is not very palatable, possesses sucrase activity, low isomaltase and maltase activity, and virtually no lactase activity. It has been shown that viable yeast cells reduce hydrogen excretion by 70%, with loss or reduction of clinical symptoms.[71]

Recently, sacrosidase (invertase), a liquid preparation produced from *S. cerevisiae* (Sucraid) that has already been used to hydrolyze unrefined sucrose solutions, has been successfully used in the treatment of patients with CSID.[72] It is a β-fructofuranosidase that is tasteless and resistant to pH changes down to 1.5, but it has no activity with oligosaccharides containing α-1,6-glucosyl bonds.

LACTASE-PHLORIZIN HYDROLASE

Structure, Biosynthesis, and Polarized Sorting

LPH (EC 3.2.1.23/62) is a type I integral membrane glycoprotein of the intestinal brush border membrane that comprises two major hydrolytic activities[73]: lactase activity, which is responsible for the hydrolysis of milk sugar lactose, the main carbohydrate in mammalian milk, and phlorizin hydrolase, which digests β-glycosylceramides, which are part of the diet of most vertebrates. A physiologic role of phlorizin hydrolase activity is still unknown.[73]

The LPH gene is located on chromosome 2 and consists of 17 coding domains, or exons.[74–76] The amino acid sequence deduced from the cDNA encoding LPH consists of 1,927 amino acids (Figure 43.1-4 summarizes the structural and biosynthetic features of LPH). The LPH molecule is highly *N*- and *O*-glycosylated. The primary sequence of the human enzyme predicted 15 potential *N*-glycosylation sites (the rabbit and rat enzymes possess 14 and 15, respectively). *N*- and *O*-Glycosylation are crucial in the folding, maturation, and enzymatic activity of LPH.[77,78] A study of the effect of glycosylation on the intracellular transport of human LPH in biopsy samples in the presence or absence of glycosidase inhibitors demonstrated that carbohydrate modification affects the transport rates from the ER to the *cis*-Golgi but is not important in the transport of LPH from the *cis*-Golgi to the cell surface.[3,78] Moreover, processing of N-linked carbohydrates has direct effects on the *O*-glycosylation of LPH, which is essential for the acquisition of LPH for an efficient enzymatic function. In fact, a mature *N*- and *O*-glycosylated LPH protein is almost fourfold more active than a LPH glycoform that is only *N*-glycosylated.[78]

All cloned LPH species have shown that the molecule consists of four highly conserved structural and functional regions.[74] These domains, denoted I to IV, reveal 38 to 55% identity to one another. The catalytic activity of lactase is localized to Gln_{1249} in the homologous region III and of phlorizin hydrolase is localized to Gln_{1773} in the homologous region IV.[79] Because of the four homologous regions, LPH may have arisen from two subsequent duplications of one ancestral gene.[74] An evolutionary and developmental example supporting this notion is given by the sequence similarities of LPH and each of its homologous regions with β-glycosidases from archebacteria, eubacteria, and fungi. It is possible that LPH belongs to a superfamily of β-glucosidases and β-galactosidases. The prokaryotic β-glycosidases are, on average, about 50 kDa in size, which corresponds to approximately one-fourth of the size of full-length LPH or roughly to the size of one homologous region (I–IV).

The biosynthesis and processing of LPH in several mammalian intestinal epithelial cells follow essentially similar pathways.[3,80,81] In what follows, the post-translational processing of the human enzyme most relevant to this book is described (see Figure 43.1-4). LPH is synthesized as a single-chain 215 kDa pro-LPH precursor that is *N*-glycosylated while translocating into the ER. *N*-Glycosylation is a critical covalent modification that is directly implicated in the attainment of pro-LPH to a correct protein folding, which, in turn, is a prerequisite for homodimerization, another modification step that takes place in the ER and is absolutely required for pro-LPH to exit the ER.[82,83] Dimerization is not only important for efficient transport from the ER, it is also crucial for the acquisition of enzymatic activity.[82] A correctly folded monomeric pro-LPH is not sufficient per se for a functional protein. The transmembrane domain of pro-LPH is a crucial structure in the dimerization event of pro-LPH, and its elimination results in a monomeric, inactive pro-LPH. Complex *N*- and *O*-glycosylation of pro-LPH in the Golgi apparatus precedes a proteolytic cleavage step that takes place in a late Golgi compartment, most likely the *trans*-Golgi network, and eliminates the large profragment

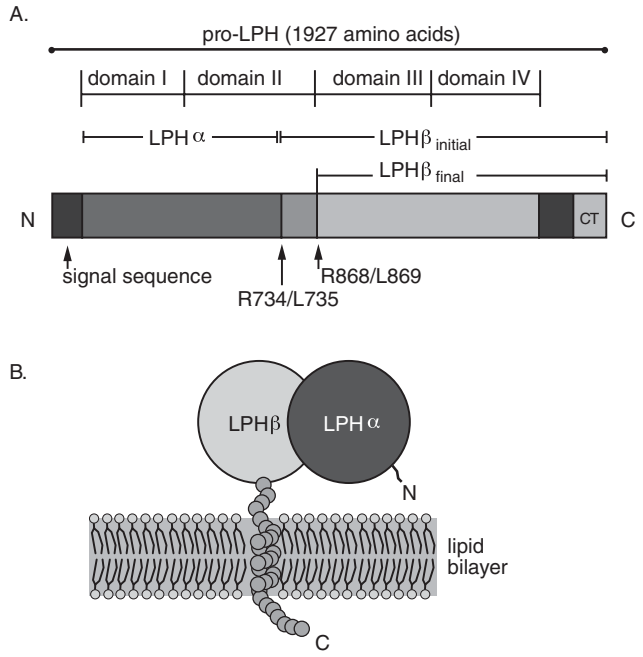

FIGURE 43.1-4 Schematic representation of the structure of pro–lactase-phlorizin hydrolase (LPH) in human small intestinal cells and the two constructs LPHβ and LPHα. *A*, Some important structural features of pro-LPH in human small intestinal cells. Shown is the cleavable sequence Met^{1}-Gly^{19} at the N-terminal. The ectodomain encompasses amino acid residues Ser^{20} to Thr^{1882}. Four internal homology domains within the pro-LPH amino acid sequence are indicated. The intracellular proteolytic cleavage takes place between Arg^{734} and Leu^{735} to generate $LPH\beta_{initial}$. The luminal extracellular cleavage occurs at Arg^{868} to generate the brush border mature enzyme, $LPH\beta_{final}$ (Arg^{868}-Phe^{1927}).[84,85] *B*, Schematic drawing of the membrane orientation of pro-LPH. The C-terminal on the cytosolic side of the membrane, the luminal N-terminal, and the subunits LPHα and LPHβ are depicted. CT = cytoplasmic tail; MA = membrane anchoring.

LPHα at Arg_{734}/Leu_{735}, leaving the membrane-bound LPH-$\beta_{initial}$, which extends from Leu_{735} to Tyr_{1927}.[84,85] LPH$\beta_{initial}$ is sorted with high fidelity to the apical membrane, where it is cleaved by pancreatic trypsin at Arg_{868}/Ala_{869} to LPHβ_{final} (Ala_{869}–Tyr_{1927}), which comprises the functional domains of the enzyme and is also known as mature brush border LPH of 160 kDa.[3,80,81] The proteolytic cleavage of pro-LPH neither activates LPH nor is required for the correct transport of LPH to the brush border membrane.[86–88] The profragment LPHα undergoes intracellular degradation immediately after intracellular cleavage, as has been shown in pulse-chase experiments of intestinal biopsy samples.[89] The LPHα polypeptide is neither *N*-glycosylated, despite the presence of five potential *N*-glycosylation sites, nor *O*-glycosylated. This, together with its high content of hydrophobic amino acids and its tendency to form a compact, rigid, and trypsin-resistant structure immediately after translation, endows LPHα with the characteristics of an intramolecular chaperone that is directly involved in the folding of the LPH$\beta_{initial}$ domain. Individual expression of a cDNA encoding LPH$\beta_{initial}$ generated a protein that was not as transport competent as pro-LPH. Only when LPH$\beta_{initial}$ was coexpressed with LPHα were correct folding and enzymatic activity of LPH$\beta_{initial}$ restored.[90] It is clear, therefore, that LPHα is required in the context of correct folding of pro-LPH. Finally, LPHα does not express enzymatic activities toward lactose, despite the strong structural homologies between regions LPHα and LPHβ.[86]

To achieve its physiologic function, pro-LPH or its membrane-bound form must be transported to the luminal surface of epithelial cells, that is, to the brush border membrane. The sorting to the apical membrane of proteins such as LPH is signal mediated. Until present, several types of signals have been described; some of them are associated with membrane detergent-insoluble glycophosphatidylinositol (DIG)/cholesterol membrane microdomains,[91] whereas some do not associate with DIGs. LPH is not associated with microdomains, and its sorting is not mediated through *O*-glycans as SI.[16,92,93] Recent observations have strongly suggested that putative sorting signals of pro-LPH are exclusively located in the domain corresponding to the brush border–associated LPHβ_{final}, precisely in the homologous region IV that also harbors the catalytic domain of LPH.[88,94]

Epidemiology of Adult-Type Hypolactasia

Primary adult-type hypolactasia affects the majority of the world's human population. More than 75% of the human adult population shows a decline to about 5 to 10% of the level at birth during childhood and adolescence ("post-weaning decline"). The incidence of adult-type hypolactasia varies between different populations because the expression of lactase is genetically determined. Up to 80% of blacks, Arabs, and Latinos, as well as up to 100% of American Indians and Asians, reveal adult-type hypolactasia; the lowest prevalences (< 5%) are detected in populations in northwestern Europe.[95] The persistence of lactase activity is most likely a consequence of a dominant gene defect with failure to repress the synthesis of lactase after weaning in regions with an abundant milk supply, which implies the "culture historical hypothesis."[96]

Molecular Basis

Genetic analysis of homozygotes and heterozygotes of lactase-persistent and lactase-nonpersistent families supported the initial idea that lactase activity is inherited as a single autosomal dominant gene.[97] There are currently several ideas that attempt to explain the late onset of lactase activity. Initial concepts suggested that lactase regulation may be a post-translational event, as is the case in CSID (see above). Alterations in the structural features of LPH itself may generate an inactive protein or lead to a defective post-translational processing and possibly intracellular degradation of the protein. This hypothesis was later discussed when the mRNA levels of lactase in the intestine of adult rats were found to be almost similar to the mRNA levels in fetal rats.[98] Another study used biopsy material to demonstrate that appreciable levels of lactase mRNA were detected in the intestines of hypolactasic individuals in southern Italy.[99] These observations were compatible with post-translational regulatory mechanisms of adult-type hypolactasia.

A study of a race- and sex-balanced cohort in which levels of jejunal lactase protein, activity, and mRNA were measured showed that black heritage predicts low lactose-digesting capacity (LDC) and white heritage predicts high LDC.[100] A decisive criterion in these studies was the assessment of the lactase-to-sucrase ratio (L:S) in jejunal biopsy specimens. All subjects with a high LDC had an L:S ratio > 0.5, immunodetectable LPH protein, and measurably higher LPH mRNA levels than subjects with low LDC. Further, LPH mRNA levels correlated highly with lactase-specific activity (r = .80) and L:S ratio (r = .88). The direct correlation between LPH mRNA levels and lactase expression argues that the gene responsible for the human lactase polymorphism regulates the level of LPH mRNA. Similarly, studies in the rat small intestine have essentially described a similar coordinate pattern of interrelationship between mRNA and protein levels of LPH.[98] The regional distribution of lactase activity, although clearly dependent on the presence of the LPH mRNA pattern, did not absolutely correlate with the mRNA and protein levels along the proximal-distal axis. This suggests that additional secondary mechanisms, perhaps post-translational, influence the lactase activity. One potential modification is *O*-glycosylation that commences in the *cis*-Golgi and increases the activity of LPH fourfold.[78] It is known that variations in the extent of *O*-glycans are associated with the differentiation state of intestinal cells to polarized enterocytes. These events are associated with dramatic alterations in the gene expression of intestinal proteins and increased enzymatic activities of typical intestinal markers such as SI and LPH. Along this, a regulatory mechanism of the enzymatic function of LPH (and also SI) that depends on the carbohydrate moiety and therefore the differentiation state of the intestinal cell cannot be excluded. Despite this, the major mechanism of regulation of LPH is transcriptional because the majority of the accumulated data have clearly demonstrated that adequate levels of LPH mRNA must be present to detect LPH

activities.[97,100,101] Meanwhile, the structure of the LPH gene has been published, as well as data on the interaction of specific regulatory elements with 5′ flanking regions of the gene. The gene for LPH is approximately 55 kb and is made out of 17 exons, which all encode LPH.[75] Transcription factors such as CTF/NF-1 and AP2 bind to regions in the LPH gene located within 1 kb of the 5′-flanking region of rat and human genes.[102] The lactase activity has been demonstrated to be enhanced in rats by glucocorticoids during the first weeks in life. Glucocorticoids also have regulatory effects on human lactase.[103] Responsive elements to glucocorticoids have not been, however, in the 5′-flanking region of LPH. Analysis of 5′-flanking sequences employed, fused to human growth hormone as a reporter gene in transfected Caco-2 cells or the nonintestinal cell line HepG2, has demonstrated the exclusive and specific function of these sequences in cells of intestinal lineage.[104] Footprint analysis of the promoter region has led to the identification of a nuclear protein (NF-LPH1) that binds to a 15 bp region just upstream from the transcription site (between –54 and –40), which is functional in the adenocarcinoma cell line Caco-2 and is probably involved in regulation of lactase activity.[102,105]

A specific region in the LPH promoter, CE-LPH1, has been identified that interacts with NF-LPH1 and is strongly associated with the regulation of gene transcription of LPH.[105,106] Nevertheless, differences in the promoter region of LPH between individuals with low LDC and those with high LDC have not been detected. Likewise, there is no evidence that describes variations in the levels of particular transcription factors associated with low or high LDC. Also, sequence analyses of the coding and promoter regions of the gene encoding LPH did not reveal DNA variations associated with low LDC. More recently, however, Enattah and colleagues identified a variant associated with adult-type hypolactasia.[107] Linkage dysequilibrium and haplotype analyses of nine extended Finnish families, the locus to a 47 kb interval on chromosome 2q21, were restricted. Sequence analysis revealed a DNA variant, C/T-13910, about 14 kb upstream from the gene locus of LPH that associates with low LDC in Finnish families and 236 individuals from four different populations. Another variant, G/A-22018, 8 kb telomeric to C/T-13910, is also associated with adult-type hypolactasia in 229 of 236 cases. Significant effects of these variants on the levels of LPH mRNA and the L:S ratio were demonstrated. Here the expression of LPH mRNA in the intestinal mucosa in individuals with T(-13910) A(-22018) alleles was found to be manyfold higher than that found in individuals with C(-13910), G(-22018) alleles.[108] Likewise, a significant elevation of the L:S ratio in the T(-13910) A(-22018) allele could be observed.

Clinical Features

Symptoms of adult-type hypolactasia increase with age, with many patients developing signs of lactose intolerance in adolescence and adulthood. They start briefly after consumption of milk, causing abdominal discomfort (colicky pain), flatulence, loose stools, diarrhea, and flatus. Symptoms are related to the amount of lactose administered, showing a broad heterogeneity in response among patients.

A major issue is the clinical relevance of lactose intolerance. The milk rejection rate has been 31% among the lactose malabsorbers and 12% among the lactose-tolerant individuals. However, 70% of the lactose-intolerant patients drink some milk.[109] Fifty-nine to 75% of lactose malabsorbers develop symptoms within 3 or 4 hours after ingesting 12 g of lactose (equivalent to 240 mL of milk) or less, 2 of 20 subjects had symptoms with 3 g of lactose, and 5 of 20 subjects had symptoms with 24 to 96 g of lactose.[109,110] Individuals with low lactase levels have revealed persistently low polyethylene glycol concentrations, representing fluid accumulation in the jejunum of 6 g of fed lactose and in the ileum of 12 and 24 g of fed lactose.[110] This study found no consistent correlation between jejunal lactase levels and the threshold for lactose-inducted symptoms. Symptoms in most lactose malabsorbers do not appear to be caused by small amounts (0.5–7.0 g) of lactose.[111] Other studies have identified lactose malabsorbers who tolerate one (240 mL) or even two cups of milk per day.[112,113]

The question has been raised as to whether symptomatic lactose malabsorbers have an additional gastrointestinal affection, such as irritable bowel syndrome.[112,114] Because about 70% of patients with milk intolerance and patients with irritable bowel syndrome revealed a positive lactose hydrogen breath test[115] and bowel transit is increased in irritable bowel syndrome,[9,10] it is hypothesized that fast intestinal transit contributes to the complaints of lactose malabsorbers.

Diagnostic Evaluation

Symptoms and nutritional history lead to the diagnosis of adult-type hypolactasia. Laboratory assessment of this diagnosis includes, preferentially, a lactose hydrogen breath test and, exceptionally, confirmation by lactase activity of a duodenal biopsy. The diagnostic values of other procedures, such as reducing substances, which are positive in the feces of patients with lactose malabsorption, implying no specificity for lactose malabsorption, are limited and depend more on the context of the patients' complaints and the results of additional laboratory evaluations. The lactose tolerance test is considered to be less sensitive than the lactose breath test. The latter does not reliably predict tolerance toward lactose in infants recovering from diarrhea.[65,116] Another study has found the relationship between the lactose breath hydrogen test and lactase activities not to be uniform.[117] A bad correlation among the lactose tolerance test, lactase activity, and hydrogen breath test has been found in children who suffered from chronic diarrhea.[65]

Lactose tolerance and hydrogen breath tests must be performed after a 6-hour (overnight) fast. Conditions that cause false-positive results are a low gastric emptying rate[58,118] and fast intestinal transit time.[119] False-negative results of the breath test are obtained by hydrogen nonexcretors or are due to prior treatment with antibiotics. Hydrogen breath tests are falsely negative in nonexcretors because hydrogen is metabolized by bacteria to methane. The daytime breath

hydrogen profile, which includes breath sampling at home at half-hour intervals during 1 day from awakening until bedtime, was abnormal in children with abdominal pain and diarrhea,[120] suggesting that the prevalence of lactose malabsorption underlying these conditions is high.

Diagnosis of adult-type hypolactasia on the enzyme level requires lactase activity in jejunal (duodenal) biopsies of less than 8 U/g protein or 0.7 U/g wet weight.[117] Disaccharidase activities are reduced in the duodenum compared with the proximal jejunum.[66,67] Other disaccharidase activities, as well as the morphology of the mucosa, must be in the normal range to exclude a secondary lactase deficiency. Therefore, the ratios of maltase to lactase and sucrase to lactase have been proposed as additional parameters.[121] Disaccharidase (lactase, sucrase, maltase) activity in aspirated fluid correlates with that measured in the mucosal microvillus membrane,[122] but this method has not been widely adopted.

Concerning the clinical relevance of lactose intolerance, it is a crucial diagnostic step to determine the response of the patient to a lactose-reduced diet. It allows one to estimate which symptoms are solely caused by lactose intolerance and not to additional affections, such as irritable bowel syndrome. The differential diagnosis of adult-type hypolactasia includes other causes of chronic diarrhea in childhood, especially infectious, immunologic (cow's milk allergy), and inflammatory disorders of the intestinal mucosa, many of them causing secondary lactase deficiency owing to mucosal damage. A reduction of duodenal lactase ($p = .024$) and pathologic lactose breath testing ($p = .005$) has been reported in patients with active Crohn disease in contrast to Crohn disease patients in remission.[123] Another study has not found lactase activity levels to be different in a cohort of patients with inflammatory bowel disease (37%) in comparison with control patients with chronic abdominal pain.[123,124]

Therapeutic Aspects

An increased frequency of osteoporosis has been reported in lactose malabsorbers that is due to the dietary calcium restriction as a consequence of milk avoidance.[125,126] Calcium is essential for adequate mineralization of bone matrix. Because lactose malabsorbers cannot use the main source of calcium, that is, dairy products, they need calcium supplementation to prevent osteoporosis.[127] Two hundred forty milliliters of milk contains 300 mg of calcium, with linkage to casein and optimal bioavailability. The National Academy of Science (NAS) recommends a dietary calcium intake of 210 mg/d for age 0 to 6 months, 270 mg/d for age 6 months to 1 year, 500 mg/d for age 1 to 3 years, 800 mg/d for age 4 to 8 years, and 1,300 mg/d for age 9 to 18 years.[128] Many lactose malabsorbers tolerate small amounts of milk without complaints so that they can improve calcium intake by solid cheeses and yogurt. Yogurt is a fermented milk product containing live active culture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, which reveal lactase activity. The lactase of yogurt, cultured buttermilk, and curds ferments lactose to lactic acid; however, pasteurization of these products reduces digestion of lactose. *Lactobacillus acidophilus* also possesses lactase activity and is available as "sweet acidophilus milk," with a taste similar to that of milk.[129] Alternatives to dairy products represent calcium-fortified fruit (orange) juice, vegetables, or lactose-free milks.

Lactase (β-galactosidase) is produced from yeast (*Kluyveromyces lactis*) or fungi (*Aspergillus oryzae*). Preincubation of milk with lactase was associated with a significant lower breath hydrogen and crying time in infants with colic compared with placebo.[130] Formulation of stable lactase microparticles with enteric release seems to preserve the activity of acid-labile lactase for enteric release.[131] However, warming of breast milk and formulas treated with lactase for 15 minutes significantly increases osmolality by 87 to 122 mOsm/kg owing to hydrolysis of lactose.[132]

Addition of lactase to a preterm formula may enhance weight gain.[133] Preterm infants have relative lactose malabsorption because lactase activity is only 30% of its full-term level by 26 to 34 weeks gestation.[134] Lactase activity is depressed in rabbit fetuses with intrauterine growth retardation.[135] Hypoxia in newborn rats causes delayed maturation of lactase.[136] Enteral feeding of preterm infants is suggested to increase lactase activity,[137] which has been confirmed in preterm pigs.[138] Sphingomyelin, the major phospholipid of human milk, reduces lactase in the intestine of 2-week-old rats, promoting enzymatic and morphologic maturation of the neonatal gut.[139]

Gene technological strategies to lower the lactose content in milk are developed[140] because more than 75% of the the adult human population is affected by hypolactasia, and long-term complications of lactose malabsorption, such as osteoporosis, represent an endemic disease. There are inconsistent studies regarding the hypothesis that lactase deficiency protects against parasite infections such as malaria.[141,142] The strategy to reduce the lactose content in milk includes benefits even for individuals without lactase deficiency, taking into account that the potentially harmful effects of lactose consumption, such as arteriosclerosis and ovarian cancer, can be overcome. A high incidence of galactose-induced senile (cortical) cataracts has been found in lactose absorbers.[143,144] Ischemic heart disease and ovarian cancer have also been attributed to the consumption of high amounts of lactose (galactose).[145–147] In contrast, there is some evidence that lactose may be beneficial against lower intestinal diseases such as Crohn disease because of its prebiotic potential.[148] Prebiotics are essential for the establishment of intestinal colonization of newborns. On the other hand, *Bifidobacterium bifidum*, as well as conventional colonization of the germ-free intestine, induces biochemical maturation of enterocytes in gnotobiotic mice, promoting the postnatal decline of lactase activity.[149]

Congenital Lactase Deficiency (Alactasia)

Congenital lactase deficiency is a rare disorder inherited by an autosomal recessive mode. Since the first report in 1959,[150] few cases of this disease have been described.[151,152] To date, the molecular basis of this deficiency is still obscure. However, post-translational mechanisms are likely to be implicated in the lactase activity in these cases, as has been described for CSID (see above).

The typical symptoms of congenital lactase deficiency start from a few days after birth with the onset of breastfeeding (or lactose-containing formula feeding). They consist of liquid and acid diarrhea, meteorism, and severe malnutrition. The patients are hungry; vomiting is rarely noted. Some patients present with hypercalcemia and nephrocalcinosis, which may be provoked by the metabolic acidosis and/or the calcium absorption–increasing effect of lactose.[153]

When congenital lactase deficiency is suspected, a lactose-free diet should be started. In the case of a positive effect, this diagnosis is further confirmed. Finally, the diagnosis needs to be based on a deficient lactase activity in a duodenal or jejunal biopsy. Other disaccharidases, as well as the morphology of the intestinal mucosa, should be in the normal range.

Because of the severe symptoms, congenital lactase deficiency requires a strong restriction to a lactose-free diet (see above). In the further course of the disease, some patients may cope well with lactase supplementation.

MALTASE-GLUCOAMYLASE

Structure and Biosynthesis

Another brush border enzyme with digestive properties similar to those of isomaltase, MGA (EC 3.2.1. 20 and 3.2.1.3) contributes to the overall digestion of α-glycosidic linkages of carbohydrates. MGA compensates the lack of SI in a number of cases of CSID. MGA activity has been proposed to serve as an alternate pathway for starch digestion when luminal α-amylase activity is reduced because of immaturity or malnutrition, whereby MGA plays a unique role in the digestion of malted dietary oligosaccharides.[21] Similar to SI, MGA is a type II integral membrane glycoprotein of the intestine. Immunoprecipitation with specific monoclonal antibodies and electrophoretic analysis of the precipitates under denaturing conditions reveal a 335 kD single polypeptide in the presence or absence of reducing agents.[1] Examination of the quaternary structure of MGA by cross-linking analyses demonstrated a monomeric form of MGA in the brush border membrane. Biosynthetic studies in intestinal biopsy samples revealed two forms of MGA: the ER 285 kD mannose-rich polypeptide and the mature, complex, glycosylated 335 kD brush border protein. Unlike SI, mature MGA does not undergo extracellular cleavage in the brush border membrane by pancreatic secretions because the 335 kD polypeptide was the dominant form in the presence or absence of pancreatic secretions. In a fashion similar to SI, MGA is heavily *N*- and *O*-glycosylated, and it is likely that its trafficking to the brush border membrane is mediated by *O*-glycan units located in the stalk region—again, a structural similarity with SI.

The cDNA of human small intestinal MGA cDNA has recently been isolated from mRNA using reverse-transcriptase polymerase chain reaction.[154] The deduced amino acid sequence of 1,857 amino acids corresponds to an unglycosylated protein of an apparent molecular mass of 210 kD. The substantial difference between this size and the biosynthetic forms of MGA in biopsy samples is probably due to glycosylation and the electrophoretic behavior of the glycoforms on sodium dodecyl sulfate–polyacrylamide gel electrophoresis. It is also possible that splice variants of MGA exist. MGA and SI are strikingly homologous (59% homology) and, correspondingly, share several common structural features, such as the presence of an *O*-glycosylated stalk region immediately following the membrane-anchoring domain, a trefoil or P domain, and two identical catalytic sites.[154]

The recent cloning and sequencing of the human MGA gene could demonstrate its close evolutionary relationship to SI. The gene is located at chromosome 7q34 and consists of 58 exons.[21] Expression of the gene product results in a protein that hydrolyzes maltose and starch, but not sucrose, and is thus distinct from SI. Another similarity of both genes is their identical exon structures. These similarities strongly support the notion that MGA and SI have evolved by duplication of an ancestral gene, which itself had already undergone a gene duplication.

Molecular Basis of Deficiency

The hypothesis that villus atrophy accounts for the reduced maltase enzyme was examined in mucosal biopsy specimens.[155] It was observed that the drastic reduction in the maltase activity and the MGA mRNA levels of about 45% paralleled the reduction in the villin mRNA used as a marker for villus integrity. Likewise, the levels of the SI mRNA were also decreased in proportion to villus atrophy of malnourished infants. Congenital cases of MGA deficiency are rare, and one case has recently been characterized at the molecular level.[69] In this case, however, sucrase deficiency and lactase deficiency have also been detected. Isolation and sequencing of the MGA cDNA revealed homozygosity for a nucleotide change in the patient that resulted in a substitution of serine by leucine at amino acid residue 542 (S542L), very close to the catalytic site aspartic acid. Analysis of the function of this mutation by its introduction into the wild-type MGA cDNA did not reveal any alteration in the biosynthesis, processing, and enzymatic activity of mutant MGA. The promoter region of MGA in this patient was also analyzed, and no nucleotide changes could be observed. This case led to the conclusion that the reduced activities of MGA, SI, and LPH are caused by shared, pleiotropic regulatory factors.

Clinical Implications

In 1994, glucoamylase deficiency was reported in a large group of children suffering from chronic diarrhea; the morphology of their mucosa was normal.[156] The incidence was estimated to be 1.8% among children with chronic diarrhea. Although these patients have revealed normal pancreatic amylase activities, other disaccharidases, most notably isomaltase, have shown marked reduced activities in addition to glucoamylase. This could be due to the known overlap of isomaltase and glucoamylase activities toward maltose as a substrate.

Primary MGA deficiency should be suspected in the differential diagnosis of chronic diarrhea of infants and toddlers. Besides diarrhea, patients present with abdominal dis-

tention and bloating. Symptoms start with the introduction of starch and formula containing short polymers of starch at 4 to 6 months of age or later until the fifth year of life.

Some of these MGA-deficient patients show a positive response to challenge with 2 to 4 g/kg starch and to re-elimination of starch. The starch digestive capacity can be evaluated by the $^{13}CO_2$ breath test using ^{13}C-starch oligomers as a loading substrate.[69,157] Reducing substances are inconsistently found to be positive. The diagnosis is established by low activity of glucoamylase of a duodenal biopsy and evidence of a normal pancreatic amylase. A high percentage of glucoamylase-deficient subjects have been found in a group of dyspeptic children with normal duodenal histology, some of them presenting additionally with low activity of sucrase and lactase.[158]

An elimination diet excluding starches and short polymers of glucose within lactose-free infant formulas should be given for 3 to 4 weeks, documenting any relief of symptoms. Patients who do not show symptoms should stick to this diet to an extent, which keeps them free of symptoms.

TREHALASE

Structure and Function of Trehalase

Trehalase is another member of α-glucosidases of the small intestine.[159] It is also present in the human serum.[160] Because plasma trehalase activity is increased in diabetes[161] and reduced in rheumatoid arthritis,[162] it is not used to diagnose trehalase deficiency. Its substrate, trehalose, is a disaccharide composed of two glucose molecules. It is contained in mushrooms, lower plants (algae), insects, *Ascaris lumbricoides*, and *Artemia salina*. Because trehalose improves the quality of dried food, it became a food additive.

A complete cDNA clone encoding human trehalase has been isolated from a human kidney library.[163] The protein is composed of 583 amino acids with a calculated molecular weight of 66,595 kD. The enzyme is a type I membrane glycoprotein that is synthesized with a typical cleavable signal peptide at amino terminus and contains five potential glycosylation sites. Trehalase is associated with the membrane via a glycosylphosphatidylinositol type of membrane anchor. The sequence similarity of the human enzyme with those of the rabbit, silkworm, *Tenebrio molitor*, *Escherichia coli*, and yeast suggested a common old ancestral gene. The small intestine trehalase is also expressed in the liver and the kidney.

Trehalase Deficiency

The first case of trehalase deficiency, which is inherited as an autosomal recessive disorder, was reported in 1971.[164] Two years later, it was described in a family.[165] An autosomal recessive inheritance has been suggested as a genetic background owing to trehalase deficiency. Other brush border enzymes are not affected. Trehalase deficiency is found in 8% of Greenlanders.[166,167] It is rare in white Americans.[43] Only a few cases have been described in other populations. The implications and significance of this observation on molecular pathophysiology of the intestine are unclear, however.

The major source of trehalose for humans is young mushrooms.[168] Symptoms resembling those of lactose intolerance consist mainly of vomiting, abdominal pain, meteorism, and diarrhea. Two trehalase-deficient patients developed no symptoms after trehalose load and proclaimed themselves mushroom tolerant.[168]

Because the increases in breath hydrogen and blood glucose did not differ between trehalose-intolerant and -tolerant subjects, oral tolerance and breath tests seem to be unsuitable for diagnostic investigation.[168] Trehalase deficiency requires the performance of a duodenal biopsy with determination of trehalase activity. The normal range (mean ± 2 SD) of trehalase activity is 4.79 to 37.12 U/g protein.[166] Trehalase deficiency is diagnosed when the activity of duodenal trehalase is less than 8 U/g protein.[167] The activities of other brush border enzymes, as well as the duodenal histology, are normal, ruling out the presence of a general mucosa damage as an underlying cause of trehalase deficiency. The few studies performed indicate that there is a poor correlation among patient complaints, results of oral tolerance and breath testing, and trehalase activities.

The only available therapy is trehalose restriction, which includes reducing the consumption of mushrooms and trehalose-containing food additives. At present, several applications of trehalose in human nutrition are considered by the food industry.[169]

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2. *Congenital Intestinal Transport Defects*

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Disturbances in intestinal nutrient and ion transport can lead to clinical symptoms that range from severe life-threatening diarrhea in a neonate to neurologic deficits in early childhood. With few exceptions, most epithelial transport disorders are both exceedingly rare and inherited in an autosomal recessive manner. Given their genetic basis, the diagnosis of a transport defect should be entertained particularly when there is a family history of consanguinity but should also be considered when similar problems have not occurred in other family members.

The most common clinical manifestation of this large group of epithelial transport disorders is chronic diarrhea.[1] Recognition of severe diarrhea during early infancy may occasionally be missed when stool is mistaken for urine; therefore, careful attention should be made to separate and measure urine and feces. Categorizing the type of diarrhea into either malabsorptive or secretory is a useful first step in the evaluation of any child with chronic diarrhea. The type of diarrhea may be assessed by performing either a trial of fasting or by analysis of stool electrolytes while the child is on a full diet.[1] To calculate the stool osmotic gap, either cations such as Na^+ and K^+ or anions such as Cl^- and HCO_3^- may be measured in liquid fecal samples.[2,3] If the sum of twice their value (either cations or anions) is significantly lower than 290 mOsm, the predicted osmolarity, an osmotic gap is present, and the patient is malabsorbing dietary nutrients. In contrast, a low osmotic gap (< 50 mOsm) is seen in secretory diarrheas, which are generally large volume (1–2 L) and fail to abate in the fasted state.

Nutrient-specific malabsorption is an important characteristic of all inherited transport disorders and is generally not a feature of some of the more common chronic diarrheal disorders. However, defects of pancreatic and brush border enzyme function share many of the clinical characteristics seen in transport defects and will also lead to malabsorption of specific nutrients. In contrast, generalized malabsorption (ie, not nutrient specific) occurs in the clinical setting, where the intestinal absorptive capacity is insufficient to appropriately handle a normal dietary load.[4] Nonspecific abnormalities of intestinal absorption most often occur when either small bowel length is inadequate or intestinal villus or epithelial microvilli are abnormal. For instance, enteropathies such as hypersensitivity to cow and soybean proteins, and even more unusual disorders, such as autoimmune enteropathy, microvillus inclusion disease (Mendelian Inheritance in Man [MIM] #251850), and immunodysregulation-polyendocrinopathy-enteropathy (MIM #304790). generally present in early infancy with features of nonspecific malabsorption.[5,6] In fact, the majority of patients with an abnormal intestinal histology have a mixed form of diarrhea that has both osmotic and secretory components. Therefore, the identification of normal small bowel histology is an essential part of the evaluation of children with congenital transport defects.

Secretory diarrhea is due to an imbalance between absorption and secretion of electrolytes. During early infancy, chronic diarrhea that is purely secretory in nature is due almost exclusively to a small number of transport disorders and, as such, is not secondary to defects of enzymatic function, as is frequently seen in malabsorptive diarrhea. Several hormone-secreting tumors may present with severe secretory diarrhea and need to be distinguished from two disorders of intestinal transport, namely congenital chloride diarrhea (MIM #214700) and congenital sodium diarrhea (CSD) (MIM #270420; Table 43.2-1).[7] Table 43.2-1 is a summary of the molecular basis of all disorders of nutrient assimilation. For each disorder, the table contains its MIM number, inheritance pattern, and chromosomal localization; the name, number, and function of the defective protein; and the primary gastrointestinal symptom.

DISORDERS OF CARBOHYDRATE ABSORPTION

Complex sugars are digested by salivary and pancreatic amylases and further by brush border enzymes into monosaccharides that are absorbed in the small intestine (Figure 43.2-1). Enterocytes, which are renewed every 6 days, express the enzymes and transporters needed for carbohydrate digestion and absorption. The enterocytes' apical brush border membrane contains the sucrase-isomaltase and lactase-phlorhizin enzymes, the Na^+-dependent glucose-galactose cotransporter sodium-coupled glucose transporter (SGLT)1, and the fructose uniporter (GLUT5). The basolateral membrane contains the glucose-galactose-fructose uniporter (GLUT2) and the Na^+, K^+–adenosine triphosphatase (ATPase) pump. Fructose is passively transported across the intestine by the brush border fructose uniporter (GLUT5) and by the basolateral GLUT2 uniporter. The pump maintains the low intracellular Na^+ concentration by extruding Na^+ into the blood in exchange for K^+. The net result is that glucose stimulates Na^+ absorption across the intestine.

Disturbances of carbohydrate assimilation may be secondary to defective enzymatic processing of complex sugars and disaccharides or failure to transport monosaccharides across the apical membrane (see Table 43.2-1 and Figure

TABLE 43.2-1 MOLECULAR BASIS OF DISORDERS OF NUTRIENT ASSIMILATION

DISORDER	MIM NO.	IP	CHR	GENE/PROTEIN (HUGO NO.)	FUNCTION	CLINICAL SYMPTOMS
Carbohydrate digestion						
Congenital lactase deficiency	223000	AR	2q21	Lactase-phlorhizin hydrolase	Hydrolyzes lactose	Lactose-induced diarrhea
Hypolactasia	223100	AR	2q21	Lactase-phlorhizin hydrolase	Hydrolyzes lactose	Lactose-induced diarrhea
Congenital sucrase-isomaltase deficiency	222900	AR	3q25	Sucrase-isomaltase	Hydrolyzes sucrose	Sucrose-induced diarrhea
Glucoamylase deficiency	~	AR	7q34	Maltase-glucoamylase	Hydrolyzes starch	Starch-induced diarrhea
Carbohydrate absorption						
Glucose galactose malabsorption	182380	AR	22q13	Na^+–glucose-galactose cotransport (SGLT1); SLC5A1	Apical glucose-galactose transporter	Glucose-induced diarrhea
Fructose malabsorption	~	AR	1p36	Facilitative fructose transport (GLUT5); SLC2A5	Apical fructose transporter	Fructose-induced diarrhea
Fanconi-Bickel syndrome	227810	AR	3q26	Facilitative glucose transport (GLUT2); SLC2A2	Basolateral glucose transporter	Diarrhea and nephropathy
Protein digestion						
Enterokinase deficiency	226200	AR	21q21	Serine protease 7	Proenterokinase—hydrolyzes trypsinogen	Protein-induced diarrhea; edema
Trypsinogen deficiency	276000	AR	7q35	Trypsinogen	Hydrolyzes endo- and exopeptidases	Protein-induced diarrhea; edema
Amino acid absorption						
Cystinuria type I	220100	AR	2p16	rBAT; SLC3A1	Heavy-chain cysteine, dibasic and neutral AA apical trans.	Nephrolithiasis
Cystinuria type II/III	600918	AR	19q13.1	$b^{(0,+)}$AT; SLC7A9	Light-chain cysteine, dibasic and neutral apical AA transport	Nephrolithiasis
Lysinuric protein intolerance	222700	AR	14q11	y^+ L-type AA transporter 1; SLC7A7	$y^{(+)}$L-type AA basolateral transport	Vomiting, diarrhea, neurologic delay
Hartnup disorder	234500	AR	5p21	?	Neutral AA transport	Dermatitis, neurologic delay
Iminoglycinuria	242600	AR	~	?	Imino AA transport	Neurologic delay
Fat digestion						
Pancreatic lipase deficiency	246600	AR	10q26	Pancreatic lipase	Hydrolyzes dietary triglycerides to fatty acids	Steatorrhea
Fat assimilation						
Abetalipoproteinemia	200100	AR	4q22	Microsomal triglyceride transfer protein	Transfers lipids to apolipoprotein B	Steatorrhea
Hypobetalipoproteinemia	107730	AD/AR	2p24	Apolipoprotein B	Apolipoprotein that forms chylomicrons	Steatorrhea
Chylomicron retention disease	246700	AR	5q31	Sar1-ADP-ribosylation factor family GTPases	Targets intracellular protein-coated vesicles	Steatorrhea
Primary bile acid malabsorption	601295	AR	13q33	Sodium–bile acid transporter; SLC10A2	Ileal Na^+–bile acid transporter	Steatorrhea/bile acid diarrhea
Tangier disease	205400	AR	9q	ATP–binding cassette transporter 1	Controls intracellular cholesterol transport	Liver, spleen, lymph node enlargement
Sitosterolemia	21250	AR	2q21	ATP binding cassette, subfamily G, member 8; ABCG8	Dietary sterol absorption	Atherosclerosis

(continued)

TABLE 43.2-1 CONTINUED

DISORDER	MIM NO.	IP	CHR	GENE/PROTEIN (HUGO NO.)	FUNCTION	CLINICAL SYMPTOMS
Ion and metal absorption						
Congenital sodium diarrhea	270420	AR	~	?	?	Sodium-secreting diarrhea
Congenital chloride diarrhea	214700	AR	7q22	Solute carrier family 26; SLC26A3	Na^+-independent Cl^-/HCO_3^- exchanger	Chloride-secreting diarrhea
Cystic fibrosis	219700	AR	7q31.2	Cystic fibrosis transmembrane regulator	cAMP-dependent chloride channel	Pancreatic insufficiency; ileus
Acrodermatitis enteropathica	201100	AR	8q24.3	Zinc-/iron-regulated transporter 4; SLC39A4	Zn^+ transporter	Diarrhea and dermatitis
Menkes disease	309400	XR	Xq12	Cu^{++} ATPase, α-polypeptide; ATP7A	Cu^{++} transporter	Neurologic delay
Primary hypomagnesemia	248250	AR/X	3q27	Paracellin 1; claudin 16	Mg^+ transporter/sensor	Seizures, deafness, and polyuria
Classic hemochromatosis	235200	AR	6p21.3	Hemochromatosis gene	Modulates endocytosis of the TfR-Fe^{++}	Cirrhosis, cardiomyopathy, diabetes
Juvenile hemochromatosis	602390	AR	19q13	Hepcidin	Hepatic antimicrobial peptide	Cirrhosis, cardiomyopathy, diabetes
Hemochromatosis type III	604250	AR	7q22	TfR2	Receptor for transferrin	Cirrhosis, cardiomyopathy, diabetes
Hemochromatosis type IV	606069	AD	2q32	Ferroportin; SLC11A3	Basolateral Fe^{++} transporter	Cirrhosis, cardiomyopathy, diabetes
Vitamin absorption						
Folate malabsorption	229050	AR		~	~	Anemia; diarrhea; neurologic delay
Congenital pernicious anemia	261000	AR	11q13	Intrinsic factor	Required for binding of cobalamin to cubilin	Anemia; neurologic delay
Imerslund-Graesbeck syndrome (Finland)	261100	AR	10p12.1	Cubilin	IF-cobalamin receptor	Anemia; proteinuria
Imerslund-Graesbeck syndrome (Norway)	261100	AR	14q32	Amnionless	Targets the IF-cobalamin and cubilin to endosomes	Anemia; proteinuria
Congenital deficit of transcobalamin II	275350	AR	22q11.2	Transcobalamin II	Primary transport protein for cobalamin in serum	Anemia; diarrhea; neurologic delay
Thiamine-responsive megaloblastic anemia	249270	AR	1q23.3	Thiamine transporter protein; SLC19A2	Thiamine transporter	Anemia; diabetes; cranial nerve defects
Familial retinol-binding protein deficiency	180250	AR	10q24	Retinol-binding protein 4	Binding protein for retinol transfer	Ophthalmologic problems
Selective vitamin E deficiency	277460	AR	8q13.1	α-Tocopherol transfer protein	Binding protein that adds vitamin E to VLDL	Vitamin E malabsorption

AA = amino acid; AD = autosomal dominant; ADP = adenosine diphosphate; AR = autosomal recessive; ATP = adenosine triphosphate; ATPase = adenosine triphosphatase; cAMP = cyclic adenosine monophosphate; CHR = chromosomal localization; GTPase = guanosine triphosphatase; HUGO = Human Genome Organization; IF = intrinsic factor; IP = inheritance pattern; MIM = Mendelian Inheritance in Man; VLDL = very-low-density lipoprotein; X = link recessive.

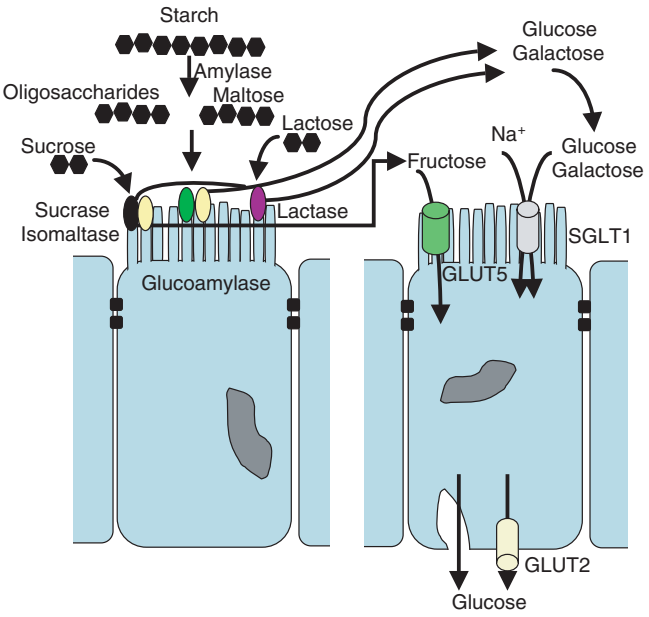

FIGURE 43.2-1 Carbohydrate assimilation. GLUT = glucose transporter; SGLT = sodium-coupled glucose transporter.

43.2-1). Abnormalities of carbohydrate assimilation may be categorized into three general forms: ontogenic forms that result from the normal immaturity of the digestive functions during the early stages of life, genetic forms that are congenital conditions, and an acquired form that is preceded by a period of normal function. The age at onset of clinical symptoms is an important characteristic to consider when evaluating the basis of carbohydrate-induced diarrhea (Figure 43.2-2).

Defective synthesis of lactase-phlorhizin hydrolase (LPH) may lead to chronic diarrhea, resulting from either primary lactase deficiency (MIM #223000) or hypolactasia (MIM #223100) (see Table 43.2-1).[8,9] Primary lactase deficiency is an extraordinary rare disorder that presents during the immediate neonatal period with diarrhea, whereas hypolactasia occurs after infancy in many individuals of African, Asian, and Hispanic origin. The presence of selective diarrhea in children on a sucrose-based diet may be secondary to abnormal synthesis of sucrase-isomaltase (MIM #222900).[10] Diarrhea induced by large complex carbohydrates is usually an indication of abnormal exocrine pancreatic function, such as is seen with cystic fibrosis, but may also be attributed to a deficiency of glucoamylase synthesis.[11,12] Distinguishing between these various disorders can be accomplished by analyzing small bowel disaccharidase activity and with carbohydrate-specific breath hydrogen testing.[13]

Carbohydrate intolerance is characterized by diarrhea that improves within hours when carbohydrates are reduced or eliminated from the diet. Therefore, abnormal carbohydrate assimilation results in the presence of major osmotic forces in colonic luminal fluid derived from oligosaccharides, lactose, sucrose, or glucose. Unabsorbed carbohydrates are fermented by the resident colonic microflora to gas (hydrogen, methane, and carbon dioxide) and volatile short-chain fatty acids (SCFAs) such as acetate, butyrate, and propionate. These SCFAs are absorbed by the colonic epithelium by poorly defined diffusion and transporter-mediated mechanisms.[14] The absorbed SCFAs are metabolized preferentially by colonic epithelia, where they are an important energy source and also facilitate the absorption of NaCl. Therefore, SCFA absorption leads to a reduction of luminal osmolarity and thereby facilitates the colonic absorption of water. Nevertheless, inadequate colonic salvage results in diarrhea when the amount of ileal effluent is too large for the normal colon and its flora to manage. The passage of unabsorbed and unfermented carbohydrates into the distal colon provides an additional osmotic load that results in the retention of water and subsequent diarrhea.

Glucose Galactose Malabsorption

Glucose galactose malabsorption (GGM) is a rare disorder that causes severe life-threatening diarrhea and dehydration during the neonatal period (MIM #182380) (see Table 43.2-1). GGM was first established when investigators demonstrated evidence of an inherited disorder of active glucose and galactose transport.[15–17] Conformation was provided by in vitro studies carried out on duodenal biopsies of patients with GGM.[18–21] In these studies, autoradiographic techniques demonstrated that epithelial cells from the patient were unable to accumulate galactose owing to a reduction in the number of transporters binding phlorhizin in the brush border membrane (Figure 43.2-3).

Genetics. The relative abundance of GGM in consanguineous unions and the absence of vertical transmission suggest an autosomal recessive pattern of inheritance. Although obligate heterozygotes may have an impaired capacity to absorb glucose, clinical evidence of malabsorption and its clinical symptoms have not been documented.

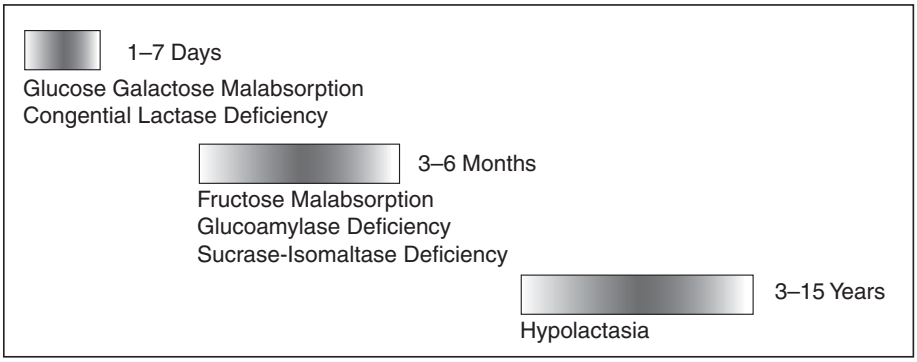

FIGURE 43.2-2 Carbohydrate assimilation: age at onset.

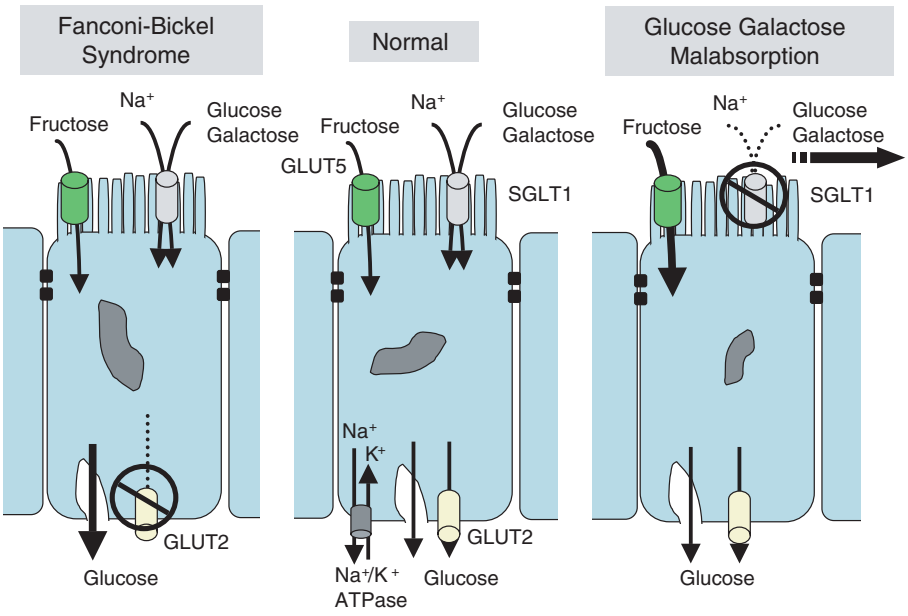

FIGURE 43.2-3 Carbohydrate absorption across the enterocyte in normal control, glucose galactose malabsorption, and Fanconi-Bickel syndrome. ATPase = adenosine triphosphatase; GLUT = glucose transporter; SGLT = sodium-coupled glucose transporter.

Although the disorder is rare, we are aware of some 300 patients, and it is most frequent in populations that have a high incidence of consanguinity. Recent genetic testing was performed on 82 patients in 74 unrelated families from the United States (of north European, African, Hispanic, and Mediterranean descent), France, the Netherlands, Japan, the United Kingdom, Belgium, and Sweden.[22–25] Analysis of these probands led to the identification of 46 mutations that account for GGM, and all but one patient were determined to have mutations. Identical mutations were identified on both alleles in 65% of our patients, and the remainder were compound heterozygotes.

No mutations were identified in only three GGM cases. Virtually every patient had a unique mutation; only one mutation, C355S, has been found in three unrelated patients (Figure 43.2-4). The majority of the mutations are missense,[22] but we have also identified six nonsense, seven frame shift, and seven splice site mutations. The nonsense, frame shift, and splice site mutations all produce truncated proteins, which are expected to be nonfunctional. The truncated proteins are nonfunctional based on assays using the oocyte expression system.

The missense mutations are distributed throughout the protein (see Figure 43.2-4). Two methods have been used to distinguish between mutations that produce GGM and those that are benign polymorphisms. The first was to express the wild-type and mutant proteins in oocytes and to measure sugar transport activity and the second was to see if the mutated reside was conserved in other mammalian members of the *SGLT* gene family. It should be noted that all mutated residues causing defects in sugar transport in oocytes are conserved within the 18 closely related genes, including eight SGLT1s from different species. However, the two missense mutations that do not impair sugar transport, F405S and H615Q, are not conserved and rarely occur in the general population. In con-

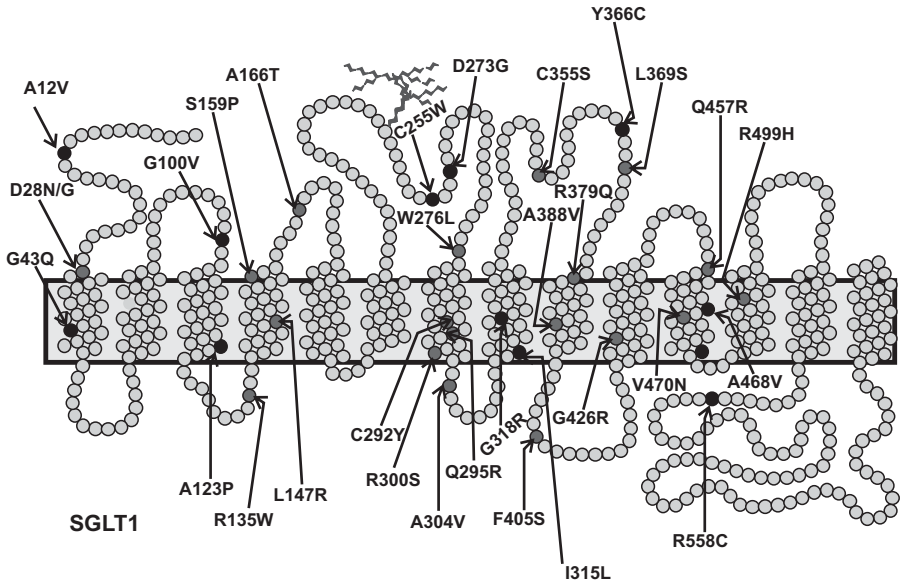

FIGURE 43.2-4 Location of missense mutations in sodium-coupled glucose transporter (SGLT)1 and patients with glucose galactose malabsorption.

trast, the N51S variant occurs in the general population at a frequency of 4% in over 552 alleles analyzed. On this basis, we also suggest that A12V is a benign polymorphism, whereas the other eight untested mutants are mutations that may be responsible for the impaired sugar transport in these GGM patients.

Molecular Pathophysiology. The primary carbohydrate present in breast milk is lactose (50–90 g/L) that is hydrolyzed by lactase on the external surface of the intestinal brush border. The liberated glucose and galactose are then transported across the brush border membrane by SGLT1 and accumulated within the enterocyte (see Figure 43.2-3). Lactose is the primary source of dietary galactose, whereas complex carbohydrates provide the major source of glucose in older children. Until recently, the facilitated carrier GLUT2 was believed to be responsible for transport of glucose and galactose across the basolateral membrane; however, this is now doubtful in light of studies on humans and mice with defective GLUT2.[26,27]

SGLT1 is responsible for the tight coupling of two Na^+ ions and one sugar molecule across the membrane during one catalytic turnover. The rate of Na^+-sugar cotransport depends on the magnitude of the glucose and galactose concentrations. The basolateral Na^+-K^+ pump maintains the low intracellular sodium concentration by pumping sodium out of the cell, and this results in the net transport of sodium (chloride or bicarbonate), sugar, and water across the epithelium.[28] Because the osmolarity of stool in both secretory and malabsorptive diarrhea is always isomolar with serum (290 mOsm), the addition of undigested solutes (glucose and galactose) and SCFAs that are present in the colon of a GGM patient on a carbohydrate meal will result in an excessive amount of fluid to maintain the required fecal osmolarity.

Although the upper and mid–small intestine has a large capacity to absorb salt and water, ~ 6 L/d, little occurs in the absence of dietary carbohydrate and protein. Glucose stimulates salt and water absorption, and this is generally explained by the coupled transport of Na^+ and glucose across the brush border and the subsequent pumping of Na^+ out of the enterocyte across the basolateral membrane by the Na^+-K^+ pump. It is then thought that water follows solute absorption by a process referred to as local osmosis. However, it has recently been postulated that there is a more direct link between sodium, sugar, and water transport across the brush border membrane, that is, water cotransport, but this hypothesis is not widely accepted.[29] Nevertheless, the link between salt, sugar, and water absorption provides the rationale for oral rehydration therapy. In principle, other sodium-coupled transporters, for example, those for amino acids, also may be used in oral rehydration therapy.

Clinical Manifestations. The majority of patients with GGM present clinically with severe life-threatening diarrhea during the neonatal period (see Figure 43.2-2). However, on rare occasions, adult patients have been identified with milder symptoms.[30] Diarrhea is most frequently detected during the first 2 days of life, while the patient is still hospitalized, and this usually results in a severe metabolic acidosis and hyperosmolar dehydration. The diarrhea in GGM results from the presence of unabsorbed carbohydrate in the gut. If not properly diagnosed in a timely manner and if dietary management is not implemented, GGM is frequently fatal.

Isolated carbohydrate malabsorption is distinctly unusual during the first several days of life, and its presence should initiate the workup for both GGM and primary lactase deficiency. The diarrhea seen in GGM patients will persist while they remain on standard formulas, including protein-hydrolysate formula, because they generally contain glucose.

Diagnostic Criteria or Laboratory Investigations. Three key features are necessary to establish the diagnosis of GGM: elimination of glucose and galactose (and lactose) from the diet results in the complete resolution of diarrheal symptoms, a positive glucose breath hydrogen test, and a normal intestinal biopsy. Specifically, the small bowel architecture should have a normal villus-to-crypt ratio and an absence of abundant inflammatory cells in the lamina propria and intraepithelial compartments.

When considering the diagnosis of GGM, several general and specific diagnostic tests may be performed. The hospitalized patients should have standard stool analysis, including pH, occult blood, and leukocyte analysis and evaluation for the presence of reducing and nonreducing sugars (Clinitest). Carbohydrate malabsorption and subsequent colonic bacterial fermentation result in the synthesis of high concentrations of SCFAs (acetate, butyrate, propionate), which leads to the acidification of stool ($pH < 5.3$).[2] Stool electrolytes (Na^+, K^+, and Cl^-) should be obtained, and a large osmotic gap of greater than 40 mOsm when compared with serum osmolarity would suggest carbohydrate malabsorption. In contrast, a low osmotic gap (< 50 mOsm) would be more consistent with secretory diarrhea. After sufficient stool samples have been obtained, and the quantity and characteristics of the diarrheal pattern have been established, a fasting trial should be performed. Diarrhea will abate during the fasting state if it is osmotic in nature. The stools of a patient with GGM should be acidic, with detectable levels of reducing substance and an electrolyte analysis consistent with osmotic diarrhea while the patient is on a glucose-based diet.

Small bowel biopsies should be performed early in the evaluation of a patient with chronic diarrhea. Analysis of the mucosa provides several important clues that would assist in establishing the diagnosis. Mucosal defects such as flattened villi and a low villus-to-crypt ratio are inconsistent with the diagnosis of GGM or any other disorder resulting from a primary transport defect. Therefore, GGM patients should have normal-appearing microvilli on electron microscopy, with normal villus architecture on light microscopy.

Mucosal biopsies can also be used to assess lactase and sucrase activity, which may be helpful in distinguishing GGM from primary lactase or sucrase deficiency. Glucose and galactose uptake studies of mucosal samples are very useful but are difficult to perform and are generally not available. Biopsy samples may also be useful in establishing GGM by immunohistochemical analysis using antisera

to SGLT1. As we reported earlier, most SGLT1 mutations result in either targeting defects to the plasma membrane or incomplete synthesis in the form of a nonsense mutation.[22] Immunohistochemical analysis of mucosa has shown an absence of SGLT1 on the apical membrane of individuals with mutations that result in improper targeting to the plasma membrane. Unfortunately, commercial antibodies to SGLT1 are generally unreliable.

The glucose breath hydrogen test is another useful test that should be performed in a patient in whom the diagnosis of GGM is being considered. Carbohydrate breath hydrogen testing is an easily performed test that may assist in the diagnosis of several types of carbohydrate malabsorption disorders, including lactose, sucrose, and fructose malabsorption. The test relies on the colonic fermentation of unabsorbed carbohydrate by bacteria that produce methane, hydrogen, and carbon dioxide gas. This gas chromatographic analysis quantifies the level of hydrogen gas in exhaled air following the administration of either glucose (or galactose) at 2 g/kg body weight or a maximum of 50 g for patients who weigh more than 25 kg. Although an elevation of more than 20 ppm above the fasting baseline is consistent with the diagnosis of glucose malabsorption, most patients with GGM have levels that are frequently higher than 100 ppm (unpublished observations of authors, 2004). Therefore, it is reasonable and probably safer to consider using a much lower glucose load (< 0.5 g/kg) when performing a breath hydrogen test in a patient who is believed to have GGM.

The recent use of antibiotics diminishes the usefulness of carbohydrate breath hydrogen tests over the short term, and lactulose, an obligate nonabsorbable carbohydrate, may be used to assess its sensitivity. More specifically, the inability to mount a significant elevation in hydrogen or methane gas after a lactulose challenge would suggest the absence of a significant amount of gas-producing colonic bacteria. The development of abdominal pain and diarrhea following a carbohydrate challenge should raise the suspicion of a false-negative breath hydrogen test. The specificity of an abnormal glucose breath hydrogen test may be confirmed by performing a fructose breath hydrogen test that should be normal in GGM patients because fructose is absorbed via the unaffected uniporter, GLUT5. However, although slight fructose malabsorption is particularly common in younger children, breath hydrogen levels in a GGM patient will be substantially higher with glucose compared with fructose administration.[31] Interestingly, 10% of healthy adults are glucose malabsorbers, as judged by breath hydrogen tests.[32]

Genetic testing for GGM is currently not justified unless the patient is related to a GGM patient with known mutations in *SGLT1*. Testing involves the screening of SGLT1's 15 exons for mutations by single-stranded conformational polymorphism and the subsequent sequencing of genomic deoxyribonucleic acid (DNA). Identified missense mutations are studied using a heterologous glucose uptake system in *Xenopus* oocytes. This conformational test has been essential to distinguish disease-causing mutations from inconsequential polymorphisms. The abundance of private mutations has made molecular testing costly, cumbersome, and rather slow. Therefore, although genetic testing can be used as a final verification for the diagnosis of GGM, it is clearly not necessary to establish the diagnosis. Genetic testing has been used successfully to perform prenatal diagnosis on a family at risk for GGM.[33]

Treatment. With GGM, as with other disorders of carbohydrate malabsorption, symptoms resolve on an elimination diet free of glucose and galactose. Several formulas are available for managing GGM patients during early infancy. Ross Carbohydrate Free Formula (Ross Products, Columbus, OH) is frequently used in North America and may be supplemented with fructose (4 g fructose per 30 mL of concentrated formula and diluted further with water to a final volume of 60 mL). In Europe, Galactomin 19 (Nutricia, Zoetermeer, The Netherlands) formula is generally used and is supplied with fructose. Care should be exercised with formulas containing fructose because fructose absorption is limited in young children. Although these formulas are necessary only for the first several years of life, they are frequently used as nutritional supplements well into late childhood.

The dietary management of GGM patients becomes particularly challenging as children enter into late infancy and begin to exert more independence. Guidance from a well-trained dietitian is frequently invaluable. Highly motivated parents will generally benefit from dietary manuals that list the sugar content of selected foods. Reviewing with parents such a dietary list will help identify particular foods that have a relatively low glucose level. Galactose in its monosaccharide form is rarely present in sufficient amounts in foods and is primarily found in lactose-containing products such as dairy products. Nevertheless, the required diet of a GGM patient is very restricted and inevitably high in fat and protein. The required lifelong glucose- and galactose-free diet may, in the long term, have significant renal and cardiovascular consequences; however, none have been reported in GGM patients up to 40 years old.

Fructose Malabsorption

Fructose malabsorption has been implicated in both toddler's diarrhea and in a rare and poorly defined autosomal recessive disorder named isolated fructose malabsorption (IFM). Distinguishing between the more common toddler's diarrhea and IFM is particularly difficult, and it is unclear if they represent distinct disorders (see Figure 43.2-2). The clinical and genetic features of IFM are not as well defined as its other counterpart carbohydrate transport disorder, GGM. Although the ability of GLUT5 to transport fructose has been demonstrated, there remains insufficient information confirming that GLUT5 represents the main fructose transporter of the intestine; nevertheless, GLUT5 remains the best candidate gene for IFM (see Figure 43.2-1).[34]

Genetics. Eight patients with clinical evidence suggestive of IFM were previously evaluated for mutations in the *GLUT5* gene.[35] The analysis included the evaluation of the originally described kindreds with IFM. Analysis of both the coding region and intron and exon boundaries of the *GLUT5* gene failed to identify a deleterious mutation that could account for defective transport. Although the study

failed to evaluate the upstream regulatory region of the gene for mutations, defects in the promoter region of genes account for only less than 5% of the disease causing mutations in other disorders.[36] Because linkage analysis examining the inheritance pattern of this specific locus has not been described, the molecular basis of IFM remains undefined.

Molecular Pathophysiology. Current data suggest that fructose absorption in the small intestine occurs via GLUT5.[37] Fructose absorption is independent of glucose absorption and remains intact in patients with GGM. Studies performed primarily in rodents have demonstrated that GLUT5 expression is regulated in a tissue- and development-specific manner and is induced by dietary fructose.[37] Specifically, *GLUT5* messenger ribonucleic acid and protein levels are found at very low levels prior to weaning and increase dramatically in the proximal small intestine in weaned animals. The molecular mechanism that controls the expression of GLUT5 has not been established to date. An analysis of the age dependency of GLUT5 levels in human intestine has not been performed; however, fructose malabsorption as determined by the breath hydrogen test is certainly more common during early infancy.[31] Moreover, whether or not the fructose malabsorption that is present in toddler's diarrhea is secondary to inadequate GLUT5 expression has yet to be formally established.

Clinical Manifestations. Fructose is the primary monosaccharide found in fruits and fruit juices. Fruit juices that contain a high proportion of fructose to glucose or an excessive amount of the nonabsorbable carbohydrate sorbitol have been associated with infant diarrhea and abdominal pain.[38–40] Malabsorption of fruit juices is dose dependent, with diarrhea developing when the daily consumption of juice exceeds 15 mL/kg body weight, and may be well tolerated at a dose of 10 mL/kg^{-1}.[39] Whether children with toddler's diarrhea have a reduced capacity to absorb fructose compared with other similarly aged children has yet to be defined. Intolerance to dietary fructose has also been identified in infants with colic and adults with irritable bowel syndrome.[41,42]

It should be noted that IFM is a disorder that is distinct from hereditary fructose intolerance (MIM #229600), which results from a deficiency of aldolase B and the subsequent accumulation of fructose-1-phosphate in the cytoplasm.

Diagnostic Criteria or Laboratory Investigations. Fructose malabsorption can be assessed by either placing the patient on a fructose elimination diet or by performing a fructose breath hydrogen test. Fructose given at 2 g/kg body weight or a maximum of 50 g can be used to perform these studies.[13] To determine whether the malabsorption is fructose specific, intestinal biopsies should reveal normal intestinal histology, and malabsorption should be limited to fructose and not other monosaccharides. Neither in vitro intestinal uptake studies nor genetic testing is currently available.

Treatment. Children experiencing significant fructose-induced diarrhea should either reduce or eliminate their dietary fructose load to resolve symptoms. It has been established in rodent models that GLUT5 levels are dependent on the chronic load of dietary fructose; therefore, it is conceivable that fructose malabsorption may resolve by initiating a feeding regimen of incrementally increasing amounts of fructose. Because fructose malabsorption is generally limited to early infancy, attempts to reintroduce fructose should be considered as patients enter into the school-age years.

Fanconi-Bickel Syndrome

Fanconi-Bickel syndrome (FBS; MIM #227810) is a very rare autosomal recessive disorder that is characterized by carbohydrate malabsorption, tubular nephropathy, hepatomegaly and abnormal glycogen accumulation, failure to thrive, and fasting hypoglycemia (see Table 43.2-1).[43,44]

Genetics. In 1997, Santer and colleagues identified homozygote mutations in the facilitative glucose transporter GLUT2 in four patients with FBS.[45] Since this original description, over 60 patients with FBS have been investigated for mutations in the *GLUT2* gene, and over 30 mutations have been identified (see Figure 43.2-3).[27] Although most of the mutations are nonsense and result in a truncated transporter, several missense mutations were also identified. In vitro functional studies have not been performed to confirm that these mutations adversely alter the transport capabilities of the GLUT2 protein.

Molecular Pathophysiology. The mechanism by which mutation in the GLUT2 transporter alters the fasting- and feeding-induced changes in serum glucose is not well understood; however, impaired hepatocyte transport and glucose-sensing mechanism by pancreatic β cells are possible explanations.[44]

In the small intestine, GLUT2 is localized on the basolateral membrane and was believed for many years to be the primary method of monosaccharide exit from the enterocyte (see Figure 43.2-3). However, a severe osmotic carbohydrate diarrhea is generally not seen in most FBS patients, and mice containing a targeted deletion of GLUT2 also retain monosaccharide transport across the basolateral membrane. It has been suggested that a GLUT2-independent pathway for glucose transport exists in enterocytes.[26,46]

Clinical Manifestations. The diagnosis of FBS should be considered in patients with hepatomegaly and evidence of a renal tubulopathy. Although carbohydrate malabsorption has been reported in some but not all cases of FBS, it is certainly not a dominant feature in most reported cases. Failure to thrive, long-term short stature, rickets, and osteoporosis are clinical characteristics that are found in most patients with FBS.[44]

Diagnostic Criteria or Laboratory Investigations. Urine analysis will demonstrate evidence of glycosuria, a generalized aminoaciduria, and excessive urinary losses of phosphate and calcium. Histologic analysis of the small bowel, liver, and kidney generally reveal excessive glyco-

gen stores. Abdominal ultrasonography is useful to confirm the relative enlargement of the liver and kidney.

Treatment. Management is primarily focused on supplementing urinary electrolyte losses. Uncooked cornstarch has been used to prevent hypoglycemia and to minimize postprandial hyperglycemia.[47]

DISORDERS OF AMINO ACID AND PEPTIDE ABSORPTION

Assimilation of dietary proteins requires initial hydrolysis by multiple gastric, pancreatic, and brush border proteases with subsequent active and passive transport of dipeptides and amino acid across the apical membrane of the enterocytes (Figure 43.2-5). Intracellular peptidases hydrolyze dipeptides to single amino acids, which efflux across the basolateral membrane through a second series of transporters. The 21 common amino acids add to the diversity of dietary proteins whose digestion requires a host of endopeptidases and exopeptidases with specific yet overlapping specificities. Passive and ion-mediated transepithelial absorption is mediated by a diverse group of recently characterized transporters with broad amino acid specificities.[48–50] The redundant function of these enzyme and transport systems minimizes the physiologic consequences of a deficiency in most components of this process.

Protein digestion is initiated in the stomach by a family of pepsin proteases that are secreted by chief cells in the inactive form as pepsinogen (see Figure 43.2-5). Pepsinogen becomes activated by the acidic content of the stomach, where it produces nonabsorbable peptides. Under most circumstances, however, gastric hydrolysis is not essential for protein digestion. Dietary proteins that reach the intestine are further hydrolyzed into smaller peptides by a family of endopeptidases (trypsin, chymotrypsin, and elastase) and exopeptidases (carboxypeptidase A and B) that are secreted in their inactive form by the exocrine pancreas. Enterokinase, a serine protease located on the brush border membrane of enterocytes in the duodenum, converts the proenzyme trypsinogen to trypsin. Trypsin subsequently cleaves the precursor form of other endopeptidases and exopeptidases into their active equivalent. The luminal oligopeptides undergo further hydrolysis by another large family of oligopeptidases (aminopeptidase and carboxypeptidase) located on the brush border membrane. These proteolytic enzymes have well-defined substrate specificity and hydrolyze the dietary protein into either dipeptides or single amino acids that are subsequently transported by a group of uniporters.

Disorders of protein digestion are rare but should be considered when evaluating a patient for a potential defect of amino acid transport. The most well-characterized disorder of specific protein malabsorption is a deficiency of enterokinase synthesis (MIM #226200; see Table 43.2-1).[51] Enterokinase is the key upstream enzyme required for activation of endo- and exopeptidases and processes trypsinogen to trypsin. Children with enterokinase deficiency present with diarrhea, failure to thrive, and hypoproteinemic edema while on a diet of intact dietary proteins, whereas symptoms improve on an amino acid–based formula. Primary trypsinogen deficiency (MIM #276000) has also been reported and results in clinical symptoms that resemble enterokinase deficiency.[52] Nearly 90% of children with cystic fibrosis (MIM #219700) have a disruption of exocrine pancreatic function that impedes the digestion of dietary protein, fat, and carbohydrates.[11,12] A similar type of generalized malabsorption occurs in the late stages of other disorders of chronic pancreatitis, including the autosomal dominant disorder hereditary pancreatitis (MIM #167800).[53]

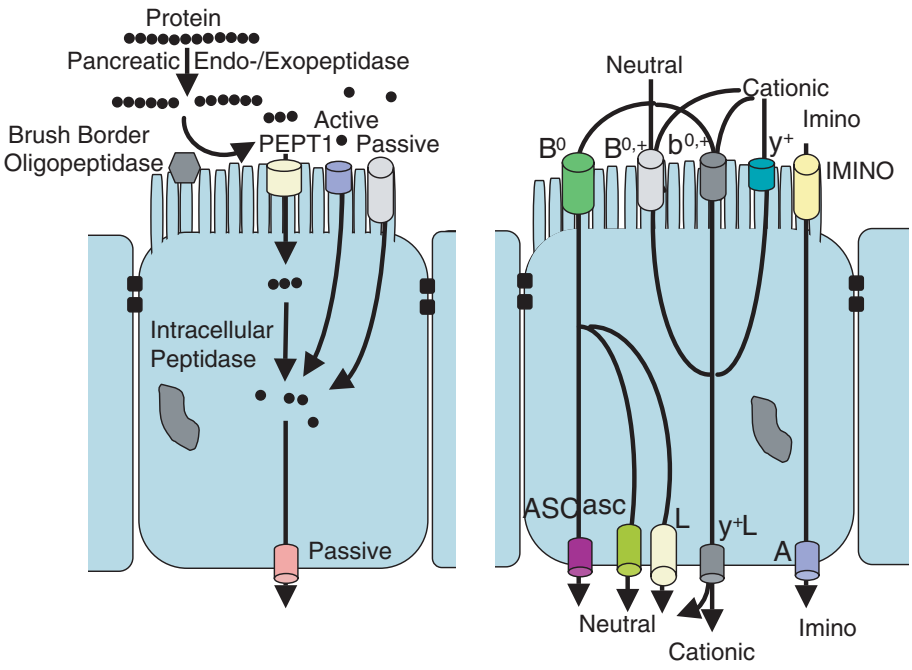

FIGURE 43.2-5 Protein and amino acid assimilation in the small intestine. Various amino acid transport systems are present on the apical (B^0, $B^{0,+}$, $b^{0,+}$, y^+, IMINO) and basolateral (ASC, asc, L, y^+L, A) Membrane. PEPTI = peptide cotransporter peptide transporter-1.

A large group of amino acid transporters have recently been cloned and characterized and are expressed either on the apical or basolateral membrane of the enterocyte (see Figure 43.2-5).[48,49] The brush border membrane contains the B^0 and $B^{0,+}$ Na^+-dependent systems that transport neutral amino acids. Anionic and cationic amino acids are selectively transported via system x_c^- and y^+, respectively. Proline and hydroxyproline are selectively transported by the imino system, which has yet to be isolated. In contrast, cationic and neutral (cystine) amino acids are transported by system $b^{0,+}$, which is defective in cystinuria (MIM #220100), an autosomal recessive aminoaciduria that is not associated with a significant gastrointestinal phenotype (Figure 43.2-6 and Table 43.2-1).[54] Although the $b^{0,+}$ system is expressed in the kidney and intestine, it has been postulated that dietary cysteine is absorbed in patients with cystinuria as a component of oligopeptides via the H^+-oligopeptide cotransporter peptide transporter-1.[55]

Intracellular neutral amino acids efflux across the basolateral membrane in a size-dependent manner; with system L carrying the larger and system ASC the smaller amino acids (see Figure 43.2-6). Small neutral amino acids also cross the basolateral membrane via a Na^+-dependent ASC system, whereas the A system is selective for both neutral and imino acids. In contrast, cationic amino acids are transported by the Na^+-dependent system y^+L, which is defective in lysinuric protein intolerance (LPI). Although the redundancy in the transport specificity of these various systems essentially minimizes gastrointestinal and nutritional consequences that are observed when an individual transport system is disrupted, LPI patients do experience significant gastrointestinal symptoms.

Lysinuric Protein Intolerance

LPI (MIM #222700) is an autosomal recessive disorder that results from mutations in the *SLC7A7* gene, which encodes for the cationic amino acid transporter y^+LAT-1 (see Figure 43.2-6 and Table 43.2-1).[56,57] The y^+LAT-1 transporter is located on the basolateral membrane of the enterocyte and renal tubules and transports cytoplasmic dibasic amino acids such as lysine, arginine, and ornithine in exchange for Na^+ and neutral amino acids.[58,59] Several gastrointestinal symptoms, including failure to thrive, diarrhea, and vomiting, are the main clinical symptoms associated with LPI.

Genetics. The *LPI* gene was discovered by positional cloning, and approximately 30 mutations have been identified in more then 100 patients with LPI.[56,57,60–62] Founder mutations have been identified in Finnish, Japanese, and Italian kindreds, in whom the incidence of LPI is particularly common.

Molecular Pathophysiology. The gene encoding for y^+LAT-1 is expressed in the small intestine, kidney, and lungs. Ornithine and arginine are important urea cycle intermediates that are not absorbed in either the kidneys or intestine of patients with LPI, and their relative deficiency, particularly during a high-protein diet meal, results in urea cycle dysfunction that leads to hyperammonemia and subsequent alterations in mental status.[63]

Clinical Manifestations. Undiagnosed patients with LPI frequently present with failure to thrive and a self-imposed restriction of dietary protein. Although diarrhea, emesis, and abdominal pain are frequent symptoms, the distinguishing feature of LPI is that the consumption of a high-protein diet results in hyperammonemia and dramatic changes in mental status. Chronic exposure to dietary protein is usually associated with various degrees of mental retardation. Marked hepatosplenomegaly and frequent bone fractures are also characteristic of LPI,[64] and patients' life spans are limited by complications associated with alveolar proteinosis and osteoporosis.[65] Recent reports have also suggested that arginine deficiency in patients with LPI may be associated with vascular endothelial dysfunction resulting from a decline in arginine-dependent nitric oxide production.[66] LPI is also associated with a relative deficiency of total and antigen-specific immunoglobulin, and although the function of T lymphocytes appears

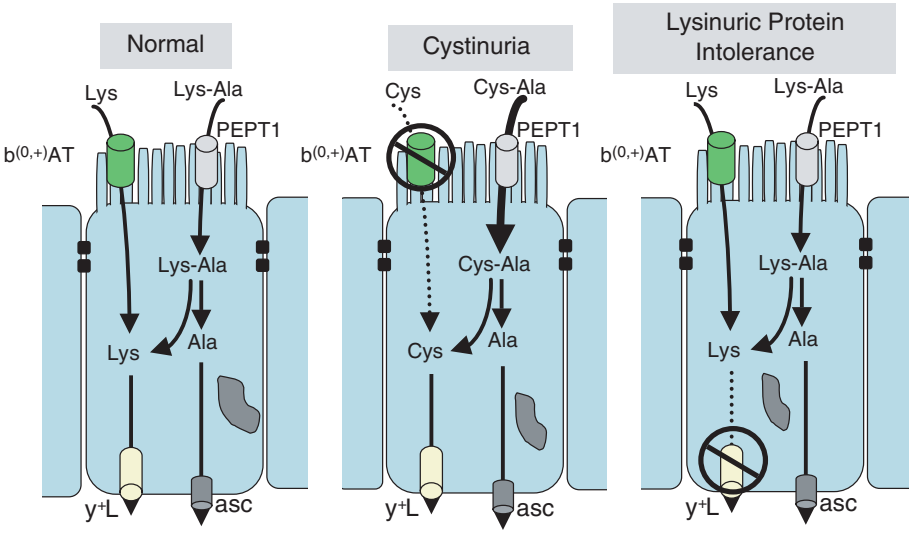

FIGURE 43.2-6 Amino acid transport in the small intestine of normal, lysinuric protein intolerance, and cystinuria patients. Lysine (Lys), alanine (Ala) and cysteine (cys) are transported across the apical ($b^{0,+}$AT) and basolateral (asc, y^+L) membrane. PEPTI = peptide cotransporter peptide transporter-1.

to be unaffected, fatal varicella infections have been described.[67,68] Hemophagocytic histiocytosis and systemic lupus erythematosus have also been described in several patients with established LPI.[69–72]

Diagnostic Criteria or Laboratory Investigations. Quantitative plasma amino acids and urinary organic acids provide useful hints into the possible diagnosis of LPI because plasma levels of diamino acids are low, whereas urinary levels of lysine and orotic acid are elevated.[58,59] Particular care should be given to the administration of an enteral bolus of a dietary protein load, which will result in the rapid impairment of motor function and mental status changes that are associated with hyperammonemia.

Treatment. Lysine is an essential amino acid, and serum levels are particularly low in patients with LPI. Short-term oral and intravenous supplements have been attempted in LPI patients, and whereas oral administration was poorly tolerated (diarrhea and vomiting), systemic administration was associated with only marginal symptoms.[63,73] Although long-term studies have not been reported, determining whether normal serum lysine levels diminishes the osteoporosis, failure to thrive, bone marrow dysfunction, and pulmonary complications that are associated with LPI would be of particular interest.

Citrulline supplements (200 mg/kg/d) and dietary protein restriction (1.5 g/kg/d) are currently used to manage patients with LPI.[63] Mealtime supplements of citrulline are readily absorbed and reduce postprandial hyperammonemia following a protein meal. Oral carnitine supplementation has been shown to be important in a subset of patients described with LPI and hypocarnitinemia.[74]

Other Defects of Amino Acid Transport

A second disturbance of amino acid transport has been characterized to date: system $b^{0,+}$, which is defective in cystinuria and is selective for cationic and neutral amino acid transport across the brush border membrane.[54] Hartnup disease (MIM #234500) and iminoglycinuria (MIM #242600) are two other autosomal recessive disorders that have been well characterized clinically; however, the molecular basis of either disorder has not been solved to date (see Table 43.2-1).[75,76] The diagnosis of these disorders can be established with analysis of urine amino acid profiles, and both disorders present with neurologic symptoms and an absence of significant gastrointestinal complications.

DISORDERS OF FAT ABSORPTION

Assimilation of dietary fats requires hydrolysis and subsequent repackaging of lipids by a complex group of intraluminal and intracellular events. The clinical consequences of disorders of fat assimilation include failure to thrive, steatorrhea, and neurologic deficits that result from the malabsorption of fat-soluble vitamins.

Lipolysis is initiated by gastric lipase in the stomach and concludes in the intestine through the action of pancreatic lipase. Because pancreatic exocrine function is particularly immature during early infancy, fat absorption in this age group primarily depends on the action of gastric lipase.[77] A congenital deficiency of pancreatic lipase (MIM #246600) is an exceedingly rare autosomal recessive disorder that leads to fat malabsorption.[78]

Fatty acid and diglyceride are the products of triglyceride hydrolysis, and their solubilization to the aqueous phase of luminal content requires adequate levels of conjugated bile salts. Various disorders of intrahepatic and extrahepatic cholestasis will impede the delivery of luminal bile salts and result in fat malabsorption. Bile salts are particularly important in the emulsification of long-chain fatty acids because of their lower aqueous solubility when compared with their medium-chain (12 to 6 carbons) counterpart.

A fatty acid transport protein (FATP4) was recently isolated, and its localization to the brush border membrane suggests that it may have a role in the absorption of luminal long-chain fatty acids.[79,80] A recent description of *FATP4*$^{-/-}$ knockout mice demonstrates a 40% reduction in fatty acid uptake and a neonatally lethal restrictive dermopathy.[81–83] In contrast, cholesterol export across the epithelial layer was recently established to occur via an adenosine triphosphate (ATP)-binding cassette transporter, ABCA1. The role of ABCA1 in cholesterol and phospholipid transport was confirmed when linkage analysis established it as the gene defective in Tangier disease (MIM #205400; see Table 43.2-1).[84,85] Tangier disease patients have a deficiency of high-density lipoproteins, which predisposes them to developing premature coronary heart disease despite hypocholesterolemia; they also accumulate cholesterol in tissue such as the liver, spleen, lymph node, and small intestine. Patients with sitosterolemia (MIM #210250) have the peculiar ability to absorb an excessive amount of the main plant sterol, sitosterol. Mutations in the ABC transporter, ABCG5 and ABCG8, have been associated with sitosterolemia.[86] These proteins form a heterodimer, which serves as the primary sterol efflux mechanism in the intestine. Despite defective processing of cholesterol and sterol, patients with Tangier disease and sitosterolemia have no significant gastrointestinal phenotype except hepatosplenomegaly. In contrast, disorders that result in defective packaging of re-esterified lipids into apolipoprotein-rich chylomicrons and very-low-density lipoprotein (VLDL) particles generally lead to fat malabsorption and failure to thrive (see Table 43.2-1).

Abetalipoproteinemia

Abetalipoproteinemia (ABL), or Bassen-Kornzweig syndrome (MIM #200100), is the classic and most well-characterized disorder of fat absorption and results from failure to reassemble dietary fat in the form of β-lipoproteins (see Table 43.2-1). Triglyceride and cholesterol esters are primarily transported in the plasma via β-lipoproteins; therefore, ABL patients have a severe deficiency of both of these neutral lipids. Patients generally present shortly after birth with failure to thrive and steatorrhea and if, untreated, will develop irreversible neurologic problems in late infancy. However, milder variants have been identified in asymptomatic adult patients who were identified by routine cholesterol screening.

Many of the original investigators studying the biology of ABL hypothesized it to be a defect secondary to the synthesis of apolipoprotein B synthesis; however, several studies failed to identify significant mutations in the *apoB* gene.[87] Subsequently, Wetterau and colleagues determined that the microsomal triglyceride transfer protein (MTP) was not detectable in the small bowel enterocytes of ABL patients and described homozygote mutations in the *MTP* gene.[88]

Genetics. MTP is a 97 kDa subunit that forms a heterodimer with a 55 kDa protein named disulfide isomerase (PDI).[89] Only a handful of mutations have been identified in ABL patients, and the majority result in the formation of a truncated and nonfunctional MTP.[90] Very few missense mutations have been isolated, and no mutations have been reported in the gene encoding the PDI protein. In fact, PDI is not required for MTP to properly bind and transfer lipids to apolipoprotein B.

Molecular Pathophysiology. Serum lipoproteins in patients with ABL are deficient in triglycerides and cholesterol-rich apolipoproteins B-100 and B-48, which form VLDL and chylomicrons. Apolipoprotein B-100 is synthesized by hepatocytes that produce VLDL, whereas the truncated protein (apolipoprotein B-48) is formed by enterocytes and is essential for chylomicron formation.

The role of MTP in chylomicron formation has been definitively established by investigating the consequences of ABL-causing mutations.[89] The role of MTP in lipoprotein formation has been performed using both in vitro cell lines and various types of MTP knockout mice.[91–93] These studies demonstrate that apolioprotein B alone is incapable of initiating lipoprotein formation and that MTP is specifically required to complete proper assembly. The protein component of apolipoproteins B-100 and B-48 is synthesized in the endoplasmic reticulum and must fold correctly to form a complex with lipids. Therefore, MTP has two distinct roles in lipoprotein assembly: first, to move lipids such as cholesterol and triglycerides into the lumen of the smooth endoplasmic reticulum and, second, to transfer small quantities of these lipids to the newly formed and unstable apolipoprotein B protein in a process that has been termed "lipidation."[92] In the absence of lipoprotein assembly, lipid droplets accumulate in the endoplasmic reticulum and are not transported to the *trans*-Golgi apparatus in the enterocyte and hepatocytes.[94] Small molecular inhibitors of MTP have been identified by high-throughput techniques, and it is anticipated that they potentially represent more potent cholesterol-lowering drug therapies.

Clinical Manifestations. As with any other autosomal recessive disorder, a history of parental consanguinity should alert the clinician to the possibility of APL. The typical clinical presentation in early infancy is one of failure to thrive, emesis, and low-volume diarrhea. Poor growth despite adequate caloric intake is an early clinical feature that should hasten the evaluation of a possible defect of fat malabsorption. In the long term, patients may develop an aversion to fatty meals as a way to minimize their diarrheal symptoms. The first evidence of neuromuscular abnormalities is usually the loss of deep tendon reflexes and results from long-term vitamin E deficiency; other neuromuscular manifestations, including retinitis pigmentosa, ataxia, and spinocerebellar degeneration, may be mistaken for various forms of Friedreich ataxia.

Diagnostic Criteria or Laboratory Investigations. Serum samples should be analyzed for evidence of β-lipoprotein (VLDL and chylomicron) deficiencies. Specifically, plasma triglyceride levels are generally < 10 mg/dL, whereas cholesterol levels range from 25 to 40 mg/dL. Acanthocytosis is usually seen in peripheral blood smears and results from abnormal lipoproteins in the plasma membrane of erythrocytes. Endoscopic evaluation of the small bowel can show yellow discoloration, and biopsies have characteristic fat-laden enterocytes located in the upper portion of the villus (Figure 43.2-7). Fat droplets within the cytoplasmic compartment of the enterocyte may be confirmed by electron microscopy.

Treatment. To minimize diarrheal symptoms, ABL patients are generally managed on a low-fat diet (~15 g/d), and serum levels of fat-soluble vitamins should be monitored periodically. Adequate serum levels of vitamins A and K can be achieved with supplementation of moderate oral doses; however, absorption of tocopherol is severely impaired and may require massive doses (2,000 mg in infants, and 10,000 mg in older children) of tocopherol to diminish the neuromuscular manifestations of vitamin E deficiency.

Hypobetalipoproteinemia

Hypobetalipoproteinemia (HBL; MIM #107730) is another very well-characterized disorder of fat absorption that results from the formation of abnormally truncated forms of apolipoprotein B (see Table 43.2-1).[95,96] The heterozygote state has been estimated to occur in approximately 1 in every 3,000 Americans.[96]

Genetics. The primary distinction between ABL and HBL is that the obligate ABL heterozygotes (biologic parents) have normal cholesterol and triglycerides levels, whereas HBL heterozygotes have low lipoprotein levels.[97] Therefore, HBL is inherited in an autosomal dominant manner; however, patients with only a single defective allele generally have no gastrointestinal and only minimal, if any, neuromuscular abnormalities. In contrast, individuals with two defective alleles are indistinguishable from ABL patients based on both physical and laboratory evaluation.

Many mutations have been described in the *APOB* gene in patients with HBL.[98] The mutations all result in proteins that are truncated either because of a nonsense or frame shift mutation that leads to a premature downstream stop codon. Several kindreds have been described that are clinically indistinguishable from HBL and ABL, yet mutations in either the apolipoprotein B or MTP alleles have not been identified.

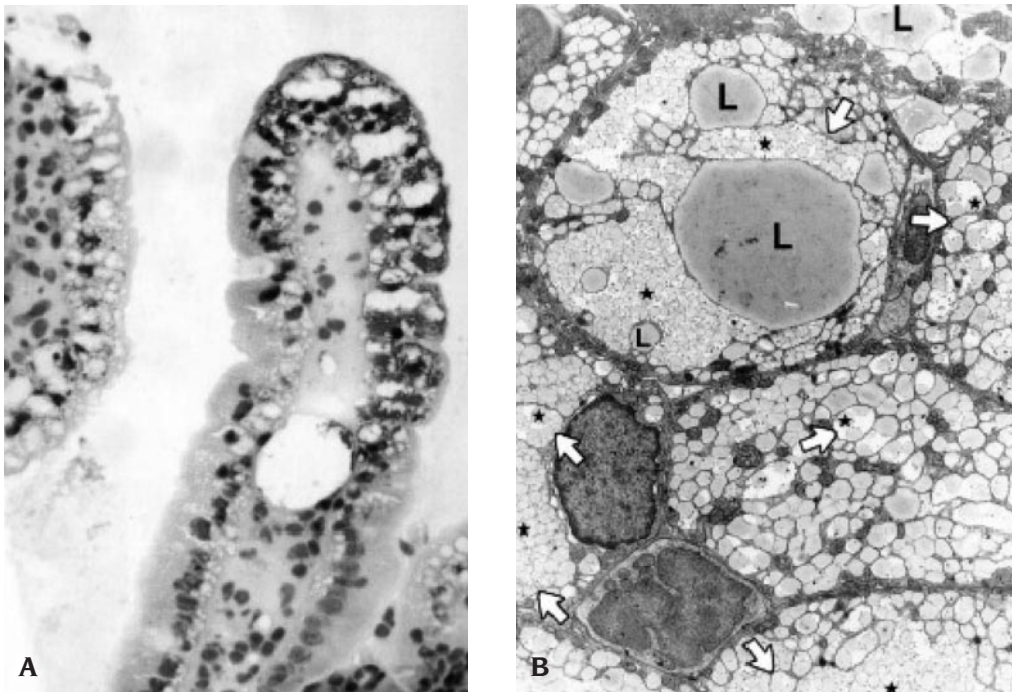

FIGURE 43.2-7 Classic lipid enterocytes seen in patients with abetalipoproteinemia, hypobetalipoproteinemia, and chylomicron retention disease. *A*, Intestinal biopsies from patients show vacuolization and marked staining with Oil Red O when examined by light microscopy (×10,003 original magnification). *B*, Electron microscopy of biopsies from patients shows the accumulation of large lipid droplets, free in the cytoplasm (L), and smaller chylomicrons and very-low-density lipoprotein–sized particles (stars) in membrane-bound vesicles (*arrows*) (×55,003 original magnification). Courtesy of Dr. Marie-Elisabeh Samson-Bouma, Faculté de Médecine X. Bichat.[94]

Molecular Pathophysiology. Various models have shown that truncation of the carboxy terminal of the apolipoprotein B protein influences the process of lipidation, as described earlier. Therefore, the inadequate addition of neutral lipids to the shortened apolipoprotein B protein leads to instability of the protein and its eventual degradation.

Clinical Manifestations. The clinical manifestations of patients with homozygote mutations of the *APOB* gene are identical to what was previously described in the ABL section of this chapter. In contrast, defects in a single allele (heterozygotes) may have subtle neurologic findings indicative of mild vitamin E deficiency.

Diagnostic Criteria or Laboratory Investigations. In heterozygote patients, cholesterol, triglyceride, and low-density lipoprotein levels are lower than in other unaffected members of the same kindred. In contrast, patients with homozygote mutations in the *APOB* allele have cholesterol, triglyceride, chylomicron, and VLDL levels that are similar to what was previously described in ABL patients. Moreover, characteristic findings such as acanthocytosis and fat-laden enterocytes are also seen (see Figure 43.2-7).

Treatment. Homozygote HBL patients should be managed as described in the ABL section, including the restriction of dietary fat and the supplementation of vitamin E. In contrast, the heterozygote patient should be given moderate doses of vitamin E.

Chylomicron Retention Disease

Chylomicron retention disease (CMRD; MIM #246700), or Andersen disease, is the least common of the classic disorders of lipid packaging in the intestine (see Table 43.2-1).[99] CMRD is an autosomal recessive disorder in which apolipoprotein B and MTP are produced; however, there is failure to secrete chylomicrons across the enterocyte's basolateral membrane.

Genetics and Molecular Pathophysiology. Recently, genome-wide screening was performed on patients with CMRD and a related disorder (Marinesco-Sjögren syndrome; MIM #607692), and a locus in chromosome 5q31.1 was identified as segregating with the disease locus.[100] A gene named *SARA2* resides in this locus and encodes for the Sar1b protein, which is a member of the Sar1–adenosine diphosphate–ribosylation factor family of small guanosine triphosphatases. This family of proteins has been shown to play an important role in the intracellular trafficking of proteins to organelles within a cell.[101] Various types of mutations (missense, nonsense, frame shift) were identified in the coding region of the *SARA2* gene that were unique to affected family members with CMRD and were not seen in either unaffected family members or the population at large. Moreover, each missense mutation alters a highly conserved residue in the guanine nucleotide-binding motif, which would presumably interfere with guanosine triphosphate binding. These data imply a role for Sar1b in the intracellular trafficking of chylomicrons to the *trans*-Golgi apparatus.

Clinical Manifestations and Diagnostic Criteria or Laboratory Investigations. Patients have been reported to present as neonates with severe diarrhea but may also first come to medical attention in early adolescence with neuromuscular manifestations of vitamin E deficiency. Interestingly, a large majority of patients with CMRD present with an intractable form of diarrhea with carbohydrate and amino acid malabsorption.[99] A low-fat diet will decrease the lipid distention of enterocytes and results in a hormalization of the tolerance for carbohydrates and

amino acids. However, the retinitis pigmentosa and neuromuscular manifestations are less severe when compared with either HBL or ABL. Enterocytes are also laden with fat, as is seen in HBL and ABL (see Figure 43.2-7).

Treatment. Treatment consists of restricting dietary fats and supplementation of vitamin E.

Primary Bile Acid Malabsorption

Efficient digestion and absorption of dietary lipids and lipid-soluble vitamins require their emulsification by bile salts (taurocholate) within the intestinal lumen.[102] The majority of dietary fats are absorbed in the jejunum, and residual bile salts are reabsorbed primarily by ileal enterocytes that express a Na^+-dependent bile salt transporter, apical sodium co-dependent bile acid transporter (ASBT) (*SLC10A2*), on its apical membrane. Primary bile acid malabsorption (PBAM; MIM #601295) is a very rare autosomal recessive disorder that results from an impairment of bile acid reabsorption secondary to a defect in the function of the ASBT transporter (see Table 43.2-1).[103,104] Taurocholate malabsorption more frequently results from the loss of an adequate amount of ileum, which may occur either because of surgical resection or because of extensive inflammation secondary to disorders such as Crohn disease.[105,106] The diarrhea that accompanies both primary and secondary forms of bile acid malabsorption may be attributed to both fat malabsorption (steatorrhea) and bile acid stimulation of colonocyte secretion of ions and fluid. Idiopathic adult-onset bile acid malabsorption has also been described in patients with presumably normal ileal function.[107]

Genetics. Oelkers, Dawson and colleagues screened the *SLC10A2* gene for mutations in patients with PBAM and identified four mutations, including three missense mutations (Ala171Ser, Leu243Pro, Thr262Met) and a splice site defect in exon 3.[103] In vitro analysis of these missense mutations failed to alter the proper targeting of ASBT to the cell membrane, however; the Leu243Pro and Thr262Met mutations impaired taurocholate transport.[108] The Ala171Ser mutation was found in 28% of asymptomatic individuals and appears to represent a benign polymorphism because bile acid absorption was not impaired by in vitro analysis. A missense mutation (Pro290Ser) was identified on a single ASBT allele (heterozygote), a patient with Crohn disease.[109] The Pro290Ser mutation abolishes taurocholate transport and in heterozygote carriers may influence cholesterol homeostasis and bile acid metabolism; however, the frequency and role of this and other polymorphisms in the general population await further investigations. No significant germline mutations were identified in a large cohort of adult patients with idiopathic bile acid malabsorption.[107]

Targeted deletion of the *SLC10A2* gene in a murine model causes only limited phenotypic changes, including fecal outputs that were indistinguishable from heterozygote ($SLC10A2^{-/+}$) or wild-type animals.[110] Fecal levels of bile acids were elevated 20-fold, whereas the size of the bile acid pool was reduced by 80%. Surprisingly, only mild steatorrhea was detected because fecal fat levels were fourfold higher in the null compared with the wild-type animals on a low-fat diet; however, more significant steatorrhea can be induced by augmenting the dietary fat content. Despite several limitations, this mouse model should improve our understanding of how the enterohepatic circulation influences absorption of dietary lipids.

Molecular Pathophysiology. The Na^+-dependent bile salt transporter ASBT is expressed on cholangiocytes and ileocytes and therefore plays an essential role in the enteric and hepatic phases of the enterohepatic circulation. Taurocholate is excreted in the biliary tree, where it is initially stored in the gallbladder and eventually enters the duodenum after cholecystokinin-induced gallbladder contraction in response to a meal. A small fraction of taurocholate is passively absorbed in the jejunum and colon. Along the horizontal axis of the intestine, ASBT expression is limited to the ileum, where it transports taurocholate via an electrogenic, Na^+-dependent mechanism. The reabsorption of taurocholate is about 95% efficient, and the 5% loss is replaced by hepatocyte conversion of cholesterol to bile salts.

Although the transport system that facilitates taurocholate transport across the basolateral membrane of the ileocyte has not been definitively established, the use of alternative splice sites (deletion of exon 2) appears to produce a truncated ASBT (348–154 amino acids) that may represent the bile acid efflux pump present on the basolateral membrane of the ileocyte. Nevertheless, bile salts enter the portal circulation and are transported across the sinusoidal membrane of the hepatocyte by a Na^+-dependent taurocholate cotransport polypeptide, NTCP (*SLC10A1*). Transport of recycled and newly formed taurocholate out of the hepatocyte occurs across the canalicular membrane via an ATP-dependent bile salt export pump, BSEP.

Clinical Manifestations. Patients with PBAM present during infancy with diarrhea, steatorrhea, failure to thrive, and low plasma levels of low-density lipoprotein cholesterol.[103,104] The diarrhea is generally secretory (persists while fasting) in character and is exacerbated by the addition of dietary fats.

Diagnostic Criteria or Laboratory Investigations and Treatment. The small intestine of patients with PBAM should be normal in length and architecture, and the extent of the diarrhea should improve with a trial of cholestyramine and a low-fat diet. Bile acid absorption by the ileum may be measured using the bile acid analogue ^{75}Se-homocholic acid–taurine test.

DISORDERS OF MINERAL AND ELECTROLYTE ABSORPTION AND SECRETION

Congenital Chloride Diarrhea

The most common cause of congenital secretory diarrhea in the presence of normal intestinal mucosa is the autosomal recessive disorder congenital chloride diarrhea (CCD; MIM #214700; see Table 43.2-1). Currently, over 250 cases of CCD has been described in the literature (Figure 43.2-8).[111]

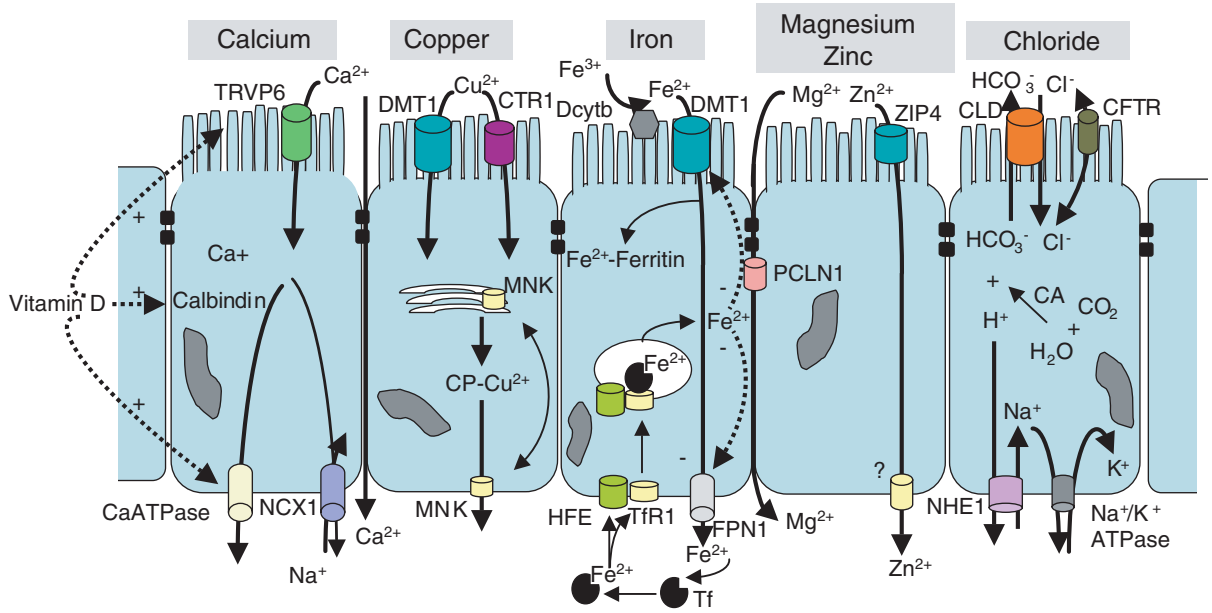

FIGURE 43.2-8 Ion and metal transport in the small intestine. ATPase = adenosine triphosphatase; CFTR = cystic fibrosis transmembrane regulator; CLD = congenital chloride diarrhea; CTR = copper transporter; Dcytb = duodenal cytochrome b; DMT = divalent metal transporter; FPN = ferroportin; HFE = haemochromatosis; IF = intrinsic factor; MNK = Menkes syndrome transport protein; NCX = Na^+-Ca^{2+} exchanger; NHE = Na^+-H^+ exchange; PCLN = paracellin; TfR = transferrin receptor; TRPV6 = transient receptor potential (vanilloid) 6; ZIP = zinc- and iron-regulated protein.

Nearly half of the reported cases have been confined to Finland, where it has been estimated to occur in 1 in 20,000 of the population, whereas it has been estimated to occur in 1 in 32,000 live births in the Persian Gulf.[112]

An earlier linkage analysis of several families identified the CCD locus to a region of chromosome 7q31 located in the vicinity of the cystic fibrosis gene, cystic fibrosis transmembrane regulator (*CFTR*).[113,114] The gene defective in CCD was originally named down-regulated in adenomas (*DRA*) and was subsequently renamed as the solute carrier family 26, member 3 gene (*SLC26A3*).[115] This gene encodes for a protein that was a viable candidate for CCD because it was expressed in colonocytes, and its chromosome localization matched previous linkage analysis.[116] The *SLC26A3* gene encodes a Na^+-independent Cl^-/HCO_3^- exchanger that is expressed primarily in the apical brush border membrane of intestinal enterocytes (see Figure 43.2-8).

Genetics. Makela and colleagues and Hoglund and colleagues identified several mutations in the *SLC26A3* gene in patients with CCD, including the prevalent Val317del mutation, which was found in a Finnish population.[111,115] Further in vitro studies demonstrated that the Val317del impaired chloride transport, confirming the role of the *SLC26A3* gene in CCD.[117] To date, over 30 disease-related mutations have been identified in over 100 patients studied with CCD.[111] One-third of the mutations are either microdeletions or missense and nonsense mutations. Three prominent founder mutations have been identified that account for the majority of cases of CCD in Finland (Val317del), Poland (I675-676ins), Saudi Arabia, and Kuwait (Gly187X). Therefore, genetic testing will be easier in a patient whose family linage originated in Finland, Poland, or the Middle East.[112] A small number of patients have been identified that fit the clinical characteristics of CCD but do not have detectable mutations in the *SLC26A3* gene. Furthermore, analysis of the precise transport function of this protein and the effects that the missense mutation has on its transport characteristics has not been described.

Molecular Pathophysiology. SLC26A3's main role as a Cl^-/HCO_3^- exchanger is to reabsorb chloride along the length of the distal intestine and colon (see Figure 43.2-8).[113] In the distal intestine and colon, salt (NaCl) absorption is secondary to SLC26A3 transport of chloride and Na^+-H^+ exchanger (NHE-3) transport of sodium. In normal physiologic conditions, the expression of NHE-3 in the proximal small intestine is low, and the Cl^-/HCO_3^- exchanger secretes bicarbonate into the lumen while absorbing chloride produced by gastric acid.[118,119] Thus, the exchanger serves the important role of neutralizing the acidity of gastric secretion once in the proximal small bowel. CFTR is a chloride channel whose primary role in the small intestine is to secrete chloride, which is then absorbed in the distal intestine and colon by the Cl^-/HCO_3^- exchanger. The impaired function of the Cl^-/HCO_3^- exchanger in CCD patients would disrupt the neutralization of gastric acid in the proximal small bowel and unabsorbable chloride levels in colon.

Clinical Manifestations. The earliest clinical symptoms may occur in utero with severe polyhydramnios and dilated loops of small bowel detectible by ultrasonography and may resemble a distal intestinal atresia.[120] The polyhydramnios and in utero diarrhea frequently result in prematurity. Patients with CCD generally present during the first week of life with severe life-threatening secretory diarrhea.

The serum electrolytes prior to treatment are unique among the various disorders of congenital diarrheal disorders and include metabolic alkalosis, hypochloremia, hypokalemia, and hyponatremia.

Diagnostic Criteria or Laboratory Investigations. The diagnosis of CCD would be suggestive if fecal chloride concentration exceeds the concentration of cations (Na^+ and K^+). This classic characteristic of CCD suggests that fecal HCO_3^- levels are particularly low and that another cation, presumably accounted for by H^+, contributes to the excessive electroneutral transfer of HCl. Patients with CCD have been found to experience intestinal and colonic ulcerations, which may be attributable to impaired HCl neutralization (personal observations, 2004).

Treatment. The mainstay of therapy is the oral or parenteral administration of KCl supplements. Although oral administration is the preferred route of electrolyte supplements, they are not well absorbed and may therefore contribute to the diarrhea. The administration of proton pump inhibitors has recently been shown to improve the degree of diarrhea, presumably by inhibiting meal-induced gastric acid secretion.[121] The investigators provided convincing data that poor absorption of gastric HCl contributes to the fecal electrolyte losses, and therefore diarrhea, in patients with CCD.

Congenital Sodium Diarrhea

CSD (MIM #270420) is an exceedingly rare autosomal recessive disorder that presents with secretory diarrhea and many of the same clinical characteristics seen in patients with CCD (see Table 43.2-1).[122,123] Currently, only six cases of CSD have been described in the literature, and the molecular basis of this condition has not been fully established.[124] Nevertheless, the available information suggests that the disorder results from impaired function of the intestinal Na^+-H^+ exchanger (see Figure 43.2-8). The gastrointestinal tract expresses primarily three Na^+-H^+ exchangers: NHE-2, -3, and -4.[125] NHE-4 is expressed largely by gastric mucosa, whereas both NHE-2 and -3 are present in the small intestine.

Genetics. A recent study of two large Austrian kindreds with five affected members confirmed that CSD is transmitted in an autosomal recessive manner.[124] The investigators performed multipoint linkage analysis to demonstrate that defects in none of the known Na^+-H^+ exchangers are responsible for the CSD in these kindreds.

Molecular Pathophysiology. Vesicle transport studies performed on brush border membrane samples confirmed that CSD is the result of a faulty Na^+-H^+ exchanger.[122] The NHE-3 exchanger is considered to be the primary transporter responsible for Na^+ absorption because deletion of the *NHE3* gene in mice results in sodium secretory that resembles CSD.[126,127] These mice also had a mild proximal tubular acidosis that was secondary to the role of NHE-3 in renal Na^+ and HCO_3^- reabsorption. Although mild diarrhea was reported in these mice, the primary finding in the gut was an excess amount of luminal fluids and an expanded surface area. Further understanding of the pathophysiology of CSD will require the elucidation of the loss of function mutations in the yet to be identified disease-causing gene.

Clinical Manifestations. Patients with CSD have high-volume secretory diarrhea that is very alkaline and contains high concentrations of Na^+. Therefore, patients generally experience hyponatremia, low or normal excretion of urinary Na^+, and metabolic acidosis. Polyhydramnios resulting from in utero diarrhea is also associated with CSD, and a recent report described choanal atresia in two patients with CSD.[124]

Although the intestinal biopsies of patients with selective impairment of transport function are generally considered to be normal, biopsies from three recently described cases that fit the clinical characteristics of CSD showed partial villous atrophy and a decreased villus-to-crypt ratio.[124] It is unclear if these abnormal histologic findings are a consequence of CSD or a result of the limited enteral intake of these patients who were on total parenteral nutrition. Electromicroscopy showed no evidence of microvillus atrophy or inclusion disease but demonstrated unexpected membranous whorls located in lysosomal bodies, vacuoles, and mitochondria.

Diagnostic Criteria or Laboratory Investigations. The diagnosis of CSD should be considered in patients who present as newborns with high-output secretory diarrhea and can be established by confirming the presence of elevated levels of fecal Na^+ and HCO_3^-. Unlike patients with CCD who have metabolic alkalosis, metabolic acidosis is typically found in CSD and in all other forms of congenital diarrhea.

Treatment. The medical management of CSD patients is to maintain their fluid and electrolyte balance with the use of oral or parenteral fluids and salts.

Acrodermatitis Enteropathica

Primary acrodermatitis enteropathica (AE; MIM #201100) is a rare autosomal recessive disorder of impaired intestinal absorption of zinc (see Table 43.2-1 and Figure 43.2-8).[128,129] Although secondary deficiencies in trace elements such as zinc and selenium are a common complication of chronic diarrhea, patients with AE present in early infancy with profound depletion of total-body zinc.

Genetics. Genome-wide screening for the AE allele was recently reported using two large consanguineous families, and a locus residing within chromosome 8q24.3 was identified.[130] Within this region of the genome resides a gene that encodes a member of the zinc- and iron-regulated transporter family of proteins (ZIP4; Figure 43.2-8).[131] Sequencing of the *ZIP4* gene identified five missense mutations in patients with AE.[132–134] Although the transport function of the mutant forms of the ZIP4 protein has not been evaluated, each mutation alters highly conserved and critical amino acid residues in the family of zinc transporters.

Molecular Pathophysiology. ZIP4 is expressed on the apical membrane of epithelia in the intestine, colon, stomach,

and kidney.[135] ZIP4 is a member of a recently described family of proteins that control the transport of zinc into the cytoplasm of various cells. Although patients with AE have a markedly reduced capacity to absorb luminal zinc, their responsiveness to high-dose oral zinc supplements raises the possibility that they retain a redundant but less efficient zinc transport mechanism in the intestine (see Figure 43.2-8).

Clinical Manifestations. During childhood, zinc deficiency may develop either because of a primary defect in absorption or secondary to either inadequate intake or excessive losses.[128,136] Zinc deficiency that is due to excessive fecal losses of zinc is a common finding in patients with acute and chronic diarrhea, and short-term oral supplements have been shown to reduce the duration and severity of diarrhea.[137] Infants who are exclusively breastfed are also predisposed to zinc deficiency because its supply by breast milk may be insufficient to provide adequate zinc requirements.[138]

AE patients generally present with anorexia, diarrhea, and severe failure to thrive.[128,129] The dermatitis located on the hands, feet, and perirectal and oral regions has a vesicobullous character, and the alopecia of the scalp and face is also a striking feature that occurs in patients with prolonged zinc deficiency. Humoral and cell-mediated immunodeficiencies have been reported in association with AE and may contribute to both poor wound healing and recurrent infections. Poorly characterized neurologic features such as mental lethargy and neurosensory abnormalities may also be present.

Diagnostic Criteria or Laboratory Investigations. Zinc deficiency results in low levels of serum alkaline phosphatase, a zinc-dependent metalloenzyme. Alkaline phosphatase levels decline with age, and lower than expected levels should raise the possibility of zinc deficiency. Both serum and urinary zinc concentrations are significantly reduced in untreated patients with AE. Radiolabeled zinc has been used to assess the absorptive capacity of the intestine using both mucosal biopsy samples and in vivo uptake studies.[128,129]

Histology of the small intestine has shown several abnormalities that may represent the consequence of inadequate zinc concentrations in patients with AE. Paneth cells and enterocytes of the small bowel have been shown to have inclusion bodies and abnormal intracellular organelles; villus atrophy has also been reported.[128,129]

Treatment. Patients with AE can be managed with large oral doses, 3 to 30 µmol zinc/kg body weight/d, of zinc supplements. Chronic supplementations with adequate doses of zinc will resolve the clinical and laboratory features of this disorder.

Calcium Absorption

Calcium absorption in the small intestine occurs by both active and passive transport mechanisms.[139] Passive absorption of dietary calcium occurs paracellularly along the entire length of the small bowel, and this pathway is predominant when the calcium supply is abundant. In contrast, the active calcium transport system is located primarily in the duodenum and is particularly important when the level of dietary calcium is low. The active transport system was recently identified as a member of the transient receptor potential vanilloid 6, or TRPV6, and is expressed primarily in the proximal small bowel.[140] This transporter has also been named ECaC2 and CaT1, and a homologous TRPV5 transporter is present primarily in the kidney (see Figure 43.2-8).

TRPV6 expression in the intestine is limited to the enterocytes, specifically the brush border membrane. Once within the cytoplasm, calcium forms a complex with a calcium-binding protein called calbindin D_{9k}, or CaBP. Calcium efflux out of the enterocyte takes place by either the CaATPase (PMCA1b) or by the Na^+-Ca^{2+} exchanger NCX1 (see Figure 43.2-8). Vitamin D (1,25-dihydroxyvitamin D_3) directly influences the expression of both TRPV5/6 and CaBP by directly activating vitamin D–responsive elements in the immediate promoter region of both genes and increasing transcription.[141] Congenital disorders that impair calcium absorption include pseudovitamin D deficiency rickets (MIM #264700), which is secondary to a defective synthesis of 25-hydroxyvitamin D_3 1α-hydroxylase.[142] Specific inherited defects in the transporters that control dietary calcium absorption have yet to be described.[143]

Disorder of Magnesium Transport

Congenital primary hypomagnesemia (MIM #248250) is a rare autosomal recessive disorder that is associated with impaired absorption of dietary magnesium and a renal tubular defect of magnesium transport (see Table 43.2-1 and Figure 43.2-8).[144] Hypocalcemia secondary to low parathyroid hormone and 1,25-dihydroxyvitamin D_3 levels is common and may result in infantile tetany. The molecular basis of primary hypomagnesemia was elucidated several years ago when linkage analysis led to the identification of a tight junction protein named paracellin 1 (PCLN1).[145] Mutations in the gene encoding the PCLN protein (also called claudin 16) were identified in 10 kindreds, and the protein was located in the tight junction region of the thick ascending limb of Henle. Whether paracellin is expressed by small intestinal epithelium has not been reported. The mechanism by which paracellin influences magnesium transport has not been identified; although paracellin may serve as a selective paracellular transporter of magnesium, it may also represent a sensor of magnesium concentration.

Disorders of Copper Transport

Copper is a critical cofactor required for the synthesis and function of numerous proteins whose roles range from antioxidants to enzymes required for mitochondrial function. The diet is an important source of copper, and our understanding of the complex process of copper absorption, intracellular processing, and efflux has improved by investigating the basis of two disorders of copper transport, Menkes syndrome and Wilson disease.[146,147] Menkes syndrome (MIM #309400) is a rare (1 in 100,000) X-linked recessive disorder of copper deficiency resulting from

defective synthesis of the Menkes syndrome transport protein (MNK) (see Table 43.2-1 and Figure 43.2-8).[148] In contrast, Wilson disease (WD; MIM #277900) is a more common autosomal recessive disorder that is the consequence of copper accumulation secondary to malfunctioning of the WD transporter.[149] Both transporters have been localized to the membranes of the *trans*-Golgi network (TGN), where they modulate copper transport into the network. When intracellular copper levels increase, both transporters redistribute to small vesicles and the plasma membrane, where they presumably control the efflux of copper out of the cell. MNK is expressed primarily by enterocytes and cells of the placenta and central nervous system, whereas expression of the WD transporter is limited mainly to hepatocytes. Given their tissue-specific pattern of expression, defects in the function of MNK result in inadequate efflux of copper from the gut, whereas an alteration of WD results in failure to excrete copper to bile, which subsequently accumulates in the liver.[146]

The mechanism of dietary copper absorption has not been definitively established; however, two active transporters located on the apical membrane of the enterocyte have been implicated (see Figure 43.2-8). The H^+–divalent metal transporter (DMT1) has a broad range of substrates, including copper, iron, manganese, and cobalt, to name a few.[150] A more specific family of high-affinity copper transporters, CTR1 and -2, has been isolated and is expressed in a broad range of tissue, including the intestine.[151]

Within the intracellular compartment of the enterocyte, copper may be either incorporated into copper-containing proteins or traffic into the TGN by way of the MNK transporter.[146,147] Copper binds ceruloplasmin within the TGN and subsequently enters the systemic circulation bound primarily to ceruloplasmin. Although copper levels do not influence the production of ceruloplasmin, the protein becomes unstable (shorter half-life) during copper-deficient states, resulting in low ceruloplasmin levels. Because defects of both MNK and WD lead to impaired trafficking of copper across the TGN membrane, Menkes syndrome and WD are both associated with hypoceruloplasminemia.

Genotype-phenotype relationships have been established in Menkes syndrome, and the clinical consequences of mutations in MNK are primarily the result of a copper-deficient state that leads to improper synthesis of copper-containing proteins. Patients generally present during early infancy with failure to thrive, various neurologic symptoms, hyperpigmentation, and morphologic changes of the hair. Most children with Menkes syndrome die by 4 years of age, and although copper supplementation has not been shown to be efficacious, recent anecdotal reports suggest that copper-histidine may delay the onset of various symptoms.[152] In contrast, WD generally presents during early adolescence with either neurologic or hepatic consequences of copper overload. Chelation therapy with D-penicillamine or ammonium tetrathiomolybdate augments renal excretion of copper while improving its positive balance in the liver and brain.[153] Oral administration of zinc has been shown to inhibit dietary intake of copper, presumably by competing for transport into the enterocyte via DMT (see Figure 43.2-8).[150,154]

DISORDERS OF IRON TRANSPORT

Our understanding of the mechanism of iron absorption has improved dramatically over the last several years with the discovery of the molecular basis of hereditary hemochromatosis (HH; MIM #235200; see Table 43.2-1).[155,156] HH is an autosomal recessive disorder characterized by excessive iron absorption by the intestine and secondary multiorgan failure related to excessive tissue iron content. Hemochromatosis is probably the most common genetic disorder among individuals of European ancestry, with an estimated carrier frequency of about 1 in 8.[155,156] The isolation of the defective gene responsible for HH was performed by linkage analysis and sequencing of an area within the major histocompatibility region of chromosome 6p. The most common form of HH is secondary to mutations in the gene that encodes a major histocompatibility class I–like protein, HFE. Several mutations, including Cys282Tyr, His63Asn, and Ser65Cys, account for the majority of abnormal alleles in the *HFE* gene.[157, 158]

The role of HFE in iron absorption was elucidated when HFE was found to interact with the transferrin receptor (TfR) with an affinity that was comparable to the diferric transferrins' interaction with the receptor (see Figure 43.2-8). Immunohistochemical analysis also confirmed that HFE and TfR were localized to both the basolateral membrane and the intracellular compartment of crypt cells of the intestine. It has been speculated that HFE facilitates the interaction of diferric transferrin and TfR, resulting in endocytosis in clathrin-coated vesicles and the eventual release of iron into the cell cytoplasm.

The primary apical iron transporter was identified by expression cloning to be the H^+-DMT1, which also transports copper (see Figure 43.2-8).[150] Because the absorption of dietary iron is limited to the reduced ferric (Fe^{2+}) form, the brush border enzyme cytochrome *b* (duodenal cytb) reduces the oxidized form (Fe^{3+}) prior to transport (see Figure 43.2-8). The efflux of iron across the basolateral membrane is mediated by a recently characterized transporter, ferroportin 1 (FPN1).[159] The transporters are both expressed primarily on duodenal enterocytes located along the upper portion of the villus, and their expression is dramatically up-regulated in iron-deficient states.

The common HFE mutation appears to inhibit its interaction with its heterodimeric partner, β_2-microglobulin, and diminish the level of HFE on the basolateral membrane. Because the endocytosis of the transferrin-TfR- Fe^{2+} complex requires adequate levels of HFE, mutations of the HFE protein would reduce cytoplasmic iron levels while augmenting the expression of DMT1 and FPN1 and increasing iron intake.[160,161] In contrast, in iron-abundant states associated with wild-type HFE, the endocytosis of the Fe^{2+}-containing complex would increase the cellular stores of iron and thereby reduce the synthesis of DMT1 and FPN1.

Deficiencies of proteins other than HFE have recently been implicated in the pathogenesis of other forms of hemochromatosis (see Table 43.2-1). Mutations in the

gene encoding ferroportin 1 have been identified in an autosomal dominant form of hemochromatosis (MIM #606069).[162] Defects in TfR 2 have also been identified in several kindreds (MIM #604250), whereas a juvenile form of hemochromatosis (MIM #602390) has been associated with mutations in hepcidin, an antimicrobial peptide synthesized by hepatocytes.[163,164]

DISORDERS OF VITAMIN ABSORPTION

Folate Malabsorption

Dietary folate is found primarily in green leafy vegetables, organ meats, and grains as polyglutamylated folates. Glutamate carboxypeptidase II, expressed on the brush border membrane of small bowel enterocytes, hydrolyzes folylpolyglutamates to a form (monoglutamyl folates) that can be transported by the reduced folate transporter 1 (RFC-1) (Figure 43.2-9). Once within the enterocyte, folylmonoglutamate is converted to 5-methyltetrahydrofolate (5-MTHF), which exits the basolateral membrane via the RFC-1 transporter. The folate-binding protein mediates the transport of 5-MTHF in hepatocytes, where it is stored in the form of polyglutamylated folates, an essential cofactor for nucleic and amino acid synthesis.

Folate deficiency generally results in hyperhomocysteinemia because 5-MTHF is required to synthesize methionine from homocysteine. A sufficient level of methionine is required for the synthesis of *S*-adenosylmethionine, an enzyme critical for DNA methylation. Congenital folate malabsorption (MIM #229050) is a rare autosomal recessive disorder associated with the clinical symptoms of diarrhea, glossitis, and seizures in the face of pancytopenia (see Table 43.2-1).[165] The underlying mechanism of the congenital form of folate deficiency has not been identified. Folic acid deficiency has been definitively associated with neural tube defects in neonates and an increased risk of cardiovascular disease in adults with elevated levels of homocysteine.[166] Serum folate and homocysteine levels are readily available assays that may be used to assess adequate folate stores.

Vitamin B_{12} Malabsorption

The absorption of vitamin B_{12} (cobalamin) is a complex process that is initiated by gastric acidity that removes cobalamin from dietary proteins and transfers it to haptocorrin (cobalamin-binding protein). Intrinsic factor (IF), produced by parietal cells, binds cobalamin after pancreatic proteases hydrolyze the cobalamin-haptocorrin complex in the duodenum. The ileal enterocytes express cubulin, which forms a heterodimer with amnionless and forms a receptor for the cobalamin-IF complex (see Figure 43.2-9).[167] Amnionless's role is to direct the receptor-vitamin complex to the endosomes, where it encounters a second receptor, megalin.[168] The cobalamin-IF complex is cleaved within the endosome, resulting in the formation of a cobalamin-transcobalamin 2 complex, which transverses the enterocyte and enters the systemic circulation.

The clinical consequences of vitamin B_{12} and folate deficiency overlap because cobalamin is a cofactor for methionine synthase, which uses a methyl group from 5-MTHF to convert homocysteine to methionine.[169] Hyperhomocysteinemia is thus the consequence of both vitamin B_{12} and folate deficiency. Defects of cobalamin absorption are, however, uniquely associated with methylmalonicacidemia because adenosylcobalamin is required for appropriate mitochondrial fatty acid metabolism.[170]

Vitamin B_{12} deficiency can be secondary to numerous problems, ranging from inadequate intake to disruption of any of the various steps required for absorption.[169] Primary or secondary achlorhydria is commonly associated with cobalamin deficiency because gastric acidity is required for the formation of the cobalamin-haptocorrin complex. Bacterial overgrowth in the small intestine may also disrupt the cobalamin-IF complex and lead to a decline in cobalamin levels. Because cobalamin is absorbed exclusively in the ileum, disorders resulting in an inadequate ileal absorptive capacity will also result in vitamin B_{12} deficiency.

There are three rare yet well-described autosomal recessive disorders associated with congenital forms of cobalamin deficiency. Congenital pernicious anemia (MIM #261000) is associated with an absence of IF, which facili-

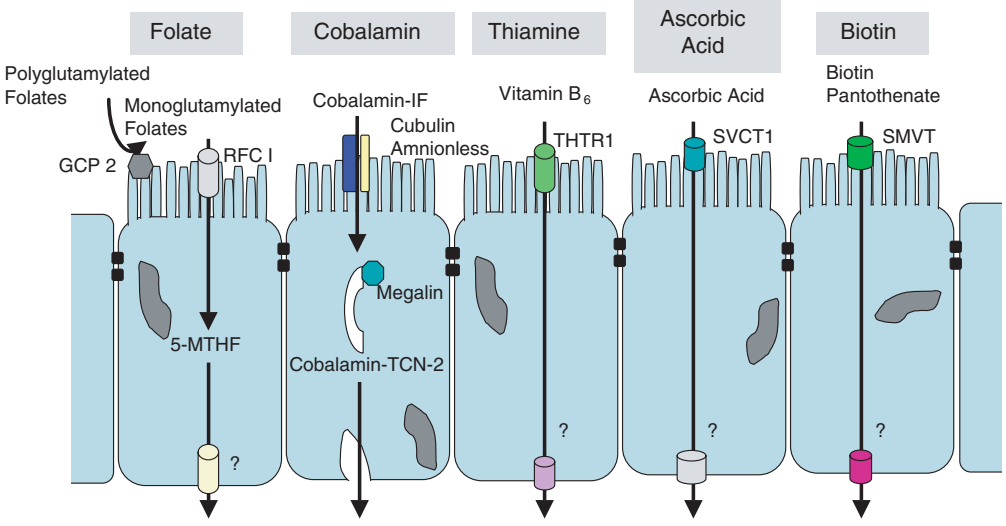

FIGURE 43.2-9 Water-soluble vitamin absorption in the small intestine. GCP = glutamate carboxypeptidase; MTHF = methyltetrahydrofolate; RFC = reduced folate transporter; SMVT = sodium multivitamin transporter; SVCT = sodium-vitamin C cotransporters; TCN = transcobalamin; THTR = thiamine transporter protein.

tates the binding of cobalamin to the cubilin-amnionless receptor.[171] Imerslund-Graesbeck syndrome (MIM #261100) was originally described in both Finland and Norway, and linkage analysis of the Finnish patients identified mutations in the cubilin receptor[172]; whereas the Norwegian cases were recently identified to be secondary to mutations in the amnionless gene (see Table 43.2-1).[167,173] Finally, congenital defects of transcobalamin 2 (MIM #275350) have also been shown to result in inadequate levels of vitamin B_{12} and in both bone marrow failure and a range of neurologic deficits.[174] Measurement of serum vitamin B_{12} levels and evidence of homocysteine and methylmalonic acid may be used to assess vitamin B_{12} stores. The Schilling test is particularly useful in assessing the various origins of cobalamin deficiency.[170]

ABSORPTION OF VARIOUS WATER-SOLUBLE VITAMINS

The molecular basis of several water-soluble vitamins has recently been elucidated. Vitamin C, or L-ascorbic acid, has been found to be transported across the intestinal epithelial layer by the Na^+-dependent vitamin C transporter sodium vitamin C transporter (SVCT)1 (see Figure 43.2-9).[175] Ascorbic acid transport via SVCT1 appears to be critical during the perinatal period because null SVCT1$^{-/-}$ knockout mice succumb immediately after birth.[176] Because the dietary deficiency of ascorbic acid has been well described, inherited forms of a congenital deficiency have not been reported. Although the pyridoxine (vitamin B_6) transporter has not been identified, recent reports have begun to determine various characteristics of this pH-dependent carrier-mediated system.[177]

Expression cloning techniques were also used to identify the biotin transporter. The Na^+-dependent multivitamin transporter sodium multivitamin transporter was also identified to transport pantothenate (vitamin B_5) and lipoate at high affinity (see Figure 43.2-9).[178] An abnormality of biotin transport was recently described in an encephalopathic child; however, the molecular mechanism has not been described.[179] Thiamine absorption across the intestinal brush border membrane is facilitated by the thiamine transporter protein 1 THTR1. The thiamine transporter resembles the structure of the folate transporter RFC-1, and mutations of THTR1 have been associated with a thiamine-responsive megaloblastic anemia syndrome (MIM #249270; see Table 43.2-1).[180,181] This is a rare autosomal recessive disorder that is associated with megaloblastic anemia, diabetes mellitus, and deafness.

ABSORPTION OF VARIOUS FAT-SOLUBLE VITAMINS

The most common cause of a generalized deficiency of fat-soluble vitamins includes specific dietary restrictions and a wide assortment of gastrointestinal disorders that impair fat absorption. All fat-soluble vitamins are absorbed by enterocytes primarily by passive diffusion, a process that is facilitated by the emulsification of fats by bile salts. Selective deficiencies are certainly more unusual and may suggest a defect in the interaction of vitamin with a specific transfer protein. For instance, vitamin A (retinol) is packaged in chylomicrons in the enterocyte and is eventually taken up by hepatocytes, where it is either stored or remains bound to retinol-binding protein for transfer to other cells. A rare inherited disorder of retinol-binding protein deficiency (MIM #180250) has been described and leads to an assortment of ophthalmologic findings and a low serum level of vitamin A.[182] A selective vitamin E deficiency (MIM #277460) has also been identified that is secondary to mutations in the α-tocopherol transfer protein and results in a form of spinocerebellar ataxia and undetectable serum vitamin E levels (see Table 43.2-1).[183]

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3. Congenital Enteropathy Involving Intestinal Mucosa Development

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The definition, presentation, and outcome of intractable diarrhea of infancy (IDI) have changed considerably during the last three decades because of major improvements in nutritional management and better understanding of the pathology of the small bowel mucosa. Originally, the syndrome of IDI was described by Avery and colleagues in 1968 based on the following features: diarrhea occurring in an infant younger than 3 months of age, lasting more than 2 weeks, with three or more negative stool cultures for bacterial pathogens.[1] Most cases were managed in the hospital using intravenous fluids while the diarrhea was persistent and intractable, with a high mortality rate from infection or malnutrition. From a multicenter French study, Ricour and colleagues reported similar features of IDI, whereas, in most cases, no specific diagnosis was provided.[2] Recently, the term "severe diarrhea requiring parenteral nutrition" was proposed.[3,4] Within this group of patients with diarrhea, it seems possible to differentiate protracted diarrhea of infancy (PDI), which resolves despite its initial severity, and IDI, which requires long-term parenteral nutrition (PN) and, in some cases, intestinal transplant. From a recent French multicenter study involving 65 cases of severe diarrhea requiring more than 1 month of PN, Tables 43.3-1 and 43.3-2 show the main characteristics of infants with PDI and IDI and the diagnosis distribution, respectively.[5]

PDI is primarily due to either a specific immunodeficiency or a sensitization to a common food protein (eg, cow's milk and gluten) or to severe infection of the digestive tract (postenteritis syndrome). The so-called "intractable ulcerating enterocolitis" of early onset is difficult to classify.[6]

TABLE 43.3-1 MAIN CHARACTERISTICS OF INFANTS WITH PROTRACTED AND INTRACTABLE DIARRHEA OF INFANCY

CHARACTERISTIC	PDI	IDI	*P*
Gestational age (mo)	38.5 ± 1.2	38.2 ± 1.7	NS
Birth weight (g)	2,910 ± 340	2,930 ± 462	NS
Onset of diarrhea (d)	120 ± 49	38 ± 17	.02
PN duration (mo)	5 ± 11	24 ± 8	.01
Mortality (%)	2.5	23.8	.05

Adapted from Goulet O et al.[5]
IDI = intractable diarrhea of infancy; NS = not significant; PDI = protracted diarrhea of infancy; PN = parenteral nutrition.

IDI with persistent villous atrophy usually starts within the first 2 years of life and is associated with abundant diarrhea that persists in spite of protracted bowel rest. It rapidly becomes life threatening, and long-term PN is required. It is associated with a persistent histologic intestinal lesion and may continue for years despite various therapeutic trials, including steroids and/or cyclosporin A or tacrolimus. These characteristics clearly differentiate IDI from PDI, which responds to bowel rest and/or enteral nutrition, or to specific treatment in case of immunodeficiency or allergy, and always recovers even after several weeks or months of parenteral and/or enteral nutrition.

CURRENT VIEW OF INTRACTABLE DIARRHEA OF INFANCY

During the early 1980s, conditions such as autoimmune enteropathy[7] or microvillous atrophy/microvillous inclusion disease (MVA/MVID)[8,9] have been characterized within the heterogeneous group of patients with IDI. An attempt to classify enteropathy-associated intractable diarrhea was proposed on the basis of immunohistologic criteria emphasizing the presence or absence of activated T cells in the intestinal mucosa.[10] A multicenter survey

TABLE 43.3-2 CAUSE AND OUTCOME OF PROTRACTED AND INTRACTABLE DIARRHEA OF INFANCY

CAUSE	PATIENTS (*n*)	DECEASED (*n*)
PROTRACTED DIARRHEA	39	1
Multiple food intolerance	15	0
Infectious enteritis	14	0
Colitis (including 2 CMV)	6	1
CDG syndrome	1	0
Ganglioneuroblastoma	1	0
Unknown	2	0
INTRACTABLE DIARRHEA	21	4
Abnormalities of the enterocyte		
Intestinal epithelial dysplasia	6	1
Microvillous atrophy	3	0
Autoimmune enteropathy	5	3
Phenotypic diarrhea	3	0
Undefined	4	0

Adapted from Goulet O et al.[5]
CDG = congenital disorders of glycosylation; CMV = cytomegalovirus.

from the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) collected cases of IDI and villous atrophy (excluding MVA/MVID) with precisely defined light microscopic characteristics to study categorization of disorders within IDI.[11] Several groups were delineated on the basis of clinical and histologic analyses. The first one presented with extradigestive symptoms suggestive of autoimmune enteropathy, including arthritis, diabetes, nephrotic syndrome, dermatitis, anemia, and thrombocytopenia, and tended to have a later onset of diarrhea, which was of larger volume, compared with a group of patients who had only gastrointestinal symptoms and gut autoantibodies. Two other groups included patients who did not exhibit mononuclear cell infiltration of the lamina propria. Some patients were small for gestational age and presented with phenotypic abnormalities corresponding to the previously described "syndromatic diarrhea."[12] Some other infants presented with mucosal changes, including mild to moderate villous atrophy, epithelial cell tufts, and branching and/or pseudocystic glands.[13] Family history was noted in 40% of these patients. The last group included infants with epithelial dysplasia and infants with villous atrophy in whom histologic analysis did not result in specific features being recognized.

Histologic analysis seems to be the most important point for the diagnosis of IDI. Patients present histologically in two clearly different forms: the first one is characterized by mononuclear cell infiltration of the lamina propria and is considered to be associated with activated T cells. The second pattern includes early onset of severe intractable diarrhea with villous atrophy without mononuclear cell infiltration of the lamina propria but specific histologic abnormalities involving the epithelium. To date, several types of primary epithelial abnormalities inducing IDI have been described. The first described was MVA/MVID and, more recently, tufting enteropathy or intestinal epithelial dysplasia.

MICROVILLOUS ATROPHY/MICROVILLOUS INCLUSION DISEASE

In 1978, Davidson and colleagues reported five infants with severe, persistent diarrhea beginning in the newborn period in which light microscopy revealed a thin mucosa, described as crypt hypoplastic villous atrophy.[14] Electron microscopic examination of small intestinal biopsies from three of the patients showed severe brush border abnormalities and increased liposome-like bodies and, in one, the features now recognized as the diagnostic features of MVA/MVID, intracytoplasmic cysts made up of brush border and increased secretory granules.[8] Further children were reported, each of whom had the characteristic microvillous inclusions and secretory granules.[8,15] From these first clinical and histologic descriptions, MVA/MVID has been established as a distinct disease within the syndrome of IDI, including characteristic diagnosis features. MVA/MVID in its typical form is a congenital disorder of intestinal epithelial cells presenting as intractable secretory diarrhea within the first days of life.[16]

The name MVID[9] was first used some years after the disease had been described as MVA.[8,15] Although it correctly highlights the presence of microvillous inclusions, it ignores the increased presence of secretory granules, which is the first abnormality to become evident in the epithelial life cycle. Indeed, the formation of microvillous inclusions may be secondary to the presence of the secondary granules. No useful purpose would be served by attempting to find a comprehensive name for the disease, and it is perhaps best to wait for the cause of the disease to be identified and to reconsider matters then.

Typical Clinical Expression of MVA/MVID

In general, infants develop severe secretory diarrhea within the first days after birth. They pass over 250 to 300 mL/kg body weight of fecal output per day containing electrolyte concentrations similar to those seen in small intestinal fluid. This disorder is particularly life-threatening because massive diarrhea leads to rapid dehydration and electrolyte imbalance, with subsequent metabolic decompensation within a few hours.[14–16] Severe watery diarrhea persists despite bowel rest. Differential diagnosis may include congenital chloride diarrhea[17] or sodium hydrogen exchange deficiency.[18] Both are usually associated with hydramnios. Blood and stool electrolyte assessment allows the diagnosis of each of these disorders to be easily made. Conversely, clinical presentation may sometimes be very atypical, with predominant occlusive syndrome with full and distended small bowel and colon. Some newborns have been thought to present intestinal pseudo-obstruction syndrome, and some have unfortunately been operated on for an ileostomy. After ruling out the diagnosis of congenital electrolyte malabsorption diarrhea, the diagnosis of MVA/MVID has to be considered, especially if the child has consanguineous parents and/or siblings have died from severed diarrhea in early infancy. In the largest ESPGHAN survey performed by Phillips and Schmitz, clinical variants were identified in which the symptoms were of late onset, that is, the onset of diarrhea was delayed after the neonatal period, and such cases have been termed "late-onset MVA."[16] It appears that diarrhea in these cases is less severe, and although secretory granules and microvillous inclusions are present, they are distributed differently to cases of congenital MVA/MVID (see below).[16]

Histologic Presentation

Histologic analysis of small bowel biopsies shows a variable degree of villous atrophy, generally without any inflammatory infiltrate. Highly characteristic for this disorder is the accumulation of periodic acid–Schiff (PAS)-positive secretory granules within the apical cytoplasm of enterocytes (Figure 43.3-1).[19–21] The abnormal staining pattern is first noted in upper crypt epithelial cells in congenital MVA but appears later in the epithelial life cycle in late-onset MVA, first becoming evident in low villous enterocytes.[21] A third, and very rare, variant, termed "atypical MVA," has the abnormal PAS stain arising in low crypt epithelial cells.[21] Apart from the increase in secretory granules, crypt cells otherwise appear near normal on electron microscopy in

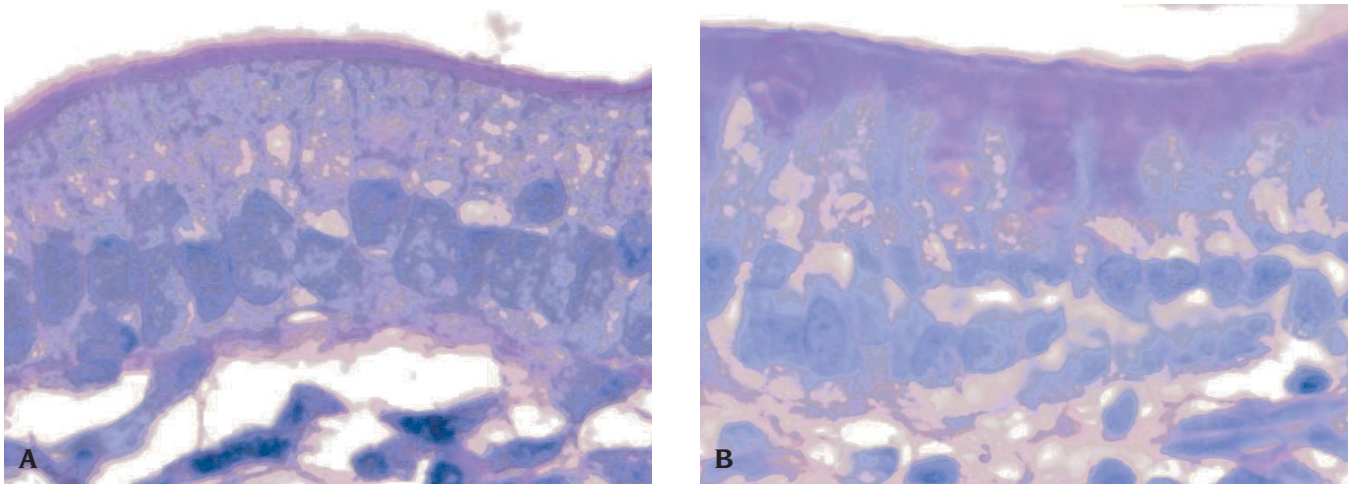

FIGURE 43.3-1 Microvillous atrophy/microvillous inclusion disease (MVA/MVID) (periodic acid–Schiff [PAS] staining; ×120 original magnification). *A*, Normal mucosa, normal PAS, brush border staining. *B*, Abnormal accumulation of PAS-positive material in the apical cytoplasm of epithelial cells in MVA/MVID.

congenital MVA/MVID, with a well-developed brush border. In contrast, in mid- to upper villous epithelial cells, there are rare or absent microvilli and an increased presence of autophagic vacuoles, along with the diagnostic presence of microvillous inclusions (Figure 43.3-2).[21] The lesion is also present in the large bowel,[8] and although it may be easier to biopsy the rectum and distal colon in early infancy, diagnostic information is not easy to discern owing to the increased presence of goblet cells.

Diagnosis can be strongly indicated by the light microscopic examination of proximal small intestinal mucosal biopsies. The thin, abnormal mucosa is not diagnostically specific on hematoxylin and eosin staining, and the description "hypoplastic" villous atrophy is incorrect because the crypts do not contain less cells than in normal mucosa, and there is a high epithelial cell turnover.[21,22] Diagnostic information can be obtained by performing PAS staining. This reveals an abnormal brush border pattern with positive staining material within the apical cytoplasm of enterocytes (see Figure 43.3-1B). It is important to realize that both secretory granules and microvillous inclusions will stain with PAS, the former producing an even apical intracytoplasmic stain, primarily in the upper crypt and low villous region, the latter giving a vacuolar and uneven pattern in older epithelial cells. The distribution of these two characteristic findings means that correct orientation of the biopsy sample is necessary for accurate evaluation.

There are reports of novel diagnostic stains that have been put forward as replacements for electron microscopy. The impetus for this comes from the consideration of the time it takes to perform transmission electron microscopy. However, it is perfectly possible to carry out rapid electron microscopy and to get an answer within 2 days of taking the biopsy. Alkaline phosphatase,[23] carcinoembryonic antigen,[24] and CD10 staining[25] have all been suggested, but the two former stains do not appear to stain secretory granules and only highlight microvillous inclusions. Indeed, it has been shown that the direct and indirect constitutive pathways in MVA/MVID are intact, and various disaccharidase and peptidase brush border enzymes are normally inserted

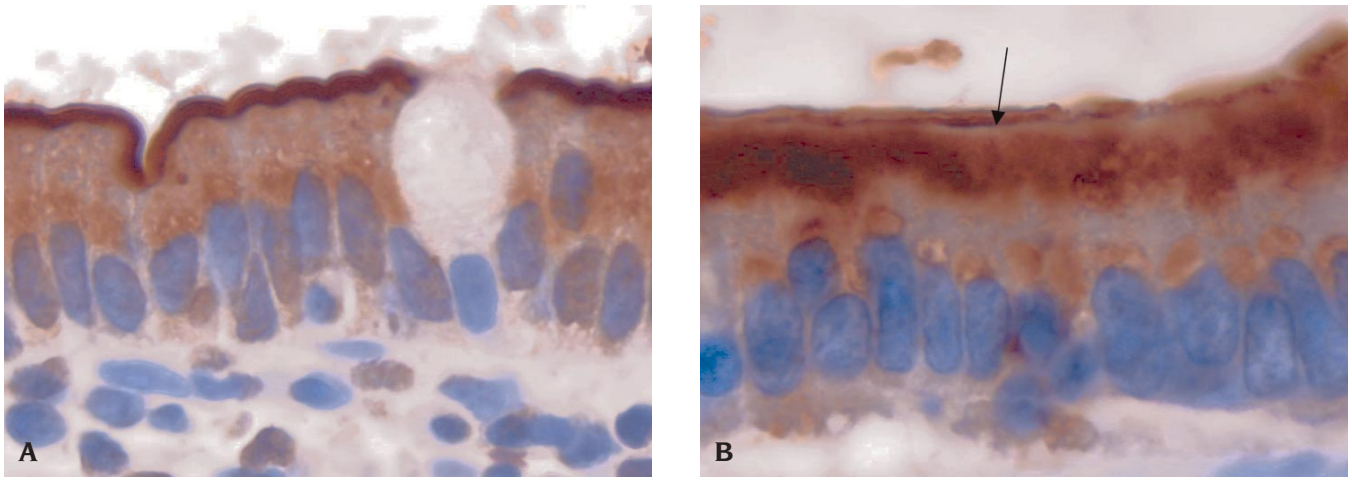

FIGURE 43.3-2 Microvillous atrophy/microvillous inclusion disease. CD10 immunostaining (×300 original magnification) in a control subject (*A*) and a patient with microvillous inclusion disease (*B*).

into the apical membrane and do not accumulate in secretory granules, although they do appear in microvillous inclusions.[26] However, CD10, a membrane-associated neutral peptidase with a linear brush border staining pattern in normal small intestine, appears to parallel PAS staining (see Figure 43.3-1B and Figure 43.3-3).[25,27]

Pathophysiology and Mode of Transmission

A defect in the membrane trafficking of immature and/or differentiating enterocytes was proposed as a potential pathogenetic mechanism of MVA/MVID,[20,26] leading, as a direct functional consequence, to complete intestinal failure. It has been speculated that the disease is associated with a disorder of the enterocyte cytoskeleton that produces an abnormal assembly of microvilli. However, morphologically normal microvilli appear in the crypt region in congenital and late-onset MVA, so it would appear logical that the microvillous changes seen in older cells are a consequence of another as yet unidentified event within the cell, perhaps linked to the accumulation of secretory granules. "Intestinal microvillous dystrophy" has been reported with the speculation that it is a hypothetical variant of MVA/MIVD.[28] However, the only reported similarity is in the abnormal microvilli, which is reported in many diseases, and MVA/MVID was excluded because there was no increase in secretory granules and no microvillous inclusions were seen. The underlying pathogenesis of MVA/MIVD is still very unclear, although a cytoskeletal myosin deficiency has been found,[29] indicating a possible molecular motor defect that could result in a trafficking abnormality. The nature of the secretory granules is not known. By using sucrase-isomaltase as a representative protein, it appears that the direct and indirect constitutive pathways are intact in MVA/MVID and that a defect in endocytosis is unlikely.[26] More recently, by investigating the glycobiologic nature of the epithelial accumulation of PAS, it is suggested that MVA/MVID involves a defect in exocytosis of the glycocalyx.[30] The absence of glycocalyx would have profound consequences on normal cell function.

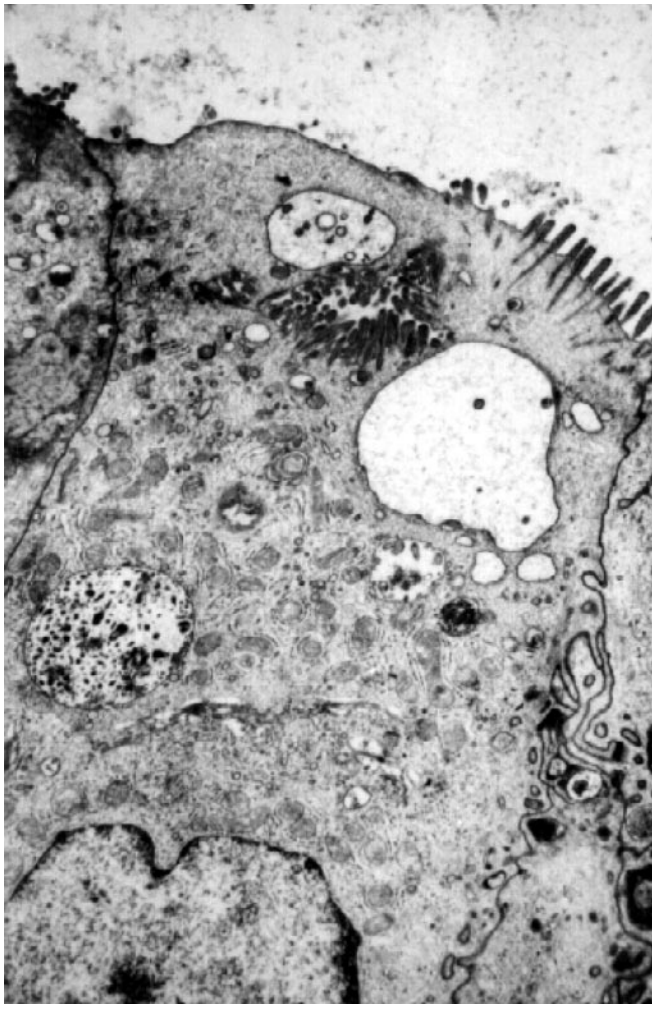

FIGURE 43.3-3 Microvillous atrophy/microvillous inclusion disease. Electron microscopy of jejunal biopsy specimen. The brush border is almost absent. The cytoplasm contains a microvillous inclusion.

Considering the number of cases with affected siblings and the frequency of consanguinity among parents with affected infants, this disease appears to be transmitted as an autosomal recessive trait.[14,16] No candidate genes for the disease have been identified to date. Affected siblings have been reported,[31] and MVID/MVA has been reported in children with autosomal dominant hypochondroplasia[32] and dihydropyriminidase deficiency.[33] The gene defect of the former disease has recently been localized on chromosome region 4p16.3[34] and the latter on 8q22[35]; thus, although they may be helpful in elucidating the genetic basis of MVA/MVID, the association may also be purely coincidental.

Long-Term Outcome

MVA/MVID is a congenital constitutive intestinal epithelial cell disorder leading, in its typical early-onset form, to definitive lifelong intestinal failure. A multicenter survey of 23 patients performed revealed an extremely reduced life expectancy in congenital MVA/MVID.[16] These patients had a 1-year survival rate of less than 25%. Most children died of septic complications, liver failure, or metabolic decompensation.

A minority of cases of MVA/MIVD may survive with a limited increased stool output and may require only partial PN.[36] Treatment with corticosteroids, colostrum, or epidermal growth factor has not been successful, but octreotide has been used with partial success in one patient.[16] In comparison with the poor initial outcome before the 1980s, PN now allows most infants and children to survive. However, complications related to inadequate PN (recurrent catheter-related sepsis, extensive thrombosis, fat overload syndrome, cholestasis) limit long-term survival. In addition, although without evidence of an associated renal disease, some of these infants and children presented with chronic electrolyte imbalance and acidosis with subsequent impaired growth. Others, because of repeated dehydration episodes associated with unchanged phosphate and calcium intake, presented with kidney calcification. Finally, even with adequate long-term PN and normal growth, most children continue to have a high and uncomfortable stool output that requires daily replacement with the daily risk of severe dehydration. Thus, intestinal

transplant became the only definitive treatment of this rare intestinal disease.

Place of Intestinal Transplant for MVA/MVID

Some cases of successful intestinal transplant for MVA/MVID have been reported following the use of a new immunosuppressive drug, tacrolimus (originally FK506).[37–40] Transplant has involved isolated intestine[37,40] or intestine combined with the liver.[38,39] In the report of these cases, there is an open discussion on whether the colon should be transplanted together with the small bowel.[37–40]

Ten consecutive patients with early-onset congenital MVA/MVID were evaluated between 1995 and 2002 at Necker-Enfants Malades Hospital (Paris) for potential small bowel transplant. Two patients died before they could be put on the waiting list, and one patient is still awaiting small bowel transplant. We performed cadaveric intestinal transplant in seven patients aged between 3 and 11 years in conjunction with the use of tacrolimus, steroids, and interleukin-2 blockers. Three isolated intestinal transplants and four liver-associated intestinal transplants were performed. Right colon transplant was performed in five cases (two isolated intestinal transplants). One patient died during transplant surgery from acute liver failure and hemodynamic shock, probably owing to a so-called reperfusion shock. The other six patients (86%) survived with a median follow-up of 3 years (range 1–8 years). Graft rejections occurred in two patients (one small bowel, one small bowel and liver) and responded favorably to methylprednisolone pulses. All patients were completely weaned from PN. The five patients with an additional colon graft were weaned off PN after a median 36 days postsurgery. In comparison, those without a colonic transplant obtained full intestinal autonomy several months after transplant. Thus, the Paris experience shows that small bowel transplant alone or in combination with liver transplant is highly successful in children with MVA/MVID, offering a longer-term perspective for patients for the first time. Additional colon grafting markedly improves outcome and quality of life after small bowel transplant in MVA/MVID. These preliminary results of intestinal transplant in this rare disease are very encouraging and demonstrate that the prognosis of MVA/MIVD has changed dramatically during the last decade.

Finally, in a child suspected of having MVA/MVID, a precise classification to one of three different variants (congenital [early onset], late onset, or atypical) should be obtained as early as possible and confirmed by an experienced pathologist according to clearly defined morphologic criteria. This is particularly important in light of the recent report of Croft and colleagues reporting on a 5-year-old girl with a late-onset form of MVA who was weaned from total PN and is thriving on a normal unrestricted diet.[41] There is some evidence to believe that this rare form of MVA has a better prognosis than the most frequent form of congenital MVA/MVID, which has a high mortality rate.[16]

Given the high success rate of small bowel transplant in MVA/MVID resulting in restored digestive autonomy, it is recommended that once the diagnosis of congenital MVA/MVID has been made and the child is in a eutrophic phase under total PN, transplant should be considered.[42–45] This approach should avoid PN-associated liver impairment. Conversely, patients with a late-onset or atypical form of MVA/MVID should not be automatically scheduled for small bowel transplant. The individual course of the disease will help to decide whether an individual child is a candidate for intestinal transplant.

INTESTINAL EPITHELIAL DYSPLASIA (TUFTING ENTEROPATHY)

In 1994, three cases of neonatal severe diarrhea with abnormal epithelial pictures were reported by Reifen and colleagues under the name of "tufting enteropathy."[46] One year later, Goulet and colleagues reported nine cases of severe neonatal diarrhea that were clearly different from MVA/MVID.[13] Further studies demonstrated that intestinal epithelial dysplasia is an epithelial disorder of the intestinal mucosa involving both small intestine and colon.[47] Intestinal epithelial dysplasia seems to be frequent in patients of Arabic, Middle Eastern, or North African origin.[13] One other characteristic of this recently described disease is its clinical and histologic heterogeneity and its association with malformation or other epithelial disease.

Clinical Expression

Typically, the patients present during the first weeks of life with severe diarrhea. Most have consanguineous parents and/or an affected sibling, some of whom have died during the first months of life with severe diarrhea of unknown origin. Most of the time, diarrhea persists in spite of bowel rest but at a lower level than in MVA/MVID patients, thus justifying attempts at continuous enteral feeding using protein hydrolysates or amino acids. Usually, continuous enteral feeding results in a worsening diarrhea; the newborn does not grow adequately and rapidly develops failure to thrive, with severe protein-energy malnutrition. The early onset of diarrhea may indicate a diagnosis of MVA/MIVD, but this is easily excluded by performing a proximal small intestinal mucosal biopsy with histologic investigations (see above). The disease is not well recognized owing to its recent description, and its clinical and histologic heterogeneity make diagnosis difficult.

Histologic Presentation

Villous atrophy is present but variable in severity. In typical intestinal epithelial dysplasia, abnormalities are localized mainly in the epithelium and include a disorganization of surface enterocytes with focal crowding, resembling tufts (Figure 43.3-4). These characteristic "tufts" of extruding epithelium, first described by Reifen and colleagues,[46] are seen toward the villus tip and may affect up to 70% of villi. The tufting process is not limited to the small intestine but involves colonic mucosa.[13] This picture can also be observed in crypt epithelium, and, in addition, crypts often have an abnormal aspect with dilatation, such as pseudocysts and abnormal regeneration with branching

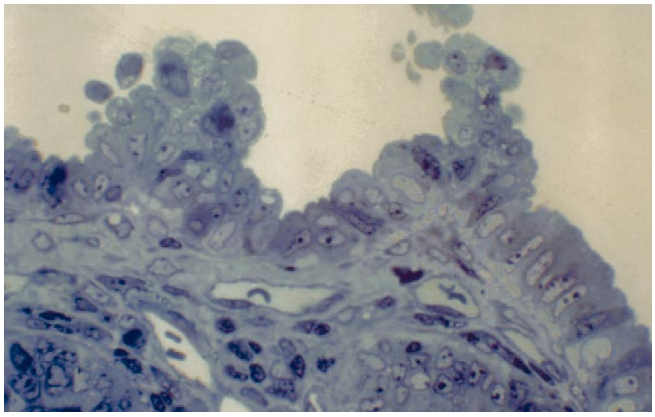

FIGURE 43.3-4 Intestinal epithelial dysplasia. Disorganization of surface epithelium showing tufts with apical rounding epithelial cells (hematoxylin and eosin; ×120 original magnification).

(Figure 43.3-5).[13] The study of basement membrane components has demonstrated an abnormal laminin and heparan sulfate proteoglycan deposition compared with biopsy specimens from patients with celiac disease or autoimmune enteropathy.[13] Relative to controls, there was faint and irregular laminin deposition at the epithelial lamina propria interface, whereas heparan sulfate proteoglycan deposits were large and lamellar, suggestive of an abnormal development of the basement membrane at the origin of the epithelial abnormalities. On the other hand, Patey and colleagues have shown an increased immunohistochemical expression of desmoglein and ultrastructural changes of desmosomes that are increased in length and number (Figure 43.3-6).[47] It is not clear if these changes are secondary or primary characteristics.

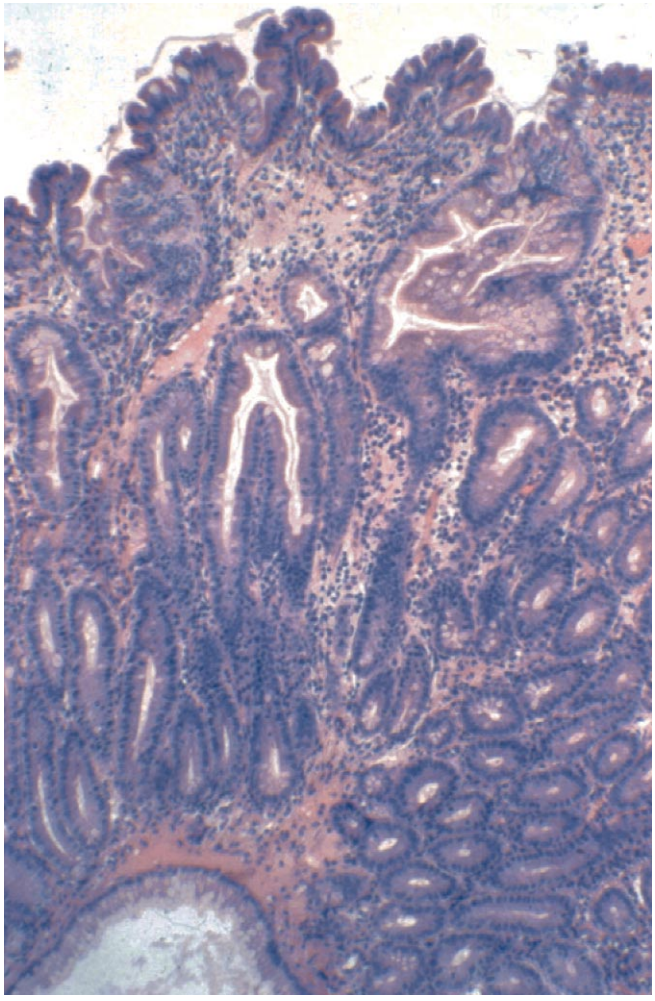

FIGURE 43.3-5 Intestinal epithelial dysplasia. Partial villous atrophy with crypt hyperplasia and/or pseudocystic crypt appearance, branching pictures, and disorganization of surface epithelium (hematoxylin and eosin; ×120 original magnification).

Difficult Diagnosis

Diagnosis of intestinal epithelial dysplasia may be difficult for several reasons. Despite the early onset of severe and permanent diarrhea and failure to demonstrate microvillous inclusion disease, the characteristic "tufts" of extruding epithelium do not appear clearly. In the initial experience, diagnosis has been made late in the illness, whereas initial biopsies performed during early life showed only nonspecific villous atrophy with or without monocellular cell infiltration of the lamina propria. Thus, only strict elimination of microvillous inclusion disease and repeated duodenal or jejunal biopsies allow diagnosis. In addition, it is rare and difficult to show any specific abnormalities of basement membrane components, integrins, or desmosomes in the mucosa in the absence tufts. The second difficulty is related to the infiltration of the lamina propria by T cells, some of which are being activated. Such infiltration suggests the hypothesis of immune-related enteropathy as previously described, particularly when tufts are missing or ignored. Defective cell adhesion toward the villus tips leads to increased intestinal permeability with subsequent severe secondary inflammation. A mouse model of dysfunctional E-cadherin clearly demonstrated that primary disorders of epithelial permeability or integrity could induce secondary T cell–mediated changes.[48] These changes were reported by Murch and colleagues in infants with epithelial dysplasia.[49] They showed that inhibition of secondary T-cell activation significantly improved enteral absorption and decreased crypt cell proliferation, although without permanent resolution of the severe diarrhea.

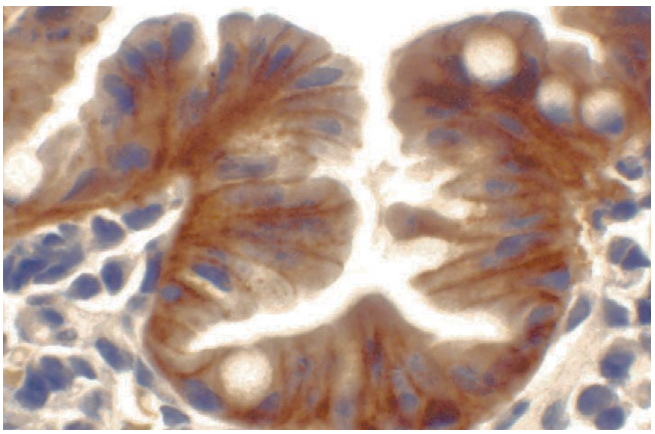

FIGURE 43.3-6 Intestinal epithelial dysplasia (desmoglein stain; ×300 original magnification).

Associated Disorders

Several cases of intestinal epithelial dysplasia have been reported as being associated with phenotypic abnormalities, for example, Dubowitz syndrome or malformative syndrome.[50,51] An association between congenital IDI and choanal atresia was recently reported in four children.[50] Djeddi and colleagues observed malformations, including rectal atresia or esophageal atresia. In addition, it became very common to detect punctiform keratitis in infants with intestinal epithelial dysplasia.[52] We recently reported an associated nonspecific punctiform keratitis in about 50% of patients with clinically and histologically recognized intestinal epithelial dysplasia. This association is important to know for diagnostic evaluation, which should include a systematic ophthalmologic examination by an experienced specialist. The fact that some children do not have such abnormalities confirms the heterogeneity of the disease.

Interestingly, Lachaux and colleagues have recently reported a case of a newborn presenting with pyloric atresia and intractable diarrhea.[53] Light microscopic examination showed extensive desquamation from the fundus to the rectum, with only a few epithelial cells remaining at the base of the crypts. Electron microscopy of the gut revealed normal desmosomes but a cleavage located between the lamina propria and the basal pole of the enterocytes. This first described disease is supposed to be related to congenital deficiency of $\alpha_6\beta_4$ integrin. This integrin is known to be defective in epidermolysis bullosa, in which gross epidermal shedding occurs, although the cutaneous expression of $\alpha_6\beta_4$ integrin appeared normal in this case of IDI. This is consistent with a mutation within an intestinal isoform of the $\alpha_6\beta_4$ integrin or a deficiency of a related and immunohistochemically cross-reactive intestinal integrin.[54,55] Rather like epidermolysis bullosa, which shares several similarities at the ultrastructural level and possibly the molecular level, there are likely to be several distinct mutations that can cause this phenotype.

Pathophysiology and Mode of Transmission

In infants with intestinal epithelial dysplasia with characteristic tufts, Goulet and colleagues reported abnormal laminin and heparan sulfate proteoglycan deposition on basement membrane compared with biopsy specimens from patients with celiac disease or autoimmune enteropathy.[13] Basement membrane molecules are involved in epithelial mesenchymal cell interactions, which are instrumental in intestinal development and differentiation.[56–59] Alterations suggestive of abnormal cell-cell and cell-matrix interactions were seen in patients with epithelial dysplasia without any evidence for abnormalities in epithelial cell polarization and proliferation.[47] Alterations included abnormal distribution of adhesion molecules $\alpha_2\beta_1$ integrin along the crypt-villous axis. The $\alpha_2\beta_1$ integrin is involved in the interaction of epithelial cells to various basement membrane components, such as laminin and collagen. There is no clear explanation for the reported increased immunohistochemical expression of desmoglein or for the ultrastructural changes affecting desmosomes, which are increased in length and number (see Figure 43.3-6).[47] Tufts correspond to groups of nonapoptotic cells that are no longer in contact with basement membrane. The increased cell-cell adhesion suggested by the modification of desmoglein distribution and the ultrastuctural changes of desmosomes in tufted cells might contribute to the inhibition of apoptosis. A mouse in which the gene encoding the transcription factor Elf3 is disrupted has morphologic features ressembling epithelial dysplasia in infants.[60] In this mouse model, there is abnormal morphogenesis of the villi while progenitor crypt cells appear normal. The enterocytes in Elf3-deficient mice produce low levels of transforming growth factor-β type 2 receptor, which induces the differentiation of immature intestinal epithelia. Currently, in human infants, the primary or secondary nature of the observed modifications remains to be determined. Both the clinical studies and the findings in experimental animal models should provide clues to the pathogenesis of these epithelial abnormalities and to the severity of this neonatal diarrhea.[60,61]

To date, the genetic origin of this disorder is suspected from the clear association of parental consanguinity and/or affected siblings. These features suggest an autosomal recessive transmission. The gene involved in this congenital inherited autosomal recessive disease remains to be found. This enteropathy, in which epithelial shedding clearly proceeds aberrantly, has been reported as a cause of IDI and may be more common than MVA/MVID, especially within the Middle East and Maltese population. The above-described associated disorders, especially punctiform keratitis, might be helpful in approaching the genetic nature of this rare and severe epithelial disease.

Outcome

This neonatal diarrhea, which resists all treatments, requires permanent PN. However, it seems that some infants have a rather milder phenotype than others.[62] Because of partial intestinal function and limited amount of stool output, some patients need only partial long-term PN with 3 to 4 times weekly infusion. However, careful monitoring should be performed to avoid progressive growth retardation. In most patients, the severity of intestinal malabsorption and diarrhea makes them totally dependent on daily long-term PN, with the subsequent risk of complications. It is thus another indication for intestinal transplant.[63–65] There is a suggestion that intestinal epithelial dysplasia may ameliorate with age,[62] but this needs further study.

SYNDROMATIC DIARRHEA

These patients present with diarrhea starting in the first 6 months of life (< 1 month in most cases) and have several features in common. They are small for gestional age and have an abnormal phenotype.[12] All have facial dysmorphism with a prominent forehead, a broad nose, and hypertelorism (Figure 43.3-7). They have a distinct abnormality of hair, tricorrhexis nodosa, in which the hair is woolly, difficult to manage, easily pulled out, and poorly pigmented even in children of Middle Eastern origin. In addition, the

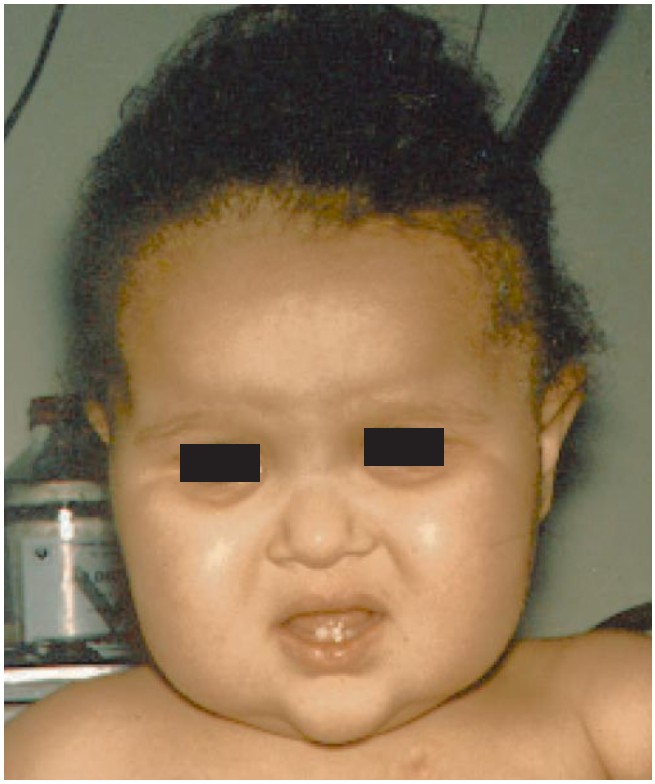

FIGURE 43.3-7 Syndromatic diarrhea. Patient with an abnormal face, a prominent forehead, a broad nose, and hypertelorism.

previously reported patients had defective antibody responses despite normal serum immunoglobulin levels and defective antigen-specific skin tests despite positive proliferative responses in vitro.[12] Small bowel biopsy specimen of the patients with syndromatic diarrhea show moderate or severe villous atrophy with variable mononuclear cell infiltration of the lamina propria and absence of epithelial abnormalities. Histologically, there are no specific abnormalities. Prognosis of this type of intractable diarrhea of infancy is poor because most patients have died between the ages of 2 and 5 years, some of them with early onset of liver disease.[12] The cause of this diarrhea is unknown, and the relationship between low birth weight, dysmorphism, severe diarrhea, trichorrhexis, and immune deficiency is unclear. Among the congenital forms of hair dysplasia, tricorrhexis nodosa is very common and can be present in several pathologic conditions.[66–70]

The coexistence of morphologic, trichologic, and immunologic abnormalities with early-onset intractable diarrhea disproportionate to the mucosal architectural abnormality (consistent with a primary enterocyte abnormality) suggests either mutation within several genes, inherited together by linkage disequilibrium, or more probably interference with a higher level of control, such as a patterning gene. The characteristic hair abnormalities may allow a more focused search for candidate mutations because relatively few patterning genes have been implicated in hair development. These include suggestively lymphoid enhancer factor 1, as well as fibroblast growth factor 4, Sonic hedgehog, and bone morphogenetic protein 2.[71,72] In addition, transgenic knockout mice null for various FGH and EGH family members exhibit a range of defects in hair development.[72]

Syndromatic diarrhea represents a separate entity within the syndrome of intractable diarrhea of infancy and seems different from trichohepatoenteric syndrome. This syndrome has been described in two siblings presenting with neonatal hemochromatosis phenotype, intractable diarrhea, and hair anomalies.[66] Prenatal symptoms were intrauterine growth retardation, hydramnios, and placental hyperplasia. Clinical anomalies included hypertelorism and sparse, thin, curly hair (trichomalacia). Clinical course was marked by intractable diarrhea, with normal histologic and enzymologic studies, cholestatic jaundice, hepatomegaly appearing after 30 days, and progressive liver failure, leading to death after a few months. The only metabolic anomaly was progressive hypermethioninemia. Pathologic examination of both children showed a similar pattern of multivisceral iron deposit compatible with a diagnosis of neonatal hemochromatosis.

Finally, dysmorphologenetic aspects, hair abnormalities, and immune function should be reassessed in other cases of syndromatic diarrhea, and relationships with other disorders should be sorted out. Immune dysfunction seems to be an integral part of the disease but needs to be further characterized.[73]

OTHER DISEASES OF INTESTINAL EPITHELIUM

The classification of IDI is probably incomplete because other forms with abnormal small bowel mucosa have been described, including mitochondrial deoxyribonucleic acid (DNA) rearrangements,[74] congenital enterocyte heparan sulfate deficiency,[75] phosphomannose isomerase deficiency, and a carbohydrate-deficient glycoprotein syndrome with hepatic-intestinal presentation.[76,77] Rare diseases involving the immune system and small bowel mucosa[78] or severe intractable enterocolitis of infancy[6] also seem clearly different from the above-described epithelial diseases.

In conclusion, progress has been made within this heterogeneous group of patients in the characterization of different conditions. When confronted with the diagnosis of IDI, clinical and histologic data should be taken into account. Such an analysis may allow a broad classification, even in the absence of immunohistochemical studies. The main clinical criteria that should be considered are birth weight, familial history, date of onset and characteristics of diarrhea (watery and/or bloody, persistent despite bowel rest), the existence of extradigestive manifestations, the presence of gut and/or other autoantibodies, and the existence of phenotypic abnormalities. Protracted diarrhea of infectious or allergic origin and/or immunodeficiency has to be eliminated. MVA/MVID can be diagnosed easily by performing PAS staining and electron microscopy. Tables 43.3-1 and 43.3-2 summarize the features of distinctive disorders within the syndrome of intractable diarrhea with persistent villous atrophy. The proposed classification is provisional but indicates that there are different pathogenetic mechanisms operating in

the clinical groups of patients with IDI and may allow specific treatment to be pursued.

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CHAPTER 44

ENTEROPATHY

1. *Celiac Disease*

Markku Mäki, MD, PhD
Olli Lohi, MD, PhD

Samuel Gee accurately described the classic form of childhood celiac disease, the malabsorption syndrome in infancy, in 1888.[1] Throughout the twentieth century, celiac disease has puzzled clinicians and investigators. The major breakthrough and milestone in understanding the disease came from pioneering clinical research performed by Dr. Willem Karel Dicke in the Netherlands. He discovered the harmful effect of dietary gluten in celiac disease.[2] He writes in his thesis summary that the harmful effect on patients with celiac disease is not caused by all cereals but specifically by wheat flour. Also, rye elicited or aggravated the clinical symptoms and signs, but wheat starch did not give rise to the phenomena. With construction of a peroral intestinal suction biopsy apparatus in the late 1950s, it became evident that gluten induces small intestinal villous atrophy and crypt hyperplasia. In the 1960s, it was shown that the mucosa heals on a gluten-free diet, and it became evident that gluten intolerance in celiac disease is permanent.[3] One important milestone in celiac disease clinics was the discovery of the circulating gluten-dependent autoantibodies,[4,5] the importance of which became evident only later. Celiac disease aggregation in families was a first clue to genetic susceptibility,[6] and evidence for a primary association of the disease with human leukocyte antigen (HLA)-DQ2 was shown.[7] In 1997, a new era in celiac disease clinics and research began with the discovery of transglutaminase 2 (TG-2; former tissue transglutaminase) as the autoantigen for celiac disease, the human antigen targeted by serum autoantibodies.[8]

DEFINITION OF CELIAC DISEASE

The synonyms in the literature for celiac disease are celiac sprue and gluten-sensitive enteropathy. Celiac disease is an autoimmune-like systemic disorder in genetically susceptible persons perpetuated by the daily-ingested gluten cereals wheat, rye, and barley with manifestations in the intestine and in organs outside the gut. Today it is understood that the nature of celiac disease is much more complex than simply intestinal malabsorption, which, as such, is, in fact, no longer essential for the diagnosis.

GLUTEN: THE MAJOR ENVIRONMENTAL TRIGGER

Cereals are cultivated almost all over the world and form one important component in human nutrition. Wheat, rice, and corn are spread worldwide, and rye, barley, oat, millet, and sorghum are of importance for specific regions. Figure 44.1-1 shows the taxonomic relationship of the gluten-containing cereal grains that activate celiac disease. Wheat, rye, barley, and oat are, by definition, "gluten." Of these, the first three with excellent baking properties belong to the tribe Triticeae, whereas oat belongs to a different tribe but to the same grass subfamily as wheat, rye, barley, and rice.

Intestinal inflammation and damage in celiac disease are tightly dependent on dietary exposure to prolamins present in wheat, barley, and rye. Prolamins are characterized by their high content in glutamine (35–37%) and proline (17–23%) residues. Wheat gluten consists of a complex mixture of many gliadin and glutenin polypeptides. Gliadins are monomers, whereas glutenins form large polymeric structures. Gliadins are 250– to 300–amino acid–long polypeptides, and on the basis of their amino acid sequences, they can be subdivided into α-, γ-, ω-gliadins and glutenins divided into high- (650–800

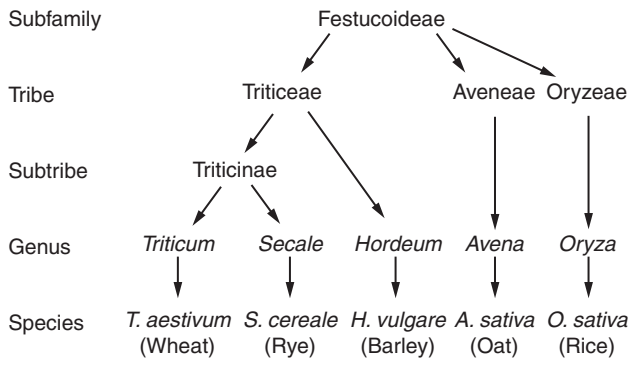

FIGURE 44.1-1 Taxonomy relationships of major cereal grains. Adapted from Kasarda DD.[9]

residues long) and low- (270–330 residues long) molecular-weight proteins. Furthermore, toxic prolamin peptide structures can be found in rye (secalins) and barley (hordeins),[9] which are similar to wheat gliadin peptides.[10] T-cell stimulatory peptides, as discussed below, are also to be found in rye and barley prolamines but not in avenins from oat.[11] However, looking at the literature in a modern, evidence-based manner, only wheat has been scientifically proven to induce disease. Regarding rye and barley, the evidence is based more on consensus, and clinical research has been scarce. Rice, corn, buckwheat, millet, and sorghum are not harmful for celiac disease patients. The case of oat, which is still under debate, is discussed separately. Also, other environmental triggers may be operative, but whatever the other "secondary hits" might be, the disease responds favorably when only one trigger is removed, the dietary gluten.

Oat and Celiac Disease

Even if oat is defined as gluten, its toxicity in celiac disease was readdressed in the early 1990s.[12] When the literature was re-evaluated, it was clear that earlier evidence was not thorough. Some studies were interpreted to prove that oat is harmful[13,14]; some stated that oat can be consumed by celiac disease patients without detrimental effects.[15–17] The reliability of these studies could be questioned because the evidence was based on a few patients, 1 to 12 studied cases, and none of the studies were controlled. In one study only, small intestinal biopsies had been performed.[17] In the study by Janatuinen and colleagues, oat proved to have no detrimental effect on nutritional status, laboratory values, or small intestinal mucosa in celiac disease patients in remission at 6 months, nor did it prevent the intestinal mucosa of newly diagnosed patients from healing after 1 year of treatment.[12] These results were later confirmed in other studies.[18–21] It has also been suggested that including oat can help celiac disease patients follow a strict gluten-free diet.[22] Dermatitis herpetiformis patients, who are thought to be very sensitive to gluten, do also seem to tolerate oat.[23,24] In the United States, Hoffenberg and colleagues first showed oat to be nonharmful for children with celiac disease.[25] In a recent 1-year randomized double-blind study comprising 93 children, a moderate amount of oat in a gluten-free diet did not prevent clinical or small intestinal mucosal healing or a humoral immunologic response.[26] Cereal toxicity studies using in vitro organ culture also point in the direction of nonharmfulness.[27,28] However, very recently, a patient intolerant to oat was identified.[29] It seems evident that, in rare cases, there are celiac disease patients who are intolerant to oat. They should be identified, and their diet should be designed not to contain oat.

GENETIC FACTORS

Celiac disease is a multifactorial disease in which, in addition to environmental triggers, inherited factors confer susceptibility. A clear observable clue to genetic susceptibility is the tendency of the disease to run in families.[6] The disease prevalence among the first-degree relatives of a proband has varied from 1 to 18%, often, as a rule, at about 10%.[30] The low-prevalence figures may be due to different screening strategies or the tendency of the disease to develop late, meaning that it is found in individuals who once had been excluded for the disease.[31,32] Celiac disease is also found in healthy relatives of multiple case families.[33,34]

The concordance and discordance rates of monozygotic and dizygotic twin pairs are often compared to define the balance between environmental and genetic factors of a disease. In celiac disease in a large twin study, Greco and coworkers found the concordance rate of monozygotic twins to be 86% compared with 20% in dizygotic twins.[35] This indicates that genes play a very important role. The literature is also knowledgeable of reported discordance in monozygotic twins, in whom concordance has developed later.[36] One should also remember that monozygotic twins are genetically not identical. The differences come from point mutations, skewed X-chromosomal inactivation, nondisjunctions, and gene rearrangements of immunoglobulin and T-cell receptors. Recently, it was clearly shown that one identical twin may have celiac disease, whereas the other suffers from dermatitis herpetiformis.[36]

The major histocompatibility complex (MHC) on chromosome 6 short arm (Figure 44.1-2) comprises a gene cluster including the HLA class II genes. The HLA genes are highly variable and polymorphic, which forms the basis for the immune system to recognize foreign and self-antigens, processed and presented by antigen-presenting cells. Certain HLA alleles are found to be associated with diseases, either in a protective or a predisposing manner, and many of these diseases are of an autoimmune nature. Celiac disease is strongly associated with the HLA class II extended haplotype DR3-DQ2 or DR5/7-DQ2. The DQ2 molecule, an α/β heterodimer, is located on the surfaces of cells involved in immune responses and is encoded by the alleles *DQA1*0501* and *DQB1*02* (in *cis*, in which both risk alleles are located in the same chromosome in linkage with DR3, and in *trans*, in which the individual is heterozygote for DR5 and DR7) (see Figure 44.1-2). Approximately 90% of the celiac disease patients carry the DQ2 molecule, and 10% of the patients are positive for DQ8 (DR4 haplotype), in which the α/β heterodimer is encoded by the genes *DQA1*0301* and *DQB1*0302*.[7,37,38] A large European multicenter study gave further evidence of the strong association to the risk alleles described above: from 1,008 celiac disease patients, 61 were identified to be negative for both DQ2 and DQ8. Fifty-seven of these encoded half of the DQ2 heterodimer. Only 4 were found to be negative for all known risk alleles.[39] Individuals not carrying any of the risk genes have a very low probability of developing celiac disease. Clinicians may take advantage of this knowledge, and a negative test for both DQ2 and DQ8 speaks against celiac disease.[40] On the other hand, even if most celiac disease patients carry the risk alleles, it must be borne in mind that in whites, 15 to 20% of the general population are DQ2 positive and another 20% are DQ8 positive. Owing to the extremely low DR3 gene frequencies in countries such as China and Japan and in black African countries, the probability of diagnosing celiac disease is very low.

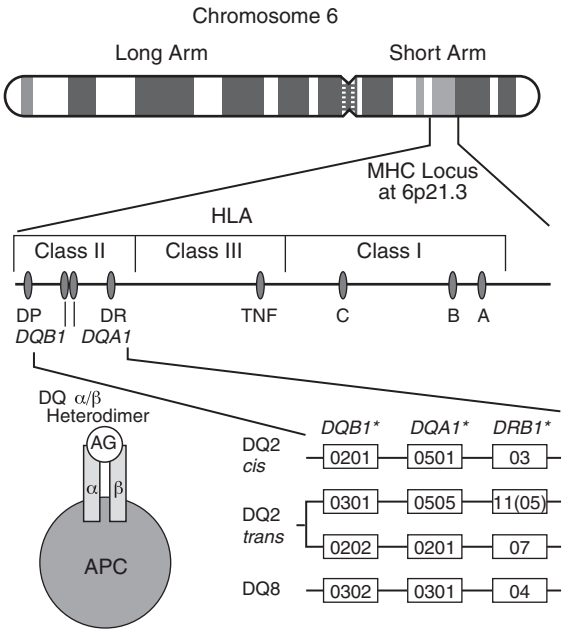

FIGURE 44.1-2 Human major histocompatibility complex (MHC) location in chromosome 6 short arm. In this locus, the key genetic risk factors for celiac disease and dermatitis herpetiformis, the human leukocyte antigen (HLA) class II genes *DQA1*05* and *DQB1*02* encoding the DQ2 α/β heterodimer molecule, are located. Individuals who have DR3 (the gene *DRB1*03*) almost always, owing to linkage disequilibrium, carry also the DQ alleles *DQA1*0501* and *DQB1*0201* in the *cis* position, that is, on the same chromosome. The genes encoding the DQ2 molecule may also be on different chromosomes, in *trans* position, *DQA1*05* in linkage with DR5 (*DRB1*11*) and *DQB1*02* in linkage with DR7 (*DRB1*07*). The genes encoding the DQ8 α/β heterodimer are located in the DR4 haplotype. The HLA class II molecules are situated on antigen-presenting cells (APC), and they have a groove where the antigen (AG) peptides are presented to T helper cells.

Disease concordance between HLA identical siblings is much lower than that in monozygotic twins, being approximately only 30%.[41] HLA genes are important but not sufficient to predispose them to celiac disease. Therefore, it is probable that other genes outside the HLA region are involved in celiac disease susceptibility. Recently, autosomal genomic screenings have provided evidence of linkage of several non-HLA loci to celiac disease.[42,43] There is now strong evidence of linkage at two non-HLA regions, chromosomes 5q31-33[44] and 19p13.1.[45] The linkage to a certain chromosome region does not, however, indicate which genes might be involved. One of the future targets for celiac disease research is to identify these genes and study their role in the disease.

PATHOGENESIS

Several major hypotheses regarding the nature of the primary host defect in celiac disease have been proposed during the past decades, namely, the missing enzyme theory, the immunologic hypothesis, the membrane glycoprotein defect, and the mucosal permeability defect. When the pathogenetic mechanisms are known, it may turn out that all of the above-mentioned and some so far unknown mechanisms are operative. Today there is evidence that celiac disease is a T cell–mediated chronic inflammatory bowel disorder with an autoimmune component, also with extraintestinal manifestations. There are three prominent features of celiac disease: (1) its remission is highly dependent on a strict gluten-exclusion diet; (2) it requires a unique genetic background for antigen presentation—expression of the HLA type II molecules DQ2 or DQ8; and (3) patients have specific circulating autoantibodies to the ubiquitous TG-2.[8] What is remarkable is the connection between these three characteristics: TG-2–deamidated (see below) gluten peptides presented in the context of DQ2 or DQ8 molecules stimulate proliferation of T cells derived from celiac patients and secretion of inflammatory cytokines, particularly interferon-γ, the expression of which is increased in active celiac disease. Parallel to this, B cells receive signals and, by an unknown mechanism, turn against the self, TG-2, and start producing autoantibodies. The role of TG-2 autoantibodies in the pathogenesis of celiac disease is unclear and controversial, although they have been shown to inhibit the differentiation of T84 crypt-like cells in a three-dimensional tissue culture model.[46] Interestingly, these autoantibodies can be found deposited in the subepithelial region of normal-appearing intestinal biopsies, targeting TG-2 even before the infiltration of T cells into lamina propria or the onset of evident celiac disease or even before the antibodies are measureable in the sera of patients.[47]

The DQ2 and DQ8 molecules are responsible for presenting celiac disease–related gluten peptides to T cells in the small intestine. When biopsies from treated celiac disease patients are challenged in vitro with gluten, the result is a strong stimulation of $CD4^+$ lamina propria T cells.[48] These T cells express α/β T-cell receptor, and they can be isolated and cultivated. Importantly, the T cells recognize only gluten peptides presented by DQ2 or DQ8 molecules.[49] The groove of HLA-DQ2 and -DQ8 molecules contains positively charged basic amino acid residues in several anchor positions and, therefore, predicts a preference for negative charges at critical positions of the bound peptides. However, gluten proteins are rich in proline and glutamine and contain few negatively charged residues. This obvious puzzle can be explained by the post-translational modification of glutamine residues in gluten by TG-2 enzyme.[50] TG-2 is a Ca^{2+}-dependent enzyme that modifies a whole range of proteins by crosslinking, transamidating, or deamidating specific polypeptide-bound glutamines.[51] It has been shown that TG-2 can deamidate gluten peptides in situ and that the lesion-derived T cells predominantly recognize deamidated gluten peptides, that is, peptides in which certain glutamine residues have been converted to negatively charged glutamic acids. Deamidation increases

the binding affinity of gliadin peptides for DQ2 and DQ8.[52] Detailed characterization of these peptides has demonstrated that there is no single pathogenic motif but instead many DQ2- and DQ8-restricted epitopes. The current estimate is that there are at least 50 T cell–activating epitopes present in gluten. T-cell populations that have several distinct gluten specificities can be derived from a single patient. On the other hand, there is a hierarchy of the epitopes in that some epitopes are recognized by T cells from almost all patients, whereas others have reactivity only in a minority of celiac disease patients.[53] One remarkable feature of T cell–stimulating gluten regions is the high content of proline residues. It is evident that prolines play a key role in determining the structure, immunogenicity, and proteolytic resistance of immunodominant gluten peptides. Experiments have shown that treating α_2-gliadin with a physiologic repertoire of digestive enzymes leads to the survival of 33-mer peptide fragment (Figure 44.1-3).[54] The 33-mer fragment has turned out to be an excellent substrate of TG-2, and it has been shown to be able to activate T-cell clones derived from celiac disease patients. It contains a cluster of partially overlapping T cell–stimulating epitopes (oligomerized epitopes). This kind of repetitive nature of epitopes enhances their T-cell activation potential. Intriguingly, degradation and loss of the antigenicity of 33-mer peptide could be induced by the addition of bacterial prolyl endopeptidase. This finding has opened up the possibility that specific prolyl endopeptidases could be used to detoxify this and potentially other proline-rich, digestion-resistant gluten peptides.

In active celiac disease, the level of TG-2 expression is increased: TG-2 is expressed at the epithelial brush border and extracellularly in the subepithelial region.[8,50] The pH in the proximal small intestine is about 6.6. This should enable the deamidation of gluten peptides in the luminal side of small intestine because it has been demonstrated that the propensity for deamidation compared with transamidation is markedly increased when the pH is below 7.3.[55] Another possibility is that TG-2 is endocytosed and active during the early stages of the endocytic process, when the pH is slightly acidic. Thus, gluten peptides could undergo TG-2–mediated deamidation during the endocytosis and before the peptides bind to the DQ2 or DQ8 molecules in the endosomes of antigen-presenting cells.

A crucial question in understanding the pathomechanisms of celiac disease is why only gluten, of the many food proteins, brings about such a harmful immune response. The increased level of TG-2 expression in the mucosa of celiac patients seems to be related to inflammation. Infection and inflammation, in turn, might breach the epithelial barrier of intestine and lead to harmful influx of gluten peptides into the lamina propria. The higher concentration of gluten peptides in the lamina propria and the abundant TG-2 would promote the formation of deamidated peptides. In experimental animal models, oral administration of antigens induces systemic hyporesponsiveness, defined as oral tolerance.[56] Although it is not clear if oral tolerance operates in humans by this definition, it is obvious that the immune system of the gut tries to ensure that immune responses to food proteins are not mounted normally. Therefore, it can be envisaged that tolerance to gluten in celiac disease is either not established or is broken in early childhood. The absence of intestinal T-cell responses to gluten in nonceliacs and the preferential recognition of deamidated gluten peptides suggest that there is, in the beginning, tolerance to unmodified gluten fragments. Accordingly, deamidation and the altered affinity of modified peptides for DQ2 and DQ8 would be the key event leading to the disruption of tolerance in celiac disease patients. Another possibility is that gluten could function as an adjuvant factor in eliciting T-cell responses, or short gluten peptides might reach the underlying lamina propria of the gut at higher concentrations than do other food protein peptides.

Recently, a novel aspect of the pathogenesis of celiac disease has been described. By using in vitro organ cultures, Maiuri and colleagues demonstrated that intestinal immune reactions in celiac disease are partly driven by the (less specific) innate immune system, which provides a quick, so-called pattern recognition response to stimuli such as viral and bacterial proteins.[57] Maiuri and colleagues' data propose that certain gluten peptides elicit an early innate immune response (the toxic peptide[10,58]; see Figure 44.1-3), whereas immunodominant peptides[59] drive much slower adaptive immunity (which is dependent on HLA presentation, T-cell recognition, and T-cell expansion), and that in the pathogenesis of celiac disease, there is an interplay between these mechanisms.

CLINICAL DISEASE

Malabsorption Syndrome

Characteristically, celiac disease manifests during infancy and before school age. In the classic form of childhood celiac disease, symptoms and signs of malabsorption become obvious within some months of starting a gluten-

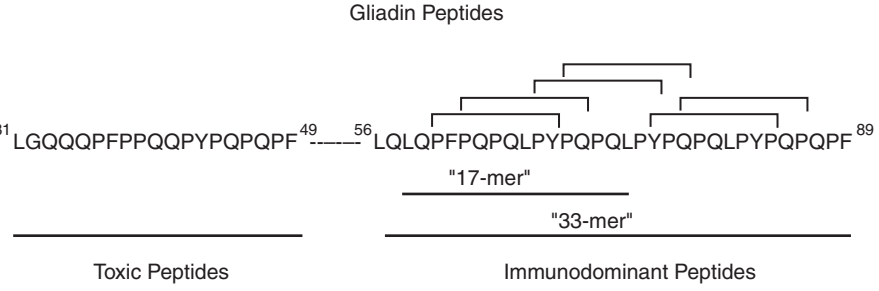

FIGURE 44.1-3 Amino acid sequences of part of domain I of A gliadin showing three proline- (P) and glutamine- (Q) rich peptides known to be either toxic or immunogenic for celiac disease (explained in the text). Within the immunodominant peptides, especially the 33-mer, several T-cell epitopes can be identified (oligomerized epitopes).

containing diet. The child has chronic diarrhea or loose stools and vomiting, and the abdomen is distended (Table 44.1-1 and Figure 44.1-4). Typically, the child presents with failure to thrive, and proximal muscle wasting may be seen. These symptoms are those of classic celiac disease (see Figure 44.1-4). Young infants with a malabsorption syndrome and prolonged diarrhea, as presenting in the 1960s,[60–63] may be hypotonic and have other symptoms of severe enteropathy, for example, dehydration, hypoproteinemia, hypokalemia, hypoprothrombinemia, and hypocalcemia. Rickets may be a presenting symptom even in one-fourth of the children diagnosed for celiac disease.[64] In some communities, the delay in diagnosis might be due to the presence of other diseases clinically resembling celiac disease.[64] Table 44.1-2 lists disease conditions that may be interpreted as celiac disease also at a small intestinal mucosal level.

Presenting signs of malabsorption in celiac disease in older children may be short stature,[65–68] delayed puberty,[67] iron deficiency anemia,[67,69,70] and osteoporosis.[71] The older the child is, the more diffuse are the symptoms, and gastrointestinal symptoms may be totally missing.

Changing Clinical Features

Nowadays, celiac disease presenting with a malabsorption syndrome is an exception, not the rule, and a changing symptom pattern has been experienced in most countries.[72] During the early 1980s, it was reported that childhood celiac disease was disappearing,[73–75] but this seemed not to be true. Rather, clinicians were experiencing a changing pattern of disease presentation with milder symptomatology and an increase in the age at diagnosis (Figure 44.1.-5).[67,76] Later evidence has verified this to have been a general trend throughout Europe.[77]

One exception existed: Sweden. Ivarsson and colleagues described a countrywide epidemic of a chronic malabsorptive syndrome in infancy during a 10-year period from 1985 to 1995.[78] The annual incidence rate increased fourfold in children less than 2 years of age, when, at the same time, such cases disappeared in a neighboring country, Finland.[67,79] Before the epidemic in 1983, the recommendations for gluten introduction were changed from 4 months to 6 months of age. Also, in 1983, the amount of gluten in the industrially produced weaning food gruels and porridges in Sweden was doubled on average. The only difference between these two countries was the amount of gluten ingested by infants. Healthy infants in Sweden ingested two to three times more wheat protein than did infants in Finland. They also consumed 50% more than infants did in 1978, which was before the onset of increase in incidence.[79] Finnish infants again received more barley and oat. An important message here is that

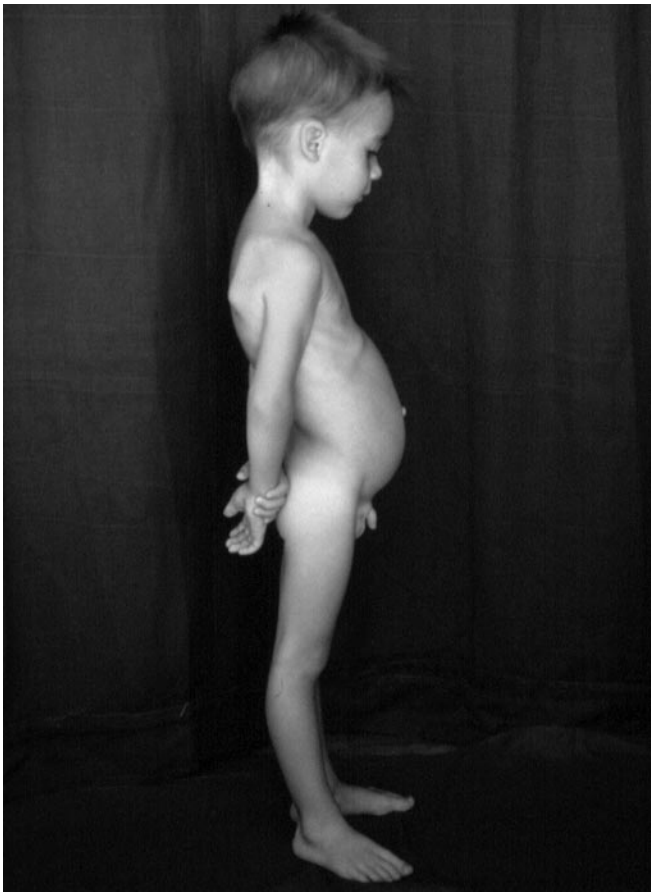

FIGURE 44.1-4 A 4-year old boy with newly diagnosed celiac disease presenting with loose stools and recurrent abdominal pain. He also had growth retardation and muscle weakness. A distended abdomen is seen. Photograph courtesy of Dr. Ilma Korponay-Szabo, Heim Pál Children's Hospital, Budapest, Hungary.

TABLE 44.1-1 DATA ON 53 CONSECUTIVE PATIENTS SUFFERING FROM CELIAC DISEASE AT ADMISSION TO THE CHILDREN'S HOSPITAL, UNIVERSITY OF HELSINKI, DURING THE 1960S

General data	
Number of patients	53
Age at onset of the symptoms (mo)	7.7
Gluten in the diet before the onset of symptoms (mo)	4.3
Age on admission (mo)	10.2
Symptoms	
Diarrhea or altered stools	87%
Vomiting	74%
Failure to gain weight	98%
Signs	
Weight below the 2.5th percentile	70%
Distended abdomen	64%

Adapted from Visakorpi JK.[60]

TABLE 44.1-2 DIFFERENTIAL DIAGNOSIS*

Cow's milk protein intolerance
Gastroenteritis, bacteria, rotavirus
Inflammatory bowel disease (Crohn disease)
Cow's milk and soy allergy
Eosinophilic gastroenteritis
Immunodeficiency states
Giardiasis
Bacterial overgrowth syndrome
Drugs
Radiotherapy

*Disease states in which small bowel mucosal inflammation or partial villous atrophy may occur (often not total villous atrophy with crypt hyperplasia).

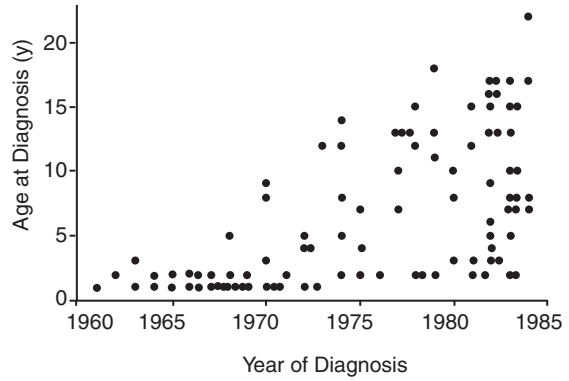

FIGURE 44.1-5 Changing pattern of celiac disease with decreasing numbers of cases diagnosed in young children and increasing numbers diagnosed at school age and adolescence after 1972. Reproduced with permission from Mäki M et al.[67]

new authority directives or other changes within the community may change the disease panorama completely even during a short time period.

Case Finding

The observation that celiac disease exists in a completely asymptomatic form was obtained from gluten challenge[63,80] and family studies.[6,30] Today it is clear that celiac disease with typical gastrointestinal symptoms represents only the tip of the iceberg (Figure 44.1-6).[81,82] In clinically silent celiac disease, a manifest mucosal lesion, which is gluten dependent, can be found on biopsy. The mucosal lesion responds favorably to a gluten-free diet. The reliable serologic screening tools, as discussed below, allow us today to detect such cases. On the other hand, one may also argue that clinically silent celiac disease, as such, is just an undiagnosed condition.

Celiac disease can be found in children with traditional symptoms and signs but in a very mild form (Table 44.1-3).[63] Abdominal discomfort, loose stools, flatulence

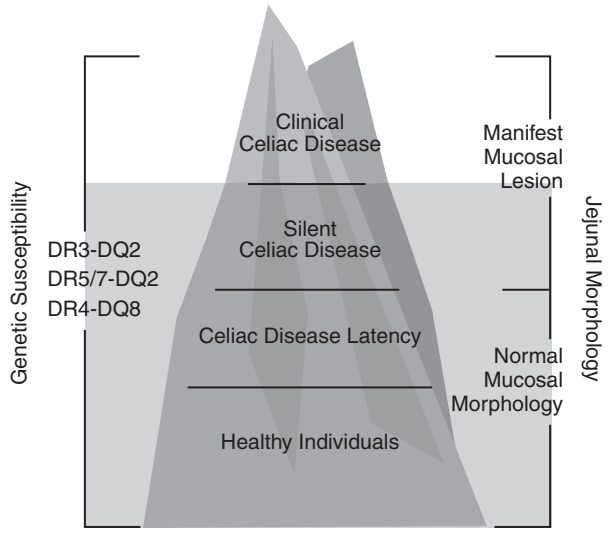

FIGURE 44.1-6 The iceberg of celiac disease. Adapted with permission from Mäki M and Collin P.[82]

TABLE 44.1-3 DIAGNOSTIC APPROACH IN FINDING CELIAC DISEASE IN INDIVIDUALS WITHOUT CLEAR SYMPTOMS SUGGESTING JEJUNAL MUCOSAL ATROPHY

Policy
High index of suspicion of celiac disease
Liberal use of serologic screening tests
Case finding in patient groups
Minor abdominal symptoms (abdominal discomfort, loose stools, recurrent abdominal pain, lactose intolerance)
Growth failure, delayed puberty, iron deficiency anemia, rickets
Other chronic or celiac disease related diseases (type 1 diabetes, thyroid disease, selective IgA deficiency)
Skin disorders, suspected dermatitis herpetiformis
Celiac-type, permanent tooth enamel defects
Joint symptoms as arthralgia, arthritis
Liver diseases
Some neurologic diseases
Down syndrome, Turner syndrome
Healthy first-degree relatives of celiac patients

problems, recurrent abdominal pain, and diagnosed lactose intolerance are conditions in which celiac disease should be sought. Arthritis and arthralgia may also be presenting symptoms in celiac disease.[83] As mentioned earlier, short stature, delayed puberty, and isolated nutritional deficiencies are already signs of malabsorption, even in cases with no gastrointestinal symptoms.

In addition to finding clinically silent celiac disease in healthy first-degree relatives of celiac disease patients,[6,30–34] the disease is highly prevalent in type 1 diabetes mellitus (2–5%),[84,85] in individuals with selective immunoglobulin A (IgA) deficiency (10%),[86] and in Down syndrome (10%).[87,88]

It is widely accepted that dermatitis herpetiformis is gluten induced, has the same genetic background as celiac disease,[36,38] and is a classic example of the extraintestinal manifestation of the disease.[89] Other linked disorders include permanent-tooth enamel defects,[90] central and peripheral nervous system involvement,[91,92] liver involvement,[93,94] and even autoimmune diseases in general.[95] A risk for a malignant complication of untreated celiac disease seems not to be as high as previously thought.[96]

DIAGNOSTIC CRITERIA

The gold standard for clinical diagnosis is the initially manifested small intestinal mucosal lesion, villous atrophy, together with crypt hyperplasia (Figure 41.1-7). Another characteristic is an increased density of intraepithelial lymphocytes.[97] Heavy cellular infiltrativity is not always present in untreated celiac disease. The European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN; formerly ESPGAN) set the criteria for the diagnosis in 1970.[98] It was stated that a patient with absent or almost absent villi who shows definite improvement on a gluten-free diet cannot be designated as having celiac disease before he has been proved to normalize on a dietary treatment, not only clinically but also histologically, and subsequently to relapse after reintroduction of gluten. This

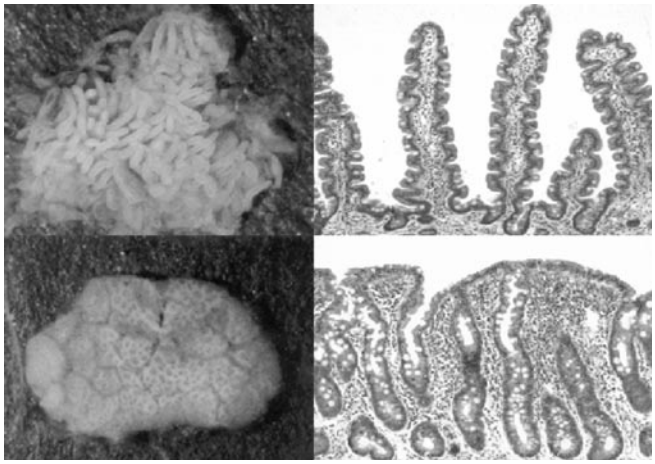

FIGURE 44.1-7 Dissection microscopic (*left*) (×50 original magnification) and histologic sections (*right*) (hematoxylin and eosin; ×280 original magnification) of small intestinal mucosal biopsy specimen with normal mucosal morphology with high villi (*upper*) and celiac disease (*lower*) (subtotal villous atrophy with crypt hyperplasia).

implies that three small intestinal biopsies should be performed. Also, a "2-year rule" was developed. It implicated that on gluten challenge, there should be a recurrence of the mucosal lesion within 2 years of gluten reintroduction. These strict criteria were later revised. However, the cornerstone remained the same, that is, in the initial biopsy, flat small intestinal mucosa should be found.[99] Neither repeated biopsy nor gluten challenge was considered mandatory in cases with full clinical remission after withdrawal of gluten from the diet. However, in uncertain cases, the old criteria may still be used, particularly when other diseases clinically complicate or resemble celiac disease (see Table 41.1-3).

Today clinicians may use new tools, serology (endomysial or TG-2 autoantibodies), and HLA-DQ typing. Positivity for the autoantibodies is an accurate indication of manifest small intestinal mucosal lesion (see below). On the other hand, negativity for the alleles encoding DQ2 and DQ8 speaks strongly against celiac disease.[40] In developing countries, the TG-2 autoantibody positivity is also highly indicative of celiac disease.[8,100] On a population-based level, the endomysial and TG-2 autoantibody positivity goes hand in hand with celiac disease–specific HLA-DQ markers.[101]

New Diagnostic Criteria Warranted

In celiac disease, small intestinal mucosal damage develops gradually, from normal morphology through inflammation to the so-called flat lesion (subtotal villous atrophy with crypt hyperplasia) (see Figure 41.1-7). This development is depicted in Figure 41.1-8, indicating the gradual process of mucosal deterioration, which may take years or even decades.[30–32,102–106] This means that when a child, an adult, or an elderly person on a gluten-containing diet has been shown to have a normal small intestinal mucosal morphology, even with no cellular inflammation, the disease is not excluded by conventional histology. The mucosa may deteriorate later.[107,108] A further demand on new criteria is obvious because patients suspected of having the disease, with correct HLA-DQ types but with only minor mucosal lesions and with an increased density of γ/δ-positive intraepithelial lymphocytes, were shown to have a gluten-dependent disease and osteopenia.[109] Oral tolerance to gluten in celiac disease may be broken in some individuals only after decades. This may be due to the amount of gluten in the diet but also because other environmental factors may act as "second hits." When gluten is introduced in the infant's diet, the ingested amounts vary. Later, daily food may contain gluten from 1 gram to up to 15 to 30 g. With this "normal" diet, the mucosal lesion may manifest at any age. Celiac disease with only low-grade inflammation should be distinguished from other diseases (see Table 44.1-2). It can be foreseen that biopsy will not be the gold standard in the diagnosis of celiac disease in the future when the diagnostic criteria will be widened toward "celiac trait" or "genetic gluten intolerance."

When performing gluten challenge studies with "normal" food, it has become evident that the individual tolerance to gluten is variable (see Figure 41.1-8). Most individuals react with a mucosal lesion within 3 months to 2 years on gluten ingestion, but it may take even longer,

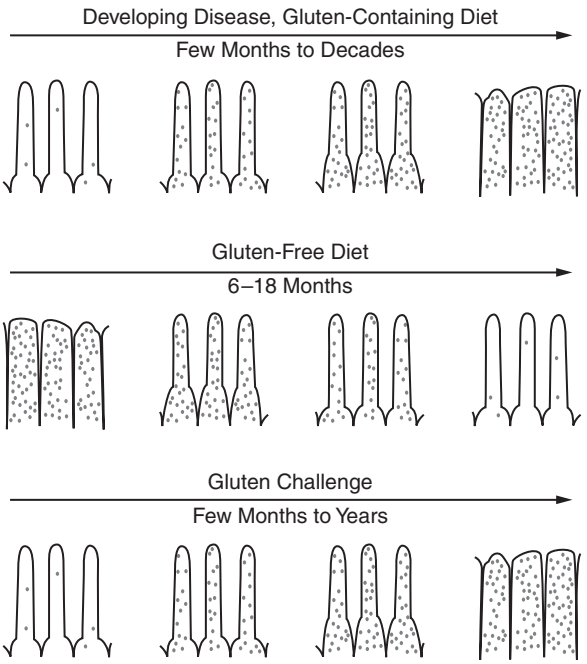

FIGURE 44.1-8 Schematic behavior of celiac disease small intestinal mucosa. *Upper row:* After starting a gluten-containing diet in infancy, the mucosa may deteriorate within a few months to that typical for celiac disease, villous atrophy with crypt hyperplasia. On the other hand, oral tolerance toward gluten may be kept for decades; the mucosa may be normal on morphology or show only a low-grade inflammation before deterioration. *Middle row:* The mucosa heals in 1 year on a gluten-free diet. *Lower row:* During gluten challenge, the mucosa may deteriorate within a few months, but it may also take years. Gluten intolerance in celiac disease is permanent, and a recurrence of the lesion will occur.

up to 14 years, before the mucosa relapses. In a Finnish series of gluten challenge performed in 29 adolescents and young adults with previously confirmed and treated celiac disease, 4 girls did not relapse within 2 years, one relapse occurred after 7 years, and another relapse manifested with dermatitis herpetiformis after 14 years of gluten ingestion.[80] In the latter case, the small bowel mucosal morphology remained normal.

SCREENING AND EPIDEMIOLOGY

Oral glucose tolerance tests, fecal fat excretion, D-xylose excretion tests, hematologic investigations, and radiologic examination of the small bowel failed to distinguish patients with suspected malabsorption from those with or without mucosal atrophy and, thus, frequently gave misleading results.[110] Intestinal permeability tests are not widely used as screening methods. However, autoantibodies have been shown to be highly predictive for untreated celiac disease. When there is a suspicion of celiac disease or the antibodies are positive, small intestinal biopsy should be the first diagnostic procedure. Often serum autoantibody tests direct the patient to the invasive diagnostic test.

In the United States, the disease is rare when the criteria for diagnosis rely on classic symptoms such as diarrhea and short stature.[111] By broadening the clinical indication, however, antibody screening seems to indicate that the prevalence in the United States is similar to that in Europe.[112] This is confirmed in recent large-scale screening programs.[113] Screening programs within childhood populations indicate that celiac disease is heavily underdiagnosed, and serologic testing using endomysial and TG-2 autoantibodies has the potential to detect otherwise undiagnosed disease in even more than 1 in 100 individuals (Table 44.1-4).[101,114–120] However, it should be remembered that subclinical cases of celiac disease will not be detected by screening only selected groups of at-risk patients.[81,101] Early detection of the disease and subsequent dietary elimination of gluten might be an appropriate method for preventing complications later in life. Today, however, we use mainly directed case finding screening. Figure 41.1-9 gives an example of the value of the use of antibody screening in one hospital: during 15 years, new cases per year have increased over 10-fold. Currently, population-based screening programs are used only in research.

TABLE 44.1-4 PREVALENCE OF CELIAC DISEASE IN CHILDREN IN VARIOUS COUNTRIES

GEOGRAPHIC AREAS	PREVALENCE	STUDY
North Africa, Saharawi people	1:18	Catassi et al[114]
Hungary	1:85	Korponay-Szabo et al[115]
Italy	1:95	Meloni et al[116]
Finland	1:99	Mäki et al[101]
Sweden	1:100	Carlsson et al[117]
United States	1:104	Hoffenberg et al[118]
Netherlands	1:198	Csizmadia et al[119]
Germany	1:500	Henker et al[120]

SEROLOGIC SCREENING TOOLS

Serum gluten and milk antibody tests, performed with immunodiffusion techniques, were already promising in an early series of celiac disease patients.[62,63] As reviewed by Mäki, various methods have been developed to determine gliadin antibodies in serum: immunofluorescence, enzyme-linked immunosorbent assay (ELISA), diffusion-in-gel ELISA, solid-phase radioimmunoassay, and strip ELISA.[121] These antibodies have frequently been found in patients with untreated celiac disease, but the sensitivities and specificities of the tests have been unsatisfactory.

The currently used autoantibody tests have been in use since 1971,[121] and by measuring the IgA class R1-type reticulin antibodies, sensitivities and specificities became high, close to 100%.[122] The endomysial autoantibody test, with human umbilical cord tissue as a substrate, was widely used and was standardized and ring-tested to detect untreated celiac disease.[123] Following the identification of tissue transglutaminase as the target in rodent and primate tissues for celiac disease–specific autoantibodies, a non–observer-dependent ELISA method was developed and was used to detect the antibodies.[8,124–128] In contrast to gliadin antibodies, the serum endomysial and TG-2 autoantibodies are genetically determined and correlate strongly with the celiac-type HLA genetics.[30,33,101]

TREATMENT

In celiac disease, a lifelong gluten-free diet is the only effective treatment. Wheat-, rye-, and barley-based products should be avoided. Even if oat, by definition, is "gluten," its prolamin avenin seems not to be disease inducing. In Finland, the United Kingdom, and other northern European countries, industrially purified wheat starch–based gluten-free flours have been accepted as part of the gluten-free diet for over 40 years. A minority of patients in Finland have been prescribed a natural gluten-free diet. On the other hand, in many countries, a zero limit is demanded, thus not allowing the use of purified wheat starch–based flours. On

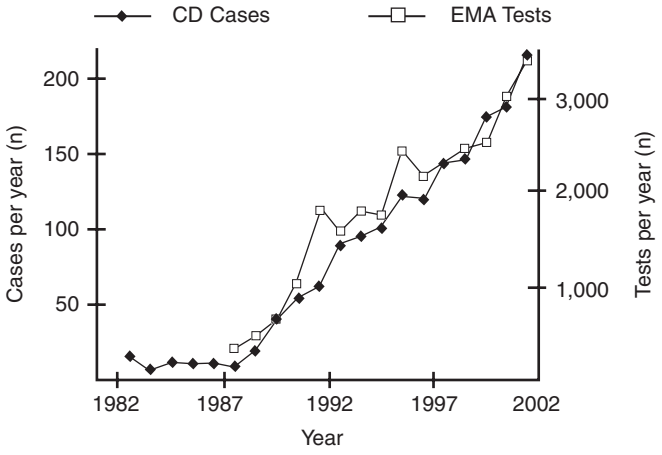

FIGURE 44.1-9 Significant increase of new celiac disease (CD) cases per year is obvious after starting the endomysial antibody (EMA) testing in 1987 in the Heim Pál Children's Hospital, Budapest, Hungary. Courtesy of Dr. Ilma Korponay-Szabo.

a long-term gluten-free diet including wheat starch–based gluten-free flours, no increased mortality, malignancies, or morbidity in the celiac disease patients over the population in general were observed.[129] On the prescribed diet, the mucosa heals and stays morphometrically normal over 10 years of flour ingestion.[130] Recently, it was concluded from a controlled study that wheat starch containing gluten-free flour products were acceptable in the gluten-free diet. No differences were observed in clinical responses; small bowel mucosal morphology; intraepithelial T-cell densities; mucosal HLA-DR expression; serum endomysial, TG-2, or gliadin antibody levels; quality of life measurements; or bone mineral densities when compared with the group on a diet that was gluten free by nature.[131]

The daily intake of gluten by the patients can be measured. The gluten levels in the products can be determined using the new R5 ELISA method, thus allowing an estimate of even trace amounts of gluten in the gluten-free diet.[132] It should be remembered that natural gluten-free products may contain gluten because international trade tolerates about 5% (50,000 ppm) extraneous grain in a cereal. On the other hand, if compliance to any given gluten-free diet is poor, the patient may ingest daily several grams of gluten.[133]

FUTURE INSIGHTS

Our increasing knowledge of the molecular and cellular details of events leading to celiac disease should benefit patients. The knowledge of the harmful gluten epitopes should facilitate our ability to genetically modify these epitopes in grains without losing their baking properties. The large number of T-cell epitopes and the complexity of wheat genetics complicate manipulation of wheat to remove toxic peptides. An alternative strategy might be to inhibit the deamidation process by developing TG-2–specific inhibitors.[134,135] However, the inhibitors should be designed to inhibit only the deamidation activity of TG-2, not the other enzymatic activities or structural properties of TG-2. It is noteworthy that celiac disease patient IgA antibodies inhibited differentiation of T84 crypt-like cells to enterocytes, and specifically designed inhibitors might do the same, that is, provoke crypt hyperplasia and villous atrophy. One approach could be to use enzyme-supplement therapy (eg, bacterial prolyl endopeptidase) to destruct the proteolytically resistant proline-rich fragments of gluten proteins.[54] This approach is attractive and practical because the protease could be ingested along with a diet containing gluten. However, the enzyme should be present in large amounts, and it should work very efficiently in the correct place (upper part of small intestine) to prevent efficiently undigested peptides from activating T cells. Interference with the stimulation of $CD4^+$ gluten-specific T cells, their eradication, or silencing could be another effective way to control the disease. Alternatively, blocking the peptide-binding sites of DQ2 and DQ8 HLA molecules could prevent the presentation of disease-inducing gluten fragments. One more strategy is blockade of signals derived from the cytokine interleukin-15 (part of innate immunity). We should not forget, however, that a gluten-exclusion diet is a safe treatment, although it is not convenient or easy to comply with. New therapeutic approaches must outweigh the current gluten-free therapy with regard to costs and safety.

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2. Food-Allergic Enteropathy

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The best described mucosal manifestation of food allergy is food-allergic (sensitive) enteropathy, in which an immunologically mediated abnormality of the small intestinal mucosa occurs. The features of this enteropathy may include excess lymphocyte infiltration, epithelial abnormality, or architectural disturbance. This may often impair absorption and less commonly causes a frank malabsorption syndrome. Such enteropathy continues while the food remains in the diet and remits on an exclusion diet but recurs on food challenge. In most current cases, formal evidence of such an allergic reaction to challenge, based on sequential biopsies, is not performed in children with clinical intolerance to dietary proteins. Diagnosis is thus based on histologic features at initial biopsy and clinical response to antigen exclusion and challenge. The consequences of enteropathy include abdominal distention, loose stools, micronutrient deficiency, and sometimes protein-losing enteropathy leading to edema. In contrast to celiac disease, these enteropathies are usually restricted to early life, and later challenge with the protein is usually tolerated. The disorder is best described for cow's milk, and cow's milk–sensitive enteropathy (CMSE) has been recognized for over two decades.[1]

RECOGNITION OF FOOD-SENSITIVE ENTEROPATHIES

The existence of food-sensitive enteropathies was initially controversial and established only after the development of the pediatric biopsy capsule. The disorders were finally confirmed by the same diagnostic techniques introduced for celiac disease, based on sequential small intestinal biopsies at diagnosis, after food elimination, and again after challenge. Small intestinal mucosal abnormalities were first suggested to occur in response to cow's milk protein by Lamy and colleagues in 1963.[2] These observations were supported by other reports of abnormal small intestinal mucosa in young children who manifested delayed clinical reactions to cow's milk and improved clinically after cow's milk exclusion.[3,4] The first formal study of the histologic response to cow's milk challenge in CMSE came from Kuitunen and colleagues,[5] who showed that symptom-free cow's milk–allergic children with normal prechallenge biopsies reacted to milk challenge by recurrence of symptoms and development of histologic small intestinal abnormality. Walker-Smith and colleagues confirmed the existence of CMSE by serial small intestinal biopsies related to dietary milk intake in five infants.[6] The mucosal lesion at the time of first biopsy was of variable severity, in part related to the length of time the patients had already been on a milk-free diet. In all children, there was improvement in morphology after a milk-free diet and in most a return to normal. In all, there was a significant deterioration after a further milk challenge. However, the mucosal lesion was markedly variable in its severity, ranging from a partly flat mucosa to a mild degree of partial villous atrophy. Disaccharidase activity rose on a milk-free diet and fell after a positive milk challenge,[6] whereas intraepithelial lymphocytes increased on challenge.[7] Comparison of biopsies taken at the duodenojejunal junction with those taken a further 15 cm distally showed that proximal abnormalities induced by milk challenge in CMSE were greater than those in the distal small intestine.[8] The lesion is thus likely to be less extensive than in celiac disease, but there is significant interpatient variability.

Abnormalities of the small intestinal mucosa have been reported in children with intolerance to a variety of other protein, including soy, wheat, oats, eggs, rice, and fish.[9–11] In some cases, these reports were corroborated by serial biopsies during challenge, but the gradual acceptance of the existence of food-sensitive enteropathy has made serial diagnostic biopsies unusual.

CLINICAL FEATURES OF FOOD-ALLERGIC ENTEROPATHY

Classic Food-Sensitive Enteropathy

The most common syndrome is CMSE. Its classic presentation is with chronic diarrhea and failure to thrive, often beginning after an episode of gastroenteritis in a formula-fed infant and recovering only after cow's milk is excluded from the diet.[1,12] Other clinical features include abdominal distention, perianal erythema, or napkin rash owing to malabsorbed sugars and dermatographia. Associated clinical features may include colic, gastroesophageal reflux, rectal bleeding, or eczema. Up to 40% of infants with classic CMSE also sensitize to soy, often after a "honeymoon period" of about 2 weeks, when it appears to be tolerated. The great majority, however, settle on extensively hydrolyzed formulas. Classic CMSE is usually a self-limiting condition, with most children tolerating reintroduction at the age of 2 to 3 years.[1,12,13] However, some children may have persistent low-grade symptoms for much longer.[14,15] There is sometimes a history of additional immediate reactions to food antigens, such as rash, urticaria, angioedema, or even anaphylaxis. However, many children suffer

enteropathy without immediate reactions, and it is important to recognize that food-allergic enteropathy can occur in the absence of any systemic signs of food allergy. Thus, skinprick tests may be negative, and specific immunoglobulin E (IgE) is undetectable.[1,12,16,17] Review of the infant's weight record often shows a period of good weight gain prior to the onset of symptoms, followed by downward drift against the percentiles until antigen exclusion is adequate. In terms of mechanism (discussed later), this presentation represents the transient loss of initially established oral tolerance.

Breast Milk Sensitization and Multiple Food Allergy

In contrast to this classic presentation, there are increasing reports of very early life sensitization in exclusively breastfed infants. Symptoms can begin very early in life, sometimes within the first 2 weeks.[16,18] In these infants, cow's milk is usually one of several antigens that induce symptoms, and many affected infants are intolerant of multiple foods. These infants have a different pattern of weight gain compared with classic CMSE in that failure to thrive is seen in only about 25%, who usually show poor weight gain from birth.[19,20] For the infants who have inherited high IgE or predisposition to immediate reactions, diagnosis is usually more straightforward because the clinical history usually indicates the causative antigen(s), and skinprick tests are usually positive.[1,16] In contrast, those without immediate responses, particularly if weight gain is good, may present great diagnostic difficulty and may not be recognized as suffering from food allergies. Endoscopic evidence of duodenal and/or ileal lymphoid hyperplasia appears to be characteristic.[21–24]

For these infants, it appears that oral tolerance mechanisms are not primarily established[17,25]; thus, sensitization occurs to trace amounts of multiple antigens eaten by their mother and passing into breast milk. The clinical picture is often dominated by dysmotility, with delayed gastric emptying and gastroesophageal reflux contributing to a severe colic-like presentation with irritability.[16] Many infants have associated eczema, and napkin rash occurs owing to malabsorbed dietary sugars.[16] The histologic findings are less striking than for classic CMSE, and this can be a condition that causes great diagnostic difficulty.

FOOD PROTEIN–INDUCED ENTEROCOLITIS AND COW'S MILK COLITIS

The food protein–induced enterocolitis syndrome (FPIES) is a particularly severe and sometimes life-threatening form of mucosal food hypersensitivity.[26,27] It is most commonly associated with cow's milk or soy ingestion but has recently been reported to be induced by a variety of foods usually thought to be of low antigenicity, including rice, oat, barley, vegetables, and poultry.[11] It is also common for such symptoms to occur in exclusively breastfed infants, triggered by milk protein in the mother's diet.[28] Negative skinprick tests do not exclude this diagnosis, and the majority of cases are, in fact, negative.[29] The infant usually presents with severe vomiting and diarrhea and may become dehydrated and shocked, requiring emergency admission to hospital.[11] Some demonstrate melena and passage of mucus per rectum. The withdrawal of milk or soy from the diet induces remission, but early challenge often induces a systemic reaction with peripheral neutrophilia, in addition to vomiting and loose stools. The condition may develop after the earlier diagnosis of cow's milk colitis, in which colonic features such as melena, diarrhea, and passage of mucus occur without the systemic manifestations, apart from thrombocytosis.[1] Inadvertent milk or soy challenge after a period of exclusion diet may then precipitate the full-blown FPIES response. Skinprick testing is usually negative, and the diagnosis may have to be based on the response to antigen exclusion and challenge. Many affected infants may respond in this manner to multiple antigens, with tolerance re-established around 2 to 3 years for FPIES[11] and rather earlier for cow's milk colitis.[1]

If colonoscopy is performed, the colitic changes are usually milder than with classic inflammatory bowel disease, and the macroscopic findings are dominated by loss of the vascular pattern, prominent lymphoid follicles with a rim of perifollicular erythema (red halo sign), and an easily traumatized mucosa.[1] Histologic changes include mononuclear cell infiltration, mucosal eosinophilia with evidence of degranulation, and the presence of lymphoid follicles in the majority of colonic biopsies. If the ileum is visualized, lymphoid hyperplasia is usually seen. This condition may also occur in older children and be unrecognized as attributable to milk allergy, leading, on occasion, to mistaken laparotomy in the investigation of rectal bleeding.[30] A trial of cow's milk exclusion is thus recommended in all children with a history of unexplained rectal bleeding.

HISTOLOGIC FEATURES OF FOOD PROTEIN ENTEROPATHIES

Early reports of the classic CMSE lesion suggested that the mucosa was flat and largely similar to that in celiac disease.[5] With improvements in the composition of infant milk formulas, this became very rare, and mucosal damage is usually now much less striking and of patchy distribution (Figure 44.2-1).[1,31] At initial diagnosis, there may be areas demonstrating mild villous atrophy, with some hyperplasia of the crypts. In exceptional cases, there may be a flat mucosa, and the lesion is typically distinguished from celiac disease by the thin mucosa and patchy distribution.[32,33] Typical histologic findings are shown in Figures 44.2-1 and 44.2-2. If the diagnosis is being considered, at least two biopsy specimens are helpful for diagnosis. Dissecting microscopic examination in the endoscopy room is helpful in detecting a patchy lesion. There may be some increase in intraepithelial lymphocyte numbers, but this is often focal and less than in celiac disease.[7] Crypt cell proliferation is only modestly increased in CMSE, to an extent less than in celiac disease.[34]

Quantitative morphometric studies during serial diagnostic milk challenges[32] confirmed no increase of mucosal thickness in comparison with control biopsies but identi-

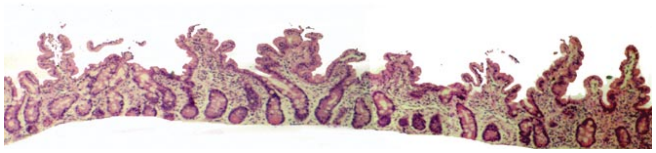

FIGURE 44.2-1 Low-power view of a small intestinal biopsy (fourth part of the duodenum) in a child with cow's milk–sensitive enteropathy. The lesion is patchy, showing areas with preserved villus architecture and a crypt-to-villus ratio greater than 2, and others with villus blunting and crypt lengthening. Such patchiness is characteristic of food-sensitive enteropathy and suggests the need for taking more than one biopsy for histologic assessment. The handling of biopsies is important, and orientation under dissecting microscopy with card mounting may be helpul in optimizing biopsy orientation.

fied an increase in intraepithelial eosinophils on milk challenge. Improvements in infant formulas have led to a change in the mucosal appearances of CMSE so that celiac-like villous atrophy is now very rare in the developed world. Morphometry of recent cases of CMSE confirms a less severe lesion than archival biopsies from the 1980s (Table 44.2-1).[16] This causes some histopathologic difficulty because there is no international consensus in reporting of subtle lesions such as villous blunting or mild mucosal eosinophilia.

One unexplained finding that may be diagnostically helpful is the accumulation of fat within the epithelium, as is also seen in postenteritis syndrome.[35] Within the lamina propria, there is often a patchy increase in the density of lymphocytes, and increased eosinophil accumulation may be seen (see Figures 44.2-2 and 44.2-3). In original descriptions, classic CMSE appeared to disappear by the age of 2 years, and milk challenge was then frequently successful.[1,12,13] However, there is now evidence that some children do not grow out of CMSE in early childhood, and an abnormal mucosa may be seen in later childhood. Kokkonen and colleagues characterized persistent CMSE in school-age children in comparison with celiac disease and control children.[14,15] Typical findings in children with CMSE were endoscopically visible lymphonodular hyperplasia of the duodenal bulb and lymphoid follicles without villous atrophy in biopsy samples.[21–23] The patients with definite CMSE showed significantly increased densities of intraepithelial T cells, particularly γδ T cells.[22] Previous studies had demonstrated variability in γδ intraepithelial lymphocyte numbers in CMSE, from a high celiac-like density to normal,[36] suggesting that this would not be a reliable marker for CMSE. The consequences of the infiltration of activated T cells in enteropathy include impairment of enterocyte absorptive mechanisms, such as decreased expression of lactase and other hydrolases, and an additional impairment of the small intestinal drive to pancreatic enzyme secretion. Thus, fecal elastase concentrations may be reduced in active disease.[37,38]

SPECIFIC FOODS CAUSING ENTEROPATHY SYNDROMES

Cow's Milk

Unlike cow's milk, human milk does not contain β-lactoglobulin, and this protein was initially implicated in the development of CMSE.[1,12,13] There is more recent evidence that allergic responses to casein may also occur and that certain epitopes may be particularly important. For systemic cow's milk allergies, IgE reaction to linear

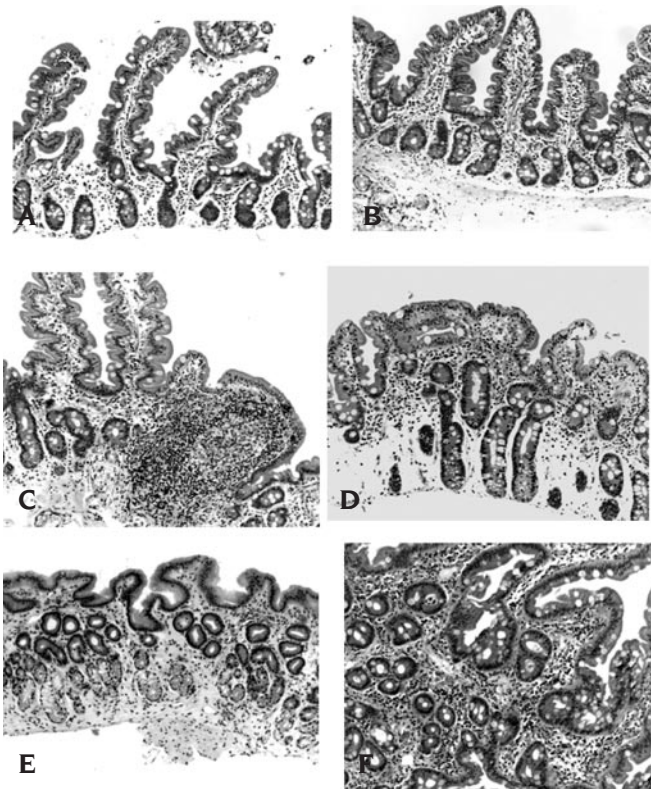

FIGURE 44.2-2 Subtle findings in food-sensitive enteropathy. *A* and *B* show biopsies within normal histologic limits from children with no eventual gastrointestinal diagnosis. *C* shows a prominent lymphoid follicle with an active germinal center in a child with cow's milk–sensitive enteropathy (CMSE). Despite the recognizable villus architecture, there is evidence of some crypt lengthening. Prominent lymphoid tissue within the duodenum and terminal ileum is a recently recognized association of childhood food allergy.[21–24] In contrast, *D* shows the major disturbance of small intestinal architecture characteristic of celiac disease, which presents little diagnostic difficulty compared with CMSE. *E* shows villus shortening in a case of cow's milk– and soy-sensitive enteropathy. The prominence of Brunner glands, however, suggests that the biopsy may have been taken in the first or second part of the duodenum, which may overemphasize villus shortening. Correct technique is to take biopsies in the fourth part of the duodenum or jejunum. *F*, Although the biopsy is cross-cut, there is a clear increase in the density of mononuclear cells within the duodenum in this case of CMSE.

TABLE 44.2-1 MORPHOMETRIC EXAMINATION OF SMALL INTESTINAL BIOPSIES IN FOOD-SENSITIVE ENTEROPATHY, COMPARING CLASSIC CMSE WITH MULTIPLE FOOD ALLERGY

PATIENT GROUP	VILLUS HEIGHT (μm)	CRYPT DEPTH (μm)	MEAN CRYPT-TO-VILLUS RATIO
Normal controls (n = 47)	345	164	2.1
Multiple food allergy (n = 45)	282	188	1.4
Classic CMSE (n = 46)	210	190	1.2
Celiac disease (n = 17)	30	400	0.0

Data from Latcham F et al.[16]
CMSE = cow's milk–sensitive enteropathy.

sequences in casein, rather than α-lactalbumin or β-lactoglobulin, are predictive of long-lasting milk allergy. Those children whose IgE bind specific sequences of α_{S1}-casein, α_{S2}-casein, and κ-casein are less likely to outgrow their allergy.[39,40] Overall, the prognosis of the child with CMSE appears good, and the great majority outgrow their sensitization by age 3 years,[13,41] although other allergies may subsequently develop in about half of the cases.

Soy

Soy-based formulas have been used for many years as a substitute for cow's milk formulas in milk-sensitized infants, although this has been controversial. Recent recommendations from the American Academy of Pediatrics (AAP) Committee on Nutrition[42] differ from those from the European Society for Pediatric Allergology and Clinical Immunology (ESPACI) and the European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN)[43] with respect to soy use. Soy-based formulae are as antigenic as cow's milk formulas[44] and cause a similar spectrum of allergic responses, including enteropathy.[1,11] A 30 kD protein in soy may induce cross-reactivity to casein in cow's milk,[45] potentially explaining why so many cow's milk–allergic children also develop soy intolerance or enteropathy. The antigenicity of soy-derived products varies substantially with the method of preparation, and children may react to some soy products but not others.[46] This may be important to consider if challenge testing is performed with a soy preparation to which the child does not react, but symptoms suggestive of soy enteropathy continue. Skinprick testing is a poor predictor of subsequent clinical reactions to soy,[47] but skin patch testing shows promise in identifying soy-sensitized children.[48]

Hydrolysates

It is important to recognize that some children can show reactions to the trace cow's milk protein present in hydrolysate formulas and thus show incomplete resolution of symptoms unless moved to an amino acid–based formula.[16,49–52] The clinical features tend to be less florid than induced by the unprocessed protein; thus, there is often some improvement in symptoms on commencing the hydrolysate. Ongoing reaction to hydrolysates may therefore be missed if the diagnosis is not considered.

Wheat Gluten

A particularly important form of food-sensitive enteropathy occurs to wheat gluten in celiac disease. The enteropathy is quite distinct to other food-sensitive enteropathies such as CMSE and is discussed separately in Chapter 44.1, "Celiac Disease."

Multiple Food Allergy

Increasing numbers of infants and children are becoming sensitized to multiple foods, making either immediate or delayed reactions or a combination. Many sensitize despite exclusive breastfeeding. Diets involving the elimination of single foods are usually ineffective, and many affected infants are intolerant of hydrolysates and require amino acid formulas.[16,49–52] This provides a particularly challenging clinical scenario and requires experienced specialist input. The antigens most likely to provoke symptoms in the multiply food-allergic child include cow's milk, soy, wheat, egg, peanuts, tree nuts, and fish, but a variety of other food antigens have been implicated.[16,29,53] If the multiply allergic

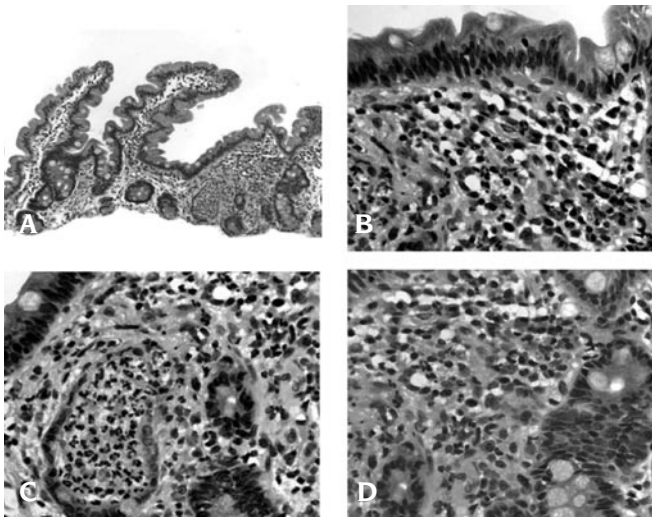

FIGURE 44.2-3 Eosinophil infiltrate within the mucosa in a case of food protein–induced enterocolitis. The low-power view in *A* shows preserved villus architecture in the duodenum, with a single crypt abscess. Higher-power views in *B* and *C* show the intense eosinophil infiltrate within the lamina propria. *C* demonstrates that the crypt abscess seen in *A* is, in fact, eosinophilic. *D* shows a sigmoid colonic biopsy from the same child, which also shows mucosal eosinophilia. This child required an amino acid formula because he was intolerant of hydrolysates.

infant remains even partially breastfed, the dietary exclusions that the mother must make may have a very negative effect, and it is often better to discontinue breastfeeding in favor of an amino acid formula if she has to exclude more than two major foodstuffs.[54] A cow's milk– and soy-free diet is just about at the level of tolerance of all but the most fanatically committed adult, and the further exclusion of egg or wheat provides not only additional complexity but a real risk of nutritional adequacy for a lactating mother.[54]

One important clinical issue in the management of the child with multiple food allergy is the maintenance of a nutritionally adequate diet, and close liaison with an experienced dietitian is mandatory. The consequences of mucosal T-cell activation in food-sensitive enteropathy include reduction in brush border disaccharidase expression,[55] leading to impaired carbohydrate absorption, and a secondary reduction in pancreatic enzyme release,[37,38] which may also contribute to malabsorption. In some cases, improvement in weight gain can be achieved by the temporary addition of pancreatic enzyme supplements. It is important to exclude cystic fibrosis in cases in which elastase is found to be low because both conditions can coexist. Many children with multiple food allergy do maintain normal growth and weight gain[20]; thus, the diagnosis may not be suspected if the child makes delayed non–IgE-mediated reactions only.[16,20] Unlike classic CMSE, it is unusual for an affected child to regain tolerance for cow's milk by the age of 2 years.[16,20]

IMMUNOPATHOLOGY

T-Cell Responses

Immunohistochemical studies during serial challenges in CMSE have identified changes induced by milk, including increased intraepithelial CD8 cells and activation of lamina propria CD4 cells, with increased human leukocyte antigen (HLA)-DR expression on surface epithelium (Figure 44.2-4).[10] The intraepithelial lymphocyte population appears to be in a state of activation and shows enhanced expression of the cytotoxic marker TIA-1.[56] Analysis of mucosal lymphocytes in CMSE or multiple food allergy confirms excess T-cell activation, with either a T helper (Th)1–dominated or a mixed Th1/Th2 response.[12,25,57–60] Thus, Th1 responses are not reduced overall within the mucosa or Th2 responses significantly increased, in contrast to findings reported in peripheral blood lymphocytes from milk-allergic infants[61] or in cloned milk-reactive T cells isolated from the duodenal mucosa.[62] Enzyme-linked immunospot analysis of children with classic CMSE showed increased interferon(IFN)-γ– and interleukin (IL)-4–producing cells, with unchanged IL-5 and reduced IL-10 in comparison with controls.[57] Th1 responses are also maintained within the Peyer patch lymphocyte population.[63] A study using reverse-transcriptase polymerase chain reaction in Finnish children with food-allergic enteropathy also identified increased IFN-γ messenger ribonucleic acid (mRNA), but with no increase in IL-4.[58] Flow cytometric analysis of intraepithelial and lamina propria lymphocytes from children with multiple food allergy did not show an increase in IL-2–, IL-4–, or IFN-γ–producing cells but showed a marked reduction in lymphocytes producing the regulatory cytokine transforming growth factor-β (TGF-β), which is also seen by immunohistochemistry and in situ hybridization.[60] Thus, although classic CMSE shows a broadly Th1-skewed mucosal lesion, the multiple food-allergic lesion may be immunologically distinct and characterized by reduced expression of the regulatory cytokine centrally associated with oral tolerance. Reduction of TGF-β expression has been seen in two further studies of milk-reactive duodenal T-cell clones from milk-allergic children[62] and of the duodenal mucosa in food protein enterocolitis syndrome.[64] This is discussed in the section on oral tolerance.

Tumor necrosis factor-α (TNF-α) production is stimulated within the mucosa by milk challenge of allergic patients,[65,66] which is again distinct from responses seen in circulating T cells from allergic children[67] but may reflect its release from mucosal mast cells. Increased TNF-α responses are also seen, in association with enhanced Th2 responses in food protein–induced enterocolitis.[68]

Mast Cells and Eosinophil Responses

One frequent association of mucosal food-allergic responses is the infiltration of both eosinophils and mast cells, both of which produce a number of proinflammatory, vasoactive, and neuroactive mediators (see Figure 44.2-3). These cells have been implicated in antigen-induced dysmotility, and their products may disturb enteric neural function.[69] In response to ingested allergens, mast cell tryptase and eosinophil cationic protein are released into the lumen and may be detected in stools.[66,70,71] In a study comparing mucosal responses in milk-intolerant infants with those in milk-tolerant infants with small intestinal enteropathy, Chung and colleagues showed that milk intolerance was associated with increased mucosal expression of eosinophil major basic protein together with up-regulation

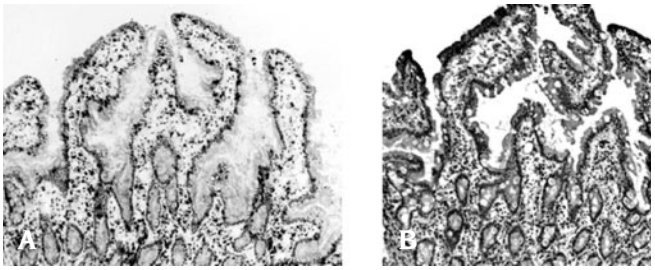

FIGURE 44.2-4 Immunohistochemical staining of frozen sections from a child with multiple food allergy. *A* and *B* are serial sections through the same villi, with *A* stained for CD3+ T cells and *B* stained for human leukocyte antigen (HLA)-DR. Positively stained cells are brown, and unreactive cells show blue counterstaining only. *A* shows a modest increase in the density of intraepithelial lymphocytes but otherwise normal T-cell density. *B* shows HLA-DR–positive epithelium at the villus tip but not in crypt epithelium. Because a major determinant of epithelial HLA-DR expression is the T helper (Th)1 cytokine interferon-γ, this positive staining is consistent with several reports of preserved Th1 responses in mucosal food allergic responses.[57–60] This contrasts with Th2 dominance of circulating lymphocytes in milk allergy.[61]

of vascular cell adhesion molecule 1.[72] In this study, the degree of villous atrophy correlated with the extent of deposition of major basic protein.

There are important differences in the time course of responses mediated by mast cells and eosinophils, despite their rather similar products. Mast cell mediators are stored preformed within intracellular granules and released rapidly through degranulation after cross-linking of surface IgE molecules. This does not require synthesis of new protein and thus ensures that mast cells play a predominant role in immediate mucosal responses. For eosinophils, there is a contrasting lag period because they need to be recruited into the tissues. Eosinophils are thus more likely to play a role in delayed responses to antigen. The three forms of response to cow's milk in sensitized infants identified by Hill and colleagues[73] of immediate, intermediate, and delayed reactions thus correlate temporally with mast cell–, eosinophil-, and T cell–mediated responses, respectively.[73]

PATHOGENESIS OF FOOD-SENSITIVE ENTEROPATHY

Control of T-Cell Responses

The intestine contains more T cells than any other organ in the body, and there are distinct roles for intraepithelial and lamina propria lymphocytes (see Figure 44.2-4). An important role of T cells in inducing the tissue remodeling characteristic of crypt hyperplastic villous atrophy in small intestinal enteropathy was made explicit by organ culture studies of human fetal intestine by MacDonald and coworkers.[74,75] Immediate hypersensitive responses in animals associated with mast cell degranulation or polymorph infiltration without T-cell activation do not cause crypt hyperplasia.

T cells within the mucosa are normally maintained in a state of tolerance, for reasons discussed in this chapter.[76] One requirement is the maintenance of a tight epithelial barrier to prevent passage of intact macromolecules to the lamina propria. The role of the epithelium in antigen presentation and cytokine production has been recognized,[77–80] and an important epithelial contribution to tolerance was suggested by Sanderson and colleagues on the basis that costimulatory ligands for T-cell activation were not expressed.[79] Recent data suggest that intestinal epithelium may directly induce a regulatory phenotype in CD8 cells.[81] It has been estimated that only peptides shorter than 11 amino acids may penetrate the epithelial barrier in health.[82] One reason for initial sensitization may be the bypassing of the normal pathways for immune tolerance through epithelial leakiness, usually owing to acute gastroenteritis.[1,25] Early studies by Gruskay and Cook showed excess egg albumin absorption in infants with acute gastroenteritis,[83] and experimental studies during viral gastroenteritis in piglets confirmed excess absorption of dietary antigens.[84] Subsequent studies of paracellular permeability in children with gastroenteritis confirmed that this is a time when antigens may penetrate in abnormal amounts.[85,86] The immune response to the penetrating antigen may then contribute to maintaining epithelial hyperpermeability and increased antigen entry.[87] Thus, a positive food challenge may induce further excess paracellular permeability.[88] The cytokines that are particularly implicated in increasing paracellular permeability in the intestine are TNF-α and IFN-γ.[65,89,90] There is an intriguing overlap of function with the cytokines central in oral tolerance, TGF-β and IL-10, because both of these cytokines inhibit IFN-γ–induced permeability,[91,92] whereas IFN-γ itself specifically blocks TGF-β signaling.[93] Thus, it appears possible that the relative local concentrations of IFN-γ and TGF-β or IL-10 may be one factor that determines when immune tolerance is regained to sensitizing antigens.

Oral Tolerance

There has been much advance in the understanding of oral tolerance mechanisms. Relevant factors include the dose of ingested antigen because low doses invoke different responses to high doses.[17,25,94,95] Bulk dietary antigen is likely to be presented by epithelium without costimulatory ligands,[79] which may induce lymphocyte anergy[79,80] by processes little understood in childhood. Murine studies suggest that specific targeting of ingested antigen occurs to late endosomes within the enterocyte and that this may be an important component of the antigen presentation process in oral tolerance.[96] There are as yet no confirmatory studies in humans. A complex state appears to be induced in lymphocytes by food administration, with an increase in both pro- and antiapoptotic factors and downregulation of T-cell receptor function.[97] A different mechanism for low doses of antigen follows its uptake by the M cells that overlie Peyer patches. The antigen induces suppressor lymphocyte formation within the Peyer patch, particularly Th3 T cells that produce TGF-β.[17,25,94,95,98] Other important regulatory T-cell populations include IL-10–producing T regulator 1 (Tr1) cells and CD4+CD25+ cells.[25,80,95] The master regulator molecule that is critical in the generation of regulatory lymphocytes is the transcription factor Foxp3,[99] and mutation in this molecule induces a severe multifocal inflammatory disease characterized by enteropathy of autoimmune basis in human infants (IPEX syndrome [immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance]).[100] Thus, Foxp3 may be a molecule that is of critical importance in maintaining oral and systemic tolerance. Several regulatory cells may be important in preventing food-sensitive enteropathy. In older patients, a population of multiply exposed CD4+CD25+ cells inhibit milk responses.[101] However, most of the circulating CD4+CD25+ in adults express the skin-homing marker cutaneous lymphocyte antigen (CLA) but not the gut-homing β_7 integrin, whereas cord blood CD25+CD4+ cells express neither marker.[102] Cord blood CD4+CD25+ cells show ineffective regulatory properties[103]; thus, neonatal animals show impaired low-dose oral tolerance.[104] Oral tolerance mechanisms in general do not appear to be fully intact at birth but develop postnatally, in response to the luminal flora. There is expression of a specific group of Toll receptors on CD4+CD25+ regulatory T cells, and their response to bacterial lipopolysaccharide is one of increased suppressive effect.[105]

The early infectious exposures of the young infant may be as important as the food antigens experienced in generating oral tolerance and preventing allergy and enteropathy.[25] TGF-β rather than IL-10 appears to be the important cytokine in preventing the development of infant food allergy. TGF-β responses but not IL-10 responses to cow's milk were impaired among cord blood mononuclear cells in the children of allergic mothers.[106] This lack of TGF-β response is seen within the mucosa of infants with CMSE and other food-related enteropathies.[60,64] The early-life generation of TGF-β1–producing Th3 cells is an area of great importance for future study of food-allergic sensitization. Infectious exposures are certainly one such factor, and blockade of innate immune responses to the enteric flora induced food-sensitive enteropathy in a notable murine model.[107] It is intriguing that duodenal TGF-β–producing cells are present at a much higher density in healthy rural African than in UK infants.[108]

On a worldwide basis, it is possible that food-sensitive enteropathy may contribute to malnutrition in young children within the tropics. Although repeated infectious challenge and caloric insufficiency are centrally implicated in the development of tropical enteropathy and the clinical syndrome of protein-energy malnutrition, there is also evidence that affected children may become intolerant of cow's milk or soy and improve on exclusion diets.[109–111] Because paracellular permeability is a major determinant of subsequent mortality in tropical infants[112] and the progression to marasmus is characterized by the increasing dominance of IFN-γ and TNF-α over IL-10 and TGF-β in the mucosa,[108] it is possible that acquired loss of oral tolerance may contribute to the high mortality from malnutrition among developing world infants. Food protein–induced enteropathy may thus be a condition of truly global importance.

B-Cell Responses

The role of mucosal IgA in the maintenance of a tolerant response to dietary antigens is probably great. The uptake of mucosal IgA by epithelium, and its transport into the lumen, is probably a key event in regulating the presentation of dietary proteins to the mucosal immune system. Food-allergic responses and low-grade enteropathy are common among adults with constitutive IgA deficiency.[113] A condition of transient IgA deficiency in infants prone to allergy was suggested by Taylor and Soothill and their colleagues as a cause of increased antigen entry and allergic sensitization.[114,115] At the whole population level, low IgA is, indeed, more predictive of overall allergic sensitization than high IgE.[116] The development of mucosal IgA responses appears to relate to an increase in the mucosal density of IgA2-producing plasma cells because IgA1 cell density is relatively stable after birth in most infants,[117] although around 10% of studied infants showed delayed maturation of all IgA responses.[118] Factors regulating IgA2 development are still poorly understood, but reduced mucosal IgA responses predispose the patient to the development of CMSE after gastroenteritis.[119] However, IgA production appears to be increased overall within the mucosa in active CMSE as a consequence of inflammation.[120] Because the cytokine responsible for the shift of B-cell isotype to IgA is TGF-β, this molecule appears to play a pivotal role in both T- and B-cell responses to dietary antigen.[25] The evidence that mucosal TGF-β production is low in infants with food-sensitive enteropathy[60,64] suggests that the mechanisms by which TGF-β is induced may be a central question in understanding the pathogenesis of food-sensitive enteropathy.

It is clear that normal infants without enteropathy may produce circulating antibodies to cow's milk proteins.[1,12,13,41] There have been few reports suggesting a pathogenic role for circulating IgG antibodies against dietary components, and the detection of IgG to cow's milk or gliadin is notoriously nonspecific. However, there have been reports suggesting the possibility of immune complex formation and complement activation in milk allergy[121] and the potential induction of antibody-dependent cell-mediated cytotoxicity to β-lactoglobulin–coated cells in CMSE,[122] although it has still not been established whether these phenomena contribute to the development of the CMSE lesion.

Transient IgE responses to foods are seen in normal children and are of doubtful relevance.[123] High-level IgE responses are usually pathologic but more important in systemic food allergies than in enteropathy. Production of IgE is favored by the dominance of Th2 cytokines, particularly IL-4 and IL-13.[124] The receptors for both cytokines share a common α chain (IL-4Rα), and increased signaling through this receptor is associated with increased atopy.[125] Blockade of either IL-4Rα or its downstream intracellular signaling molecule Stat-6 (signal transducer and activator of transcription 6) may inhibit IgE-mediated allergic reactions.[126,127] IgE may be produced within the mucosa, even in the absence of a systemic IgE response, and may be delivered into the gut lumen by a mechanism distinct from IgA transport (Figure 44.2-5).[128,129] There is up-regulation of expression of the IgE receptor CD23 on the enterocytes in active CMSE in infants, although little is known about the functional responses to mucosal IgE production by the small intestinal epithelium.[130] It is thus possible that mucosal IgE responses may be elicited by dietary antigen, even if skinprick tests are negative and circulating specific IgE is undetectable.[131]

Mast Cell and Eosinophil Responses

As mentioned above, it is common to see focal increase of eosinophils within the mucosal lesion of food-sensitive enteropathy. There has been an important recent advance in determining the mechanisms of eosinophil recruitment, and studies by Hogan and colleagues and others have clarified the central role of two mediators, IL-5 and eotaxin (Figure 44.2-6).[132–135] Studies using mutant mice, with targeted deletion of these genes, have identified separate roles for both molecules in the antigen-driven recruitment of eosinophils to the intestinal mucosa. Mice sensitized to ovalbumin and then challenged with oral administration of ovalbumin-coated beads made an allergen-specific Th2 response, characterized by increased circulating IgE and IgG1 with mucosal eosinophilia.[132] However, mice defi-

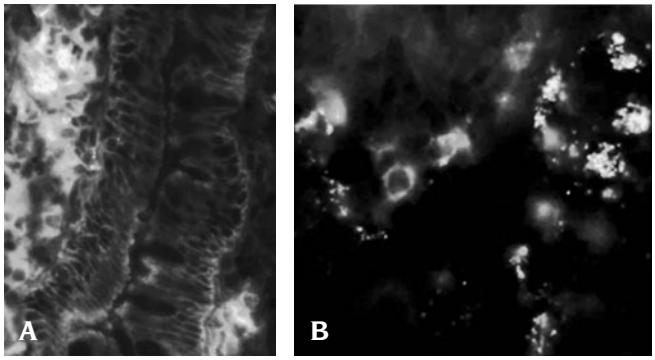

FIGURE 44.2-5 Immunofluorescence for IgA and IgE in a case of cow's milk–sensitive enteropathy. *A* shows details from two adjacent villi, with the epithelium and luminal glycocalyx outlined by IgA, whereas strongly staining IgA plasma cells are seen in the adjacent lamina propria. *B* shows two types of IgE-positive cells: round plasma cells with large unstained nuclei and homogeneously staining periphery and intensely staining mast cells showing the characteristic granular deposition. There is evidence of some mast cell degranulation, with non–cell-associated deposition of IgE. This child had a normal total IgE, undetectable milk-specific IgE, and negative skinprick tests, demonstrating the compartmentalization of IgE responses that can occur within the intestine.[25,70,128–130]

cient in IL-5 had reduced circulating eosinophils, whereas those deficient in eotaxin could not recruit eosinophils to the mucosa, although they showed evidence of systemic sensitization with both IgE and IgG1 responses.

A particular role for eosinophil recruitment in the disturbance of gut motility frequently seen in food allergies was provided by the same group in similar studies of gastric eosinophil recruitment.[133] Using ovalbumin-coated beads, it was possible to confirm that antigen induced delayed gastric emptying in sensitized animals and that this was dependent on antigen-specific recruitment of mucosal eosinophils. Further studies using these animals have identified the esophagus as a particular target for eosinophil recruitment, both by dietary antigen and by less predictable stimuli such as inhaled aeroallergens[134] or increased systemic IL-5 release.[135]

Human relevance of these murine findings is suggested by evidence of increased expression of IL-5 mRNA within the small bowel mucosa after challenge of food-allergic patients but not in atopic or nonallergic controls.[136] Circulating T cells from food-allergic children produce IL-5 on food challenge, whereas those from tolerant children do not.[67,137] Eotaxin expression is increased in the esophageal basal epithelium in infants with gastroesophageal reflux secondary to CMSE compared with those with a primary mechanical reflux.[138] These studies suggest that IL-5 and eotaxin are two potential targets for specific immunotherapy in food-allergic enteropathy.

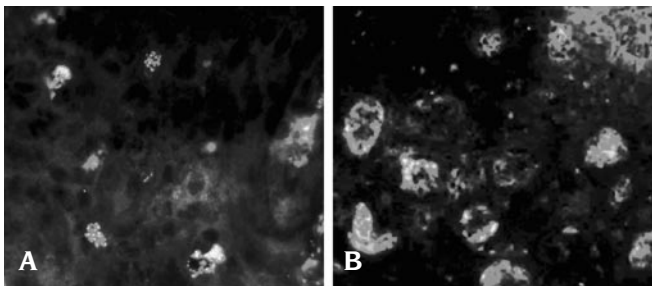

FIGURE 44.2-6 The major mediators of eosinophil recruitment in food-allergic enteropathy.[132–136,138] *A* is a high-power view showing interleukin (IL)-5 immunoreactive cells with intense granular IL-5 expression. Both mast cells and eosinophils were shown to be IL-5 positive on double staining. *B* shows intense eotaxin expression within crypt epithelium, with weaker staining on the surface epithelium in the top-right.

DIAGNOSTIC STRATEGIES FOR FOOD-ALLERGIC ENTEROPATHY

Food Challenge Testing

The core requirement for diagnosis of food allergy is response to an elimination diet with recurrence of symptoms on challenge.[139,140] Other diagnostic tests may support the diagnosis but are secondary to this. In the circumstance of allergy to a single food, complete exclusion should induce relief of all symptoms and restore normal growth if this was impaired. For a secure diagnosis, the second requirement is a recurrence of symptoms on challenge with the food antigen. In some cases, however, parents may refuse challenge if their child has improved dramatically and diagnostic tests such as skinprick tests were positive. Insurance companies often require positive food challenges for confirmation of diagnosis.

In addition to the initial diagnostic challenge, later challenges may be performed when it is reasonably likely that tolerance has been regained. This is usually after the age of 2 years, and tolerance may be regained later than this in children with multiple food allergies. The ESPGHAN recommendations for investigation of possible food-sensitive enteropathy included the need for small bowel biopsy in cases of failure to thrive or diarrhea but stated that blinded challenge was not always necessary in younger children.[141] In cases of multiple food allergies, response to elimination of just one or two antigens will be incomplete, and it may be quicker to move initially to a very restricted diet, which can subsequently be broadened. It may be difficult to persuade some parents to broaden the diet if there has been a very clear clinical response or if they have received advice from some alternative practitioners or Internet sites. In cases of exclusively non–IgE-mediated allergy, it may become very difficult to obtain a clear picture of the true level of sensitization.[16,29] The associated minor immunodeficiency may increase the chance of viral infection during prolonged food challenges.[16,115,116] It is thus important not to perform food challenges while a child is systemically unwell. For multiply sensitized children, it can be logistically difficult to perform lengthy blinded challenges.

Open food challenges are performed either in the outpatient clinic or in a day ward, depending on the severity and type of reaction expected.[29,142] It is usual to perform a graded challenge in cases of immediate reaction, beginning with single drops and moving to increasing amounts, with a period of several minutes between stages. It is mandatory to have adequate medical supervision and appropriate

resuscitation facilities in cases of immediate reaction. In cases with a history of anaphylaxis, an intravenous line should be inserted.

For the child who makes delayed reactions only, as in classic CMSE, challenges have to be extended over several days and are usually completed at home. In a recent large study of 370 challenges in 242 children, 5 children demonstrated mild reactions immediately after intake, but only on the second day at home and not at initial administration.[143] These reactions were subsequently confirmed by the skinprick test and double-blind placebo-controlled food challenge (DBPCFC). Hill and colleagues identified early reactions to milk (within 1 hour), intermediate reactions (up to 1 day), and very late reactions (up to 3 days).[49,73] For the very late reactions, the cumulative intake of milk was high—up to 120 mL. There are reports of even later responses to cow's milk challenge, with gastrointestinal, respiratory, or cutaneous responses beginning a week after challenge.[144] Thus, the interpretation of food challenges is by no means always straightforward, particularly in the case of non–IgE-mediated delayed symptoms. Whereas some cases of food-sensitive enteropathy have associated immediate reactions, many do not. There is a difficult balance between missing true late responses and colluding in the overdiagnosis of food allergy.

The "gold standard" challenge test is the DBPCFC. This is much easier for immediate reactions than delayed ones and can be quite cumbersome if several antigens need to be tested over a prolonged period. However, it does provide a degree of certainty that other challenges do not.[1,29,140,142] For immediate allergies, DBPCFCs are highly specific and of high prognostic value, with a suggested false-positive rate below 1% and a false-negative rate below 5%.[140] No universally accepted protocol exists,[140] but suspected allergens or placebo are given in a hidden form, either in liquid or inside a capsule, over a period of around 2 hours. The child should not have been given the antigen for at least 2 weeks and should be well and not receiving antihistamines. For cases of food-sensitive enteropathy, it may be necessary to exceed the total 10 g usually administered and to continue the challenge for at least 3 to 4 days.

Skinprick Testing

Skinprick tests are much less useful in straightforward food-sensitive enteropathy than in immediate allergies.[16,142] These tests are performed by placing a drop of the potential antigen on the skin and introducing it into the skin with a lancet. Both positive and negative controls are used (histamine and saline, respectively), and the results are read at 15 to 20 minutes. Sensitizing antigens cross-link surface IgE on cutaneous mast cells and induce mast cell degranulation. The size of wheal elicited may be of prognostic value. Although a wheal of greater than 3 mm is usually regarded as positive, in young infants, the histamine response is smaller because there are fewer cutaneous mast cells.[47] For immediate reactions, larger skinprick wheals (> 8 mm) may predict positive food challenges.[145,146]

The atopy patch test may potentially offer a better insight into food-sensitive enteropathies because the cellular mechanisms of this skin reaction are more similar to those in enteropathy. In the atopy patch test, the antigen in question is maintained for 48 hours against the skin in a sealed patch before being removed, and the skin response is studied after a further 24 hours.[48] A positive test is characterized by erythema and induration at this stage. The test appears to be mediated by a specific T-cell response to dietary antigen,[147] in contrast to the simple mast cell responses in the skinprick test.

The combination of patch testing with skinprick testing or specific IgE serology may detect food-sensitized children who were negative on classic testing.[148,149] Despite an apparently high sensitivity for delayed responses of up to 89%,[48,148–150] the atopy patch test has not yet become widely used and remains under evaluation. There are still few data on the relevance of patch testing in food-sensitive enteropathy, in contrast to eczema. It cannot be assumed that these two delayed hypersensitivity reactions will have identical skin patch testing results because the T cells that infiltrate the skin during the test are likely to express skin-homing markers such as CLA, whereas those involved in CMSE are likely to express the gut-homing integrin $\alpha_4\beta_7$ and not CLA. There are also possible logistic difficulties because reading of the test requires a second assessment after 3 days, and there is the possibility of artefactual induction of local erythema while unsupervised in cases of factitious illness.

Specific IgE Testing

Testing of specific IgE production to individual foods can be helpful, providing results complementary to skinprick testing. For immediate allergies, the information provided can be clinically predictive, although they are less useful in straightforward enteropathies. Positive predictive values of 95% were found for egg, milk, peanut, and fish reactions, at different thresholds.[151] The tests are reported on a semiquantitative scale of 0 to 6, with a likelihood of immediate reaction at level 3 or more. Higher titers of specific IgE for milk, casein, or β-lactoglobulin increase the likelihood of long-lasting sensitization.[152]

Study of epitope sequences bound by food-specific IgE may give prognostic information. Binding to linear peptide sequences within the egg antigen ovomucoid was associated with long-lasting allergy, whereas binding to discontinuous sequences (only bound by IgE because of protein folding) was seen in those who outgrew allergies.[39] Thus, some children will react to raw but not cooked egg, where the tertiary sequence has been disrupted. Similarly, IgE reaction to linear sequences in α_{S1}-casein, α_{S2}-casein, and κ-casein appears to be predictive of long-lasting milk allergy.[39,40] In future, epitope sequencing may identify infants at risk of long-lasting food allergies and thus potentially likely to benefit from immunotherapy.

In Vitro testing

Lymphocyte function tests are still useful only in research rather than diagnosis, and in vitro stimulation tests have not proved as useful as once hoped. Analysis of cytokine production patterns in response to food antigens may provide one future approach, particularly if surface mark-

ers characteristic of either sensitized or tolerant cells can be identified. Peripheral blood lymphocytes from food-allergic children produce a Th2 response to antigen, dominated by IL-4, IL-5, and IL-13, whereas the response from healthy controls is more Th1 skewed, dominated by IFN-γ.[137] Mucosal T cells show a different pattern of reactivity, without the Th2 dominance. A promising technique involves tagging peripheral blood mononuclear cells with carboxyfluorescein succinimidyl ester, which allows recognition by flow cytometry of antigen-specific T-cell responses.[67] This confirmed that peripheral T-cell responses to peanuts in children with peanut allergy are indeed skewed toward Th2 in contrast to findings of a Th1-dominated response in healthy controls or those who had outgrown their allergies.[67] The authors report similar skewing in milk and egg allergy, suggesting a common mechanism in IgE-mediated food allergies. Thus, it is the host immune response, rather than specific effects of food antigens, that determines whether allergy is induced. As mentioned above, the mucosal pattern in food-sensitive enteropathy is less straightforward and is characterized by preserved Th1 responses but decreased Th3-type regulatory responses.[57,60,64]

MANAGEMENT OF FOOD-SENSITIVE ENTEROPATHY

Primary Prevention

It remains controversial whether food-sensitive enteropathy and other food allergies can be prevented by stringent avoidance of allergies in early life.[153] Two position statements by the AAP[42] and the ESPACI with ESPGHAN [43] differ in some important respects. Neither considered food-sensitive enteropathy alone but rather the broader topic of overall food allergies. Both statements suggest that primary prevention should be limited to high-risk infants only and recommend use of hypoallergenic formulas, usually extensively hydrolyzed, rather than soy milk for bottle-fed high-risk infants. The degree of risk is defined on family history grounds, the AAP statement requiring two first-degree relatives with atopic disease and the ESPACI/ESPGHAN statement only one. Neither recommend maternal exclusion diets during pregnancy, whereas both recommend exclusive breastfeeding but for slightly different periods (AAP 6 months, ESPACI/ESPGHAN 4–6 months). The AAP, but not ESPACI/ESPGHAN, recommends exclusion of peanuts and nuts during lactation. The AAP also recommends much later introduction of cow's milk and eggs than the European statement.

A large study from the German Infant Nutrition Intervention study group randomly assigned 2,252 at-risk infants at birth to receive one of four blinded formulas (cow's milk based, partially hydrolyzed whey, extensively hydrolyzed whey, or extensively hydrolyzed casein).[154] The primary end point at 1 year was the presence of one or more of atopic dermatitis, gastrointestinal food allergy, or urticaria. The study had a high attrition rate owing to exclusive breastfeeding in 865 cases and noncompliance or dropping out in 442 cases. Study of the 945 remaining infants showed a protective effect of extensively hydrolyzed casein compared with unmodified cow's milk (9% vs 16% had allergies). Atopic dermatitis was reduced in infants fed extensively hydrolyzed casein or partially hydrolyzed whey formulas.[154]

Treatment of Established Food-Sensitive Enteropathy

For treatment of established food allergies overall, both the AAP[42] and ESPACI/ESPGHAN[43] statements recommend complete exclusion of the causative antigen. For the treatment of the milk-sensitized infant, both recommend an extensively hydrolyzed but not a partially hydrolyzed formula. The AAP guidelines but not the ESPACI/ESPGHAN guidelines also recommend soy as an alternative, whereas both recommend an amino acid formula for the infant who is intolerant of hydrolysates. Neither recommend the use of unmodified goat's or sheep's milk. For the infant who sensitizes while breastfed, both statements support maternal exclusion of the relevant antigen, whereas the AAP further recommends weaning to an extensively hydrolyzed formula or soy milk. With respect to probable food-sensitive enteropathy, both recommend extensively hydrolyzed or amino acid formulas. Thus, in cases of diagnosed or probable CMSE, soy milk feeds are not recommended as an alternative.[42,43]

FUTURE POSSIBILITIES FOR FOOD-SENSITIVE ENTEROPATHY

As mentioned above, there is evidence that the normal flora plays a role in the induction of tolorogenic lymphocytes and the establishment of oral tolerance.[155] Indeed, oral tolerance cannot be established normally in germ-free mice without a gut flora.[156] Handling of the newborn infant was shown in a study of the 1970 UK national birth cohort to affect the incidence of allergic disease in young adulthood.[157] The early gut colonization of allergic infants differs from those without allergies.[158] Thus, one major question is whether the "clean child hypothesis"[159] may apply at the level of the normal gastrointestinal flora and whether the major changes in early colonization in the last half-century[25,160] have played a role in the increasing incidence of allergies and the novel forms of food allergy such as breast milk sensitization. Because interaction between gut bacteria in early infancy and innate immune cells may determine whether a response sufficient to induce tolerance occurs, probiotic organisms represent a potentially important form of immunomodulator, particularly in infancy. However, much work remains to be done to determine the ideal dosage, timing, and type of probiotic.[160]

The potential role for probiotics in prevention of food allergies in susceptible infants is thus likely to be based on the role of luminal bacteria in inducing a tolerant lymphocyte response.[25,60] Genetic variation in receptors for bacterial products, such as Toll-like receptors and NOD proteins, is known to occur and is very likely to be related to allergic sensitizations,[161] particularly because Toll receptors are

also expressed on mast cells.[162] Thus, the early results using probiotics neonatally, suggesting that non–IgE-mediated allergies in particular may be reduced by *Lactobacillus GG* or *Escherichia coli*, are of particular relevance for the future.[163–165] The use of probiotics at birth may be more effective because it may allow stable long-term colonization, which does not occur if probiotics are administered after the first 6 months of life.[166]

An alternative approach to whole probiotic organisms is to attempt to induce tolerance with bacterial products. A suspension made from killed *Mycobacterium vaccae* reduced airway eosinophilia in murine asthma by inducing a TGF-β– and IL-10–dependent regulatory T-cell response that was allergen specific.[167] More safety data need to be accumulated and placebo-controlled trials completed, but such approaches appear promising for the future.

In the treatment of established food-sensitive enteropathy, there may be potential benefit from supplementing dietary ω-3 fatty acids, and a murine model of ovalbumin-induced enteropathy was attenuated by reducing intake of ω-6 fatty acids or by supplementing ω-3 intake.[168] There are also murine data to suggest that the induction of regulatory lymphocytes necessary for oral tolerance may be enhanced by administration of vitamin D receptor ligands such as 1,25-dihydroxyvitamin D_3.[169]

Other potentially novel therapies for food allergies include immunotherapy, which depends on gradually increasing the dose of antigen until a Th1 response is made.[170] Future targets include small peptide vaccines, foods with altered protein sequences, deoxyribonucleic acid (DNA) immunization, and IgE-blocking agents. The small peptide vaccines are based on synthesizing 10 to 20 amino acid portions of a known allergen, which are able to block IgE binding sites but are too short to cross-link IgE and trigger mast cell degranulation.[170] Mutations in food proteins may also prevent T-cell or mast cell responses, which are so far best studied for the major peanut proteins Ara h1-3, whose T-cell and IgE binding domains have been established and mutated.[171]

There are potentially important monoclonal antibodies that may play a role in attenuating severe food allergies. Although anti–IL-5 therapy appears to be a logical choice in eosinophil-mediated enteropathy, clinical trials in asthma have been disappointing, with a reduction in peripheral eosinophilia in asthma without an effect on bronchoconstriction.[172] In addition to IL-5 monoclonals, eotaxin blocking therapy would appear to be a logical choice for eosinophilic enteropathy in cases in which food exclusion alone is insufficient. There have also been promising results with anti-IgE monoclonal therapy in peanut allergy,[173] which is of more relevance at this stage to anaphylaxis than enteropathy.

One future challenge may be the induction of food-allergic responses, including enteropathy by genetically modified foods. In one notable case, the Brazil nut 2S albumin protein was introduced transgenically into soy to increase the protein content of cattle food. This protein turned out to be a previously unsuspected Brazil nut allergen, and the transgenic soy induced reactions in Brazil nut–allergic patients.[174] Many potentially allergic molecules are large, heavily glycosylated, and resistant to digestion.[175] It has been suggested that novel proteins should be tested by immunoassay against sera from allergic patients.[176] However, this may not detect the epitopes more likely to cause small intestinal enteropathy, and it is possible that in vitro organ culture may provide a better guide to the responses within the specialized microenvironment of the intestine that may lead to an enteropathic response to novel antigens.[131]

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3. *Autoimmune Enteropathy*

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Over the past few years, major advances in the understanding of the pathophysiology of intractable diarrhea of infancy (IDI) were made, allowing a new conceptual view of this heterogeneous group of different diseases. The term "intractable diarrhea of infancy with persistent villous atrophy" is based on the clinical observation of a diarrhea starting within the first 2 years of life, which is abundant (> 100 mL/kg/d) and persists despite bowel rest, along with a flat intestinal mucosa. Diarrhea rapidly becomes life threatening, and long-term total parenteral nutrition (TPN) is required. A clearly distinct presentation of IDI is in the form of autoimmune enteropathy (AIE). The first report of AIE in the literature goes back to 1978.[1] McCarthy and colleagues reported on an adolescent boy with immunoglobulin (Ig)A deficiency who showed a severe enteropathy with total villous atrophy and circulating gut autoantibodies.[1] The term AIE was introduced by Unsworth and Walker-Smith for severe persistent diarrhea in the absence of immune deficiency but associated with autoimmune disorders.[2] One characteristic of AIE is the presence of specific complement-fixing circulating antibodies directed against small intestinal and colonic epithelial cells.[3] A historic keystone in the description of protracted, immune-mediated diarrhea is the report of Powell and colleagues, who observed in the same family over three generations 17 boys with various autoimmune disorders. Eight of them died of severe protracted diarrhea.[4] This and other observations clearly suggest an X-linked mode of transmission.[2,5–10] However, some rare cases of girls presenting with AIE have been reported.[11–13] Meanwhile, there are several reports on immune-mediated profuse diarrhea occurring within the first few months of life, often preceded or accompanied by insulin-dependent diabetes mellitus or other endocrinopathies, indicating a systemic rather than an isolated gastrointestinal disease.[8–10]

Based on recent advances on the genetics, pathophysiology, and clinical presentation, AIE can be classified into three different types: the classic form of AIE, type 1, which is identical to the so-called IPEX syndrome (immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome), type 2 (without extraintestinal manifestations), and type 3 (in girls). AIE has to be clearly distinguished from profuse diarrhea occurring in different forms of immunodeficiency syndromes.

IPEX SYNDROME (AIE TYPE 1)

IPEX is a rare disorder, and clinical experience is based on a limited number of cases. To date, no estimates of incidence have been proposed. The main clinical characteristic of IPEX is the combination of early-onset type 1 diabetes mellitus, severe immune-mediated enteropathy, and eczema-like dermatitis (see below). IPEX is usually fatal during early infancy. Recently, with the characterization of specific mutations in the *FOXP3* gene, a first clue to the understanding of this X-linked disorder was found.[14,15]

Pathophysiology

First insight into the pathophysiology of IPEX came from scurfy mice, which are a naturally occurring X-linked mutant presenting with symptoms very similar to human IPEX, that is, massive lymphoproliferation, scaly skin, diarrhea, intestinal bleeding, anemia, thrombocytopenia, and hypogonadism.[16–18] Symptoms occur only in hemizygous mutant males; female carriers are asymptomatic. In scurfy, CD4+CD8– T cells are chronically activated and play a key role in the development of the disease.[19,20] These cells are characterized by an up-regulation of the cell surface antigens CD69, CD25, CD80, and CD86, a marked hyperresponsiveness to activation via the T-cell receptor (TCR), and a decreased requirement for costimulation through CD28. In scurfy, high levels of cytokines, such as interleukin (IL)-2, -4, -5, and -10 and tumor necrosis factor (TNF)α, were produced by these cells.[19,20] The gene mutated in scurfy mice was recently identified (*FOXP3*) and cloned on the X chromosome.[16,21] It encodes a 48 kD protein of the forkhead (FKH)/winged helix transcription factor family, named scurfin.[21] This protein is expressed at low levels in CD4+ T cells, with a more abundant expression in the CD4+/CD25+ T-cell subgroup. The structure of the protein suggests that it has deoxyribonucleic acid (DNA) binding activity. Schubert and colleagues recently showed that scurfin acts as a repressor of transcription and regulator of T-cell activation.[22] Intact scurfin represses transcription of a reporter containing a multimeric FKH binding site. Such FKH binding sites were identified adjacent to nuclear factor of activated T cells (NFAT), regulatory sites in the promotors of IL-2, or granulocyte-macrophage colony-stimulating factor enhancer. This observation indicates that intact scurfy is capable of

directly repressing NFAT-mediated transcription of the IL-2 gene in CD4+ T cells upon activation.[22] The exact molecular mechanisms leading to scurfy are not yet fully understood. However, there is experimental evidence that the biologic activity of scurfin depends on a functional FKH domain. In scurfy mice, a 2 bp insertion results in a premature stop codon leading to a loss in the C-terminal domain.[21] The loss of function of scurfin may result in abnormal (nonsuppressed) T-cell reactivity, leading to an uncontrolled inflammatory reaction.

Genetic Background

The overwhelming clinical similarities between scurfy mice and patients led to an exhaustive genetic analysis in IPEX families. Genetic mapping in these families allowed identification of the human IPEX locus to chromosome Xp11.23-q13.3.[23–25] The gene was named *FOXP3* (or *JM2*), and its protein product was named scurfin.[23] As discussed, scurfin is implicated in the regulation and suppression of T-cell activation.[22] Wildin and colleagues recently reported that in 10 of 11 screened IPEX syndrome families, mutations were found within the coding region, potentially leading to a scurfin protein product with loss of function.[26] In the eleventh family, a mutation in the 3′ untranslated region of *FOXP3* was observed. Several IPEX mutations were within the winged helix domain of scurfin, altering its DNA binding capacity.[14] In two patients with typical clinical symptoms of IPEX, no mutations were found within the coding regions of *FOXP3*,[14,15] suggesting that regulatory or conditional mutations may occur outside *FOXP3*, such as described by Bennett and colleagues in the polyadenylation signal following the final coding exon of *FOXP3*.[27] This may result in a decreased *FOXP3* messenger ribonucleic acid (RNA) expression, probably owing to nonspecific degradation of the aberrant RNA. In addition, it is possible that other forms of IPEX (AIE with extraintestinal manifestations) may also occur in boys. The molecular mechanisms of IPEX without mutations in *FOXP3* remain to be elucidated. Females are obligate carriers of these IPEX mutations without any clinical symptoms.[28] None of the mutations described in IPEX patients were observed in 240 nondisease, ethnically diverse individuals, excluding the possibility of gene polymorphisms unrelated to IPEX.[14]

Clinical Presentation

IPEX can be considered a classic, full presentation of AIE (Table 44.3-1). The major clinical symptoms are the association of a severe enteropathy with insulin-dependent diabetes mellitus along with eczema, hematologic abnormalities, and, eventually, other endocrinopathies.[26,29–31] In many cases, the onset of diabetes is prior to the first intestinal symptoms. Levy-Lahad and Wildin suggested that diabetes is the result of a complete inflammatory destruction of pancreatic islet cells.[32] Equilibration of diabetes with insulin in these patients is sometimes rather difficult.

The onset of diarrhea is often within the first 3 months of life; however, later onset was occasionally described.[6,33,34] Characteristically, diarrhea is of a secretory nature with stool sodium > 100 mEq/L, and it persists despite bowel rest. In our experience, stool volumes are approximately 150 mL/kg/d, with sometimes mucus or blood discharge. Patients usually develop a protein-losing enteropathy with a markedly increased clearance of α_1-antitrypsin and hypoalbuminemia. Severe clinical expression is most often a sign of a poor prognosis. Electrolyte imbalances are secondary to diarrhea. Electrolyte correction and stabilization of IPEX patients can initially be achieved only by bowel rest and TPN.

Another clinical feature of IPEX is eczema (atopic dermatitis), which is often severe in presentation. Eczema is most often diffuse, involving the entire skin, and may show follicular dermatitis. It was recently reported that eczema is present in about two-thirds of patients.[26] Further symptoms, such as thyroiditis, hematologic abnormalities (Coombs-positive anemia, neutropenia,[34] and thrombocytopenia), and diffuse lymphadenopathy, are markedly less frequent.[26,34] Thyroiditis most often presents in the form of hypothyroidism, requiring substitutive treatment. In our personal experience, hypothyroidism occurred in 2 of 13 AIE patients followed at Necker-Enfants Malades Hospital. Renal involvement, in the form of glomerulonephritis, tubulopathy, or nephrotic syndrome, was also described in a subset of IPEX patients.[9,35,36] In our patients, two cases presented with biopsy-proven membranous glomerulonephritis.

Other organ systems that are occasionally involved are the liver, ranging from normal liver function to acute or chronic hepatitis,[4,7,10,37] and the lung. In three patients, diffuse pulmonary interstitial lymphoid infiltrates were noted at autopsy.[3,7,38]

Endoscopy

Upper and lower endoscopies are mandatory when AIE is suspected. The entire gastrointestinal tract, from the stomach to the rectum, can be involved. In most cases, there is a marked discrepancy between macroscopic, endoscopic, and histologic findings. Macroscopically, the mucosa of the stomach, duodenum, jejunum, and ileum shows only mild abnormalities, that is, a variable degree of enhanced mucosal granularity along with erythema and sometimes erosions. Normally, no ulcerations are observed. Macroscopic signs of small bowel mucosal atrophy are rather discrete. Colonic lesions are more pronounced, that is, loss of the normal vascular pattern owing to inflammatory edema, along with erythema, potentially involving the entire colonic mucosa. In addition, a marked granular aspect of the mucosa is regularly observed. These findings are most

TABLE 44.3-1 CLINICAL MANIFESTATIONS OF IPEX

Diarrhea
Atopic dermatitis
Food intolerance
Hypothyroidism
Malnutrition
Glomerulonephritis, tubulopathy
Growth retardation/failure
Hemolytic anemia, thrombocytopenia
Diabetes mellitus
Lymphadenopathy

IPEX = immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome.

often homogeneous along the entire colon and not distributed in a patchy manner with normal mucosa interposed. However, rectosigmoidal sparing can occur. Similarly, as in the upper gastrointestinal tract, no ulcerations are observed in the colon.

Histology

Obligate histologic findings in IPEX are the combination of villous atrophy (moderate to severe) and a massive mucosal mononuclear cell infiltration (Figure 44.3-1). This infiltrate consists predominantly of T lymphocytes and is a main feature of AIE, helping to distinguish this entity from other cases of IDI related to constitutive, inherited defects of enterocytes.[38] In most cases, severe to total villous atrophy is associated with crypt hyperplasia. Total villous atrophy on duodenal biopsies can initially mislead the physician to suspect celiac disease. However, in celiac disease, villous atrophy is associated with a striking increase in the number of intraepithelial lymphocytes.[39] In contrast, T-cell infiltration in immune-mediated enteropathy predominates in the lamina propria, with no or only a moderate increase of intraepithelial lymphocytes.[40] Whereas in celiac disease, a marked increase in T lymphocytes expressing the TCR of the δ type was observed,[41] in AIE, TCR expression is restricted to the α/β+ subset. In some patients, villous atrophy is associated with epithelial cell death and crypt abscess formation (see Figure 44.3-1B). Recent studies indicate that enterocyte cell death occurs via apoptosis, probably induced by activated, cytotoxic lymphocytes. The number of goblet cells is also reduced, and, in some cases, goblet cells are almost absent.[40,42]

Mononuclear cell infiltration within the lamina propria includes mainly CD4+ T lymphocytes and macrophages, with numerous cells expressing CD25. Human leukocyte antigen (HLA)-DR expression is markedly increased on crypt epithelium.[38] These inflammatory changes are largely observed in small bowel mucosa, but they can also be seen in other parts of the digestive tract, such as the stomach or colon.[37] All along the entire intestinal tract, the same type of lesions can be observed, including epithelial cell apoptosis, crypt abscess formation, and mononuclear cell infiltration of the lamina propria. Extensive digestive involvement is usually associated with a poor outcome.

Laboratory Findings

Peripheral blood lymphocyte counts are normal, as are the T- and B-cell subsets in almost all patients (Table 44.3-2). In addition, the CD4-to-CD8 ratio was also reported to be normal, except in one observation with a slightly increased CD4 count.[10] T-cell proliferation assays (mitogens, antigens) are within the low normal range or completely normal in the vast majority of patients. Humoral immunity is normal, with normal vaccination titers and normal immunoglobulin levels for IgG, IgM, and IgA. In clear contrast, IgE levels are often dramatically raised and can be considered as one diagnostic criterion of IPEX. In addition, persistent or periodic eosinophilia is also frequent, reflecting an atopic-allergic background. In fact, skin tests for immediate hypersensitivity are often pathologic in IPEX.[24,38]

Autoantibodies of the antienterocyte or anticolonocyte type are present in the vast majority of IPEX patients; however, in some patients, the search for autoantibodies was

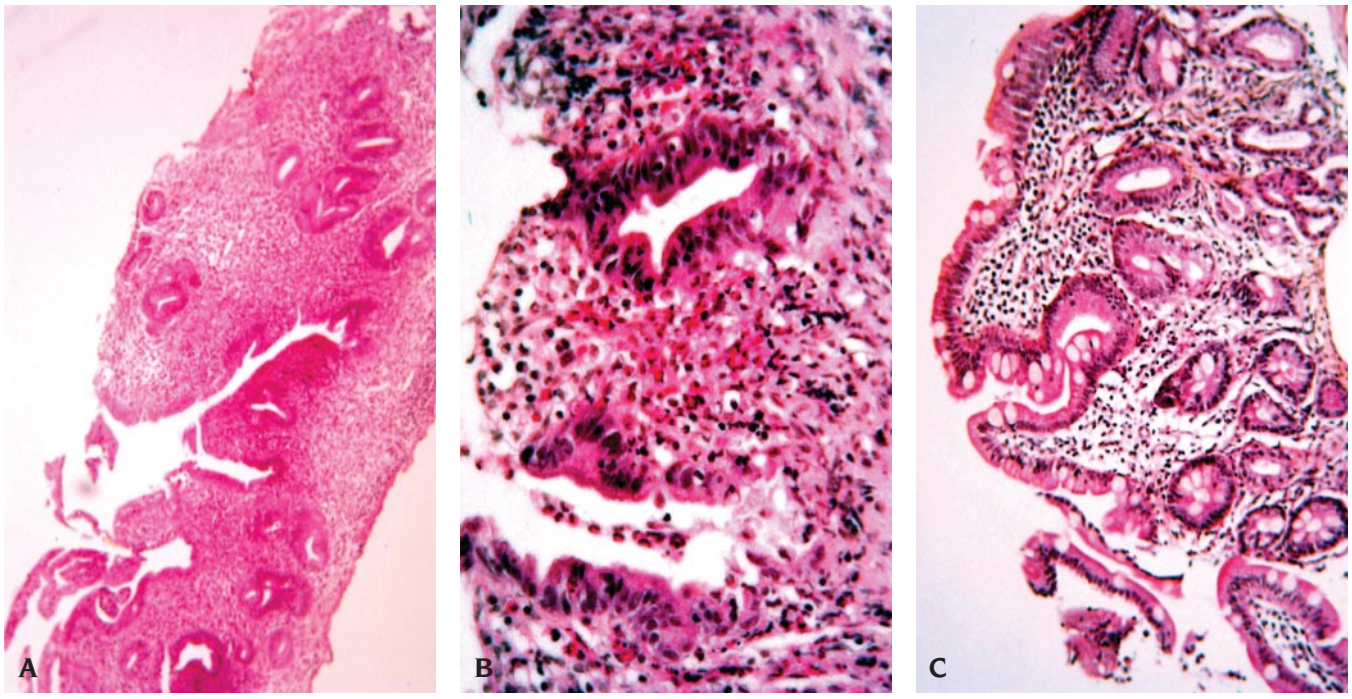

FIGURE 44.3-1 Duodenal biopsy of a patient with autoimmune enteropathy type 2 showing the dense mononuclear infiltrate of the lamina propria. Note the marked villous atrophy and dedifferentiation of the intestinal epithelial cells. *A* and *B*, At the time of diagnosis (before treatment). *C*, After 4 weeks of treatment with tacrolimus, methylprednisolone, and azathioprine, the inflammatory infiltrate is markedly reduced, and a normal intestinal architecture is visible (hematoxylin and eosin; ×10 original magnification).

TABLE 44.3-2 LABORATORY FINDINGS IN IPEX

BIOLOGIC PARAMETERS	FINDING IN IPEX
White blood cells	Normal; sometimes eosinophilia
CD4-to-CD8 ratio	Normal or CD4+ slightly increased
IgG, IgA, IgM	Normal
IgE	High–extremly high
Antienterocyte antibodies	Present (IgG type, sometimes IgA or IgM)
Anticolonocytes antibodies	Present (IgG type, sometimes IgA or IgM)
Anti–goblet cell antibodies	Present or absent
Anti-AIE-75 antibodies	Present or absent
Anti-GAD	Present or absent
Anti-SMA	Present or absent
Anti-DNA	Present or absent
Albumin	Always (markedly) decreased
Liver enzymes	Can be increased
α_1-Antitrypsin clearance	Pathologic

AIE = autoimmune enteropathy; DNA = deoxyribonucleic acid; GAD = glutamic acid decarboxylase; Ig = immunoglobulin; IPEX = immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome; SMA = smooth muscle antibodies.

negative.[9,34,43] On the other hand, antienterocyte antibodies at very low titers were also observed in other inflammatory gut conditions, such as Crohn's disease, ulcerative colitis, or cow's milk allergy.[9] In the context of IDI, the presence of antienterocyte (human duodenum, jejunum) and/or anticolonocyte antibodies of the IgG type are thus highly suggestive of AIE. Antienterocyte antibodies of the IgA or IgM type were also described in a subgroup of patients.[9] High titers and the complement-fixing ability of antienterocyte antibodies indicate a poor prognosis. Indirect immunofluorescence studies showed that these autoantibodies are directed against components of the cytoplasm of mature enterocytes, with an increasing intensity toward the villus tip (Figure 44.3-2). Positive staining of the intestinal brush border membrane can also be observed. A pathogenetic role in the onset of intestinal inflammation—as suspected in the past—is rather unlikely.[2,3,6,44,45] The occurrence of antienterocyte/anticolonocyte antibodies is rather an epiphenomenon of intestinal inflammation. A precise kinetic study performed in one patient showed that autoantibodies occurred after the onset of intestinal lesions.[44] Anti–goblet cell antibodies are also encountered in AIE patients.[42] Another highly specific antibody often detected in IPEX patients is directed against a novel gut- and kidney-specific 75 kD antigen, named AIE-75.[35,46] This antigen shares over 99% identity with NY-Co-38, a colon cancer–related autoantigen.[47] Other circulating autoantibodies observed in IPEX are antibodies against gastric parietal cells, pancreatic islets, insulin, glutamic acid decarboxylase, and anti–smooth muscle, antiendoplasmic reticulum, antireticulin, antigliadin, antiadrenal, anti-antinuclear antibodies, and anti-DNA antibodies. Finally, antithyroglobulin or antimicrosomal antibodies can be seen without the clinical symptoms of hypothyroidism.[3,4,13,43–45,48–50]

No particular or pathognomonic laboratory abnormality is observed in IPEX. Clinical laboratory tests are within the normal range, apart from signs of diabetes mellitus, protein-losing enteropathy, hypothyroidism, and, eventually, cytopenia. Electrolyte disturbances are secondary to secretory diarrhea and hypoproteinemia, and decreased albumin levels indicate the degree of protein-losing enteropathy. Abnormal biochemical liver tests (increased transaminase activity and cholestatic markers) were reported[4,7,37]; however, this can also be secondary to TPN.

Highly elevated stool levels for TNF-α or calprotectin, as markers of intestinal mucosal inflammation, are regularly encountered in IPEX patients (personal unpublished data, 2004). With the induction of remission, these inflammatory fecal markers return to normal. A reliable marker for the degree of protein-losing enteropathy in AIE is an increased clearance of α_1-antitrypsin in the stool. Indeed, whereas, normally, this clearance in the stool is less than 20 mL/d, in our IPEX patients, α_1-antitrypsin clearance was pathologically elevated.

AIE TYPE 2: WITHOUT EXTRAINTESTINAL MANIFESTATIONS

A few patients were described in whom the disease was exclusively limited to the gastrointestinal tract in the form of secretory diarrhea with positive circulating antienterocyte/anticolonocyte antibodies and positive anti-AIE-75 antibodies. In these patients, no extraintestinal manifestations were reported.[6,37] A priori, *FOXP3* mutations are not a characteristic of these patients. However, in the past, mutational analyses of the *FOXP3* gene were not performed; therefore, it is unclear if these children do not present with a minor or monosymptomatic form of IPEX. On the other hand, other mutations in regulatory genes controlling T-cell functions on the level of the intestinal mucosa are very likely. It would be interesting to study if the long-term follow-up of these patients reveals the occurrence of extraintestinal symptoms. In our personal experience, three boys presented with an isolated intestinal manifestation of AIE without any notion of endocrinopathy, hematologic abnormalities, or renal involvement. *FOXP3* mutations were negative in all patients. However, all three patients had a

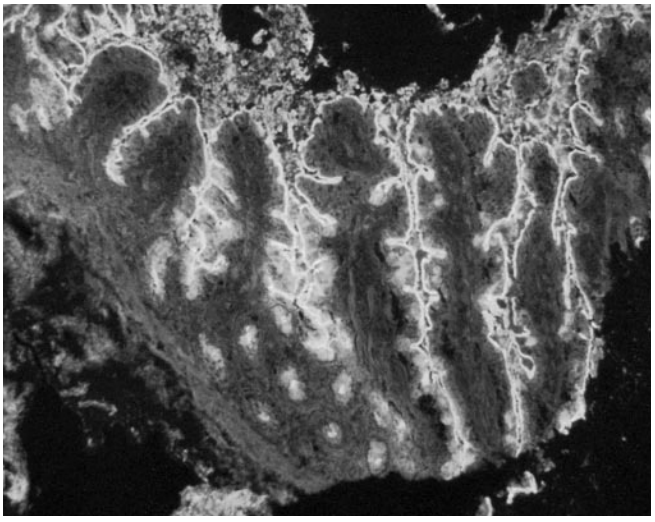

FIGURE 44.3-2 Immunofluorescence staining pattern of circulating antienterocyte antibodies, which are directed against the brush border membrane of intestinal epithelial cells.

marked eczema-like dermatitis. In this patient group, the clinical presentation of secretory diarrhea within the first few months of life and endoscopic and histologic findings were not different from typical IPEX patients (see above). In particular, the histologic picture of a marked mononuclear cell infiltration of the intestinal lamina propria, along with villous atrophy and epithelial cell apoptosis, was identical to that described in IPEX. The diagnosis of AIE type 2 is made by the presence of antienterocyte and/or anticolonocyte antibodies, along with positive anti-AIE-75 antibodies. Antienterocyte/anticolonocyte antibodies fix against intracytoplasmic compounds of mature enterocytes and, in some cases, against intestinal brush border membrane compounds (as discussed above). Future genetic analyses in these patients have to be performed to clarify if these patients carry a specific gene defect, presumably also implicated in the regulation of T-cell activation, which is different from *FOXP3*.

AIE TYPE 3: IN GIRLS

The observation of rare cases of AIE in girls[6,11–13] suggests that there might be a different non–X-linked mode of the disease. In most cases, these girls present with extraintestinal autoimmune manifestations, such as thyroiditis or diabetes. In the report by Mirakian and colleagues, one girl presented with Still disease, diabetes mellitus, and AIE with positive antienterocyte antibodies.[6] Bousvaros and colleagues described one girl showing typical symptoms of autoantibody-positive AIE associated with diabetes mellitus, indicating a strong autoimmune background.[11]

DIFFERENTIAL DIAGNOSIS

As discussed, AIE and IPEX have to be clearly distinguished from other forms of diffuse diarrhea occurring in the context of primary or secondary immunodeficiencies. Therefore, cellular and humoral immune functions have to be characterized during the initial diagnostic workup of patients in whom IPEX is suspected. In addition, some syndromatic disorders have some overlap with IPEX; for instance, Wiskott-Aldrich syndrome, which is characterized by eczema, thrombocytopenia, and autoimmunity, can also present with a variable degree of enteropathy.[51] This disorder is clearly different from IPEX in that CD8+ counts are low, and signs of a combined immune defect with recurrent bacterial infections are detectable. The search for mutations in the *WAS* gene further helps to distinguish these disorders.[52] The so-called APECED syndrome, an acronym for autoimmune polyendocrinopathy, candidiasis, and ectodermal dystrophy,[53,54] can occasionally resemble IPEX. Children with APECED syndrome develop diarrhea with malabsorption along with diabetes and/or hypothyroidism. However, in contrast to IPEX, symptoms occur later in life. APECED syndrome is inherited on an autosomal recessive basis, with a known mutation in the *AIRE* gene.[53,54] Furthermore, Omenn syndrome is characterized by erythrodermia with T-cell infiltration, lymphadenopathy, diarrhea with failure to thrive, and raised IgE with eosinophilia.[55] This syndrome can be easily distinguished from IPEX in that B-cell counts are low or even zero, and T-cell proliferation responses are poor. Diabetes is not associated with Omenn syndrome, which is characterized by mutations in the *RAG1* or *RAG2* genes.[55,56]

TREATMENT AND OUTCOME

Mortality was reported to be extremely high in patients with IPEX.[57] All patients are initially dependent on parenteral nutrition. Management by TPN can be additionally complicated in patients presenting with diabetes. In the past, different treatment strategies were attempted; however, to date, only two approaches proved to be successful: immunosuppressive medication or bone marrow transplant (BMT). Immunosuppression is quite difficult because various reports clearly indicate that the response to only one immunosuppressive drug alone is rather disappointing.[4,43–45] Over the past few years, some experience was gained in treating AIE patients with steroids alone or in combination with azathioprine, cyclosporine A (CSA), or tacrolimus.[10,11,25,58–60] Other approaches include the use of immunoglobulins, antilymphocytic immunoglobulins, or cyclophosphamide, which were all rather inefficient.[61] Recently, Vanderhoof and Young reported the successful use of anti-TNF antibodies in the treatment of a patient with AIE.[62] However, on three injections, the treatment was stopped, and high-dose steroid medication was again required to maintain remission. Therefore, no information on the long-term beneficial effect of anti-TNF treatment exists. Using immunosuppressive medication, a first series of patients was treated with CSA, but only in some of them with some benefit.[58] One patient of the series by Powell and colleagues is still alive and doing well on chronic CSA medication.[4] However, many patients responded only partially or not at all to CSA. In fact, T cells from scurfy mice are highly resistant to CSA suppression, indicating that agents inhibiting TCR signaling may be of limited benefit.[21] With the introduction of tacrolimus, some advances in the care of AIE patients were made, significantly improving the outcome of AIE.[11,24,59,60] Chronic immunosuppression was effective in some patients but ineffective in others. In our experience, remission can be most often induced by the combination of methylprednisolone and tacrolimus. As indicated in Table 44.3-3, initially, steroid pulses (25 mg/kg) were applied on 3 consecutive days, followed by 2 mg/kg/d, concomitant with tacrolimus. The doses of tacrolimus are adjusted to achieve and maintain blood levels between 8 and 12 ng/mL. To maintain remis-

TABLE 44.3-3 NECKER-ENFANTS MALADES HOSPITAL TREATMENT SCHEMA

TREATMENT	MODALITIES
Bowel rest and TPN	Until remission
Corticosteroids	3 pulses, eventually repeated
Tacrolimus	Blood levels 8–12 ng/mL
Azathioprine	2 mg/kg/d

TPN = total parenteral nutrition.

sion, we use a triple therapy approach, combining tacrolimus, prednisolone, and azathioprine. If the response to the first steroid pulse is insufficient, a second pulse is tried. The outcome measure of immunosuppressive treatment and ultimate medical aim is to stop secretory diarrhea, allowing the child to be subsequently weaned from TPN and to start normal oral or enteral nutrition. However, chronic immunosuppression may also increase the risk for viral, bacterial, fungal, or opportunistic infections, such as *Pneumocystis carinii* pneumonia. Therefore, we put our patients on trimethoprim-sulfonamide prophylaxis. A close follow-up of IPEX and AIE patients is required to avoid or at least minimize complications related to long-term immunosuppressive medication.

In our series, the induction of remission using this triple immunosuppression (methyprednisolone, tacrolimus, and azathioprine) was successful in 7 of 8 consecutive patients. In the past (before tacrolimus was available), however, all patients treated with CSA failed to come into remission and subsequently died. In the case of nonresponse to immunosuppressive drugs, including tacrolimus, we now consider these patients as candidates for HLA-identical allogenic BMT. The first successful BMT was performed in our center on a 4-month-old boy who failed to respond to tacrolimus.[30] The donor was his HLA-identical 18-year-old sister. BMT was followed by a complete remission over 2.5 years. It is important to underline that the conditioning regimen itself already controlled the disease, further confirming that IPEX is a T cell–mediated disease.[20,57] After BMT, not only the enteropathy remained in remission, but also the diabetes and eczema were clinically silent. Insulin therapy could be stopped 7 days after BMT, and parenteral nutrition was reduced 4 weeks after BMT and stopped within 6 months. Unfortunately, 29 months after BMT, the child died of a rapidly progressive hemophagocytotic syndrome, which remained completely unexplained. Meanwhile, two other boys received BMT as the ultimate treatment option for IPEX.[26] In both cases, BMT was initially successful; however, lethal, infectious complications occurred 3 and 5 months post-BMT, respectively. These three cases indicate that BMT is potentially successful in otherwise treatment-resistant IPEX. However, this treatment remains quite difficult. Therefore, further experience with BMT has to be gained by particularly experienced teams before it could be recommended as rescue therapy for AIE. On the other hand, with the increasing understanding of the pathophysiology of IPEX, there is hope that in the near future, new immunosuppressive strategies will be available to treat this T cell–mediated autoimmune disorder.

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CHAPTER 45

INTESTINAL TUMORS

1. *Intestinal Polyps and Polyposis*

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Polyps refer to any mass projecting into the lumen of the gastrointestinal tract. However, when one refers to intestinal polyps, one usually thinks of an epithelial lesion. Histologically, epithelial polyps may be divided into two major groups: neoplastic and non-neoplastic. Neoplastic polyps include benign adenomas and malignant carcinomas. Non-neoplastic types include hamartomas as juvenile polyps, hyperplastic polyps, and inflammatory polyps. However, the term "polyp" encompasses many gastrointestinal lesions (Table 45.1-1) as submucosal lesions that also may impart a polypoid appearance to the overlying mucosa.

Occasionally, polyps in children may occur in the context of a genetic gastrointestinal polyposis disorder characterized by the presence of multiple polyps throughout the gastrointestinal tract, their histopathology, their heritability within a family, and an increase in the lifetime risk of cancer in the gastrointestinal tract and other organs. Two major categories of polyposis are recognized: adenomatous polyposis syndrome and hamartomatous polyposis syndromes (Table 45.1-2).

TABLE 45.1-1 CLASSIFICATION OF COLORECTAL POLYPS

Epithelial polyps
Adenomas
Hyperplastic polyps
Juvenile polyps
Peutz-Jeghers polyps
Nonepithelial polyps
Submucosal leiomyoma
Lymphoïd polyps
Paraganglioma
Carcinoid tumor
Submucosal lipoma
Submucosal neurofibroma
Submucosal schwannoma
Ganglioneuroma

COLONIC POLYPS

Juvenile Polyps

Solitary polyps of the large intestine are common during childhood, usually presenting with painless rectal bleeding. These lesions, known as juvenile polyps, are benign and carry no long-term risk of neoplasia.

Frequency. Juvenile polyps, single or multiple (< 3), are the most frequent type of gastrointestinal polypoid lesion encountered in pediatric practice (97% of colonic polyps in our personal registry).[1] However, the true incidence of polyps in childhood remains unknown.[2] Most often, juvenile polyps are diagnosed in the first decade of life. The peak incidence occurs between 2 and 6 years of life.[1,3–5] Polyps are rare in the first year of life and much less common in children older than 10 years of age. In all studies, a male preponderance is noted.[1,5] All ethnic groups can be affected.[1]

Pathology. The various appellations given to juvenile polyps reflect some of the areas of uncertainty about the current understanding of the pathogenesis of these lesions:

TABLE 45.1-2 CLASSIFICATION OF POLYPOSIS SYNDROMES

Adenomatous polyposis syndromes
Familial adenomatous polyposis
Gardner syndrome
Turcot syndrome
Hamartomatous polyposis syndromes
Juvenile polyposis
Bannayan-Riley-Ruvalcaba syndrome
Cowden disease
Peutz-Jeghers syndrome
Mixed polyposis syndrome
Hyperplastic polyposis

juvenile retention polyp, juvenile inflammatory polyp, and juvenile hamartomatous polyp.

Grossly, juvenile polyps usually have a smooth, bright red, friable surface that bleeds easily when traumatized. Initially sessile, 90% of juvenile polyps appear pedunculated, spherical, and mushroom-like and are attached to the colonic mucosa with a narrow stalk (Figure 45.1-1). These lesions measure 1 to 2 cm in average diameter—rarely, 2 to 4 cm. The length of the stalk varies from 0.5 to 1.5 cm. The base of insertion is always broader than the distal extremity. The stalk has the same color as the colonic mucosa. The colonic chicken skin mucosa, characterized by a pale yellow, speckled pattern of the colonic mucosa, resulting from lipid accumulation in lamina propria macrophages, is observed only on larger juvenile polyps in the rectosigmoid colon, most concentrated at the base of the stalk and extending to the surrounding mucosa.[6] A cut section of polyps demonstrates numerous large cystic spaces of variable size, filled with a grayish or yellowish mucus surrounded by reddish stroma—hence the term "retention" polyp.

Microscopically, juvenile polyps have a characteristic Swiss cheese appearance created by dilated mucinous lakes widely separated by abundant stroma (Figure 45.1-2). The lamina propria is infiltrated by numerous inflammatory cells, consisting of neutrophils, eosinophils, lymphocytes, plasma cells, and histiocytes with, in some cases, lymphoid follicles. In contrast to the hamartomatous polyps of Peutz-Jeghers syndrome (PJS), smooth muscle cells are not present in the stroma.[7] However, bands of smooth muscle associated with mucosal blood vessels, serrated or stellate glands with epithelial infolding, and osseous metaplasia are occasionally seen. A single layer of cytologically bland, cuboidal to columnar, mucus-secreting epithelium lines the glands. Paneth cells are present but sparse. Some glands, filled with mucus, become cystically dilated. Epithelial cells demonstrate no atypia. Focal ulceration and hemorrhage may be seen.

Etiology. The etiology of the common juvenile polyp is unknown. If the usual mutations found in preneoplastic or neoplastic lesions are not found,[8] gene mutations implicated in juvenile polyposis (see "Hamartomatous Polyposis Syndromes") have been identified in solitary juvenile polyps but not in the somatic cells of patients.

Clinical Features. Intermittent painless rectal bleeding is the most common presentation. Rectal bleeding occurs during defecation. Major rectal bleeding responsible for acute anemia is a rare event, likely the result of autoamputation of pedunculated polyps. However, iron deficiency anemia is found in as many as one-third of cases.[9] Rectal bleeding is recurrent, present for over 3 months in 55% of cases.[1] Additional manifestations are unusual, including colicky abdominal pain, diarrhea with mucus, autoamputation with spontaneous extrusion, prolapse of rectal polyp, and prolapse of rectum.[9,10] Colocolic intussusception rarely occurs.[11]

Diagnosis. By anorectal examination, lower rectal polyps are felt as firm, moderately mobile, pedunculated masses.

Colonoscopy is the procedure of choice for diagnosis of colorectal polyps of all sizes. It allows resection of most polyps.[1,5,12,13] Endoscopic evaluation of the colon requires cleansing. The most effective preparations appear to be polyethylene glycol (PEG) electrolyte lavage solution, PEG alone, and Fleet Phosphosoda oral laxative.[14] Although juvenile polyps may be distributed throughout the colorectum, they have a distal predominance within the rectosigmoid (70%).[1,5,15] They are more often single (73%) than multiple.[1,5] Because juvenile polyps are often found in the rectosigmoid but also in the proximal colon, in front of recurrent painless rectal bleeding, and without anoperineal lesions, the preferred method for diagnosis and treatment is pancolonoscopy under anesthesia followed by snare polypectomy. Finding a polyp in the rectum does not relieve the endoscopist from the responsibility of pancolonoscopy to identify and remove additional lesions.

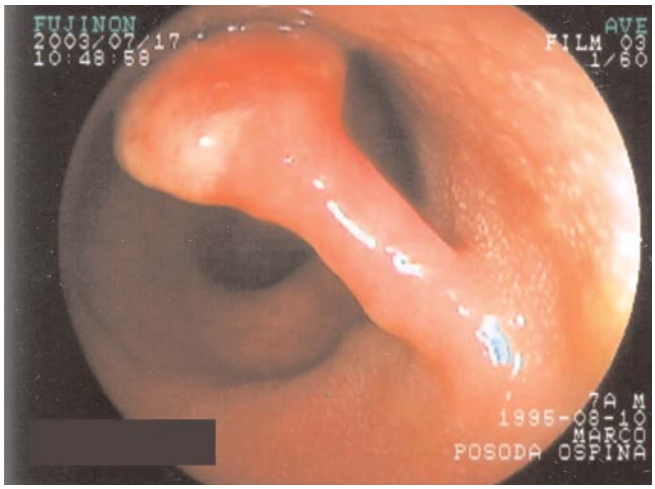

FIGURE 45.1-1 Pedunculated solitary juvenile polyp with a smooth, bright red, friable surface.

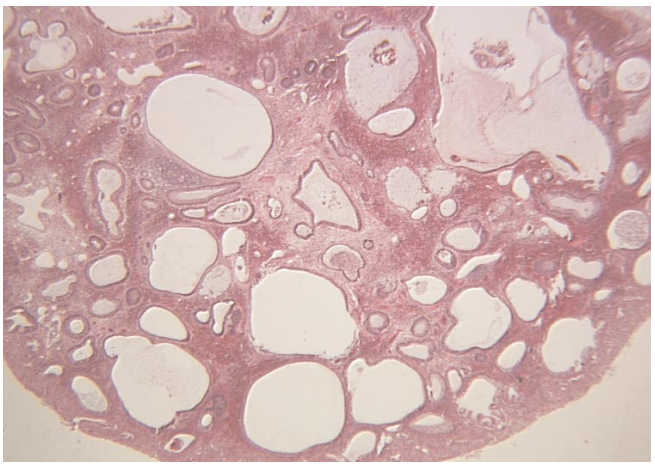

FIGURE 45.1-2 Juvenile polyp. Light microscopy: Swiss cheese appearance created by dilated mucinous lakes widely separated by abundant stroma. Lamina propria infiltrated by numerous inflammatory cells. Single layer of cytologically bland, cuboidal to columnar, mucus-secreting epithelium lines the glands. (Hematoxylin and eosin; ×75 original magnification.)

Treatment. Polypectomy is performed in a medicosurgical environment under anesthesia. Cold forceps resection is feasible only for tiny (1–2 mm) colorectal polyps. Removal with hot biopsy forceps can be used for polyps less than or equal to 4 mm in size.[16] However, the hot biopsy forceps technique anecdotally has been associated with an unexpected high rate of perforation and delayed postpolypectomy hemorrhage. Moreover, the use of hot biopsy forceps in the right colon is five times more likely to result in complication than their use in the left colon.[17] So, the monopolar hot biopsy forceps has severe risks and has no indication in this context. An alternative is to guillotine small polyps using a snare without an electric current. Tiny snares have greatly facilitated the use of snares for removal of small sessile polyps.[18] Polyps 5 mm or larger in size should generally be removed using monopolar snare cautery. Application of detachable snare loops (Endoloop, Olympus, Tokyo, Japan) to a very large stalk (> 10 mm) of pedunculated polyps has reduced the risk of delayed hemorrhage.[19] Immediate hemorrhage from pedunculated polyps can be managed by grasping the residual polyp stalk and holding it with a snare or by application of either a detachable snare or metallic clips (Endoclips, Olympus).[20] To stop more severe bleeding, an argon plasma coagulator may be useful. A 1:10,000 solution of epinephrine can be injected into the bleeding site to promote hemostasis. Postpolypectomy perforation (Table 45.1-3) occurs more frequently in children than in adults.[1,12,13,21] More than 200 procedures are reported for an endoscopist to be technically competent, and a higher complication rate appears to occur in an examiner's first 500 procedures.[14] Resected polyps or multiple polyp fragments are collected for histopathologic examination by suction into a trap if they are small or by using a Roth or a tripod basket, and, if unsuccessful, soon after colonoscopy by colorectal enema.[5] Although the risk of development of malignancy in a solitary polyp is very small, such polyps should be removed, even when discovered incidentally. It is important to emphasize that polyp histology cannot be predicted by gross appearance alone. Forceps biopsies performed through the endoscope only have a yield of correct histologic diagnosis in 75% of patients.[22]

In general, juvenile polyps do not tend to recur. However, in two large pediatric retrospective series, recurrence was observed in 4 to 7%.[1,5] The risk of malignant change for a solitary juvenile polyp is almost negligible. There are only eight cases of patients developing neoplasia in the literature.[23] In addition, a review of 82 patients with a solitary juvenile polyp showed no increased risk for colorectal cancer or of dying as result of the polyp.[24] So, if a polyp is found to be solitary after full colonoscopy and if there is no relevant family history, endoscopic polypectomy is sufficient treatment. Parents must be aware that juvenile polyps may be the first feature of juvenile polyposis. If fresh symptoms arise, the child should be reinvestigated. However, when there is a positive family history or when multiple juvenile polyps are found, the possibility of juvenile polyposis syndrome (JPS) is raised. Multiple juvenile polyps are associated with a risk of colon neoplasia,[23,25] but the precise number that statistically increases cancer risk is unknown. Three or more juvenile polyps or any number of polyps occurring in the context of a family of juvenile polyposis or colon cancer have been proposed as a criterion for a risk of colon cancer.[23]

Hyperplastic Polyps

Hyperplastic polyps rarely occur in children: 3% in our personal series of single and multiple polyps.[1] They appear as single or multiple dewdrop mucosal elevations with a smooth convex surface, arising on the mucosal crest, usually less than 5 mm, and mainly in the rectosigmoid. These small lesions are asymptomatic. When a small polyp (< 5 mm) is encountered during flexible sigmoidoscopy, it should be biopsied to ascertain whether it represents a true hyperplastic polyp or an adenoma.

Microscopically, the colonic crypts are elongated. The epithelial cells assume a characteristic papillary configuration. Absorptive and goblet cells are well differentiated. True hyperplastic polyps do not have intrinsic malignant potential. A hyperplastic polyp is not, by itself, an indication for further colonoscopy or follow-up in childhood.[26]

Polyps that display both hyperplastic and adenomatous features have been very rarely observed in childhood. When this type of polyp exhibits larger-size, prominent architectural distortion, nuclear atypia, and increased mitoses, the lesion is considered more adenomatous than hyperplastic and is termed a "serrated adenoma" (Figure 45.1-3). In such a case, the follow-up must be the same as that for adenomas.

Isolated Adenomas

Whereas in adult patients, the relationship between adenomatous polyps and adenocarcinomas has been extensively studied, isolated adenomas are extremely rare in pediatric practice. Knox, as reported in Mougenot and colleagues[2] and Rodesch and Cadranel,[5] did not observe such adenomas in patients younger than 15 years. One to three cases have been reported in a series of, respectively, 50, 77, and 129 consecutive patients with polyps.[1,2]

Pathology. Adenomas are neoplastic epithelial polyps. All adenomas feature dysplastic aspects, that is, cellular atypia, increased mitotic activity, and nuclear hyperchromatism owing to abnormal cellular differentiation and renewal. The dysplasia can be graded as low (Figure 45.1-4) or high grade (Figure 45.1-5). In high-grade dysplasia, the nuclear abnormalities are marked, and the

TABLE 45.1-3 COMPLICATIONS OF COLONOSCOPIC POLYPECTOMY

RETROSPECTIVE STUDIES IN CHILDREN	HEMORRHAGE	PERFORATION
Williams et al (1982)[13]	0/81 (0%)	4/81 patients (5%)
Mougenot et al (unpublished, 1996)	1/340 patients (0.29%)	3/340 patients (0.88%)

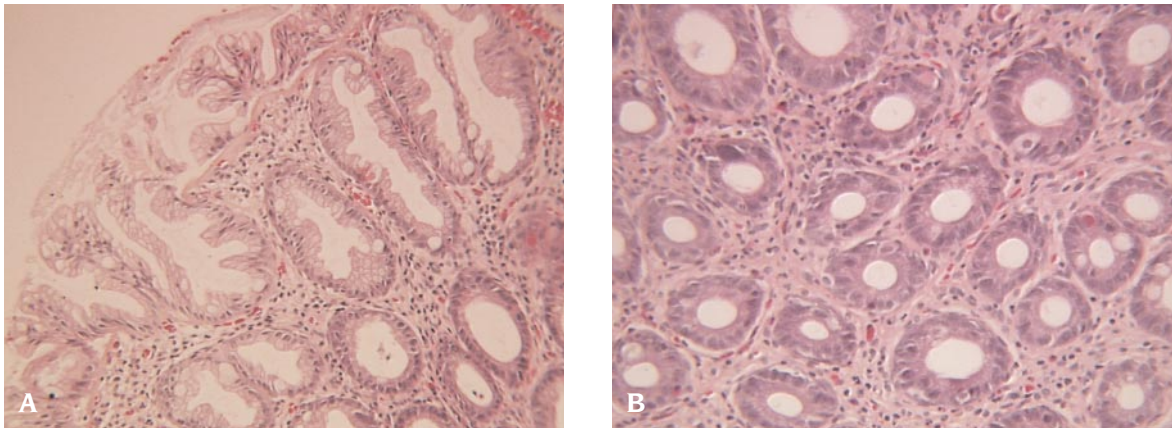

FIGURE 45.1-3 Low-power photomicrograph of a serrated adenoma showing a saw-toothed surface and stellate luminal profiles (*A*). However, the nuclei are mildly pseudostratified (*B*). (Hematoxylin and eosin; ×250 original magnifications.)

nuclei are stratified with nucleoli and uneven coarse chromatin. When cells appear overtly malignant but confined within the basement membrane, the lesion can be designated as carcinoma in situ. In practice, the term "high-grade dysplasia" encompasses severe dysplasia and carcinoma in situ. If the malignant cells extend beyond the basement membrane into the lamina propria, the diagnosis of intramucosal carcinoma must be used. In the colon, lymphatics extend only in the submucosa, which explains the absence of a risk of metastasis associated with high-grade dysplasia or intramucosal carcinoma. Invasive adenocarcinoma is defined by extension of malignant cells through the muscularis mucosa into the submucosa.

Two overall glandular patterns are separated: tubular with an organized glandular pattern (Figure 45.1-6) and villous with a frond-like pattern that is more likely present in large adenomas and is associated with a greater risk of high-grade dysplasia and invasive cancer, independent of polyp size. Adenomas demonstrating more than 75% tubular elements are called tubular adenomas. Those demonstrating more than 75% villous elements are called villous adenomas, and those with less than 75% of each are designated tubulovillous adenomas.

Adenomas originate through the process of gene mutation. Sporadic adenomas begin most commonly with somatic mutations in both alleles of the adenomatous polyposis coli gene (see "Familial Adenomatous Polyposis"). Subsequent accumulation of additional mutations results in the development of cancer. Most adenomas do not develop sufficient mutations to generate into cancer. Development of cancer requires an average time of 7 to 10 years.

Endoscopy. Only dye spraying (0.5% indigo carmine solution) in combination with high-resolution endoscopy offers the opportunity to distinguish adenomas from hyperplastic polyps in real time. Hyperplastic polyps demonstrate the normal honeycomb or evenly dotted pattern of the background colonic mucosa. All other patterns on the surface of the polyp favor an adenomatous histology. A cribriform pattern is commonly evident in adenomas when visualized with high-resolution endoscopes, even without chromoscopy.[27]

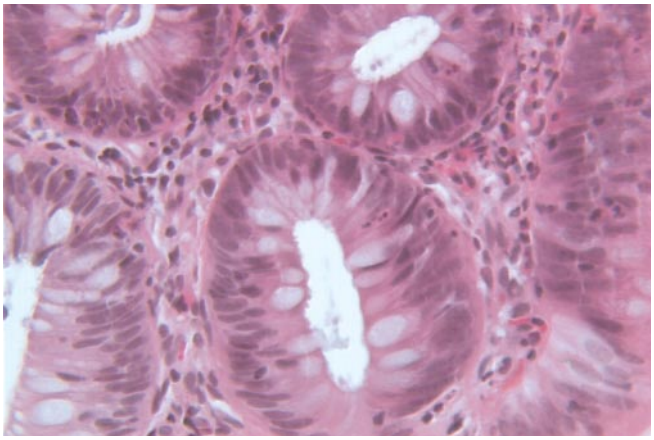

FIGURE 45.1-4 Colonic adenomatous polyp showing low-grade dysplasia with nuclear abnormalities, consisting of elongated, crowded, cigar-shaped nuclei. (Hematoxylin and eosin; ×500 original magnifications.)

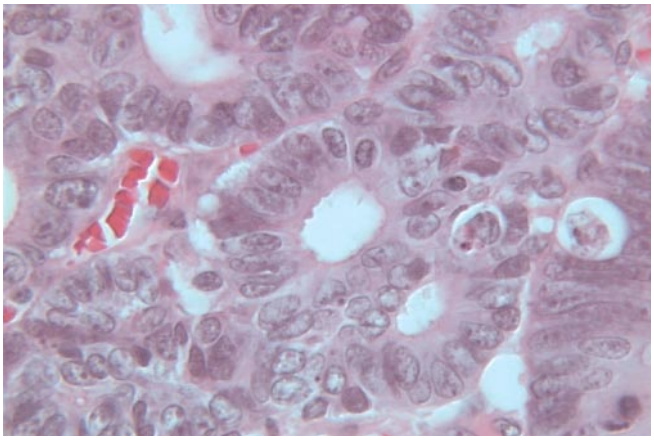

FIGURE 45.1-5 Colonic adenomatous polyp showing high-grade dysplasia with loss of goblet cell vacuoles, pleomorphic nuclei, prominent nucleoli, and an increased nuclear-cytoplasmic ratio. (Hematoxylin and eosin; ×500 original magnifications.)

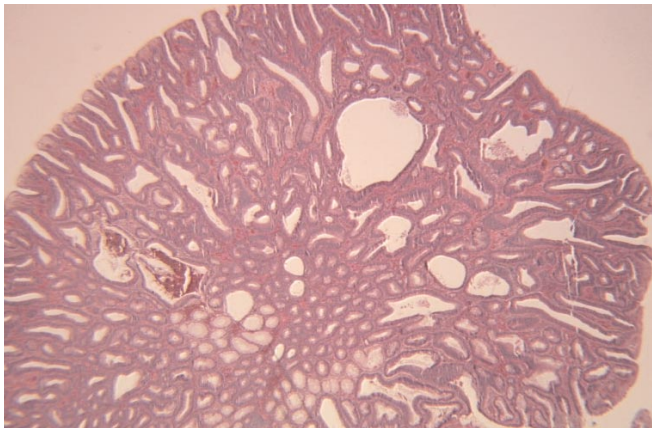

FIGURE 45.1-6 Low-magnification micrograph of a typical colonic tubular adenoma. (Hematoxylin and eosin; ×75 original magnifications.)

Pediatric Management. If an adenoma histopathologic diagnosis is made for an isolated or small number of polyps in a child or an adolescent, the following recommendations should be considered: (1) looking for a history of familial adenomatous polyposis (FAP), although a family history may be absent in sporadic forms of this disease; (2) searching for a history of familial colorectal cancer; and (3) employing postpolypectomy surveillance colonoscopy as proposed for adults by the Agency for Health Care Policy and Research Consortium (Table 45.1-4).[28]

Polyps and Ureterosigmoidostomy

After ureterosigmoidostomy, adenomas and colorectal cancer near, or distal to, the stoma have been observed in at least 29% of patients. The delay varies from 2 to 38 years after surgery: 20 years for the development of adenoma and 28 years for arising colorectal cancer.[29,30] Juvenile polyps also have been seen at sites of previous surgery, including ureterosigmoidostomy.[31] A colonoscopy follow-up at regular intervals is indicated in such cases.

Inflammatory Polyps

Inflammatory polyps arise during the healing phase of severe colitis, following full-thickness ulceration. These polyps, which have no intrinsic neoplastic potential, may mimic a neoplastic mass in diseased colon, ulcerative colitis, and Crohn colitis and chronic schistosomiasis in endemic countries.[32] Because patients with these diseases are at increased risk for developing colon cancer, careful examination of these lesions has to be considered.[33]

TABLE 45.1-4 POSTPOLYPECTOMY SURVEILLANCE COLONOSCOPY RECOMMENDATIONS OF THE AGENCY FOR HEALTH CARE POLICY AND RESEARCH CONSORTIUM

FINDINGS AT INDEX EXAMINATION	SURVEILLANCE
Single tubular adenoma	5 yr
Multiple adenomas or villous histology	3 yr
Numerous adenomas	Consider 1 yr
Large sessile adenomas	3–6 mo to examine the site

Adapted from Winawer SJ et al.[28]

POLYPOSIS SYNDROMES

Recently, major progress has been made in understanding the molecular pathogenesis of polyposis syndromes. (Table 45.1–5).[34–39] A gastrointestinal polyp results from a defect in the highly regulated counterbalance of cellular growth promotion and cellular growth inhibition. This may be the result of either aberrant gain of function in a growth-promoting protein (oncogene, most often activated) or loss of function in a growth-inhibiting protein (tumor suppressor gene, most often inactivated). In the genetic gastrointestinal polyposis syndromes, a germline inactivating mutation of a tumor suppressor gene is the crucial event. Loss of normal growth regulation results from an eventual somatic mutation of the normal allele. Progression of the polyp to gastrointestinal cancer requires further acquisition of additional genetic defects.

Inherited Adenomatous Polyposis Syndromes

The inherited adenomatous polyposis syndromes are characterized by (1) the development of a large number of adenomas in the colorectum and (2) extracolonic features (ie, gastroduodenal polyps; benign soft tissue tumors; osteomas of the mandible, skull, and long bones; and congenital hypertrophy of the retinal pigmented epithelium [CHRPE]. FAP is the most common polyposis syndrome in children. It is also known as familial polyposis for patients without extracolonic manifestations or Gardner syndrome for patients with extracolonic manifestations.[40] Identification of the adenomatous polyposis coli (*APC*) gene as the gene mutated in the germline deoxyribonucleic acid (DNA) of patients affected by FAP has been a major development in understanding the pathogenesis of colorectal cancer[34–36,41] and has allowed proper classification of Gardner syndrome,[40] attenuated FAP, and some cases of Turcot syndrome as variants of classic FAP coli (Mendelian Inheritance in Man [MIM] 175100).

Familial Adenomatous Polyposis. FAP is characterized by the progressive development of hundreds to thousands of adenomatous polyps in the large intestine (Figure 45.1-7).[7,42–44] The disease begins in younger patients with a small number of tiny polyps less than 5 mm (Figure 45.1-8).[2] Half of FAP gene carriers will have polyps at colonoscopy by approximately age 15 years. The number of polyps increases progressively, with all of the colonic length being covered by countless adenomas. In all cases, the vast majority of polyps are less than 10 mm (Figure 45.1-9).

At histologic examination, all varieties of adenomatous polyps may be seen, including tubular, tubulovillous, and villous adenomas. In addition, light microscopy reveals numerous microscopic adenomas, the smallest of which may involve a single colonic crypt (Figure 45.1-10). The dysplasia exhibited by all adenomas is now categorized as low (mild and moderate dysplasia) or high grade (severe dysplasia and carcinoma in situ).

The lifetime risk for colorectal neoplasia is 100%. Although the mean ages for adenoma and colon cancer development in FAP are 16 and 39 years, respectively,[44] reports exist of colon cancer in children as young as 5 years

TABLE 45.1-5 POLYPOSIS SYNDROMES AFFECTING CHILDREN

SYNDROME TYPE	GENE	CLINICAL FEATURE	CANCER RISK
Adenomatous			
Familial adenomatous polyposis (MIM 175100)	*APC*	GI polyposis, CHRPE	Colon 100%, periampullary thyroid, hepatoblastoma, other
Gardner syndrome	*APC*	Colon adenomas, desmoids, dental anomalies, osteomas, epidermal cysts	As for FAP above
Attenuated FAP	*APC*	Reduced adenomas number	Colon
Turcot syndrome type II (BTPS) (MIM 276300)	*APC*	GI adenomas, CNS tumors	Colon, brain
Hamartomatous			
Peutz-Jeghers syndrome (MIM 175200)	*LKB1/STK11*	GI hamartomas, mucocutaneous pigmentation	GI tract, pancreas, ovary, breast, cervix, testicle
Juvenile polyposis (MIM 174900, 601299)	*SMAD4*, *MPSH*, *BMPR1A*	Colon/stomach hamartomas, congenital heart disease, cleft lip/palatine, malrotation	Colon, stomach, duodenum, pancreas
PTEN hamartoma tumor syndromes			
Cowden disease (MIM 158350)	*PTEN*	GI hamartomas, macrocephaly, mucocutaneous pigmentation, thyroid disease, fibrocystic breast disease, endometrial fibroids, urinary/uterine abnormalities	Breast, thyroid, skin
Bannayan-Riley-Ruvalcaba syndrome (MIM 601728)	*PTEN*	GI hamartomas, macrocephaly, speckled penis	Breast, thyroid

BTPS = brain tumor polyposis syndrome; CHRPE = congenital hypertrophy of the retinal pigment epithelium; CNS = central nervous system; FAP = familial adenomatous polyposis; GI = gastrointestinal.

of age,[45] with an estimated incidence of 1 case younger than 20 years per 157 families.[46] Colorectal cancers in FAP have the same localization as sporadic cancer, but in 26 to 50% they are multiple and synchronous.[2,42]

Recently, attention has been paid to adenoma variants such as serrated adenomas, flat adenomas, and foci of aberrant crypts. Endoscopic/histopathologic correlations focusing on the differentiation of neoplastic from non-neoplastic lesions and identification of early malignant transformation have been supported in adult FAP by using new high-resolution videoendoscopes combined with optical zoom and dye spraying to define the mucosal pit pattern.[47]

Genetics: APC Gene and FAP-Associated Germline Mutations. ***Germline Mutations in the* APC *Gene.*** Germline mutations in the *APC* gene on chromosome 5q21[34,35] are responsible for FAP, an autosomal dominant disease that affects approximately 1 in 13,500 individuals.[48] Somatic *APC* mutations are found in the vast majority of sporadic colorectal cancers regardless of the histologic stage, which places *APC* at the very start of the adenoma-carcinoma sequence in humans.

APC *Gene.* The *APC* gene includes 21 exons contained within a 98 kb locus. The largest, exon 15, comprises more than 75% of the 8,535 basepairs of the coding sequence (Figure 45.1-11) and is the target of most germline mutations in FAP patients. The *APC* gene on chromosome 5q21 encodes a large protein consisting of 2,843 amino acids with a predicted molecular weight of greater than 309,000 kD. Studies indicate that *APC* partic-

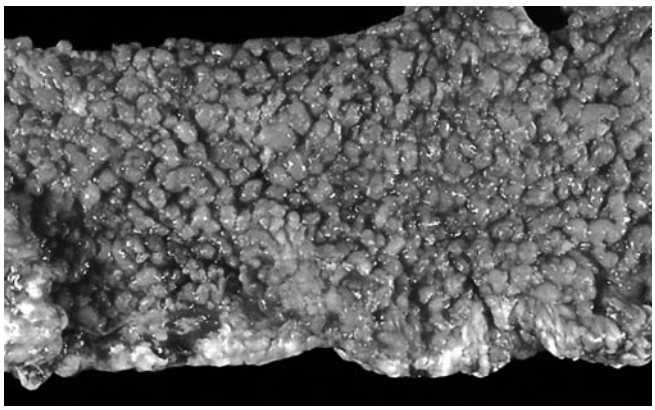

FIGURE 45.1-7 Familial adenomatous polyposis. Macroscopic aspect of resected colon with multiple adenomatous polyps carpeting the colonic surface.

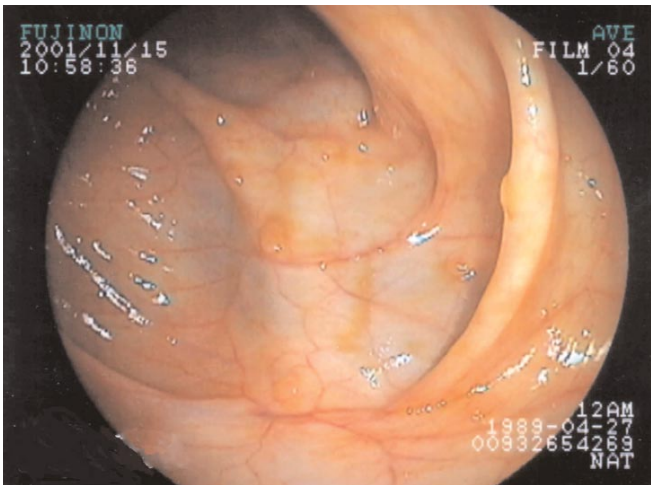

FIGURE 45.1-8 Familial adenomatous polyposis. Tiny colonic adenomatous polyps less than 3 mm.

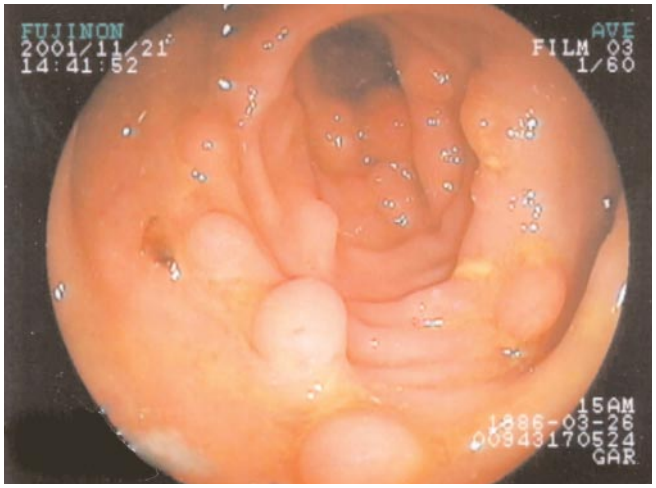

FIGURE 45.1-9 Familial adenomatous polyposis. Multiple adenomatous colonic polyps less than 10 mm.

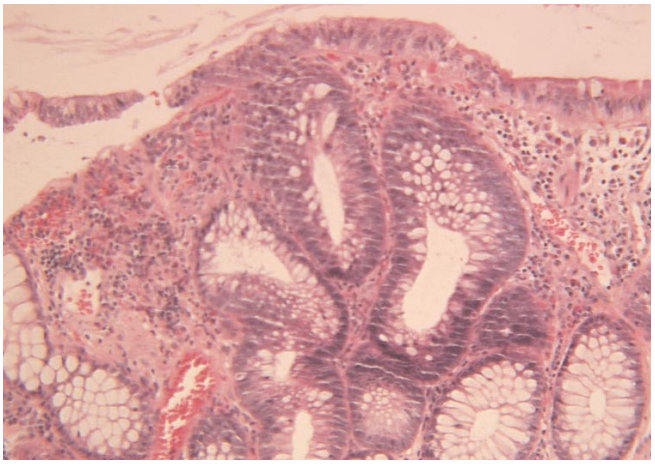

FIGURE 45.1-10 Familial adenomatous polyposis. Light microscopy reveals microscopic adenomas in a few crypts showing reduction of goblet cell vacuoles and crowded nuclei. (Hematoxylin and eosin; ×250 original magnifications.)

ipates in a variety of cellular functions, including proliferation, differentiation, apoptosis, adhesion, migration, and chromosomal segregation.[49]

Mutation Penetrance. More than 300 different disease-causing mutations of the *APC* gene have been identified. Most FAP patients carry germline mutations scattered in the 5′ half of the *APC* gene. Two codons, 1061 and 1309, are mutational hot spots and account for approximately 11 and 17% of all germline mutations. About 95% of *APC* germline mutations are either nonsense (28%) or truncating frameshift (67%) mutations, which result in truncated gene products without the C-terminus.[50]

The heterogeneity of the spectrum of *APC* germline mutations and the phenotypic variability observed among FAP families have allowed the establishment of genotype-phenotype correlations at this locus. Germline mutations between codons 168 and 1680 are associated with classic FAP,[51] whereas germline mutations between codons 1250 and 1464, especially around codon 1300, are associated with the highest number of polyps, thousands rather than hundreds of colorectal adenomas with an earlier onset of the disease.[52] The expression of some extracolonic features correlates with specific *APC* germline mutations. CHRPE is associated with germline mutations between codons 457 to 1444[53–55] but is also occasionally described in patients with germline mutations in exon 9.[56] Mutations downstream of codon 1444 correlate with the highest frequency of mandibular osteomas and desmoid tumors (DTs).[57,58]

Attenuated adenomatous polyposis coli (AAPC) is characterized by multiple adenomas, late onset of carcinoma, and, frequently, the absence of extracolonic features.[58,59] This phenotype is associated with germline mutations occurring in the 5′ (codons 78–167) and 3′ (approximately codons 1581–2843) regions of the *APC* gene and in exon 9.[58] The penetrance of AAPC, although lower than that of FAP, might still be high. An attenuated phenotype has also been reported in some families with a complete deletion of one copy of the *APC* gene.[60,61] Some mutations associated with AAPC lead to an unstable messenger ribonucleic acid (mRNA) or protein. Other mutations in alternatively spliced exons, such as those in exon 9, are spliced out of at least some mRNA species, resulting in a nearly full-length protein lacking the exon carrying the mutation.[62]

The established genotype-phenotype correlations might have implications for clinical practice. For example, the

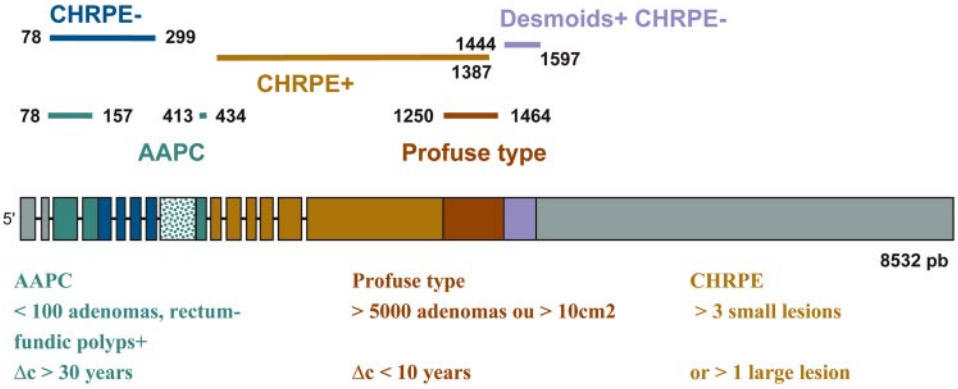

FIGURE 45.1-11 APC protein domains and familial adenomatous polyposis phenotype association with germline mutation position. AAPC = attenuated adenomatous polyposis coli; CHRPE = congenital hypertrophy of the retinal pigmented epithelium.

finding of CHPRE in a family can direct mutation analysis to the exons associated with these lesions. Moreover, the genotype-phenotype correlation regarding DTs has implications for prophylactic surgery because DTs often arise as a consequence of tissue trauma.[63–65]

Nevertheless, considerable phenotypic variability may occur even among individuals and families with identical genotypic mutations.[59,63] In genetic terms, this variability can be explained by the effects of several, as yet undetermined, genetic and environmental modifying factors.

APC *Functional* Domains. The large APC protein comprises several functional domains. Heptad repeats at the amino-terminal end mediate APC homodimer formation.[35,64] Amino acids 453 to 767 show some homology to the central repeat region of the *Drosophila* segment polarity protein armadillo, which controls cell adhesion and motility via modulation of the actin cytoskeleton.[65] A summary of key interactions of APC and β-catenin in the cell, including the Wnt-1 signaling pathway, where binding of Wnt-1 to the frizzled receptor activates disheveled, which inhibits glycogen synthase kinase (GSK) 3β phosphorylation of β-catenin, preventing its proteosomal degradation, is shown in Figure 45.1-12. This leads to the dissociation of the complex formed by axin/conductin, APC, and GSK, resulting in the accumulation of free cytoplasmic β-catenin. β-Catenin is then translocated to the nucleus, where it forms a complex with T-cell factors (Tcfs),[66] resulting in the activation of gene transcription, including the proto-oncogenes cyclin D_1 and c-*myc* and subsequent cell proliferation. Mutations in *APC* have the same effect as Wnt signaling in destabilizing the axin-APC-GSK complex. Also shown in Figure 45.1-12 are the interactions of APC with the microtubule and actin cytoskeleton and the interaction of β-catenin with the E-cadherin cell adhesion system and Tcf transcription factor. Interspersed between these motifs are three SAMP (serine-alanine-methionine-proline) repeats that mediate axin binding.[67,68] APC mediates microtubule binding when transiently overexpressed in epithelial cells and triggers tubulin polymerization in vitro. Further signals are present in the β-catenin and microtubule binding domains, which are thought to mediate nuclear localization and export of APC.[69–72] The C-terminus of APC (residues 2560–2843) interacts with the microtubule-associated protein EB1 and also binds hDLG (human homolog of the *Drosophila* disks large tumor suppressor protein) and the protein tyrosine phosphatase PTP-BL.[73,74]

Recent studies have shown that the C-terminus of APC is involved in chromosomal stability at mitosis.[67,75] APC localizes at the kinetochore of metaphase chromosomes, and this location is likely to be dependent on the interaction between APC and EB1. On the one hand, loss of the former function will lead to nondysjunction and tetraploidy; on the other hand, defects of the latter result in mitotic cells with multipolar spindles that exert multidirectional forces on the kinetochore, resulting in chromosomal breakage and fragmentation.

APC protein shows a diffuse cytoplasmic distribution, accumulating along lateral margins or subapical regions of certain cells, in particular surface cells. However, epithelial cells of the same lineage may show striking differences in the subcellular localization of the APC protein. For example, enterocytes at the base of intestinal crypts are almost always APC negative, whereas expression increases toward the upper third of the crypt and the luminal surface, where all cells are positive. In addition, enterocytes on the luminal surface show accumulation of the APC protein along their apical surfaces. The expression of APC thus seems to increase with enterocyte maturation during the migration of cells from the crypt base to the luminal surface.[76,77]

Model of Carcinogenesis. A general picture is emerging from the analysis of the essential roles of the Wnt signal transduction pathway in providing selective advantage to the nascent tumor cell and in exerting genetic instability to ensure both tumor progression and malignant transformation: the *APC* gene, because it encompasses both functions, plays a central initiating and promoting role in colorectal cancer. Its inactivation and the resulting constitutive activation of the Wnt pathway provide a strong selective advantage by affecting cell proliferation, migration, apoptosis, and, possibly, differentiation of the intestinal stem cell. Subsequently, other synergistic mutational events may allow the mutant *APC* to induce chromosomal insta-

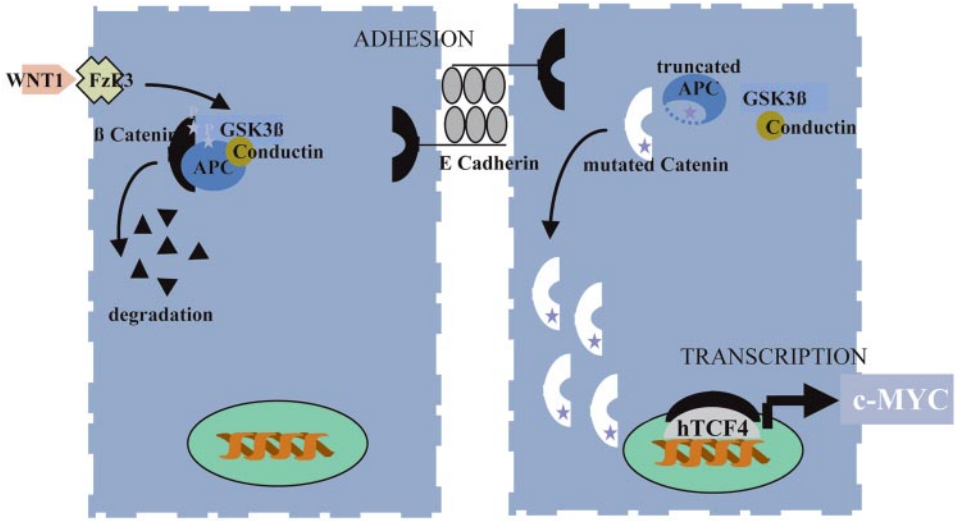

FIGURE 45.1-12 A summary of key interactions of *APC* and β-catenin in the cell, including the Wnt-1 signaling pathway to the frizzled receptor activates disheveled, which inhibits glycogen synthase kinase 3β phosphorylation of β-catenin, preventing its proteosomal degradation. Also shown is the interaction of β-catenin with the E-cadherin cell adhesion system and T-cell factor transcription factor.

bility and accelerate tumor progression along the adenoma-carcinoma sequence.

Based on the above considerations, *APC* has been proposed to have a rate-limiting role in tumor initiation and progression.[78] Loss of β-catenin regulation by *APC* provides the intestinal cell with a selective advantage and allows the initial clonal expansion. At this stage, chromosomal instability caused by loss of the C-terminus functional motifs of APC is latent owing to surveillance by the cell cycle and mitotic checkpoint machinery. The early activation of the oncogenes K-*ras* (by point mutation) and c-*myc* (as a downstream target of the Wnt pathway) will synergize with *APC* in triggering chromosomal instability and the subsequent allelic imbalances at chromosomes 17p and 18q. Additional synergisms between *APC* and other tumor suppressor genes in eliciting aneuploidy and chromosomal instability will progressively lead to malignant transformation and metastasis.

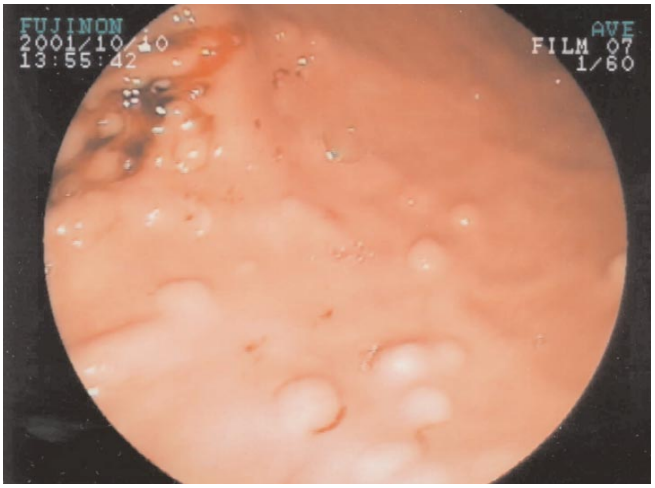

FIGURE 45.1-13 Fundic gland polyps in a child with familial adenomatous polyposis.

Clinical Features. ***Colorectal Adenomatous Polyps.*** FAP is clinically characterized by the occurrence of hundreds to thousands of colorectal adenomatous polyps at an early age and the inevitable development of colon cancer unless colectomy is performed.[79] Accounting for 1% of all colorectal cancer patients, FAP affects both genders equally and has a worldwide distribution.[48] The average age at diagnosis ranges from 34 to 43 years; the average age at colorectal cancer diagnosis is 39 years.[55,80] In pediatric practice, FAP is recognized through family screening. Rarely, rectal bleeding can reveal a sporadic or a familial case.[42]

***Extracolonic* Manifestations.** In addition to polyposis coli, patients with FAP can develop a variety of benign extracolonic manifestations and, infrequently, other cancers, as shown in Table 45.1-6.[81–91]

Extracolonic Adenomas. Fundic gland polyps are occasionally observed at pediatric age, in contrast to antral adenomas (Figure 45.1-13). They do not have a great potential for malignancy change. Subtle flat adenomas can be detected in the duodenum of children with FAP at the same time as colonic adenomas. In adults with FAP, duodenal adenomatous polyps have an average incidence of 61% (Figures 45.1-14 and 45.1-15).[92–94] The relative risk of duodenal cancer and periampullary malignancy is enhanced. At least 1% will develop duodenal cancer, diagnosed at an average age of 47 years.[95] Spigelman and colleagues have elaborated a staging of duodenal polyposis that may help to assess the risk of malignant transformation (Table 45.1-7).[96] Gastric adenomas, usually located in the antrum, infrequently occur in adult patients, but they may be a precursor for malignancy.

Other Manifestations. The extraintestinal manifestations of FAP, first attributed to Gardner syndrome, are listed in Table 45.1-6. Some of these manifestations may contribute to identify at-risk individuals.

Mandibular osteomas are sought by simple and noninvasive screening as orthopantomography.[97]

CHRPE, defined as multiple and bilateral pigmented ocular fundus lesions, is found with increased frequency in some FAP kindreds (Figure 45.1-16).[98,99] It has been identified in infants as young as 3 months old. This abnormality, which generally does not affect vision, is a reliable early marker for gene carriage in FAP, and its absence indicated lack of carriage in families affected with CHRPE.[100]

DTs may dramatically complicate the course of FAP and represent, since the widespread acceptance of prophylactic colectomy, a major cause of death.[101] The lifetime cumulative risk of developing DT is approximately 21%, with a female preponderance (female-to-male ratio 1.5), mainly during the third decade of life. The risk is 2.5-fold

TABLE 45.1-6 EXTRACOLONIC FEATURES IN FAP

CANCERS (LIFETIME RISK)	OTHER LESIONS
Duodenal (1–5%)	CHRPE
Pancreatic (2%)	Nasopharyngeal angiofibromas
Thyroid (2%)	Osteomas
Brain (medulloblastoma) (< 1%)	Radiopaque jaw lesions
Hepatoblastoma (0.7% of children < 5 yr old)	Dental abnormalities
	Lipomas, fibromas, epidermoid cysts
	Desmoid tumors
	Gastric adenomas/fundic gland polyps
	Duodenal, jejunal, ileal adenomas

Adapted from Cruz-Correa M, Giardello FM. Diagnosis and management of hereditary colon cancer. Gastroenterol Clin North Am 2002;31:537–49.
CHRPE = congenital hypertrophy of the retinal pigment epithelium; FAP = familial adenomatous polyposis.

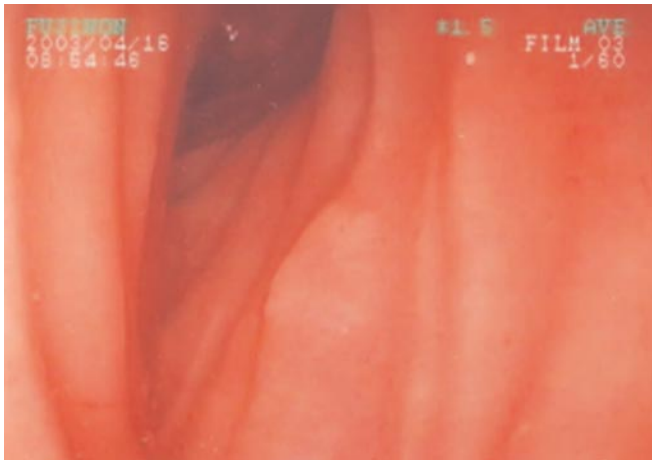

FIGURE 45.1-14 Familial adenomatous polyposis. Tiny, flat, superficially spreading duodenal adenomas.

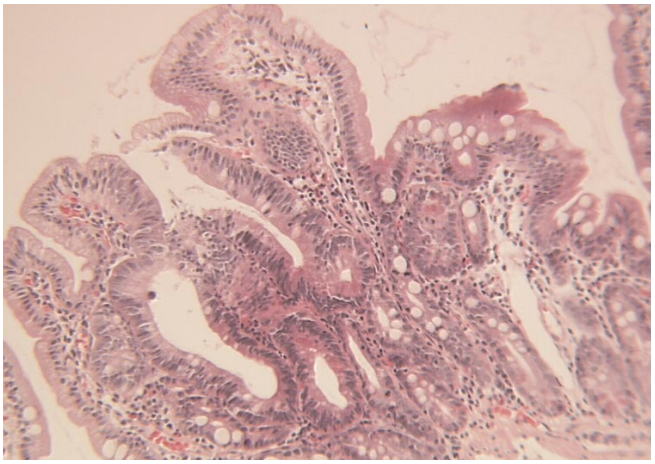

FIGURE 45.1-15 Familial adenomatous polyposis. Light microscopic aspect of duodenal adenomas. (Hematoxylin and eosin; ×250 original magnifications.)

greater in first-degree relatives of FAP patients with DT than FAP patients in general. Eighty percent of DTs occur after prophylactic colectomy within 5 years on average. They consist in desmoplastic mesenteric infiltration or progressively growing masses within the mesentery, retroperitoneum, and abdominal wall. They do not metastasize but are prone to local invasion, causing small bowel obstruction, hydronephrosis, vascular obstruction, and bowel perforation. Their usual development after surgical trauma and their high potentiality of recurrence must be taken into account in therapeutic indications.

Hepatoblastomas occur in 1.6% of children born to a parent with FAP, a relative risk approximately 850-fold greater than the general population, leading some experts to recommend annual serum α-fetoprotein determination and hepatic ultrasonography in at-risk children between 0 and 6 years of age.[102,103] All six children with the FAP mutation who recovered from the hepatoblastoma have developed colorectal adenomatous polyposis.

Diagnosis. The diagnosis of FAP among individuals with a family history is confirmed by the presence of 100 or more adenomatous polyps on colonoscopic examination.[47] However, in children, the finding of even one adenomatous polyp should alert the pediatrician to the possibility of a FAP (Figure 45.1-17). Adults with attenuated FAP exhibit fewer than 100 colorectal adenomas, whereas those with hereditary nonpolyposis colorectal cancer usually exhibit only a few colorectal adenomas, generally localized in the right colon. The simultaneous inheritance of both a germline frame shift mutation in the *APC* gene and a germline splice-site mutation in the *MLH1* gene was recently described in a 10-year-old boy with rapidly progressive FAP.[104]

To differentiate FAP from the other polyposis syndromes and from nodular lymphoid hyperplasia or hyperplastic polyps, which may mimic FAP endoscopically, histology is the key. The diagnosis can be confirmed by mutation analysis. Additionally, in at-risk individuals, the presence of more than three pigmented ocular fundic lesions on ophthalmologic examination confirms the diagnosis of FAP.[100]

Screening. An *APC* gene mutation can be identified in 80 to 90% of FAP families. The integration of genetic testing (Table 45.1-8) into clinical practice provides multiple benefits with earlier detection of lesions and prevention of cancer, removal of patient uncertainty, and the elimination of unnecessary screening. In the setting of a known mutation in a family, genetic testing of relatives can discriminate

TABLE 45.1-7 STAGES OF SEVERITY IN DUODENAL POLYPOSIS

CHARACTERISTICS OF POLYPS	GRADE (POINTS)		
	1	2	3
Number of polyps	1–4	5–20	> 20
Size of polyps (mm)	1–4	5–10	> 10
Histology	Tubular	Tubulovillous	Villous
Dysplasia	Mild	Moderate	Severe

Adapted from Spigelman AD et al.[96]
Stage 0: 0 points; stage I: 1–4 points; stage II: 5–6 points; stage III: 7–8 points; stage IV: 9–12 points.

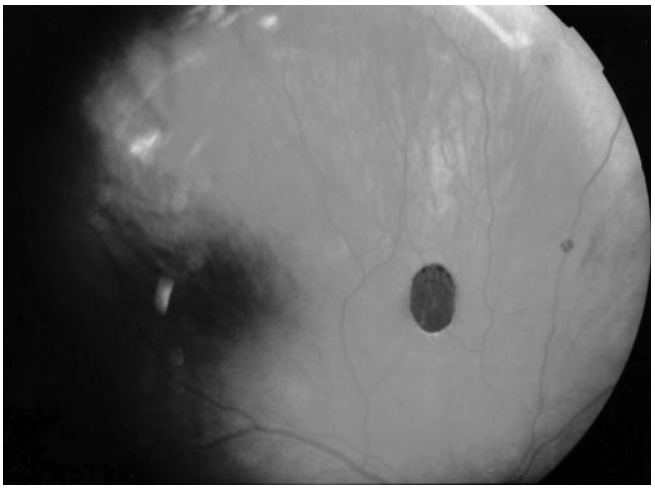

FIGURE 45.1-16 Congenital hypertrophy of the retinal pigmented epithelium.

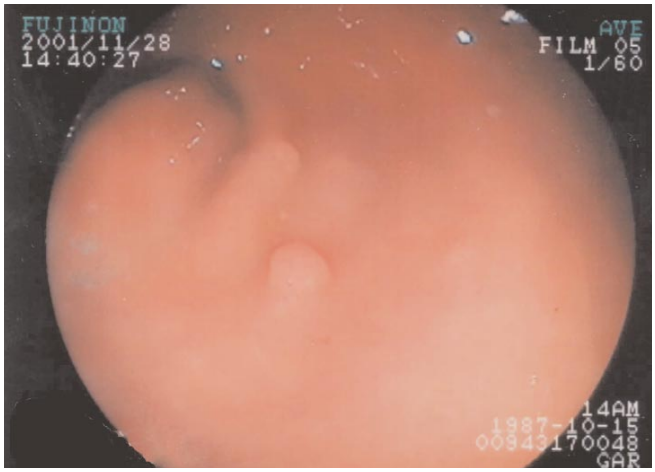

FIGURE 45.1-17 One or two adenomatous polyps at pancolonoscopy may be the early alert leading to the diagnosis of familial adenomatous polyposis in a child.

between affected and unaffected individuals with a high degree of certainty. Nevertheless, inappropriate use of genetic testing has the potential to misinform affected patients with false-negative results.[41] Written informed consent for genetic testing must be obtained from the patient and/or parents.[105,106] Taking into account that 1% of the FAP patients will develop colorectal cancer between ages 15 and 20 years, genetic testing can be proposed at 10 to 12 years of age.[107] Genetic diagnosis can be achieved by detection of *APC* mutations in DNA from peripheral blood lymphocytes using a commercially available protein truncation assay or by direct DNA sequencing.[108] A child found to be mutation negative in a family with an identified mutant *APC* allele has the same colorectal cancer risk as the general population.

If the pedigree mutation is not found or if informative genetic testing cannot be done, all first-degree family members should undergo endoscopic screening.[28,63,109] Formal recommendations exist for surveillance (Table 45.1-9) of at-risk individuals.[107] In addition, upper endoscopy, surveillance of the stomach, duodenum, and periampullary region is recommended with front-viewing and side-viewing endoscopes.[96] Owing to the increased risk of hepatoblastoma in patients with FAP, screening with α-fetoprotein levels and ultrasound imaging of the liver may be prudent in the children of affected parents from infancy to 7 years of age.[110]

Treatment. ***Colonic Polyposis.*** Colectomy is the recommended treatment for FAP patients to eliminate the risk of colorectal cancer. Timing and extent of surgery, that is, subtotal colectomy with ileorectal anastomosis (IRA) versus proctocolectomy and ileal-pouch-anal anastomosis (IPAA), have been widely debated.[63] IPAA has various disadvantages: (1) a risk of severe postoperative complications necessitating removal of the pouch and construction of an ileostomy (< 5%) and (2) a worse functional outcome compared with that of IRA, although the quality of life after IRA and IPAA seemed to be the same.[111] If FAP has a clinical expression during childhood and adolescence, the decision of an IPAA seems to be the best surgical option because the remaining risk of developing rectal cancer increases over time,[42,112,113] and a secondary proctectomy is needed in approximately half of the cases because of uncontrollable polyposis.[42] Management of young patients is summarized in Figure 45.1-18. After IPAA, the risk of developing one or more adenomas in the ileal pouch at 5, 10, and 15 years is 7%, 35%, and 75%, respectively.[114] There is also a substantial risk for the development of polyps at the anastomotic site; the cumulative risk of developing a polyp at the anastomotic site is 8% at 3.5 years and 18% at 7 years, which can be partially, but not totally, reduced by handsewn anastomosis with mucosectomy.[115] All FAP patients who undergo an IPAA procedure, irrespective of the applied surgical technique, should undergo endoscopic IPAA surveillance at regular intervals of at least once a year.[114,115]

Only patients with a few rectal polyps from families with a similar mild phenotype might be selected for IRA. The results of molecular genetic testing might be used to identify such patients.

Several studies have shown that treatment of FAP patients with a nonselective or selective cyclooxygenase-2 inhibitor (eg, sulindac or celecoxib, respectively) leads to reduction in the number and size of the colonic and rectal adenomas in both short- and long-term studies.[116–118] However, administration of sulindac for primary chemoprevention of FAP failed to prevent the development of adenomatous polyposis in gene mutation carriers,[119] and rectal cancer has been reported after prolonged sulindac chemoprevention.

TABLE 45.1-8 INDICATIONS FOR *APC* GENE SCREENING

> 100 colorectal adenomas
First-degree relatives of patients with FAP
> 20 cumulative colorectal adenomas
First-degree relatives of patients with attenuated FAP

FAP = familial adenomatous polyposis.

TABLE 45.1-9 SCREENING/SURVEILLANCE GUIDELINES IN FAP PATIENTS

At-risk individuals	
Genotyping	*APC* gene mutation (+): flexible colonoscopy annually starting at age 10–12 yr
	APC gene mutation (–): flexible sigmoidoscopy at age 25 yr
If genotype not available	Flexible sigmoidoscopy or colonoscopy annually starting at age 10–12 yr, then 2 yr starting at age 35, then as per the guidelines for average-risk individuals starting at age 50
Affected individuals	Upper gastrointestinal surveillance every 3–4 yr and annually if upper tract polyps
	If retained rectum or J pouch, flexible sigmoidoscopy every 6 months or 1 to 2 yr, respectively
	Annual physical examination and routine blood tests

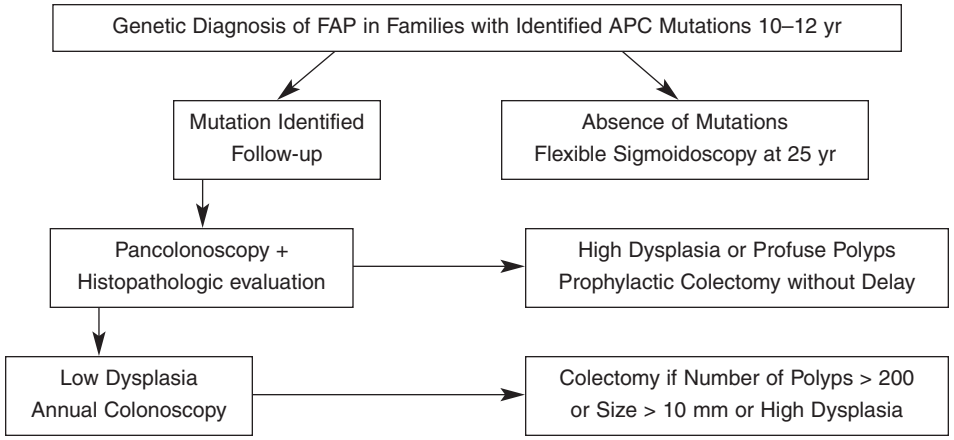

FIGURE 45.1-18 Management of at-risk familial adenomatous polyposis (FAP) patients in childhood and adolescence. Adapted from Hyer W et al.[214]

***Duodenal* Polyposis.** The therapeutic challenge of duodenal polyposis arises in adulthood.[120] Chemoprevention by nonsteroidal anti-inflammmatory drugs is not effective.[121] Endoscopic procedures have limitations, and the only treatment is prophylactic duodenal resection.[122] However, this operation has considerable potential morbidity and mortality.

***Abdominal* DTs.** In FAP families with associated DTs, this additional risk has to be taken into account in surgical management. Timing for surgery as late as possible, at least in patients with a smaller number of polyps and an expected later onset of disease, and performing the colectomy procedure in one stage must be considered. In such families, we prefer to perform an IPAA procedure as the initial prophylactic colorectal procedure because conversion of an IRA to an IPAA may be precluded by mesenteric DTs. Before surgery, abdominal computed tomography is performed in clinically indicated cases and in cases with a family history of desmoid disease.

Other Variants of FAP. Other variants of FAP include attenuated familial adenomatous polyposis (AFAP)[123] and Turcot syndrome, defined as typical FAP together with central nervous system malignancies, in particular medulloblastoma.

***Attenuated* FAP.** The clinical characteristics of AFAP include oligopolyposis, usually less than 100 colorectal adenomas at presentation, which are mostly right-sided, and a delayed onset of colorectal cancer occurring on average more than 12 years later than in classic FAP.[58] Total colonoscopy should be considered in families with an atypical form of polyposis because adenomas may be located in the proximal part of the colon. In such families, the endoscopic examinations may be started at a later age (18–20 years).[63] The adenomas have a flat rather than a polypoid aspect, leading to the initial description as "hereditary flat adenoma syndrome."[123] This condition should not be confused with hereditary nonpolyposis colorectal cancer. In patients with AFAP, fundic gland polyps and duodenal adenomas are more prominent than colonic polyps. Recently, Attard and colleagues described the first pediatric patient with AFAP diagnosed at age 9 in the setting of a strong family history of gastric carcinoma who had multiple fundic gland polyps with severe dysplastic changes requiring prophylactic gastrectomy at age 11.[124]

Turcot Syndrome. Turcot syndrome, or brain tumor polyposis syndrome, is characterized by multiple colonic adenomas associated with a primary brain tumor of various histopathologic types.[91,125,126]

Molecular genetic studies have established a new classification of Turcot syndrome kindreds into two groups according to the type of brain tumor and the genetic defect.[91] The more common group (Turcot syndrome type II) has germline mutations of the *APC* gene and medulloblastoma, which precedes the diagnosis of polyposis in some cases.[127] The other group with the family originally described by Turcot (Turcot syndrome type I) includes patients with glioblastomas and germline mutations in DNA base mismatch repair genes also implicated in hereditary nonpolyposis colon cancer.[91]

Hamartomatous Polyposis Syndromes

Hamartomatous polyposis syndromes are characterized by an overgrowth of cells or tissues native to the area in which they normally occur.[128] This overgrowth of cells or tissues, at least initially, has no presumed neoplastic potential. However, several of these syndromes are associated with an increased lifetime risk of both intestinal and extraintestinal malignancies.

Hamartomatous polyposis syndromes include JPS, Cowden disease (CD), Bannayan-Riley-Ruvalcaba syndrome (BRRS), or Ruvalcaba-Myrhe-Smith syndrome (RMSS) and PJS. In JPS, polyps involving the gastrointestinal tract are the major manifestation of the disease. In contrast, for the other syndromes, they are a component among a variety of extraintestinal features.[129] The mechanism of inheritance for these syndromes is autosomal dominant with variable penetrance. It must be emphasized that, in children, it may be difficult to distinguish patients with JPS and those with CD because extraintestinal manifestations in the latter condition are an age-related phenomenon. Rigorous long-term follow-up in clinical studies of these syndromes is needed to achieve phenotype-genotype correlations.

Juvenile Polyposis Syndrome. First described by McColl and colleagues in 1964,[130] JPS is the most common of the hamartomatous syndromes characterized by multiple gastrointestinal polyps in the absence of the extraintestinal features that are classic for other hamartomatous polyposis syndromes. Most authors support the diagnosis criteria outlined by Giardiello and colleagues.[23] These criteria include either three or more juvenile polyps of the colon or polyposis involving the entire gastrointestinal tract or any number of polyps in a proband with a known family history of juvenile polyps. JPS is inherited in an autosomal dominant manner with variable penetrance, with approximately 25% of cases having a family history of juvenile polyposis.[131]

Presentation. In children, JPS is always symptomatic (in contrast to FAP).[15] Symptoms include rectal bleeding, rectal prolapse, cramping abdominal pain, and intussusception.[2] Most cases of JPS come to medical attention between 2 and 12 years of age,[15] although rare cases of failure to thrive, anemia, and severe hypoalbuminemia resulting from a protein-losing enteropathy have been described in infants.[132,133] Polyps vary in number from 3 to 200[15] and show great variation in size and configuration. In most cases, juvenile polyps are found only in the colon.[131] In a review of 272 cases of JPS by Höfting and colleagues, the most frequently affected site was the colorectum (98%), followed by the stomach (14%), jejunum and ileum (15%), and duodenum (2.3%).[134] When polyps are numerous, follow-up should include serum albumin and α_1-antitrypsin fecal clearance.[135,136] There is an association with congenital birth defects in 15% of cases, including malrotation of the midgut, genitourinary defect, and cardiac defects.[2] The majority of congenital defects have been reported in individuals with the nonfamilial variant of the disease.

Pathology. The typical gastrointestinal polyp in JPS has the same histopathologic aspects as solitary juvenile polyp. In contrast to the polyps of PJS, muscle fibers are not present in the stroma. As hamartomatous polyps enlarge and the mesenchymal component expands, they take on a more serrated or villous-type configuration associated with a more marked degree of epithelial dysplasia.[9,25]

Molecular Genetics*: JPS *and the* SMAD *Pathway. There is evidence of genetic heterogeneity in JPS. Hereditary juvenile polyposis is not linked to *APC*. Subsets of JPS families have mutations in the tumor suppressor gene phosphatase and tensin homolog (*PTEN* 601728) located at chromosome 10q23.3.[137] *PTEN* is a ubiquitously expressed dual-specificity phosphatase that acts as a tumor suppressor and is mutated in several sporadic tumor types.[37] Mutations in this gene are also important in a subset of familial thyroid carcinoma, CD, and BRRS.[37,138] The shared clinical features of CD/BRRS and JPS, coupled with coincident somatic mutation data in juvenile polyps, raise the possibility that *PTEN* defects could cause all of these syndromes.[139,140] However, if about 60% of BRRS and more than 80% of CD demonstrate germline mutations in *PTEN*, germline mutation of *PTEN* as a cause of JPS in a child is controversial because extraintestinal manifestations that would exclude JPS could appear after adolescence, CD having a penetrance well below 10% under 15 years of age, altering an early clinical diagnosis.[141] Moreover, a proportion of JPS patients do not have germline mutations in *PTEN*. Three groups have found no evidence of germline *PTEN* mutations in 21 JPS families and 16 sporadic cases.[142,143] In a recent review of *PTEN*, JPS is not considered a so-called *PTEN* hamartoma-tumor syndrome (PHTS).[141] In summary, the association of germline *PTEN* mutations as a cause for JPS is not yet clearly substantiated. Nevertheless, Huang and colleagues identified a germline mutation of PTEN in a family in which identical twin 6-year-old girls were diagnosed with JPS.[144] Their 55-year-old father lacked manifestations of CD or BRRS and had the same mutation in *PTEN*.

Recently, constitutional mutations in small mothers against decapentaplegic 4 (*SMAD4*), located at chromosome 18q21,[38,145,146] and bone morphogenetic receptor 1A (*BMPR1A*) were shown to cause JPS.[38,145–148] *SMAD4* mutations seem to be the most relevant mutations in JPS patients without stigmata of other polyposis syndromes and account for approximately 50% of the reported familial cases of the syndrome.[38] These genes, which code for proteins involved in transforming growth factor-β (TGF-β) signal transduction, are mutated in a number of gastrointestinal cancers. Other members of the SMAD family (*SMAD1*, *-2*, *-3*, or *-5*) are not involved in the pathogenesis of JPS. No consistent mutations of the deleted in colon cancer gene (*DCC*), which is located at the same locus as *SMAD4*, have been found.[149] *BMPR1A* and its receptors belong to the TGF-β and TGF-βR superfamily, and the recent finding of four JPS kindreds with *BMPR1A* mutations further supports the importance of TGF-β superfamily mutations in JPS.[147]

SMAD proteins transduce signals from TGF-β ligands that regulate cell proliferation, differentiation, and death through activation of receptor serine/threonine kinases. Thus, TGF-β is a potent immunosuppressor, and perturbation of TGF-β signaling is linked to autoimmunity, inflammation, and cancer. Activated SMADs regulate transcription of target genes, including cell-cycle inhibitors such as *p21*, which mediate the antiproliferative response and partially explain the tumor suppressive action of the TGF-β pathway. At late stages of tumor progression, TGF-β promotes tumorigenesis via suppression of the immune system and changes in cell differentiation of epithelial tumor cells. Phosphorylation of receptor-activated SMADs (R-SMADs) leads to formation of complexes with the common mediator SMAD (Co-SMAD), which are imported to the nucleus. Nuclear SMAD oligomers bind to DNA and associate with transcription factors to regulate the expression of target genes. Alternatively, nuclear R-SMADs associate with ubiquitin ligases and promote degradation of transcriptional repressors, thus facilitating target gene regulation by TGF-β. SMADs themselves can also become ubiquitinated and are degraded by proteasomes. Finally, the inhibitory SMADs (I-SMADs) block phosphorylation of R-SMADs by the receptors and promote ubiquitination and degradation of receptor complexes, thus inhibiting signaling.[150]

Multiple somatic genetic alterations, including *APC*, *MMR*, and K-*ras* mutations, seem to play a role in the neoplastic transformation of juvenile polyps coli.[8]

Neoplastic Risk. In a large retrospective review from St Mark's Polyposis Registry published in 1988, 1,032 juvenile polyps from 87 patients were examined.[25] Pathologic specimens were available for re-evaluation from 80 patients. Twenty-two percent of the patients subsequently developed colorectal cancer. The mean age at the time of diagnosis was 34 years. Some patients also developed upper gastrointestinal malignancies.[151] In 1993, Höfting and colleagues found 48 cases of gastrointestinal cancer (18%) among 272 patients with JPS.[134] In 1995, Desai and colleagues re-evaluated the data from the St Mark's Polyposis Registry and estimated that the projected incidence of colorectal cancer alone by the age of 60 was approximately 68%.[131]

Management and Surveillance. Recommendations for endoscopic screening and treatment in patients with JPS are summarized in Figure 45.1-19.[108,129] The proband also should undergo upper gastrointestinal endoscopic screening. It is not clear whether upper and lower endoscopic surveillance is adequate to prevent malignancy. If surgery becomes mandatory, the extent of rectal polyposis is a major consideration in determining the modality of colectomy.[15] There are insufficient data to justify coloproctectomy or prophylactic colectomy solely for the risk of colorectal carcinoma.[23] The therapeutic role of cyclooxygenase inhibitors in pediatric JPS is also unclear.

In three of eight JPS families, polyps were identified in asymptomatic first-degree relatives.[23] Accordingly, first-degree relatives of patients with JPS should be screened by upper and lower endoscopy starting at 12 years of age, even when the subject is asymptomatic. Howe and colleagues recommend incorporating genetic testing into the screening algorithm.[38] However, given the presumed genetic heterogeneity of this syndrome, failure to show a mutation in *SMAD4* does not support lengthening the surveillance interval in asymptomatic first-degree relatives.[38]

Juvenile Polyposis of the Stomach. Juvenile polyposis involving the stomach without intestinal polyps at initial presentation (10–63 years with 4 of the 12 patients younger than 20 years of age), first described by Watanabe and colleagues, may be regarded as a clinical entity separate from generalized gastrointestinal polyposis.[152,153] Anemia (89%) and hypoproteinemia with most patients requiring gastrectomy (67%) are the most striking clinical features. Three subjects developed gastric cancer from 32 to 65 years.

Phosphatase and Tensin Homolog Hamartoma-Tumor Syndromes. PHTS, that is, CD[154] and BRRS,[155] is a rare autosomal disorder characterized by multiple phenotypic abnormalities and hamartomas in the intestine and other tissues. Recent nomenclature favors the term "PHTS" because germline mutations in the *PTEN* gene account for up to 80% of cases of CD and 60% of BRRS patients. Clinical differences are likely caused by allelic variations.[140,141] PTEN is a major dual lipid and protein phosphatase that signals apoptosis and mediates cellular growth arrest by inhibition of phosphoinositol 3-kinase.[156,157] Furthermore, the protein phosphatase, with the ability to dephosphorylate both serine and threonine residues, regulates cell survival pathways, such as the mitogen-activated kinase (MAPK) pathway.

Cowden Disease. CD (MIM 1583350) is an autosomal dominant condition characterized by multiple hamartomas that affect derivates of all three germ layers with an increased risk of breast, thyroid, and endometrial neoplasias.[141,158] The diagnostic criteria for CD have been revised recently (Table 45.1-10).[159] Eighty percent of patients present with dermatologic manifestations among which tricholemmomas are very suggestive of CD. Approximately 40% of affected individuals develop disease of the central nervous system. Macrocephaly is frequently observed.[160] CD in concert with cerebellar gangliocytomatosis is referred to as the Lhermitte-Duclos syndrome.[161] Only 35 to 40% of patients who meet the diagnosis criteria for CD have gastrointestinal polyposis.[130] Polyps can be typical juvenile polyps, ganglioneuromas, or adenomas. It is noteworthy that CD has a

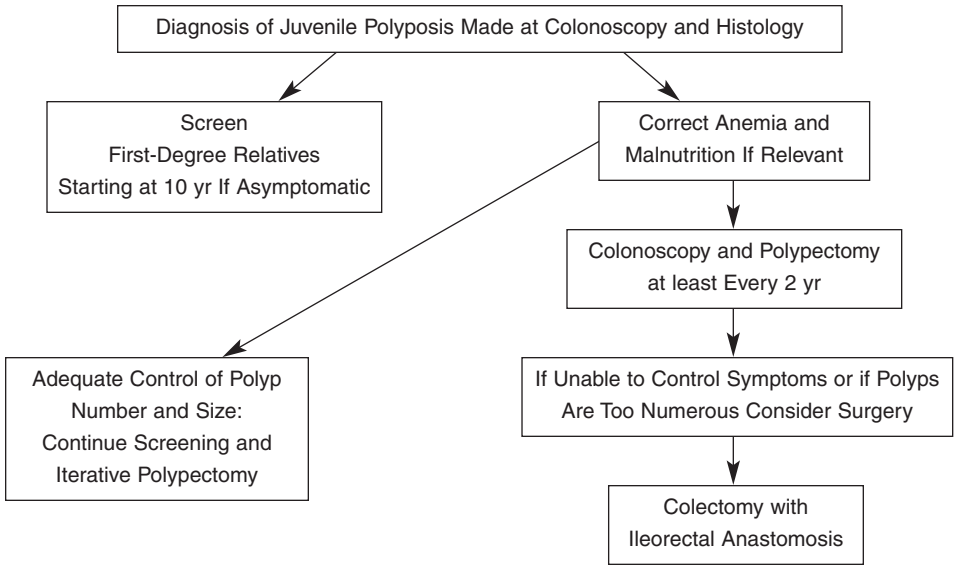

FIGURE 45.1-19 Management and surveillance in young patients with juvenile polyposis syndrome.

TABLE 45.1-10 INTERNATIONAL COWDEN CONSORTIUM OPERATIONAL DIAGNOSTIC CRITERIA

Pathognomonic criteria (mucocutaneous lesions)
Facial trichilemmomas
Acral keratoses
Papillomatous papules
Mucosal lesions
Major criteria
Breast carcinoma
Thyroid carcinoma (nonmedullary), especially follicular thyroid carcinoma
Macrocephaly (megalencephaly)
Lhermitte-Duclos syndrome
Endometrial carcinoma
Minor criteria
Other thyroid lesions (eg, adenoma or multinodular goiter)
Mental retardation
Gastrointestinal hamartomas
Fibrocystic disease of the breast
Lipomas
Fibromas
Genitourinary tumors (eg, renal cell carcinoma or uterine fibroids) or malformation

great variation in expression and carries an age-related penetrance (10% below age 15 years, 90% at age 20 years). There is no reported increase in the risk of invasive gastrointestinal malignancies.

Gastrointestinal polyposis should be addressed by endoscopic surveillance. Although no definite increased risk of colorectal carcinoma has been documented, the true risk may be unrecognized because of the rarity of the syndrome. Screening for breast and thyroid malignancies should begin in the teenage years.

Germline mutations in *PTEN* have been found in 80% of probands with CD, especially in exons 5, 7, and 8, when operational criteria (International Cowden Consortium) are applied to the diagnosis of CD (see Table 45.1-10).[158] The majority of CD cases appear to be isolated; 10 to 50% are familial.

Bannayan-Riley-Ruvalcaba Syndrome. Hamartomatous polyposis is also seen as a component of BRRS associated with macrocephaly, a speckled penis, delayed development in childhood, lipomatosis, and hemangiomatosis. Sixty percent of patients with BRRS have germline *PTEN* mutations.[162] The mutational spectra of BRRS and CD seem to overlap. For some authors, there is a higher frequency of breast tumors, fibroadenomas, and lipomas among the mutation-positive group of patients with BRRS who meet published diagnosis criteria than among the 40% of mutation-negative patients.[163] It is not known if genes other than *PTEN* may also be responsible for BRRS.[164]

Peutz-Jeghers Syndrome. PJS (MIM 1752001) is another hamartomatous gastrointestinal polyposis syndrome associated with a risk of gastrointestinal and extraintestinal cancers. The incidence of PJS is estimated as 1 in 120,000 births.[165] Mucocutaneous melanin deposition, recognized by early investigators,[166,167] is a hallmark for this syndrome resulting from mutations of the *LKB1/STK11* gene, located on chromosome 19p 13.3.[39,168–170]

Clinical Manifestations. Skin pigmentation (Figure 45.1-20) consists of small (1–5 mm) pigmented macules, primarily clustered around the mouth, eyes, and nostrils and sometimes also the perianal area. The buccal mucosa is also affected. These pigmented lesions occur before gastrointestinal polyps but are rarely observed during early infancy. The cutaneous pigment pattern often fades with age, but buccal mucosa lesions tend to persist.[166,167,171,172] PJS is a clinically heterogeneous disorder, and cases of individuals from Peutz-Jeghers kindreds having pigmentation without polyposis, and the converse, have been reported.[167,171,173]

One-third of PJS patients will experience symptoms during the first decade of life,[167] and 50 to 60% of patients will experience them before age 20.[174] Patients present most commonly with abdominal pain secondary to obstruction or impending obstruction with polyp intussusception or gastrointestinal bleeding leading to anemia.[175] According to series, Peutz-Jeghers polyps are preferentially located in the small intestine (more frequently in the jejunum than in the ileum and than in the duodenum)[172–174] or equally distributed in the stomach, colorectum, and small bowel.[176] The small and large bowel polyps tend to be pedunculated (Figures 45.1-21 and 45.1-22), whereas stomach polyps are sessile (Figure 45.1-23). The polyps grow to a very large size and, combined with their pedunculated aspect, can result in recurrent intussusception.

Pathology. In contrast to the inflammatory appearance of juvenile polyps, hyperplasia of the smooth muscle layer occurs in Peutz-Jeghers polyps. Hyperplastic smooth muscle extends in a tree-like manner toward the epithelial layer (Figure 45.1-24). The extensive dilation of cystic-filled spaces, pathognomonic for the juvenile polyp, is not seen in PJS. Invagination of the epithelium will result in islands of epithelial cells trapped within the underlying smooth muscle, resulting in "epithelial cell misplacement" in the absence of cellular atypia and an increased mitotic rate.[175,177] Histologic evidence of hamartomatous-adenomatous-carcinomatous evolution has been demonstrated for stomach, small bowel, and colorectal polyps in PJS.[178,179] In addition, there is evidence that PJS patients are prone to develop adenomatous as well as hamartomatous polyps, particularly in the large intestine.[172,178]

Genetics. Germline mutations in the *STK11/LKB1* gene are reported as the molecular cause in 70% of PJS

FIGURE 45.1-20 Mucocutaneous buccal melanin pigmentation in a patient with Peutz-Jeghers syndrome.

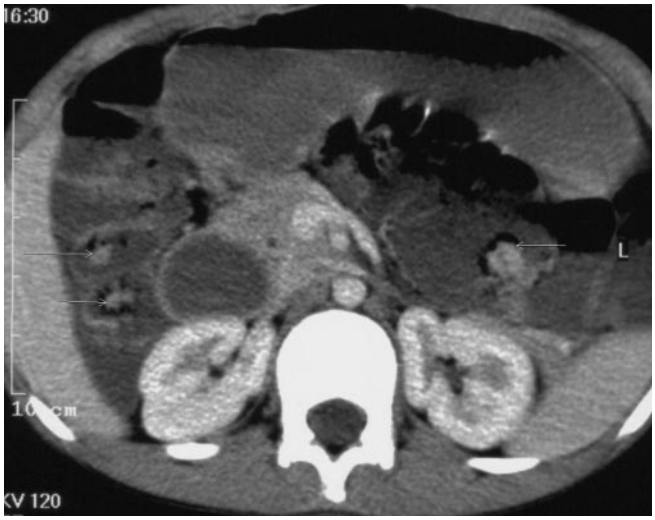

FIGURE 45.1-21 Peutz-Jeghers syndrome: abdominal pelvic computed tomographic scan showing jejunal polyps.

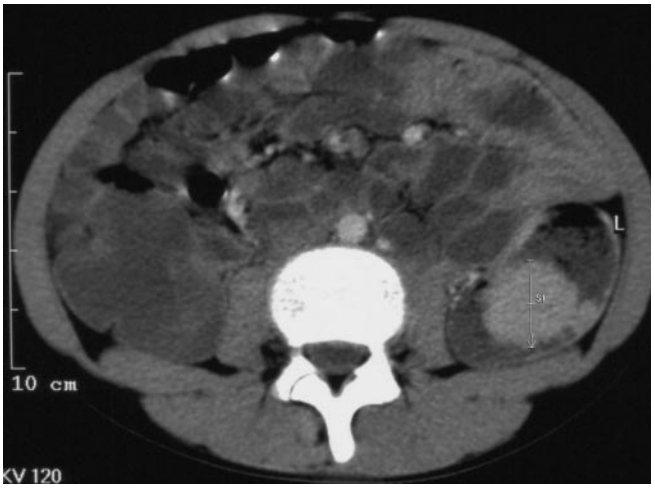

FIGURE 45.1-22 Peutz-Jeghers syndrome: abdominal pelvic computed tomographic scan showing a very large colonic polyp at the splenic flexure.

families and 50% of sporadic PJS patients.[169,180–184] The gene is divided into nine exons that encode a 433–amino acid protein containing a serine/threonine kinase domain, a nuclear localization signal in its N-terminus domain, and a prenylation consensus sequence in its C-terminus. The protein is located in the nucleus of cells but is also detected in the cytoplasm and at cell membranes.[185,186] *LKB1* is widely expressed during embryonic development. Immunostaining of the small intestine reveals that *LKB1* is expressed in two distinct topographic regions: the crypts that contain rapidly dividing stem cells and the top of the villi, where cells undergo apoptosis.[187,188] It has been proposed to act as a tumor suppressor. *LKB1* is also involved in *tp53*-mediated apoptosis.[187] *LKB1* phosphorylates *tp53* at low levels, which might be required for *tp53* activation. *LKB1* also controls cell proliferation. It interacts with the chromatin remodeling protein brahma-related gene-1 (*BRG1*)[189] and with the cell-cycle regulatory proteins LKB1-interacting protein 1 (LIP1) and WAF1.[190–192]

The *LKB1* mutations (truncated protein with incomplete catalytic domains) lead to loss in kinase activity,[180] whereas other cancer susceptibility syndromes are associ-

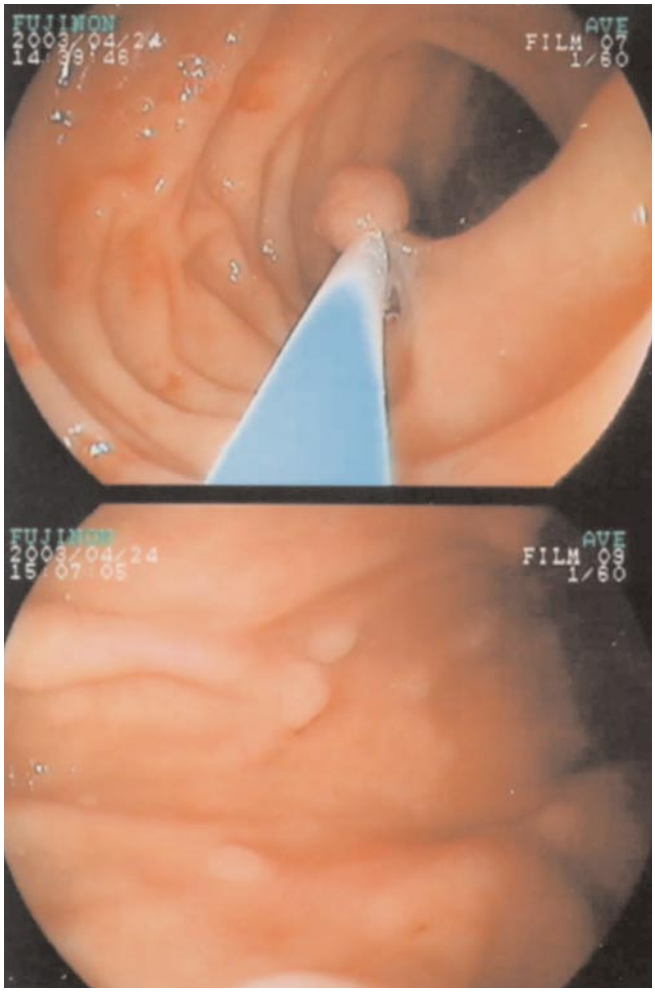

FIGURE 45.1-23. Peutz-Jeghers syndrome: endoscopic view of sessile and pedunculated stomach polyps from the same patient as in Figures 45.1-20 and 45.1-21.

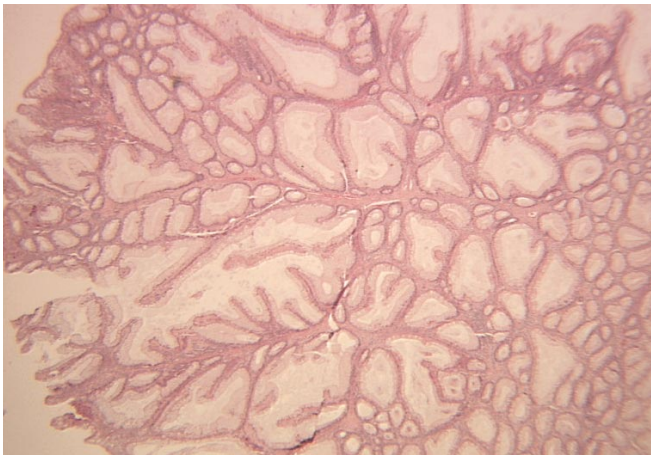

FIGURE 45.1-24 Low-power photomicrograph of Peutz-Jeghers rectal polyp showing bands of smooth muscle extending in a tree-like manner toward the epithelial layer. (Hematoxylin and eosin; ×75 original magnifications.)

ated with activation of kinase activity.[39,193] Allelic imbalance has previously been reported in a number of PJS polyps and found in a colonic adenoma from a PJS patient, strongly suggesting the existence of a hamartoma-carcinoma sequence in tumorigenesis.[194]

There is marked inter- and intrafamily phenotypic variability of expression in Peutz-Jeghers kindreds. The availability of predictive genetic testing may have some value but cannot determine the likely severity of the phenotype.[181] Moreover, not all PJS patients have demonstrable mutations in the *LBK1/STK11* gene.[39,195] However, if the gene mutation is known for previous affected cases in the family, it might have a role in presymptomatic testing for family members without pigmentation.

Cancer Risk. The risk of developing malignancy, both in the gastrointestinal tract and at extraintestinal sites, is increased in adults with PJS.[181] Moreover, malignancy can evolve as early as childhood and adolescence. In a recent meta-analysis of 210 PJS patients, the relative risk of stomach, small bowel, and large bowel cancer developing in PJS male patients was estimated at 235, 279, and 98, respectively, compared with that in the general population.[105] In a series of 222 Japanese patients, with 28 cases of intestinal malignancy, 3 teenagers with advanced gastric cancer were included. Of 70 patients under 16 years identified by a literature search, 5 had tumors, 2 of which were adenocarcinomas (1 gastric, 1 jejunal).[117] The risk of developing pancreatic cancer in PJS is estimated to be increased 100-fold.[196] Metastatic pancreatic cancer was reported in a 14-year-old boy with PJS.[197]

Gonadal tumors have been reported at an increased frequency in females and occasionally in male patients with PJS.[198] Ovarian sex cord tumors with annular tubules (SCTAT), usually found in young adult women,[199] may cause sexual precocity in young girls.[200] Analogous tumors have been identified in male patients, referred to as either large cells calcifying Sertoli cell tumors or as testicular tumors resembling SCTAT.[198,201–203] The clinical presentation is gynecomastia and rapid growth with advanced bone age in prepubescent boys.[199,203] Although often bilateral and multifocal, SCTATs usually have a benign course in the setting of PJS. Other neoplasms of the genital tract have been described in PJS females, but the major risk is breast cancer.[105]

Clinical Management. The gastrointestinal polyposis of PJS carries the dual risk of repeated resections for infarction secondary to intussusception and malignancy. More than three-quarters with PJS had one or more laparotomies owing to the recurrence or progression of polyps.[204] This high reoperation rate might be reduced in skilled hands by removal of other polyps through upper and lower endoscopy and intraoperative enteroscopy. Intraoperative small bowel endoscopy identifies 38% more polyps at laparotomy compared with external palpation and small bowel transillumination.

Children with well-defined mucocutaneous pigmentation should be enrolled into a screening program, as outlined in Table 45.1-11, and an aggressive therapeutic approach taken if symptomatic.[204–210] Every 2 years, the symptomatic PJS patient should have upper and lower endoscopies with polypectomy[206] and some form of examination of the small bowel, such as barium study by enteroclysis or wireless capsule enteroscopy in children over 10 years old. The size of the polyps and their location influence the clinical management (Figure 45.1-25). Patients with symptomatic midgut polyps greater than 1.5 cm in the jejunoileum should be referred for laparotomy with intraoperative endoscopy. During laparotomy, the surgeon can assist in telescoping the endoscope over the entire small intestine. A noncrushing clamp can be placed over the cecum to prevent large bowel distention.[205] Smaller polyps can be removed by an electrocautery snare. Larger, broad-based polyps will require an enterotomy. If needed, a sterile endoscope can also be advanced through the enterotomy site to aid endoscopic examination of the entire small bowel followed by snare resection of polyps. For children who are asymptomatic with small polyps (ie, < 1 cm in size), parents should be counseled about the risk of intussusception. If the child later develops relevant symptoms, he or she should also be referred for consideration for enteroscopy. Extensive small bowel surgical resection should not be undertaken in order to preserve gut function and limit the risk of short-bowel syndrome.[129]

Bourneville Tuberous Sclerosis. Tuberous sclerosis is a dominantly inherited disease with a variable penetrance characterized by the classic triad of mental retardation,

TABLE 45.1-11 SURVEILLANCE GUIDELINES FOR PEUTZ-JEGHERS SYNDROME

SITE	PROCEDURE	ONSET (YR)	INTERVAL (YR)
Stomach	Upper endoscopy	10	2
Small bowel	Small bowel follow-through	10	2*
Colon	Pancolonoscopy	10	2*
Breast	Breast examination	25	1
	Mammography	25	2–3
Testicle	Testicular examination	10	1
Ovary	Uterus pelvic examination	20	1
	Pelvic ultrasonography	12	1
Pancreas	Endoscopic ultrasonography (if available) or abdominal ultrasonography	30	1–2

*May consider lengthening interval based on clinical history.

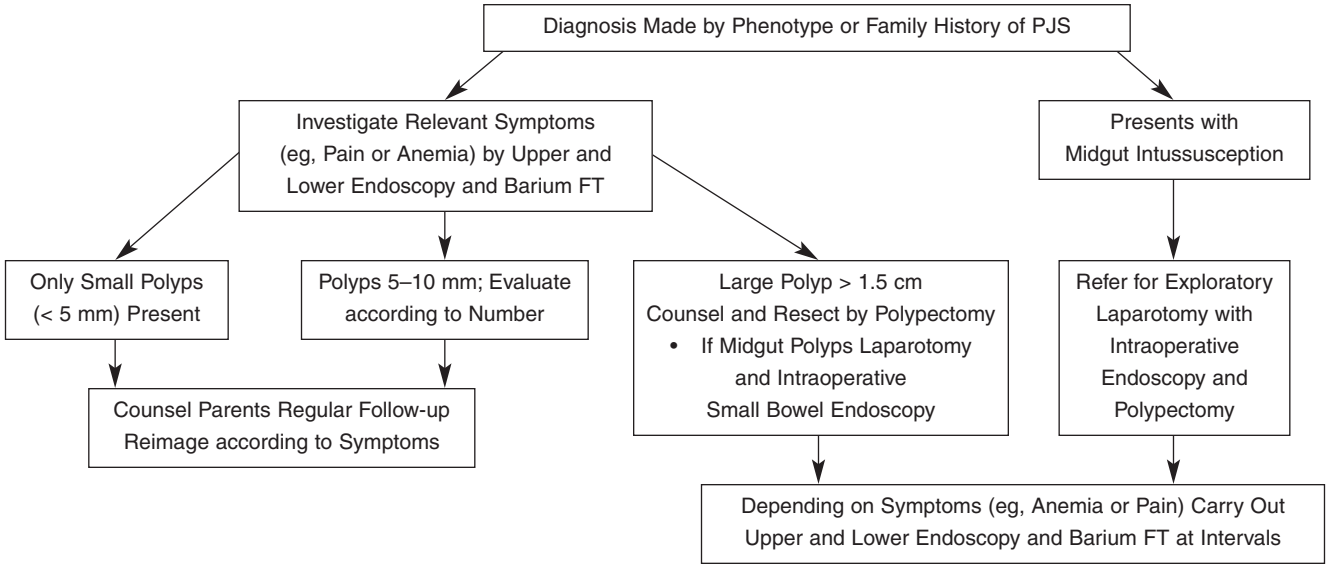

FIGURE 45.1-25 Management and surveillance in patients with Peutz-Jeghers syndrome (PJS). Adapted from Hyer W et al.[214] FT = follow-through.

epilepsy, and adenoma sebaceum in the presence of hamartomatous lesions. Mutations have been identified in two genes: tuberous sclerosis locus 1 (*TSC1*) and tuberous sclerosis locus 2 (*TSC2*). Hamartomatous polyps resembling Peutz-Jeghers polyps diagnosed at a mean age of 12 years, as well as adenomatous polyps diagnosed at a mean age of 45 years, may occur and are often located within the distal 25 cm of the colon.[210] An invasive adenocarcinoma was reported in a 17-year-old girl in association with adenomatous polyps throughout the proximal and distal colon without a family history of colorectal neoplasia.[211]

Hereditary Mixed Polyposis Syndrome

Hereditary mixed polyposis syndrome (MIM 601228) is characterized by atypical juvenile polyps, with mixed features of hamartomas and adenomas located in the colon with the risk of subsequent colorectal cancer. The locus maps to chromosome 6q16.[212]

Hyperplastic Polyposis

Hyperplastic polyposis is an uncommon condition in which large numbers of hyperplastic polyps are present throughout the colon. Keljo and colleagues described a rectal cancer developing in an 11-year-old girl with hyperplastic polyposis.[213]

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2. Other Neoplasms

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Neoplasia represents a major proportion of gastrointestinal disease in adults, but the finding of intestinal tumors in the pediatric age group is usually unexpected. Some familiarity with these rare tumors is advisable, however, because a delay in diagnosis can adversely affect patient outcome.

Rather than provide an encyclopedic list of all forms of intestinal neoplasia, this discussion focuses on three forms: carcinoma, lymphoma, and leiomyosarcoma. These tumors constitute the bulk of intestinal neoplasia in the pediatric age group, and the clinical material presented provides an approach that can be used in diagnosing rarer tumors as well. Where appropriate, a comparison with the corresponding tumors occurring in adults is made. Leiomyomas of the gastrointestinal tract are considered here and in Chapter 28, "Congenital Anomalies of the Stomach and Duodenum," and polypoid benign tumors, including adenomas and hamartomas, are discussed in Chapter 45.1, "Intestinal Polyps and Polyposis." Secretory tumors affecting the gut, including carcinoid tumors, are discussed in Chapter 47, "Secretory Tumors."

BENIGN INTESTINAL TUMORS

Adenomas are by far the most common benign intestinal tumors, followed by leiomyomas and lipomas. Adenomas and lipomas are found primarily in the colon but also can appear in the small intestine, where they present with lower tract bleeding, with abdominal pain, or as the lead point for an intussusception. Lipomas typically present in older adults but also can occur in children.[1] About 70% of intestinal lipomas arise in the colon and roughly 25% occur in the small intestine. Only rarely do lipomas develop in the stomach or esophagus.[2] In contrast, smooth muscle tumors are far more common in the proximal gut, where benign leiomyomas are prominent at all ages; only isolated cases of colonic leiomyomas are reported in children.[3,4] Distinguishing benign from malignant smooth muscle tumors of the intestine can prove difficult. Accordingly, a wide surgical resection is recommended.

ADENOCARCINOMA OF THE INTESTINE

Carcinoma of the Colon

Epidemiology. The overall incidence of carcinoma of the colon and rectum continues to increase, and, in 1999, approximately 129,000 new cases were diagnosed in the United States, where the cumulative lifetime risk is about 6%.[5] Estimates suggest that nearly 147,000 people will be diagnosed in 2003.[6] The incidence of colorectal carcinoma is highest in developed countries in northwest Europe, the United Kingdom, Canada, and the United States and lowest in Africa and Asia.[7] In the United States, the disease is more common in blacks than in whites, until 60 years of age.[6] Individuals from low-risk areas who migrate to high-risk areas develop an incidence of colon carcinoma similar to that of people residing in the high-risk area.

Although there are fewer than 200 cases reported in the pediatric literature, carcinoma of the colon is the most common primary malignant solid tumor of the intestinal tract in childhood. In several large series, approximately 1% of malignant tumors in children are colonic. The frequency of colon cancer increases with age: about 1% of cases of carcinoma of the colon occur in individuals less than 30 years of age.[8] The incidence of colorectal carcinoma in individuals younger than 20 years of age is less than 0.1 cases per million and rises to about 1 case per million in individuals between 10 and 19 years of age.[6,9] Even an infant, however, can develop colon carcinoma; the youngest reported patient was only 9 months old at the time of diagnosis.[10] A slight male predominance of carcinoma of the colon in children has been reported in some series, with a male-to-female ratio of approximately 1.5:1.[11–13] In adults, the incidence of carcinomas of the colon and rectum is also slightly higher in men.[6] Although rare, colon cancer in children demonstrates some unique features, which are discussed below in detail.

Etiology. Much research focuses on identifying the environmental agents associated with the development of colon and rectal cancer. The most convincing evidence comes from large population-based studies that have shown that diets high in fiber reduce the risk of colorectal cancer.[14,15] Fiber is hypothesized to dilute and bind potential carcinogens, reduce the carcinogen exposure time by increasing transit, and decrease the formation of secondary bile acids, among other mechanisms.[16] Small uncontrolled reports also suggest that the risk of colorectal carcinoma is associated with a diet high in calories, fat, and cholesterol, but this has not been confirmed in large well-controlled studies. Prospective studies have shown, at best, a moderate reduction in risk of colorectal adenoma formation with calcium supplementation.[17,18] Even less evidence is available to support anecdotal reports of protection from cancer with several micronutrients, including selenium and folic acid.[19] Exposure to radiation and chemicals, such as

organic solvents, also may play an etiologic role. In one series, 10 of 13 adolescent patients presenting with colon carcinoma had exposure to agricultural chemicals.[20]

As shown in Table 45.2-1, a number of predisposing conditions, including inflammatory bowel disease and the hereditary polyposis syndromes, confer a high risk for colon carcinoma. Lynch classified the development of colon cancer into four types: (1) sporadic, accounting for 70 to 80% (if familial predisposition to the presumed precursor adenomas are considered, heredity may play a role in a high percentage of cases of colon cancer currently categorized as "sporadic"); (2) familial, representing patients with at least two first-degree relatives with a history of colon cancer but no definable genetic inheritance pattern, making up 10 to 20%; (3) hereditary polyposis syndromes with cancer, less than 1%; and (4) hereditary cancer without polyposis syndrome, 5 to 6%.[21] Colon cancer in patients with inflammatory bowel disease constitutes less than 1% of the total.[22] Owing to the relatively small number of cases reported in children, the contribution of predisposing conditions is not well defined, but the incidence of preexisting polyposis or colitis in children with colon cancer is approximately 10%, a frequency that is higher compared with that in adults.[23]

Cancer is a complication of inflammatory bowel disease. In ulcerative colitis, the total duration of the colitis rather than an early age at onset is an important determinant of risk.[24] After the first 8 to 10 years of disease, the cumulative risk of colorectal cancer increases by about 0.5 to 1% per year, reaching a cumulative risk of 16 to 27% by 28 years of age. The anatomic extent of the colitis is another determinant. Patients with ulcerative pancolitis have a 15- to 19-fold risk of colorectal cancer when compared as a standardized incidence ratio with the rate in the general population. Those with left-sided disease have a 2- to 4-fold risk.[25] Colorectal cancer associated with ulcerative colitis has several unique features, including an increased likelihood of multiple lesions, the tendency for a malignancy to arise from flat mucosa rather than an adenoma, and the occurrence in a relatively younger population (mean age in the thirties).[26]

The risk of colorectal cancer in patients with Crohn disease also is increased and is related to the duration and extent of colonic disease.[27] The relative risk of developing small bowel cancer in Crohn disease patients is greatly increased over that of the normal population; however, given the small incidence of small intestinal adenocarcinoma, the absolute risk remains very low.[28] In ulcerative colitis, virtually all intestinal cancers occur at sites of active or past colonic inflammation, whereas in Crohn disease, one-third of digestive tract cancers occur in grossly normal segments of intestine. Additionally, strictures in ulcerative colitis have a high likelihood of being malignant, whereas they are largely due to fibrostenosis in Crohn disease. Many cancers in Crohn disease arise in bypassed segments of bowel. Therefore, in the setting of Crohn disease, the ability to predict the site of cancer may be diminished.

TABLE 45.2-1 CONDITIONS ASSOCIATED WITH COLON CARCINOMA

ENVIRONMENTAL FACTORS
Diet high in calories, fat, and cholesterol and low in fiber
Exposure to radiation or chemicals
DISEASES WITH HERITABLE COMPONENTS
Inflammatory bowel disease (ulcerative colitis, Crohn disease)
Polyposis syndromes: (familial polyposis coli, Gardner syndrome, juvenile polyposis, Peutz-Jeghers syndrome)
Hereditary syndromes without polyposis (Lynch I, Lynch II, familial predisposition to adenoma formation)
ACQUIRED DISEASES
Association with previous ureterosigmoidostomy
Bacteremia or endocarditis secondary to *Streptococcus bovis* infection

Some of the hereditary polyposis syndromes carry a high risk for the development of colon carcinoma. The most notable among these are familial adenomatous polyposis (FAP) and Gardner syndrome. Both of these autosomal dominant disorders are characterized by the presence of adenomas in the colon and, to a lesser extent, the small intestine and stomach. The lifetime risk of colon cancer approaches 100%.[29]

The latency period between the appearance of the adenomas and the development of colon cancer averages 10 years,[30] although carcinoma of the colon in association with this syndrome has occurred as early as in the first decade of life.[31] As might be expected, the frequency of multiple lesions is greater in those with FAP than in the general population with colon cancer.[32]

Hamartomatous polyp syndromes also are associated with an increased risk for the development of colon cancer.[33] Peutz-Jeghers syndrome is associated with both an increased risk of gut luminal cancers (small and large intestine) and extraintestinal cancers (ovarian sex cord tumors, Sertoli testicular cancer, breast cancer); the relative risks of dying from these cancers are about 13 and 9 times that of the normal population, respectively. Peutz-Jeghers syndrome arises owing to mutations in the serine/threonine kinase (*STK11*) gene, also known as *LKB1*, and diagnostic and genetic testing is now possible.[34]

The risk of colon cancer is increased in juvenile polyposis coli, with the carcinoma arising in coexisting adenomas or mixed juvenile adenomatous polyps.[35,36] In one series, 18 of 80 patients with juvenile polyposis developed colorectal carcinoma at a mean age of 34 years. However, insufficient information exists to clearly define the magnitude of the risk.[37] Close endoscopic surveillance is advised, and if numerous polyps exist, subtotal colectomy could be considered. These polyposis syndromes are discussed in more detail in Chapter 45.1.

Autosomal dominant inheritance of the tendency to develop colon cancer can also occur in the absence of hereditary polyposis syndromes. Indeed, hereditary nonpolyposis colorectal cancer (HNPCC; Lynch syndrome) is approximately five times more common than FAP-associated colon cancer.[38] Kindreds with HNPCC can be divided into two syndromes: (1) Lynch syndrome I, or hereditary site-specific colon cancer, which is characterized by an increased incidence of cancer in the colon only, and (2) Lynch syndrome II, or the cancer family syndrome,

which is associated with an increased risk of extracolonic and colonic adenocarcinomas, including endometrial, ovarian, gastric, small bowel, pancreatic, bile duct, and urinary tract cancers. The unique features of HNPCC include a propensity for proximal lesions, an increased incidence of simultaneous and subsequent colon cancers, and a relatively early age at onset (mean age of 45 years). The lifetime colorectal cancer risk approaches 85%; thus, many carriers of the disease opt for prophylactic colectomy in lieu of intensive endoscopic surveillance regimens.[39] Colon cancer may develop even in childhood; one member of a family with HNPCC was diagnosed with adenocarcinoma of the colon at 14 years of age.[40]

An unusual, intriguing predisposing condition for the development of adenocarcinoma of the colon is antecedent ureterosigmoidostomy performed for exstrophy of the bladder (Figure 45.2-1). In a series of 93 patients with ureterosigmoidostomies, 5% developed carcinoma of the colon at the level of the ureteral implants after a mean follow-up period of 18 years. The neoplastic pathogenesis is thought to be due to nitrosamine production from the interaction of colonic bacteria on urine, as well as the production of reactive oxygen radicals by neutrophils at the healing anastomotic site, with subsequent deoxyribonucleic acid (DNA) damage. Careful surveillance of these patients with annual colonoscopy beginning 5 to 6 years after surgery and consideration of colon resection have been suggested.[41]

Although not yet reported in the pediatric age group, an association of *Streptococcus bovis* bacteremia or bacterial endocarditis and carcinoma of the colon has been observed in adults.[42] Subsequent reports have confirmed the need to consider the possibility of a coexisting malignancy in individuals with these infections. A study investigating the fecal carrier rate of *S. bovis* found a similar rate in patients with benign polyps (n = 63) and controls (n = 62) but a higher carrier rate in patients with tubulovillous and villous adenomas (n = 18).[43] However, a smaller prospective study of 19 patients with colorectal cancer compared with 23 age- and sex-matched control patients did not find any difference in fecal carrier rates.[44] More research is required, therefore, before the reasons for the association between *S. bovis* and colon carcinoma are understood.

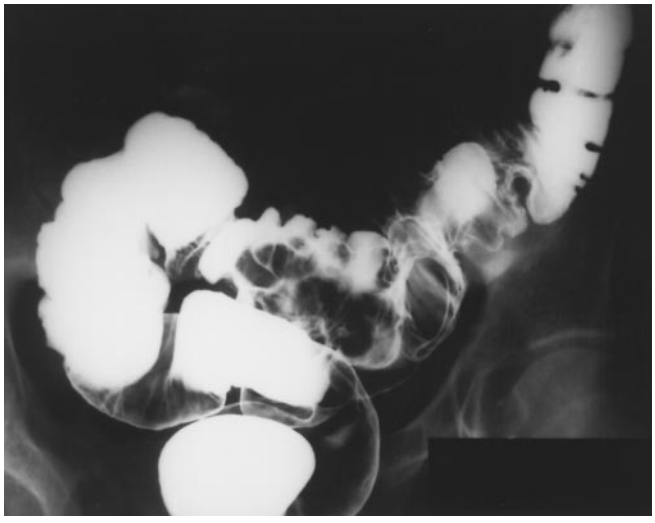

FIGURE 45.2-1 View of the rectosigmoid colon during a barium enema in a 33-year-old patient who had undergone ureterosigmoidostomy in infancy for repair of bladder exstrophy. A polypoid tumor is seen in the sigmoid colon. Reproduced with permission from the Teaching Collection, Department of Radiology, The Children's Hospital, Boston.

Molecular Biology. Recognition of the genetic component of colorectal cancer is growing rapidly.[45] Colorectal carcinoma is thought to be the consequence of several sequential genetic alterations, known as the adenoma to carcinoma sequence. These may be congenital germline mutations, as in adenomatous polyposis coli, or they may be acquired from the mutating actions of carcinogens. The effects of such genetic mutations can be categorized as activation of proto-oncogenes (as is the case for the *Ras* point mutation found in about half of larger adenomas), inactivation of tumor suppressor genes (adenomatous polyposis coli [*APC*] gene, or deleted in colon cancer [*DCC*] gene), or abnormalities in DNA mismatch repair.

Whereas no specific pattern of mutation explains all colorectal tumors, three chromosomal regions are important in colorectal carcinogenesis. First, the mutated in colorectal cancer (*MCC*) and *APC* genes are located on chromosome 5q. The *MCC* gene is mutated or deleted in some sporadic colorectal cancers but not in hereditary cases, whereas the *APC* gene has undergone point mutations in FAP and Gardner syndrome patients and may be somatically altered in tumors from sporadic colorectal cancer patients. These mutations seem to occur early in the adenoma to carcinoma sequence. Second, the *DCC* gene on chromosome 18q is involved in intermediate stages of carcinogenesis, such as the progression from dysplasia to adenomas.[46] K-*ras* inactivation is also thought to play a role in the intermediate stages of carcinogenesis. Third, the *P53* tumor suppressor gene on chromosome 17p tends to be mutated late in colorectal carcinogenesis. The current hypothesis suggests that these genetic alterations accumulate to transform normal epithelium to adenoma and then to carcinoma.

Another molecular pathway involved in colorectal cancer development involves genes that control the repair of mismatched DNA. Mutations in these genes lead to a phenotypic condition called microsatellite instability. This pathway is found in approximately 15% of sporadic cancers and 85% of HNPCC patients, suggesting that defects in mismatch repair may be one of the most common causes of inherited disease.[47] Six genes involved in the repair of mismatched DNA have been identified to date: *hMLH1*, *hMSH2*, *hPMS1*, *hPMS2*, *hMSH6*, and *hMLH3*. The mutated genes cause cancer by allowing other mutations to occur thousands of times more frequently than those in normal cells. In the great majority of tumors with microsatellite instability, a secondary mutation results in the inactivation of the transforming growth factor-β receptor II, a tumor suppressor gene in colonic epithelium.[29]

Several studies indicate that germline defects in mismatch repair genes occur in up to 60% of young patients with

sporadic colorectal cancer. Almost half of young patients with microsatellite instability have detectable germline mutations in a mismatch repair gene.[48,49] Such heritable defects provide a likely explanation for the varying clinical patterns of disease among younger patients with colorectal cancer.[50]

Pathology. Unlike carcinomas in adults, in whom lesions are more commonly in the left colon and rectum, cancers in children and adolescents tend to be more evenly distributed throughout the large intestine. Up to half of young patients have a primary lesion in the ascending or transverse colon compared with a 40% rate of right-sided lesions in older subjects.[12,51]

Carcinomas of the large bowel in adults are usually moderately to well-differentiated adenocarcinomas. Several subtypes of adenocarcinoma are recognized, however, and include a mucinous or colloid variety characterized by large collections of extracellular mucin (Figure 45.2-2). In adults, this tumor represents up to 15% of colorectal carcinomas.[52] Mucinous and signet ring histology are found in a higher proportion of pediatric cases, representing up to 50% and 10%, respectively, of childhood colorectal carcinoma.[53]

Cancers of the colon and rectum grow locally by transmural invasion, eventually penetrating through the bowel wall. Regional lymphatics and, subsequently, distant lymph nodes then become seeded by tumor. The most common sites of hematogenous spread are to the liver, lungs, and bone. The most commonly employed scheme for classifying the progression of local and regional invasion of colonic cancers is a modification of the Dukes' system by Astler and Coller.[54] Tumors in class A are limited to the mucosa; class B1 tumors have extended into, but not through, the muscularis propria; B2 tumors have penetrated the bowel wall through serosa but do not involve lymph nodes; C1 tumors are limited to the bowel wall and involve lymph nodes; C2 tumors have penetrated the bowel wall through serosa and involve lymph nodes; and D tumors have distant metastases.

Younger patients tend to have more advanced carcinomas at initial diagnosis. Enker and colleagues compiled data from a number of reports of colon cancer in younger patients (Table 45.2-2): in this scheme, 2% of patients had tumors classified as Dukes' class A, 16% as class B, 47% as class C, and 34% as class D.[55] This may be due to vague symptoms at presentation, a delay in diagnosis, and a higher incidence of aggressive tumor pathology.

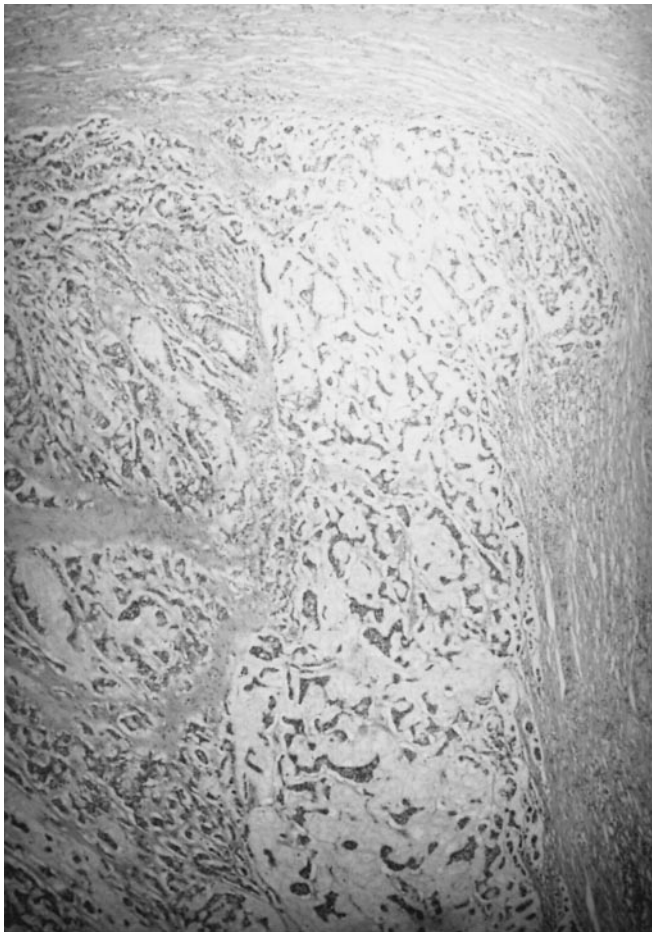

FIGURE 45.2-2 Mucinous carcinoma of the colon. The tumor cells seen infiltrating the wall of the colon are surrounded by abundant amounts of extracellular mucin. Courtesy of Dr. Donald Antonioli.

Clinical Features. The most common presenting symptom in children with carcinoma of the colon is abdominal pain, reported by approximately 95% of patients. Vomiting, weight loss, rectal bleeding, and changes in bowel habits also are frequent symptoms.[12,13] Lesions of the right colon typically become large before causing symptoms of obstruction or bleeding. In contrast, more distal tumors frequently produce concentric narrowing of the lumen, with resultant constipation and blood loss. As noted in Table 45.2-3, lesions of the left colon in childhood result in presenting symptoms of constipation and rectal bleeding more frequently than do right-sided lesions.[13] Overall, the presenting symptoms are somewhat different from those of adults, in whom abdominal pain is less common and in whom rectal bleeding, weight loss, and changes in bowel habits are more frequent.[13] This may relate to the predominance of left-sided lesions in adults compared with the more uniform location of cancers in children. The most common physical findings in children with colon cancer are abdominal masses and distention, which are apparent at presentation in over 50% of patients.[13] Other physical findings are summarized in Table 45.2-4.

Diagnosis. Because carcinomas are distributed throughout the colon in children, many lesions are too proximal to

TABLE 45.2-2 STAGING OF ADULT VERSUS YOUNG PATIENTS WITH COLON CANCER

STAGE	YOUNG PATIENTS (%)	ADULT PATIENTS (%)
A (limited to the mucosa)	2.2	
B (through mucosa but no nodes involved)	16.2	60
C (nodes involved)	47.6	
D (distant metastases)	34.0	40

TABLE 45.2-3 PRESENTING SYMPTOMS OF CHILDREN WITH COLON CANCER BY SITE OF TUMOR

	SITE OF TUMOR	
SYMPTOM	RIGHT (%)	LEFT (%)
Pain	93	87
Vomiting	52	29
Constipation	17	32
Weight loss	21	19
Blood in stool	14	23
Mass	10	0

be seen during flexible sigmoidoscopy. Colonoscopy is more sensitive than barium contrast studies and provides the advantage of allowing biopsy specimens to be obtained.[56] Ultrasonography, nuclear medicine, computed tomography, magnetic resonance imaging, and positron emission tomography studies each may be used in demonstrating metastatic disease.[57]

The use of carcinoembryonic antigen (CEA) levels for the management of adult patients with colon cancer is well established.[58] However, little information is available about the merits of using this serologic marker in children. One study noted that 4 of 20 children with advanced colon cancer had normal CEA levels.[12] Another study calculated that CEA testing (using adult normal ranges) has a specificity of 77% and a sensitivity of 64% for recurrent colorectal cancer in patients under 18 years of age. Carbohydrate 19-9 antigen is not sufficiently accurate to prove clinically useful.[59] The use of molecular markers in fecal DNA for the detection of colon cancer in children and in adults warrants further study.[60]

Differential Diagnosis. The differential diagnosis of childhood carcinoma in the colon includes intestinal carcinoids, tuberculosis of the bowel, inflammatory bowel disease, appendicitis, and enteric duplication cysts or other benign causes of intestinal obstruction. Other malignant tumors can be considered, but they are extremely rare.

Treatment. The primary treatment for children with carcinoma of the colon is surgery. A wide resection of the involved segment of bowel with removal of the lymphatic drainage should be performed. In one series, however, complete resection was possible in only 40% of children because of the advanced tumor stage.[12] Resection of the omentum and ovaries should be considered owing to the high rate of recurrence from lymphatic spread.[12,61]

TABLE 45.2-4 PHYSICAL FINDINGS OF CHILDREN WITH COLON CANCER

SYMPTOM	PATIENTS (%)
Mass	59
Distention	48
Emaciation	23
Tenderness	14
Anemia	14

Adjuvant chemotherapy or primary chemotherapy for patients with metastatic disease has provided little benefit in young patients with colon carcinoma. Most often, 5-fluorouracil, either alone or in combination with agents such as nitrosurea, levamisole, and interferon-α, is employed.[62] Most responses to chemotherapy are modest (ie, less than 50% reduction in tumor size) and transient.[12] Early studies of camptothecan derivatives (topotecan and irinotecan) have shown activity in pediatric xenographs, including colon cancer,[63] and clinical pediatric trials are ongoing.[64] Other chemotherapeutic agents, such as oxaliplatin and capecitabine, have not been examined in children. In the near future, molecular markers may well provide predictors of response to therapies[65,66] and thereby provide the opportunity for more individualized treatment regimens.

Radiation therapy improves symptoms, resectability, and survival in adult patients with advanced rectal cancer.[67] Few studies are available in children, but in one series, two patients were treated preoperatively with success in conversion to a resectable cancer.[12] The precise roles of chemotherapy, radiation therapy, combination therapy, and newer therapeutic modalities in the treatment of colon cancer in the pediatric age group await further study.

Unfortunately, the prognosis for children with colonic carcinoma remains dismal. Five-year overall survival rates of less than 3% are reported.[12] Long-term survivors are those patients presenting with less advanced disease. Contributing to the poor prognosis is the pattern of delayed recognition of colon cancer in children and young adults and the predominance of aggressive pathologic types.[51]

Conclusions. Although colonic adenocarcinoma is the most frequent primary intestinal malignant tumor in childhood, it is still uncommon. As a result, diagnosis is often delayed. Future efforts should focus on developing prevention strategies[68] and enhancing early detection. A search for an underlying hereditable risk factor also should be undertaken.[69] Evaluation of the role of nonsurgical therapies in the management of children with established colon cancer must be considered in the context of clinical research trials.

Adenocarcinoma of the Small Intestine

Even in adults, adenocarcinoma of the small intestine is rare, although it does account for approximately half of malignant neoplasms in the small intestine.[70] Such tumors are most commonly found in the duodenum. Adenocarcinomas of the small intestine in childhood should prompt a search for an underlying predisposition, including, for example, Peutz-Jeghers syndrome, Gardner syndrome, and small bowel Crohn disease. Adenocarcinoma of the small intestine complicating Crohn disease was reported in a 21 year old whose inflammatory bowel disease–related symptoms began at 10 years of age.[71] Of 36 cases of adenocarcinoma of the small bowel arising in patients with regional enteritis described by Nesbit and colleagues, 28 occurred in the ileum and 11 developed in previously bypassed bowel.[72] The location of small intestinal adenocarcinomas makes the surveillance of subjects at high risk particularly

difficult. Unexplained bleeding or symptoms of obstruction call for consideration of this diagnosis. Contrast studies remain the primary investigative tool.

INTESTINAL LYMPHOMA

Primary small intestinal lymphomas account for up to 20% of all small bowel malignancies in Western Europe and North America.[73] The incidence of non-Hodgkin lymphoma (NHL) increases with chronologic age. Striking differences exist between these tumors in adults and children with regard to histology, site of occurrence, clinical behavior, and recommendations for treatment. Important differences also are noted between intestinal lymphomas reported in Western populations and those in other populations.[74]

Epidemiology. Lymphoma is the third most common malignant neoplasm of children in the United States, accounting for 10% of all cancers diagnosed in children under 19 years of age.[6] In the developed world, 40% of children with NHL present with abdominal tumors.[75] Primary involvement of the gastrointestinal tract probably occurs in less than half of all patients with NHL. Unlike adults, in whom the stomach is most often involved,[76] the most common sites in affected children are the distal ileum, cecum, and appendix. The ratio of affected males to females in children is approximately 2:1. This gender difference diminishes with increasing age,[77] although male predominance for primary gastrointestinal lymphoma persists through all age cohorts.[78]

The spectrum of gastrointestinal lymphoma varies geographically, likely owing to altered frequency of risks factors. An extraordinarily high rate of Burkitt lymphoma exists in equatorial Africa. Immunoproliferative small intestinal disease (IPSID), also known as Mediterranean lymphoma, is clustered in the Middle East and North Africa.[79] A case series of 37 Iraqi children with primary intestinal lymphoma included 11 children with Mediterranean lymphoma. The remaining 26 had lymphomas localized to the ileum or ileocecal region; half of these tumors were characterized as Burkitt lymphoma.[80]

Pathology. Multiple typing systems currently are employed for the classification of lymphomas. The need for international standards has driven the development of a consensus classification scheme by the International Lymphoma Study Group. This scheme is reproducible and clinically relevant.[81] Nevertheless, most childhood lymphomas still are classified by more traditional histologic typing methods, such as the Luke-Collins or Rappaport systems. About 60% of childhood lymphomas are of the non-Hodgkin type,[82] which comprise the bulk of lymphomas affecting the intestine. The childhood lymphomas are subclassified into three main categories: small noncleaved cell, large cell, and lymphoblastic (Table 45.2-5). In the Rappaport classification, the small noncleaved cell category is known as "undifferentiated." Abdominal lymphomas in children are usually of the small noncleaved cell or undifferentiated histologic type, whereas a smaller proportion are of the large cell type. Small noncleaved cell lymphomas can be subdivided into Burkitt and non-Burkitt types.[83] Burkitt lymphoma may be the endemic form seen in equatorial Africa, which accounts for 50% of all childhood cancers in the region,[84] or the sporadic form seen in Europe and the United States. These histologic types of gastrointestinal NHL have similar staging and treatment approaches. IPSID is an exception and is discussed separately.

Risk Factors. Predisposing conditions include both inherited and acquired immunodeficiency states. The former include ataxia-telangiectasia, Wiscott-Aldrich syndrome, common variable immunodeficiency, X-linked lymphoproliferative syndrome (Duncan syndrome), and severe combined immunodeficiency syndrome. Post-transplant lymphoproliferative disease also results in intestinal lymphoma. Currently, these predisposing conditions are recognized only in a minority of children with NHL. Nevertheless, the presence of an intestinal lymphoma should prompt the careful assessment of immune function in all affected children.

The association between immunodeficiency and intestinal lymphomas likely relates to specific infectious triggers. Indeed, in the X-linked lymphoproliferative syndrome, Epstein-Barr virus infection triggers the development of B-cell lymphoma.[85] Epstein-Barr virus infection also is closely associated with the development of lymphomas in immunosuppressed transplant patients.[86] Epstein-Barr viral DNA is present in 95% of endemic Burkitt lymphomas compared with 15% of sporadic Burkitt lymphomas.[82] Recent evidence indicates that Epstein-Barr virus infection is also related to Hodgkin lymphoma in young persons.[87]

Intestinal lymphomas occur with increased frequency in adults with unrecognized and untreated celiac disease.[88] Most of these tumors are aggressive T cell–derived

TABLE 45.2-5 CLASSIFICATION OF CHILDHOOD NON-HODGKIN LYMPHOMAS

LUKES-COLLINS CLASSIFICATION	RAPPAPORT CLASSIFICATION	IMMUNOPHENOTYPE
DIFFUSE		
Small noncleaved cell	Undifferentiated	Mostly B cell
Burkitt		
Non-Burkitt		
Lymphoblastic	Lymphoblastic	Almost all T cell
Large cell	Histiocytic	B, T, or null cell
NODULAR (HODGKIN DISEASE)	—	Reed-Sternberg cell (B-cell origin)

NHLs. They usually involve the small intestine but may occur in other extranodal tissues. One study suggested that a strict gluten-free diet can protect against the development of lymphoma in patients with celiac disease.[89] Such an association also has been reported in children with celiac disease,[90] but these findings require confirmation from other centers.

Crohn disease in adults also is complicated by an increased incidence of small bowel lymphoma.[91] Although lymphoma of the colon is uncommon, 24 cases of primary colonic lymphoma associated with ulcerative colitis have been reported, suggesting an association between the two disorders.[92]

Molecular Biology. Karyotype translocations are frequently observed in NHLs. For instance, there is a consistent alteration in the transcription of the c-*myc* oncogene in Burkitt lymphomas. In these lymphomas, genetic material on chromosome 8, including c-*myc*, is translocated in proximity with the genes encoding immunoglobulins (Igs) on chromosomes 14, 2, or 22.[82] The c-*myc* gene is thereby deregulated, probably by virtue of its proximity to elements that normally regulate Ig transcription. Overexpression of c-myc, in turn, represses the transcription of tumor suppressor genes. In sporadic Burkitt lymphoma, the translocation itself results in the deregulation of c-*myc*, whereas in endemic Burkitt lymphoma, c-*myc* is deregulated only by the combination of Epstein-Barr virus DNA and an otherwise silent translocation.[84]

Clinical Features. Children with abdominal lymphoma frequently have presenting symptoms of abdominal pain, distention, a change of bowel habits, and nausea or vomiting. A right lower-quadrant mass occasionally may be palpable. Because primary intestinal lymphomas often involve the terminal ileum, cecum, and appendix, they occasionally result in ileocecal intussusception (Figure 45.2-3). With extensive involvement, ascites may be present. Bone marrow involvement occurs in roughly one-third of patients with undifferentiated lymphomas but is rare in endemic Burkitt lymphoma. The endemic form is also characterized by a high frequency of involvement of the mandible (70% in children younger than 5 years), as well as spinal or central nervous system involvement (30%, respectively).[93] In contrast, involvement of the bone marrow is less common than in the sporadic form of Burkitt lymphoma. In both populations, relapse in the meninges remains a serious problem for children with advanced-stage abdominal undifferentiated lymphomas.[77]

Diagnosis. Diagnosis of NHL requires biopsy for histopathologic examination, immunophenotyping, and cytogenetics. With extensive disease, a bone marrow aspirate and biopsy or cytocentrifugation and examination of ascitic fluid may prevent the need for laparotomy. Findings on barium follow-through may be confused with Crohn disease, certain cases of appendicitis, and various benign lesions, including leiomyomas and enteric duplications (Figure 45.2-4).

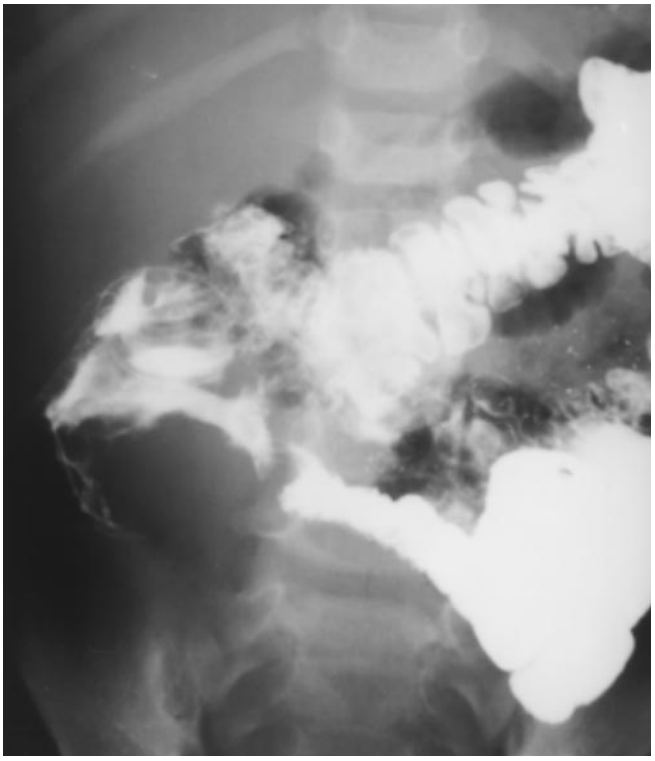

FIGURE 45.2-3 A barium enema study in a 4-year-old boy with a 2-month history of abdominal pain. The barium column stops abruptly in the ascending colon at the site of an ileocecal intussusception. Laparotomy revealed a Burkitt lymphoma of the ileocecal valve. Reproduced with permission from the Teaching Collection, Department of Radiology, The Children's Hospital, Boston.

Formal staging studies should be performed expeditiously because the progression of disease can be rapid, and therapy should be initiated without undue delay. Investigations should include chest radiography, a nuclear medicine gallium scan, computed tomography or magnetic resonance imaging of the abdomen, a spinal tap, and a bone marrow aspirate and biopsy. Leukoerythroblastosis on peripheral blood smear suggests bone marrow involvement. Serum lactate dehydrogenase levels are nonspecific but may correlate with tumor burden. Lymphocyte-derived factors are under investigation as possible prognostic markers.[93]

Treatment. If an evaluation does not reveal extensive abdominal involvement or ascites and if the bone marrow studies yield normal results, the primary bowel lesion and associated mesentery should be resected and a primary anastomosis performed. Mesenteric lymph nodes should be removed for histopathology. Surgical resection likely is of benefit only if most of the tumor burden can be removed and if it does not delay initiation of chemotherapy.[94]

Most chemotherapy protocols include cyclophosphamide, methotrexate, vincristine, prednisone, and an anthracycline. Patients with lymphoblastic lymphomas do better with the inclusion of a consolidation phase of cytarabine, L-asparaginase, 6-thioguanine, and carmustine. Induction therapy is followed by maintenance treatment in all affected children; although those with localized or completely resected intra-abdominal tumor require less intensive

FIGURE 45.2-4 Contrast study of the small bowel in a 3-year-old boy with a 6-week history of abdominal discomfort and malaise. His mother had noticed an abdominal mass when bathing him. The ileum is noted to be nodular and narrowed. At surgery, an ileal Burkitt lymphoma was seen. Reproduced from the Teaching Collection, Department of Radiology, The Children's Hospital, Boston.

treatment. Central nervous system prophylaxis is indicated in all patients with advanced disease but can be omitted in those with completely resected ileocecal primary tumors.[94] All patients with a large tumor burden are at risk for rapid tumor lysis during initial chemotherapy, with resulting metabolic derangements. Therefore, these children should receive allopurinol, vigorous hydration, and urinary alkalinization. Although there is no role for radiation therapy, even in localized intestinal disease, most patients with involvement of the central nervous sytem are given cranial irradiation.

Prognosis. In patients with abdominal disease without bone marrow or central nervous system involvement, the expectancy of cure with combined surgical resection and combination chemotherapy is up to 95%.[95] Chronologic age and gender do not have prognostic significance, except in Burkitt lymphoma, for which younger children tend to have a more favorable prognosis. The presence of bone marrow involvement or cranial disease at the time of initial diagnosis confers a poorer prognosis. Intestinal perforation complicating surgical exploration or chemotherapy with tumor necrosis also seems to be associated with a higher mortality.[88]

Immunoproliferative Small Intestinal Disease

Also known as Mediterranean lymphoma and α-chain disease, IPSID occurs in the Mediterranean basin, Iran, Pakistan, Taiwan, and South Africa and is reported only very rarely outside these areas. It is characterized by the proliferation of IgA-secreting B lymphocytes throughout the small intestine. High levels of IgA heavy chains can be detected in serum.[80,81] The pathogenesis of IPSID has not been entirely delineated, but one current hypothesis suggests that repeated enteric infections result in antigenic stimulation and immune cell proliferation. A recent study indicates that *Campylobacter jejuni* infection could well be involved in the etiology of α-chain disease.[96] Most cases of IPSID occur in patients between 10 and 40 years of age who live in conditions of poor sanitation; younger children occasionally are affected.[80] Patients with IPSID present with symptoms of malabsorption and a protein-losing enteropathy. Most have chronic diarrhea, abdominal pain, and weight loss.

A diffuse infiltrate of plasma cells is present throughout the lamina propria of the small intestine. The villus-crypt structure can be distorted to such a degree as to mimic gluten-sensitive enteropathy. In advanced IPSID, a large cell immunoblastic lymphoma develops that is frequently multifocal.

Early in the course of this disease, antibiotics may induce remission,[97] but molecular analyses suggest that even benign-appearing plasma cells represent a neoplastic process.[79] The combination of surgery, radiation therapy, and chemotherapy is used to treat advanced stages. No large clinical trials have been conducted owing to the rarity of the condition. Although long-term remissions have been recorded, long-term survival rates generally vary between 23 and 67%.[79]

LEIOMYOSARCOMA

Intestinal leiomyosarcoma is extremely rare in childhood, accounting for 0.3% of all neoplasms in children less than 15 years of age.[98] About half of childhood cases occur during infancy.[99] Less than half of reported childhood leiomyosarcomas occur in the digestive tract. A review of 47 cases in the literature suggested a slight female predominance.[100] Leiomyosarcomas present with intestinal bleeding, bowel obstruction, or intussusception. Only 4 of 47 cases of pediatric gastrointestinal leiomyosarcoma presented with an intussusception, whereas almost 30% of adults with an intussusception have a leiomyosarcoma as the lead point.[100]

Pathologic features and the treatment of leiomyosarcomas are discussed in more detail in Chapter 28 with tumors of the stomach (where they arise most commonly).

SUMMARY

Knowledge of those disorders predisposing the patient to the development of intestinal malignancies, such as inflammatory bowel disease, hereditary polyposis syndromes, and immune deficiencies, is essential for the timely diagnosis of intestinal tumors in children. In addition, it provides for the institution of a surveillance program for high-risk family members entering adulthood.[101,102] For both diagnosis and

management strategies, a multidisciplinary approach involving experts from radiology, pathology, surgery, and oncology is critical. Major advances in the care of these patients await further progress in the basic science laboratory and the translation of such findings to the bedside.

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CHAPTER 46

HYPOMOTILITY DISORDERS

1. *Idiopathic Constipation*

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A regular pattern of defecation is considered by many to be a sign of good health. In fact, many parents of children with constipation believe that a backup of stools can lead to accumulation of toxins in the blood. It is not surprising, therefore, that constipation has been found to be the most common digestive complaint in the United States. Surveys reveal that cathartics and laxatives are prescribed to about 3 million Americans yearly by family physicians and internists.[1] The exact prevalence of constipation in children is not known, but the problem leads to approximately 3% of visits to the general pediatric outpatient clinic[2] and 10 to 25% of visits to the pediatric gastroenterologist.[3,4]

DEFINITION

The definition of constipation varies among individuals. To some, it is hard stools; to others, it is large stools; and to many more, it is infrequent stools. Of 100 mothers in a rural Michigan maternity ward surveyed by Potts and Sesney, 45% defined constipation as "not being able to have a bowel movement," 23% defined it as "hard stools," 10% defined it as "no stool in several days," and 6% defined it as "pain or pressure while stooling."[5] These mothers erroneously estimated the frequency of stools in the first week through 1 month of age, when their expectations were compared with published data. Because the word "constipation" has different meanings for different people, it has been difficult to compile data on normal and abnormal patterns in children.[6] A good working definition includes all of the definitions given by the mothers in the above survey: it would slightly modify the definition in Webster's English dictionary to read "a term used to describe the subjective complaint of passage of abnormally delayed or dry, hardened feces, often accompanied by straining and/or pain."[7] The guidelines of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition similarly define constipation as "a delay or difficulty in defecation, present for two or more weeks and sufficient to cause significant distress to the patient."[8] It is easier to set standards in terms of stool frequency than in terms of size, consistency, degree of straining, or pain. In adults, the normal frequency of defecation ranges from three per day to three per week.[9,10] Lemoh and Brooke studied 55 children, aged 3 days to 2 years, and showed that stool frequency gradually decreases from a mean of four per day during the first week of life to about two per day at 2 years of age.[11] Weaver and Steiner reported that stool frequency in 350 English preschool children aged 1 to 4 years declined from an average of 1.6 per day at 1 year of age to 1.2 per day at 4 years of age.[12] In this latter group, 96% had stool frequencies ranging from three per day to three per week. Weaver's group also studied 240 infants aged 2 weeks to 20 weeks, half of whom were breastfed and the other half formula-fed, and found that 93% of these infants passed one to seven bowel movements per day.[13] Breastfed infants had more frequent stools initially, but by 16 weeks of age, both groups were passing an average of two stools per day.

One can surmise then that normal stool frequency ranges from an average of four per day during the first week of life to two per day at 1 year of age. The normal adult range of three per day to three per week is attained by 4 years of age. These data reflect the average stool frequency in normal infants and children in industrialized countries, not in developing countries, where the normal diet is rich in fiber and normal stool frequency may be different.

NORMAL DEFECATION

Stool is normally propelled down the colon to the anorectum, where it is stored until it can be eliminated in a socially acceptable manner. The anorectum stores and eliminates stool through a complex mechanism involving muscles of the pelvic floor, the autonomic and somatic nervous systems, and the group of muscles controlling the anal sphincters. These interactions have become understood as techniques to study anorectal physiology, such as anorectal manometry, electromyography (EMG), and defecography, have become available. The internal and external anal sphincters surrounding the anal canal form an angle (the anorectal angle) with the puborectalis mus-

cle (Figure 46.1-1). This angle is approximately 85 to 105° at rest.[14] The bolus of stool is propelled into the anorectum during defecation, where distention of the wall results in a temporary reflex relaxation of the internal anal sphincter, allowing stool to come in contact with sensitive receptors in the anal canal. The external sphincter simultaneously contracts, giving the individual time to decide if circumstances are appropriate to allow stool to escape. If the individual decides to allow stool to escape, increased intrarectal pressure from straining moves the fecal material toward the anal canal and the puborectalis muscle relaxes, allowing the pelvic floor to descend. Descent of the pelvic floor straightens the anorectal angle, the external anal sphincter is inhibited, and the fecal material is evacuated. If defecation is to be deferred, voluntary contraction of the puborectalis muscle and the external anal sphincter muscle decreases the anorectal angle to less than the usual 85 to 105°; defecation is prevented, and the rectum accommodates its contents.[15] In newborn babies and very young infants, the role played by the cerebral cortex in these normal events is not yet developed; therefore, defecation occurs when the internal sphincter relaxes.

PATHOGENESIS AND MECHANISMS OF CONSTIPATION

Difficulties with defecation may result from dysfunction in any portion of the normal mechanism of defecation. Such dysfunction may result from aberrations in anatomy or physiology. Constipation is termed idiopathic when it cannot be explained by any anatomic, physiologic, radiologic, or histopathologic abnormalities. Although the exact mechanism of idiopathic constipation is not known, it is generally believed that a multiplicity of factors may be involved. The final common pathway is likely to be a decrease in propulsive forces, impaired rectal sensation, or a functional outlet obstruction. A decrease in propulsive forces may result from a genetic predisposition or a structural abnormality of the colon. Abrahamian and Lloyd-Still obtained a family history of constipation in greater than 40% of 186 constipated children.[16] Concordance for constipation is reported to be six times greater among identical twins than among fraternal twins,[17] and evidence for a congenital syndrome of early-onset constipation has been identified in adults using dermatoglyphics.[18] Recently, Hubner and colleagues described four patients with severe constipation and abnormal gastrointestinal motility who lacked the connective tissue layer between the circular and longitudinal muscles of the bowel on resected specimens in addition to having abnormal submucosal ganglia.[19] They referred to these findings as "desmosis of the colon."[19] Hutson and colleagues found reduced numbers of excitatory substance P–immunoreactive nerve fibers in circular muscles of laparoscopic colon muscle biopsies obtained from a number of children with slow-transit constipation.[20]

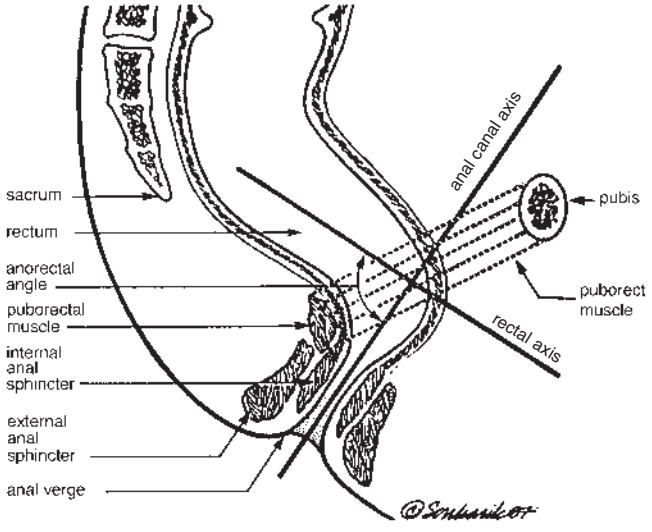

FIGURE 46.1-1 Anatomy of the anorectal region.

Impaired rectal sensation, whether primary or secondary, may lead to chronic constipation. It is known, for example, that children with spinal cord lesions may develop constipation because of impaired rectal sensation. Meunier and colleagues found that 65% of 144 children with severe constipation had significant impairment of rectal sensation, which may have been secondary to the megarectum resulting from chronic fecal retention.[21] It could be argued, however, that impaired rectal sensation itself could be the primary event leading to constipation in some children. Functional outlet obstruction leading to constipation may result from spasticity of the levator ani or impaired relaxation of the puborectalis muscle.[22] It is commonly believed that dietary fiber promotes laxation.[23] Although there are no randomized controlled studies in children to support this belief, some case-control studies have shown an association between decreased fiber intake and constipation in children.[24,25]

Although all of the above mechanisms could lead to chronic constipation, it is believed that most childhood chronic constipation results from intentional or subconscious withholding of stool. Usually, an acute episode precedes the chronic course. The acute episode may occur as the diet is changed from human milk to cow's milk, either because of a higher protein-to-carbohydrate ratio in cow's milk or, possibly, because of allergy to cow's milk protein.[26] The stool, which becomes firm and smaller in quantity, is passed less frequently and with great effort. Anal irritation, and often an anal fissure, develops, and defecation becomes painful. If this acute problem is not treated promptly, a pattern of withholding develops as the passage of stool becomes associated with pain.[27] At the urge to pass stool, the infant typically screams, stiffens the body, and tightens the gluteal muscles, while making a great effort to prevent escape of stool. The face might become flushed during this process, and parents often misinterpret these signs as an extreme effort to pass stool.

In toddlers, conflict arising out of coercive or inappropriately early toilet training is an additional factor that may initiate a pattern of stool retention.[28,29] In older children, a retentive pattern may be initiated by situations that make stooling inconvenient or uncomfortable, such as a school with unpleasant toilet facilities[30] or group A β-streptococcal

anusitis.[31,32] Toddlers and older children tend to cross their legs, stand rigidly upright, squat quietly in corners, walk on tiptoes, or hold onto furniture as they wait for the call to stool to pass.[2,27] The urge to defecate passes as the rectum accommodates to its content. A vicious cycle of retention develops as increasingly larger volumes of stool, desiccated by colonic absorption of the water content, must be expelled, often with increasing difficulty and pain. Prolonged stretching of the rectal walls, associated with chronic fecal retention, leads to an atonic and desensitized rectum, which perpetuates the situation because large volumes of stool must now be present in the rectum to initiate the call to stool. Some of these patients tolerate rectal distention volumes in excess of 500 mL at anorectal manometry.[33,34] This functional megacolon, which can be demonstrated on a barium enema, may be confused with the megacolon associated with Hirschsprung disease by the untrained eye.

When large volumes of stool in the rectal vault stretch the rectum, the internal anal sphincter relaxes while the anal canal is shortened, as demonstrated by Loening-Baucke and Younoszai.[35] Eventually, the external anal sphincter is no longer able to function adequately when the fecal mass pushes against it. Unformed stool, escaping around the impaction, leaks uncontrollably into the undergarment. This condition is referred to as encopresis.[36]

ENCOPRESIS

Encopresis is incontinence of stool not resulting from organic defects or illness.[36] This fecal incontinence characteristically ceases for several days following expulsion of a megastool. The term encopresis was introduced in 1926 by Weissenberg to describe the fecal equivalent of enuresis.[37] Although encopresis is considered by some to be caused by emotional upheavals,[38] rarely is a nonconstipated child with severe psychological disturbances seen who passes stool into clothing.[27] Furthermore, psychotherapy has been unsuccessful as a sole treatment for encopresis.[35] It is now generally accepted that chronic constipation resulting from functional fecal retention, often called psychogenic constipation, is the major cause of encopresis. Fecal incontinence owing to organic disorders, including spinal cord lesions or anatomic lesions of the anorectum, is not encopresis.

Encopresis rarely occurs before 3 years of age. In 102 encopretic patients seen at Boston Children's Hospital Medical Center over an 18-month period in the early 1970s, the mean age was 7 years, 4 months.[39] At the James Whitcomb Riley Hospital for Children/Indiana University Medical Center in Indianapolis, 274 children with constipation were seen in the gastroenterology clinic in l992.[40] Seventy-five of these children had fecal soiling. The median age of these encopretic patients was 9 years (Figure 46.1-2). Boys are more likely to experience encopresis than girls. The male-to-female ratio in the Boston study was 5.8:1[39]; in the Indianapolis study, it was 2:1.[40]

Encopresis is a source of considerable embarrassment for the child, who must deal with taunting by peers. These patients may suffer significant emotional setbacks as a result of this problem,[16,41] with loss of self-esteem and confidence.[32] Encopresis also is the source of a good deal of frustration for the parents who must deal with the smelly, dirty laundry and often angry school officials. These parents are convinced that their child has an anatomic abnormality of the colon by the time they arrive at the office of the pediatric gastroenterologist. Often punishment and psychotherapy have been tried, without beneficial results. Many parents are visibly relieved when the pathophysiology of the condition is explained to them. A few demonstrate initial skepticism, finding it difficult to accept the simple explanation for a problem that they have become convinced has an organic basis.

Encopresis often resolves spontaneously before late adolescence,[42] but Rex and colleagues have reported four patients with encopresis whose ages ranged from 16 years to 20 years, showing that this condition may persist into young adulthood.[34]

CLINICAL SIGNS AND SYMPTOMS OF CHRONIC CONSTIPATION

In many children, longer intervals between bowel movements may be the only complaint. Symptoms and signs associated with chronic constipation include abdominal pain, anorexia, vomiting, abdominal distention, excessive flatulence, and blood-streaked stools. Constipation in infants and toddlers may come to parental attention because of increasing irritability, because of a poor appetite associated with the lack of bowel movements, or when they demonstrate withholding behavior. Constipation in most older children comes to attention because of fecal soiling. Initially, the parents assume that the soiling of underclothes is due to reluctance to use the toilet, and they consult a physician only after negative reinforcement has failed. Occasionally,

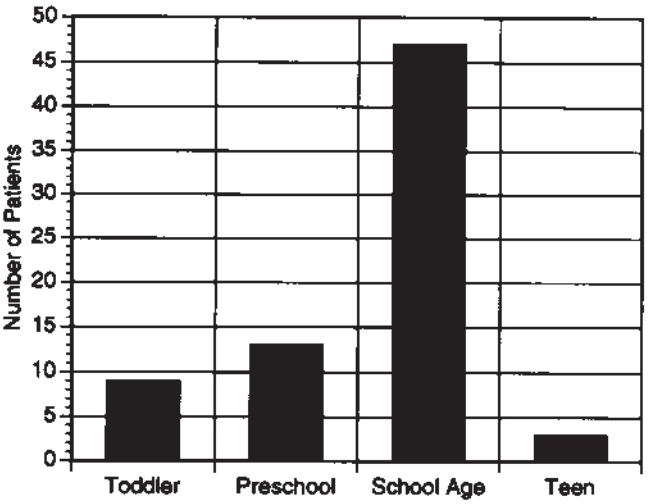

FIGURE 46.1-2 Age distribution of 72 children referred to the James Whitcomb Riley Hospital for Children for management of encopresis in 1992. Encopresis rarely occurs before 3 years of age or after 12 years of age. The median age in this group of encopretics was 9 years.

patients referred for chronic diarrhea are found to have retained stool and incontinence on rectal examination. Enuresis and recurrent urinary tract infections may be associated with encopresis, particularly in girls.[43,44]

DIFFERENTIAL DIAGNOSIS

Constipation is a symptom, not a disease. As such, it may be seen in a heterogeneous group of patients. Although no organic cause is identified in most cases, it is important to be able to identify the many conditions whose symptom complex may include constipation. Table 46.1-1 lists some of these conditions. The most common condition that must be differentiated from idiopathic constipation is Hirschsprung disease, a colonic motility disorder resulting from segmental colonic aganglionosis, with a prevalence of 1 in 5,000 live births and a male-to-female ratio of 4:1.[45] It is believed to account for 20 to 25% of all cases of neonatal intestinal obstruction[27] and 3% of constipated children referred to the gastroenterologist.[32] It can lead to severe enterocolitis with fever, diarrhea, and severe prostration, which may be fatal if the diagnosis is not recognized early.[46] Most affected infants develop difficulties with defecation during the first few weeks of life. Other signs and symptoms associated with the condition include abdominal distention, refusal to feed, and bilious vomiting. In the older infant or child in whom the diagnosis is not made earlier in life, there may be persistent abdominal distention, recurrent fecal impaction, and failure to thrive. Examination of the rectum in patients with Hirschsprung disease usually reveals an empty vault, although stool is palpable in the abdomen. A gush of air and liquid stool may follow withdrawal of the examining finger. In some patients with short-segment or ultrashort-segment Hirschsprung disease, the diagnosis may not be made until later in life.[47,48] These patients have long histories of chronic constipation and may have ganglion cells on rectal biopsy, despite anorectal manometric findings consistent with Hirschsprung disease. They are thought to have impaired innervation of some length of the sphincter mechanism.[47] Contrary to the earlier teaching that fecal soiling is not seen with Hirschsprung disease, these patients may soil, but the soiling is much less severe than one would expect from the degree of rectal impaction.[32]

TABLE 46.1-1 SOME ORGANIC CAUSES OF CONSTIPATION

ABNORMALITIES OF COLON AND RECTUM	
Chronic intestinal pseudo-obstruction	
Anal stenosis	
Anal or colonic stricture—post NEC or IBD	
Postsurgical repair of imperforate anus	
Ectopic anus	
SPINAL CORD LESIONS	
Spina bifida	
Meningomyelocele	
Sacral agenesis	
Diastematomyelia	
Spinal cord tumors (lipomas, cysts, teratomas)	
NEUROPATHIC LESIONS OF THE GASTROINTESTINAL TRACT	
Hirschsprung disease	
Intestinal neuronal dysplasia	
SYSTEMIC DISORDERS	
Diabetes mellitus	Multiple endocrine neoplasia
Diabetes insipidus	Pheochromocytoma
Hypothyroidism	Amyotonia congenita
Panhypopituitarism	Neurofibromatosis
Hypocalcemia	Infectious polyneuritis
Hypercalcemia	Prune-belly syndrome
Dermatomyositis	Scleroderma
Myotonic dystrophy	Cerebral palsy
Multiple sclerosis	
DRUGS	OTHERS
Analgesics	Celiac disease
Antacids	Cystic fibrosis
Anticholinergics	Lead toxicity
Bismuth	
Iron	
Cholestyramine	
Psychotropics	

IBD = inflammatory bowel disease; NEC = necrotizing enterocolitis.

EVALUATION

Clinical assessment is all that is needed for diagnosis and treatment of most children with chronic constipation. A thorough history and physical examination will identify most patients with an organic cause for constipation, leading to appropriate laboratory and imaging studies to make a diagnosis. Attention should be paid to age at onset of constipation and frequency of bowel movements. A dietary history may identify inadequate fluid intake, undernutrition, or a diet deficient of bulk. Often one elicits a history of constipation beginning when an infant was weaned to a cow's milk–based infant formula or was changed from infant formula to whole cow's milk. In an older child, a change in school or travel away from home may precede the onset of constipation. Inquiry into perinatal history, particularly the age at passage of meconium, and family history may be helpful in excluding Hirschsprung disease. Most patients with Hirschsprung disease fail to pass meconium within the first 48 hours of life,[49] and 20% of children with Hirschsprung disease have a family history of the condition.[45] A history of stool withholding decreases the likelihood of an organic cause for constipation. We have found it helpful to describe to parents the various postures assumed by children who withhold because many parents may not be aware of the significance of these postures and, therefore, may not volunteer this information. Inquiry into the size of bowel movements may also help differentiate between idiopathic constipation and constipation attributable to an organic lesion. The child with anal stenosis or Hirschsprung disease may pass pencil-thin stools, whereas the child with constipation secondary to voluntary withholding usually passes large stools, which may clog the commode. The presence of vomiting, abdominal distention, failure to gain weight, and weight loss suggest an organic cause for constipation and should lead to a thorough investigation.

A thorough physical examination is essential to eliminate a major illness complicated by constipation. The

abdomen is only mildly distended in functional constipation, even when large volumes of stool are retained, because gas is not retained with the stool. A fecal mass is palpable in the suprapubic region and the left lower quadrant in about 40% of patients.[50] Bowel sounds are normal. Examination of the perianal region may reveal evidence of fecal soiling. There may be stool in the underwear. Careful examination of the perianal region may identify ectopic placement of the anus, a correctable problem when recognized and operated early.[51,52] The anogenital index can be calculated when necessary. This is the distance in centimeters from the vagina or scrotum to the anus, divided by the distance from the vagina or scrotum to the coccyx. The normal anogenital index in females is 0.39 ± 0.09, whereas 0.56 ± 0.2 is normal for males.[53] An anal fissure, significant circumferential perianal erythema indicative of streptococcal anusitis, or anal trauma suggestive of sexual abuse may be identified as a reason for withholding. Scybalous stool may be palpated within the rectum or rock-hard stool may be palpated just within the internal anal sphincter. An empty rectum in the presence of a palpable fecal abdominal mass is highly suggestive of Hirschsprung disease. The lumbosacral region of the back should be examined for evidence of myelodysplasia or sacral agenesis, and a neurologic examination of the lower extremities should be performed to exclude an occult spinal cord lesion. A patulous anus, sacral dimple beneath a tuft of hair, flat buttocks, or absent reflexes in the lower extremities suggest an underlying neurologic disorder.[27] Patients with a systemic disorder are identified by the presence of other abnormal physical findings.

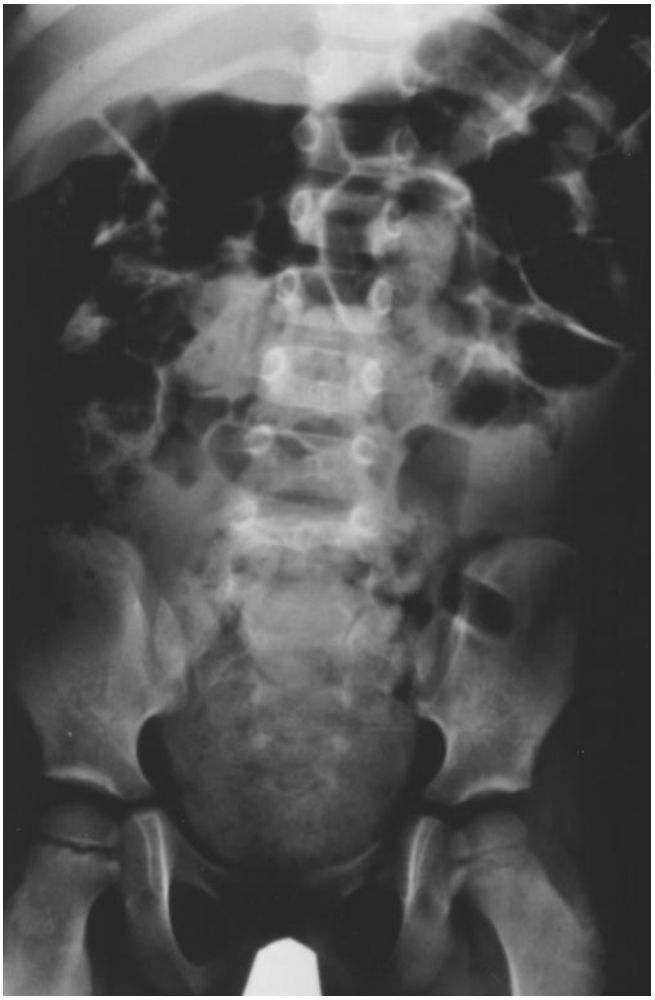

FIGURE 46.1-3 A plain radiograph of the abdomen showing a dilated rectum impacted with stool in a child with constipation.

INVESTIGATIONS

Most infants and children with chronic constipation require no laboratory investigation. In a small proportion of these children, clinical evaluation alone is insufficient and/or simple treatment measures are ineffective. In this small group of patients, diagnostic tests such as plain radiographs of the abdomen, barium enema, anorectal manometry, and rectal biopsy may be useful. A urinalysis and urine culture may be indicated in the patient presenting with accompanying complaints such as abdominal pain, enuresis, dysuria, urgency, or increased urinary frequency.

Plain radiographs of the abdomen may be necessary to establish a fecal impaction in the child who resists rectal examination and in the obese child when abdominal and rectal examinations are suboptimal. The rectum of the impacted fecal-retentive child is dilated and filled with stool to the anal verge (Figure 46.1-3).

The barium enema should be performed on an unprepared colon if the intent is to detect the transition from aganglionic to ganglionic bowel, which is typical of Hirschsprung disease (Figure 46.1-4). Usually, the ganglionic segment is dilated with stool, creating a rectosigmoid index (obtained by measuring the maximal diameter of the rectum and comparing it with a similar measurement of the sigmoid colon) of less than 1, with normal being greater than 1. If stool is removed before the barium enema, this transition zone may be difficult to identify, and the rectosigmoid index may be deceptively normal. A transition zone may not be seen in infants simply because there has not been enough time to distend the ganglionic portion of the colon with stool.[49] Loening-Baucke and colleagues calculated the sensitivity and specificity of barium enema in the diagnosis of Hirschsprung disease in the neonate to be 75% and 67%, respectively.[54] If the initial barium enema is not diagnostic, 24- and/or 48-hour follow-up plain radiographs of the abdomen, which should include a direct lateral radiograph of the rectum and sigmoid, may demonstrate delayed excretion of the barium and suggest the diagnosis.

Anorectal manometry may reveal abnormalities of defecatory function not detectable by physical examination.[55] To perform these studies, most use a manometric balloon or balloons attached to a catheter and connected to a physiograph or computer via a transducer.[14] The two most common manometers are the open-tipped perfusion manometer and the closed triple-balloon manometer (Schuster-type balloon manometer) (Figure 46.1-5). The perfusion manometer is probably the most accurate for recording resting and squeeze pressures of the anal canal. Pressures are measured using a 1 cm station pullout or a continuous pullout technique. Distention of a balloon at

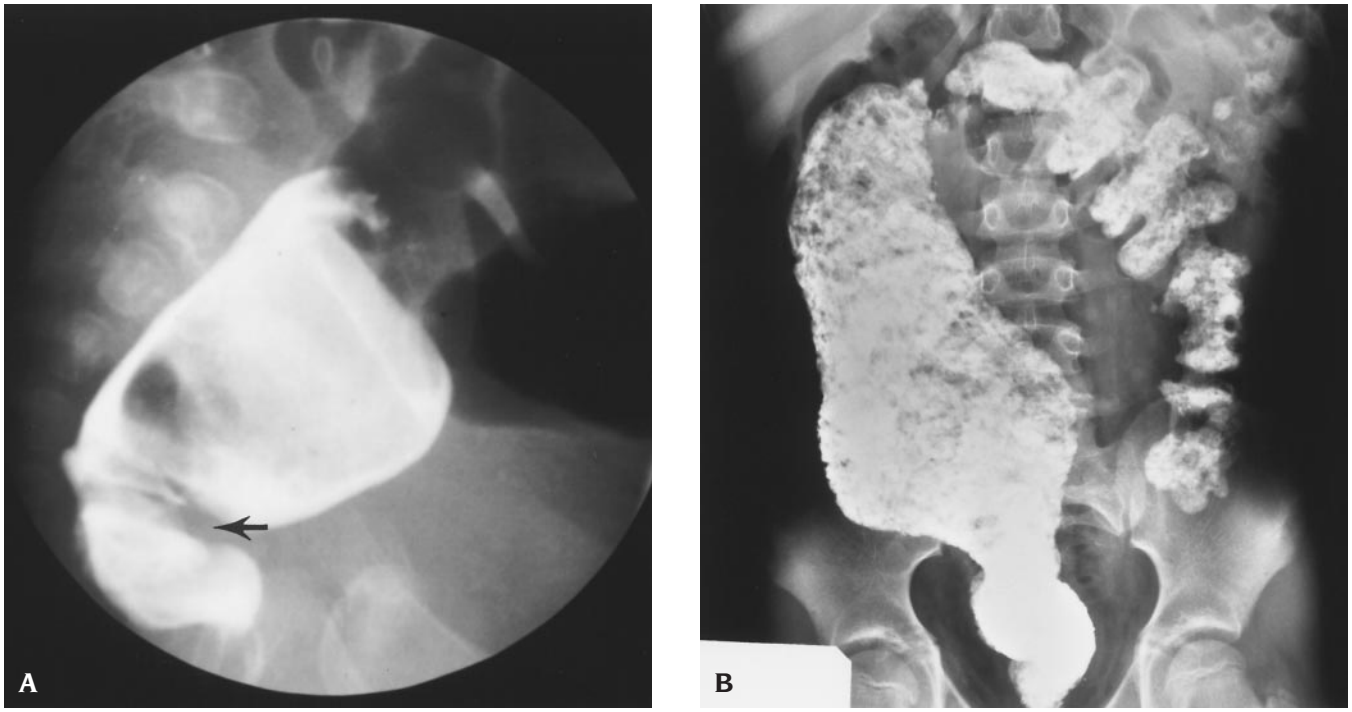

FIGURE 46.1-4 *A,* Barium enema demonstrating the transition from aganglionic to ganglionic bowel in a patient with Hirschsprung disease. Arrow points to transition zone. Courtesy of Dr. Susan White, James Whitcomb Riley Hospital for Children, Indianapolis, Indiana. *B,* A 24-hour follow-up radiograph of the abdomen demonstrating retention of barium in a patient with Hirschsprung disease. The ganglionic proximal segment is dilated with stool and barium, creating a rectosigmoid index of < 1.

the tip of the catheter with different volumes of air allows one to determine rectal sensory thresholds and elicit reflex relaxation of the internal anal sphincter. The triple-balloon system consists of a hollow cylinder surrounded by two balloons designed to sit in the internal and external anal sphincters. A rectal distention balloon passes through the hollow cylinder. This apparatus allows for simultaneous pressure recordings from the proximal rectum, internal anal sphincter, and external anal sphincter. It is most efficient for evaluating internal anal sphincter and external anal sphincter responses to rectal distention and for evaluating a subject during simulation of defecation.[14]

Anorectal manometry allows one to determine the threshold for rectal sensation, that is, the smallest volume of distention that is sensed by the subject; the threshold for internal sphincter relaxation, which is the smallest volume of rectal distention required to produce relaxation of the internal anal sphincter; the threshold for constant sensation of urgency (critical volume), which is the volume of rectal distention required to produce a persistent urge to defecate; the maximum tolerable volume, that is, the volume of rectal distention at which the subject refuses further distention; and maximal squeeze pressures of the external anal sphincter.[56] Many investigators have performed these studies,[21,50,57–59] but a lack of standardization in methods of performing anorectal manometry has resulted in inconsistencies. Efforts are now being made to standardize the performance of this and other motility tests in children.[60] Some normal values established by consensus of an International Working Group[56] are as follows: threshold for rectal sensation: ≤ 10 mL in a laboratory using rapid balloon inflation by handheld syringe, 30 mL if the inflation is gradual, 25 mL or less for children; threshold for internal sphincter relaxation: 10 to 20 mL; threshold for constant urge to defecate: ≤ 220 mL with an average of 115 mL; and maximum tolerable volume: 140 to 440 mL in adults and 110 to 280 mL in children.[33] Benninga and colleagues studied 13 healthy children aged 8 to 16 years using the perfused catheter technique.[59] They reported normal values for maximal anal resting tone of 33

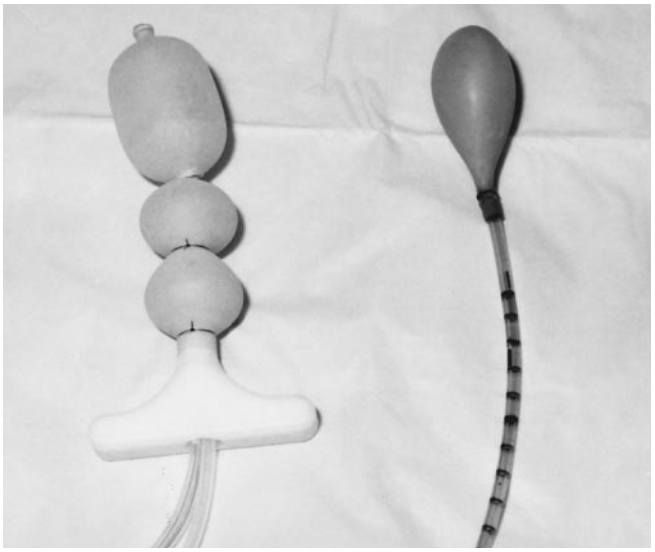

FIGURE 46.1-5 Anorectal manometry catheters. The open-tipped water-perfused catheter is on the right. The Schuster-type triple-balloon catheter is on the left.

to 90 mm Hg and for maximal squeeze pressure of 81 to 276 mm Hg; a threshold for rectal sensation of 5 to 50 mL; a threshold for internal sphincter relaxation of 5 to 40 mL; and critical volume of 90 to 180 mL.

Despite these inconsistences, anorectal manometry is a good screening test for Hirschsprung disease. A fall in internal anal sphincter pressure is seen after distention of the rectum with air, the rectoanal inhibitory reflex. This reflex is present in healthy infants and children, with the probable exception of neonates delivered before 39 weeks of gestation.[61,62] It is absent in Hirschsprung disease (Figure 46.1-6) because the aganglionic segment blocks the normal intramural pathway from rectum to internal sphincter.[63]

In the short- and ultrashort-segment forms of Hirschsprung disease, the rectoanal inhibitory reflex is absent, despite the presence of normal ganglion cells on rectal biopsy. The rectoanal inhibitory reflex may also be absent in patients with hyperganglionosis.[64,65]

Anorectal manometry has identified functional abnormalities in some children with chronic idiopathic constipation, including an increased rectal sensory threshold, decreased rectal contractility on attempted defecation, and paradoxical contraction of the external anal sphincter and puborectalis muscles during attempts at defecation.[21,40,57,66,67] The latter condition is called anismus[68] or pelvic floor dyssynergia.[56] Loening-Baucke and Cruikshank found failure of relaxation of the external anal sphincter in 50% of a group of chronically constipated children with encopresis.[50] Meunier found similar abnormalities in 77% of a group of constipated children.[69] Wald and colleagues found that 36% of children with encopresis exhibited an inappropriate contraction of the external anal sphincter during simulated defecation compared with 10% of control children.[57] Keren and colleagues found similar abnormalities in 78% of 18 constipated and encopretic children.[58] Whereas Meunier and colleagues[21] and Molnar and colleagues[70] found a significantly higher threshold for rectal

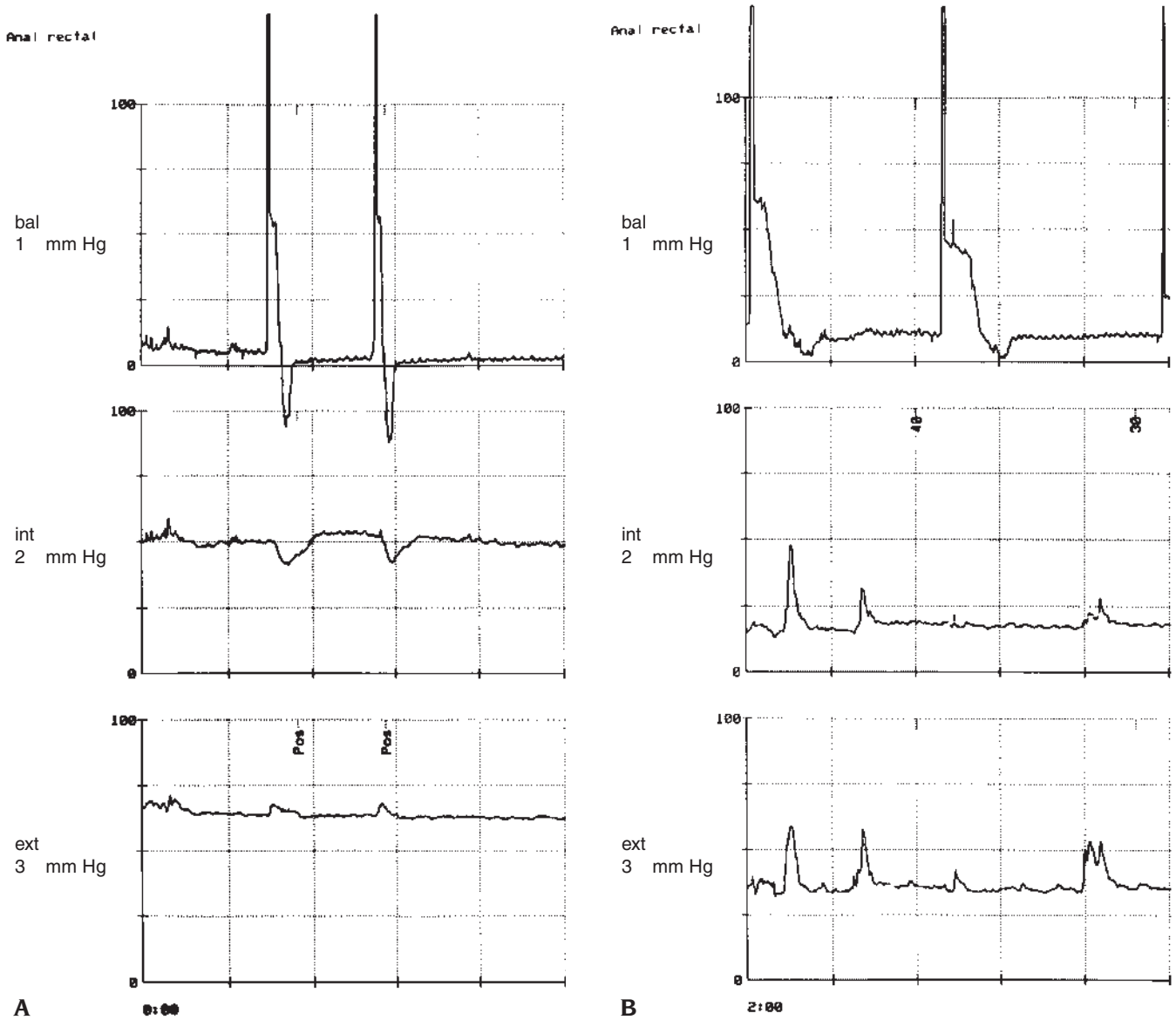

FIGURE 46.1-6 Anorectal manometric tracing demonstrating: *A,* Normal relaxation of internal anal sphincter and contraction of external anal sphincter on rectal distention. *B,* Absence of relaxation of internal anal sphincter in a patient with Hirschsprung disease.

sensitivity in constipated children compared with controls, Wald and colleagues[57] found no significant difference in mean rectal sensory threshold between encopretic children and control children. The difference between the two groups, however, appears to be related to the different methods of bowel preparation employed by the individual investigators prior to manometry.

These manometric abnormalities in children with chronic idiopathic constipation have responded to biofeedback treatment in some cases. It is presumed, for example, that paradoxical contraction of the external anal sphincter during attempts at defecation is the result of self-conditioning brought on by previous painful defecation and that it should respond to a retraining program.[57,58]

Anal sphincter EMG may be performed at the time of manometry to evaluate activity of the external anal sphincter and puborectalis muscles and for biofeedback treatment in children.[40] It involves the use of surface or needle electrodes connected through an EMG analyzer to a monitor or physiograph.

Defecography is a radiographic means of studying defecation dynamics. A barium paste is introduced into the rectum, and the patient is instructed to evacuate the paste while a series of radiographs are obtained. This study allows one to measure the anorectal angle at the time of straining and to assess the activity of the puborectalis sling.[71] Because of the need for exposure to radiation, this test has not been used routinely to evaluate children with constipation.

Colorectal transit studies obtain objective data to support a suspected dysfunction in colonic motility. In the original technique described by Hinton and colleagues, the subject ingests 20 radiopaque markers on a single day.[72] Daily radiographs of the abdomen are obtained to follow the progress of the markers through the colon until all have been expelled, up to a maximum of 7 days. One may radiograph the stool for markers, but this technique fails to identify segmental abnormalities. To minimize exposure to radiation, Metcalf and colleagues introduced a modification of the Hinton technique.[73] The patient ingests 20 markers at the same time each day for a period of 3 days. A single radiograph is obtained on the fourth day. A second radiograph may be obtained on day 7 in borderline cases and to verify apparent segmental delay. By either technique, markers are counted in the right colon, left colon, and rectosigmoid colon, using the spine and pelvis as landmarks.[74] Segmental transit is obtained by multiplying the number of markers in the segment of interest by 1.2 (the number of hours in a day divided by the number of markers ingested). Total colonic transit is obtained by counting markers in the entire colon and multiplying by 1.2. If the Hinton technique is used, the number of markers present in a segment of interest or in the entire colon on each radiograph is counted and the sum total is multiplied by 1.2.[72] With the Hinton technique, many normal adults pass the first marker within 3 days and 80% of the markers within 5 days.[72] The upper limit of normal for total colonic transit time is about 68 hours in adults[73] and between 50 and 62 hours in children, depending on the technique used.[75,76] These studies allow one to identify patients who may have abnormal motility in particular segments of colon and to differentiate between three groups of patients with chronic idiopathic constipation.[74] In one group, markers are retained too long in the entire colon (slow-transit constipation, a subgroup of which has delay in the right colon and is said to have colonic inertia). In a second group, there is a holdup in the left colon and rectosigmoid (distal constipation, a subgroup of which has normal transit through the colon with virtually no transit through the rectum and is said to have outlet obstruction). Such a situation may be seen in children with retentive constipation or paradoxical anal contraction during defecation.[77] The third group has a normal colorectal transit time (normal-transit constipation). Transit studies are performed frequently in adults. They have been found helpful in selecting and monitoring therapy.[78] They may be helpful in providing objective information in children who report or whose parents report constipation but who have no objective findings of constipation on repeated physical examination. They may also be helpful in classifying children with constipation into the subgroups described above.[77]

Colonic motor function can be precisely evaluated by colonic manometry. Manometry helps to identify children in whom a neuropathy or myopathy may be the cause of intractable constipation.[79] In some patients, colonic manometry may identify segmental abnormalities that may be amenable to surgical management.[80]

Because the diagnosis of Hirschsprung disease depends on histologic evidence of aganglionosis, a rectal biopsy is performed in those patients in whom Hirschsprung disease is strongly suspected. These would be patients with intractable constipation who have an abnormal barium enema or absence of the rectoanal inhibitory reflex on anorectal manometry. The mucosal suction biopsy that does not require anesthesia is of little or no risk. In our experience, the depth of these biopsies, except in infants, is often not sufficient to allow adequate study of the submucosa for ganglion cells, often necessitating repeat biopsies. Beyond infancy, we prefer the forceps biopsy technique done under general anesthesia. If properly performed, this technique almost always provides adequate tissue depth. We use propofol for anesthesia, and endotracheal intubation is usually unnecessary. A small risk of bleeding exists when an abnormally dilated internal hemorrhoidal vein is accidentally injured during the procedure. We obtain two biopsy specimens from the lateral walls of the rectum approximately 3 cm from the anal margin.

To the trained eye, a simple hematoxylin-eosin stain allows identification of ganglion cells in the submucosa if they are present. The absence of ganglion cells in the submucosa, often accompanied by the presence of hyperplastic neural elements, is characteristic of Hirschsprung disease, except perhaps in premature newborns. The hyperplastic neural elements associated with Hirschsprung disease may be enhanced by staining with acetylcholinesterase.[81] Other abnormalities of the enteric plexus causing constipation that may be diagnosed from a good biopsy specimen include the hyperganglionosis seen in association with intestinal neuronal dysplasia[64,65] and hypoganglionosis.[82]

TREATMENT AND PREVENTION OF CHRONIC IDIOPATHIC CONSTIPATION AND ENCOPRESIS

For most children with chronic constipation, a well-organized treatment strategy is all that is required once constipation has been confirmed. Such a program should be designed to clear any existing impaction, prevent reimpaction, and establish a regular bowel habit.

We outline the age-related treatment strategy that has been successful at our institution.

Infants on breast milk are less likely to develop constipation than their counterparts on regular cow's milk–based formula, possibly owing to the higher carbohydrate-to-protein ratio of breast milk. Occasionally, a solely breastfed infant is referred for evaluation of infrequent stools; these infants are usually perfectly healthy, with a small amount of salve-like stool in the rectal vault. We reassure the mothers, explaining that the infant's infrequent stools relate to almost complete absorption of their breast milk, leaving very little residue for stool formation.

For most infants with simple, acute constipation, dietary measures, including an increase in fluid and carbohydrate intake, often correct the problem. In infants with persistent hard stools, one to two teaspoons of maltsoup extract (Maltsupex) or dark karo syrup added to a feeding two to three times per day may be helpful. If an anal fissure develops, it should be treated promptly. An ointment such as petroleum jelly should be applied liberally to the fissure to reduce irritation from friction during the passage of stool. For older infants, increasing the intake of fluids containing sorbitol, such as apple, prune, or pear juice, may help soften the stool. A stool softener such as docusate sodium or an osmotic laxative such as lactulose or sorbitol may also be prescribed to soften the stool. Prompt treatment of a fissure is essential to prevent the development of a tendency to withhold stool. We have seen infants as young as 6 weeks of age withhold stool. For such infants, the treatment measures described above may be ineffective. We have safely treated infants over the age of 6 months with a mineral oil–based preparation in a dose of 10 cc twice per day, and there is no risk for pulmonary aspiration. In younger infants or older infants with a vomiting history and, therefore, at risk for pulmonary aspiration, we have tended to use a senna preparation in a dose of one-third to one-half of a teaspoon at bedtime until regular, soft stools are passed. Electrolyte-free polyethylene glycol (PEG) may also be used in this group of patients.

Toddlers and older children with simple acute constipation usually respond to increased fluids, especially sorbitol-containing fruit juices, and increased dietary fiber intake. Most toddlers and older children with chronic constipation referred to the pediatric gastroenterologist are voluntary stool-withholders. In many school-age and teenage children, withholding may have been ignored or not noticed during the preschool years, and encopresis is the reason for concern. Where there is a clear history of withholding, treatment should begin with a careful explanation of the basis of the problem, an intervention termed "demystification" by Levine and Bakow.[83] We describe to the parents and child the circumstances leading to a tendency to withhold stool and how fecal soiling results. This careful explanation is a very important part of a successful management strategy. The older child is intrigued that someone finally understands his or her problem, and the parents are often relieved that the child does not have a serious organic problem. Following the demystification process, we outline our strategy for management to the parents and child. Phase 1 is to clear existing impaction with three hypertonic phosphate enemas, approximately 3 cc/kg (maximum 135 cc) each over a 36-hour period. Excessive administration of phosphate enemas may result in systemic absorption of phosphorus (and sodium), leading to hyperphosphatemia and hypocalcemia, and even death, in very young infants and in patients with Hirschsprung disease.[84,85] Enemas must be used with caution in this group of patients. Occasionally, a patient with an unusually large impaction who has not responded to enemas and/or large-volume intragastric infusion of an isotonic PEG solution is manually disimpacted under anesthesia.

Phase 2 of our treatment strategy is designed to overcome the tendency to withhold stool and prevent reimpaction. Mineral oil has traditionally been prescribed in sufficient doses to overcome withholding. The authors prescribe $\frac{1}{2}$ to $2\frac{1}{2}$ ounces twice daily, depending on the patient's age. The mineral oil is given between meals, preferably in the midafternoon and at bedtime, so as to not interfere with the absorption of fat-soluble vitamins. Evidence indicates that there is no interference with fat-soluble vitamin absorption if the mineral oil is given at these times.[86,87] Electrolyte-free PEG 3350 (MiraLax, Braintree Labs, Braintree, Massachusetts) is quickly becoming first-line therapy for childhood constipation among many physicians. Studies in adults have suggested that a PEG-electrolyte solution administered in low doses is safe and efficacious for the long-term treatment of chronic constipation.[88–91] The limitation of this solution is its salty taste. DiPalma and colleagues were the first to show that an electrolyte-free PEG solution is equally safe and efficacious in adults with chronic constipation.[92] Two recently published studies looked at the safety and efficacy of electrolyte-free PEG 3350 in children with chronic constipation. Loening-Baucke compared 21 children treated with magnesium hydroxide with 28 children treated with electrolyte-free PEG 3350 at doses of 0.6 ± 0.2 g/kg/d.[93] At 12 months, 61% of the group on PEG 3350 and 67% of the group on magnesium hydroxide were doing well. Soiling was more common in the group of children treated with PEG 3350. All of the children on PEG 3350 were compliant with the medication, whereas 33% of the children on magnesium hydroxide refused to take the medication. There were no significant side effects with the PEG. Pashankar and Bishop treated 24 children with PEG 3350 for 8 weeks starting at an initial dose of 1 g/kg/d and having the patients adjust the dose as necessary to obtain two soft stools per day.[94] Twenty children completing the study demonstrated an increase in stool frequency from 2.3 ± 0.4 per week to 16.9 ± 1.6 per week. Four of nine with soiling were

cured, whereas the others improved. The effective dose was 0.8 g/kg/d. The children were very compliant with the medication and preferred it over their previous laxatives. The data on PEG 3350 are quite promising. More studies comparing PEG 3350 with other commonly used laxatives are needed to determine if this new laxative is superior to other currently available, cheaper laxatives. Our division is in the middle of one such study designed to compare PEG 3350 with three other commonly used laxatives in children with retentive constipation.

Phase 3 of our program aims at helping the older, toilet-trained child establish a regular bowel habit. The child should sit on the toilet, with proper foot support to allow for hip flexion and to help leverage, for 5 to 10 minutes after breakfast and after the evening meal to take advantage of the gastrocolic reflex to evacuate the rectum. Rewards such as stickers may be used to provide positive reinforcement.[95] We stress the importance of this aspect of the management because a regular bowel habit should evolve and persist during the period when the medication is being weaned and after it is discontinued. Advice is also given at this time on increasing water and fiber intake. We provide a fiber-content sheet to the family and establish a goal of age plus 5 g/d for the child. Unflavored psyllium may be used to reach the daily fiber goal. Finally, we provide the family with reading material and an outline of the treatment strategy to reinforce our discussion and encourage them to communicate any problems they encounter with our office.

It is important to monitor these patients initially at 1-month intervals. If the patient's interval history is satisfactory and rectal examination reveals loose or no stool in the rectal vault, the mineral oil is reduced or the PEG 3350 solution is continued. A return of soiling indicates recurrence of impaction and necessitates restarting the treatment protocol. We occasionally encounter a patient with megarectum who has a large amount of loose stool in the rectal vault on examination. It is obvious from the loose stool that the patient is compliant and is taking the medication as prescribed. In these patients, the addition of a nighttime dose of a colon stimulant, such as senna, might hasten the return of normal colonic tone. The stimulant laxative is weaned as quickly as possible to prevent dependence. The mineral oil should be slowly tapered over 4 to 6 months. The PEG 3350 solution should be taken for 3 months and then tapered over 1 to 2 months.

A common pitfall is premature discontinuation of medications. We no longer schedule return appointments when the patient is having a soft stool daily, is no longer withholding stool, and is no longer soiling. Parents are advised to treat acute recurrences of constipation promptly to prevent the reappearance of a withholding pattern. Adequate fluid and fiber intake is again encouraged.

Table 46.1-2 summarizes the treatment protocol used in our institution for children with chronic retentive constipation. Other laxatives used by pediatric gastroenterologists include magnesium hydroxide (1–3 cc/kg/d in two divided doses),[2] sorbitol (1–3 cc/kg/d in two divided doses), lactulose (1–3 cc/kg/d in two divided doses), and cisapride (0.2 mg/kg/d), although the data on the efficacy of this medication are conflicting.[96–100] Cisapride is currently no longer available in the United States. We have had little experience with magnesium hydroxide or cisapride and very little success with lactulose, sorbitol, and other stool softeners in children who *clearly* withhold. This, we suspect, is because any water gained during transit through the small bowel and proximal colon with these agents is lost in the distal colon, where it is reabsorbed with ongoing stool avoidance. Continued withholding is impossible with appropriate doses of mineral oil and PEG 3350 solution because of their ability to retard colonic absorption of fecal water.

The child with infrequent bowel movements and no history of withholding who continues to have problems despite compliance with prescribed therapy may need testing to exclude occult organic disease. Serum calcium, thyroxine/thyroid-stimulating hormone, and antiendomysial antibody (or antitissue transglutaminase) titer may be requested to exclude idiopathic hypocalcemia, thyroid disease, and celiac disease.[101–104] Some of these patients may benefit from a short course of a stimulant laxative such as senna or a bulk laxative such as psyllium. Also, some of these patients may benefit from PEG 3350 solution.

Patients with obvious emotional disturbances and those with fecal soiling and no documented impaction on repeated examinations are best treated by a psychiatrist or behavioralist.[36]

For those patients not responding to the treatment program outlined above, anorectal manometry and/or rectal biopsy should be performed to exclude Hirschsprung disease. An unprepared barium enema is a reasonable alternative if facilities for performing a rectal biopsy or manometry are not available. It should be noted that short- or ultrashort-segment Hirschsprung disease may be diagnosed only by anorectal manometry, where failure of relaxation of the internal anal sphincter on rectal distention is documented. In some patients undergoing anorectal manometry, abnormal defecation dynamics, such as a decreased sensory threshold to rectal distention or paradoxical contraction of the external anal sphincter and puborectalis muscle during simulated defecation, may be uncovered.[21,33,58,66,105,106] Such patients may benefit from biofeedback training to help them relax the external anal sphincter muscles when straining to defecate and to decrease the threshold for rectal sensation.

BIOFEEDBACK TREATMENT

Biofeedback therapy has been used in the last two decades to manage various disorders of defecation in both adults

TABLE 46.1-2 TREATMENT STRATEGY FOR CHRONIC RETENTIVE CONSTIPATION

Careful explanation of the mechanism of constipation
Hypertonic phosphate enemas every 12 h × 3 to clear impaction
Mineral oil, 0.5 to 2.5 ounces twice daily (between meals) or electrolyte-free PEG 3350 (MiraLax), 1 g/kg/d in $^1/_2$ ounce water per g qd or divided bid
Encourage patient to sit on toilet for 5 to 10 min after breakfast and the evening meal with proper foot support
Monitor patients at monthly intervals
Continue treatment for 4 to 6 mo

and children. It is based on operant conditioning, the pavlovian principle of learning through reinforcement, and was first used by Kohlenberg to correct fecal incontinence.[107] Patients are shown a tracing of normal defecation dynamics during biofeedback training. They are then allowed to see their own tracing, and the abnormalities are pointed out to them. Using the monitor or the physiograph for feedback, these patients are encouraged to correct the abnormalities on their tracings by trying different maneuvers (Figure 46.1-7). Sensory retraining is achieved by training the patient to sense progressively smaller volumes of rectal distention using the rectal balloon. Several studies now suggest that biofeedback therapy is beneficial in treating some patients with chronic constipation and encopresis. Wald and colleagues reported 67% success in those patients treated with biofeedback compared with 33% in those treated with mineral oil.[108] Keren and colleagues treated 12 patients with chronic constipation who paradoxically contracted their external anal sphincter while straining to defecate.[58] All 12 children successfully learned to relax their anal sphincter during defecation, and their bowel habits improved. Loening-Baucke studied 43 children, 20 of whom were assigned to conventional therapy, including disimpaction, followed by magnesium hydroxide (2 cc/kg/d) and a bowel regulation program.[109] The remaining 23 children received this conventional therapy and biofeedback training. Eighty-six percent of the patients receiving biofeedback treatment learned to relax their external anal sphincter during straining. At follow-up after 7 months, one patient in the conventionally treated group had recovered compared with 12 of the biofeedback-treated group. At 12 months, 16% of the conventional group had recovered compared with 50% of the biofeedback group. Others have reported similar successes in both adults and children.[106,110–113] These studies indicate that biofeedback treatment is effective in a subpopulation of children with chronic idiopathic constipation and encopresis who are found to have abnormal defecation dynamics on anorectal manometry, at least in the short term. Some recent studies have not found it to be significantly different from conventional therapy in the long term.[114,115] Biofeedback should be considered in patients failing conventional therapy because a small group of patients with intractable constipation and/or encopresis may benefit from this therapy.

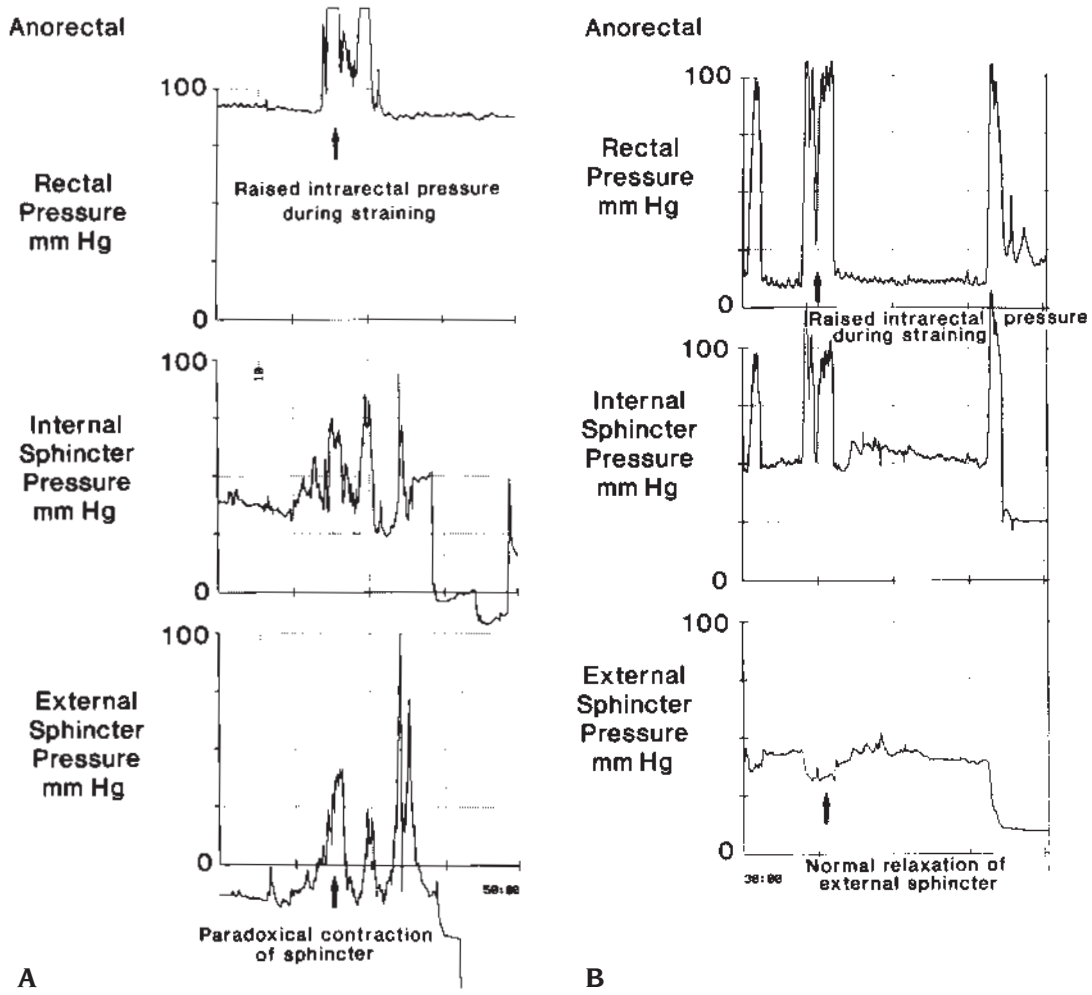

FIGURE 46.1-7 *A,* Manometric tracing of a patient demonstrating paradoxical contraction of the external anal sphincter during simulated defecation. *B,* Same patient after 3 sessions of biofeedback training. Note relaxation of the external anal sphincter during simulated defecation. (The internal sphincter tracing may reflect intrarectal pressure during straining and, therefore, should be ignored during simulated defecation).

In patients with persistent problems despite adequate treatment, it may be necessary to proceed to further studies, including magnetic resonance imaging of the lumbosacral spine to exclude occult spinal cord abnormalities and colonic manometry to exclude occult myopathy or neuropathy.

SURGICAL TREATMENT

Surgical procedures ranging from anorectal myectomy to proctocolectomy have been used to treat some adults with intractable constipation. These procedures have a very high complication rate and often have failed to correct the problem.[116–118] Obviously, there is no place for proctocolectomy in the management of children with chronic idiopathic constipation, except in a very small number of patients with debilitating symptoms and persistently abnormal colonic manometry in a nondilated colon.[80] Myectomy should be reserved for patients with well-documented absence of relaxation of the internal anal sphincter on rectal distention and a normal rectal biopsy indicating short- or ultrashort-segment Hirschsprung disease (recently also referred to as anal achalasia).[119,120]

The Malone appendicocecostomy for antegrade colonic enemas (MACE procedure), initially designed for treatment of patients with intractable fecal incontinence, may be used to manage children with intractable constipation. This procedure uses the appendix as a conduit to the cecum. After wrapping the cecum around the base to prevent reflux, the appendix is brought out to the abdominal wall as a stoma through which the colon can be irrigated with tap water or saline in an antegrade fashion (Figure 46.1-8).[121–126] To avoid complications associated with appendicocecostomies, cecostomy buttons can be placed endoscopically for the delivery of antegrade enemas.[127]

PROGNOSIS

The long-term outcome in children with chronic idiopathic constipation, with or without encopresis, is not well established. Several studies have been reported, and treatment approaches were similar except for the type of laxative used. Davidson and colleagues monitored 99 patients treated with high-dose mineral oil, a bowel training program, and close follow-up over several years and reported a 90% success rate.[86] Levine and Bakow followed 110 encopretic children treated with a similar program over a 1-year period and reported a 78% success rate.[83] In their study in which a group of children with encopresis was randomized to receive biofeedback treatment or mineral oil treatment,

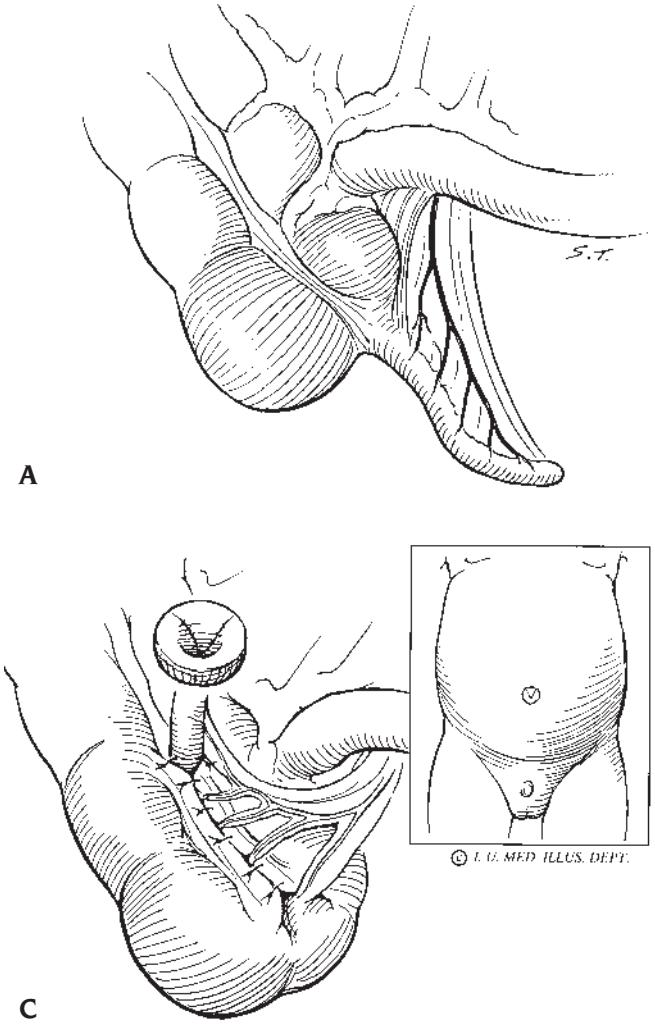

FIGURE 46.1-8 *A,* Anatomy of the cecum and appendix. *B,* The cecum is wrapped around the appendix to create a nonrefluxing appendicocecostomy. *C,* A nonrefluxing appendicocecostomy ready for antegrade enemas. Courtesy of Drs. Anthony Casale and Richard Rink, James Whitcomb Riley Hospital for Children, Indianapolis, Indiana.

Wald and colleagues reported a 71% success rate at 12 months in patients treated with mineral oil who did not have abnormalities of defecation dynamics on manometry.[108] On the other hand, Abrahamian and Lloyd-Still reported only a 47% success rate based on a 40% response to questionnaires sent to 186 patients seen over a 7-year period.[16] Most of these patients were treated with syrup of senna in combination with dioctyl sodium sulfosuccinate. Their follow-up was left to the referring physicians. In this group, the presence of soiling at the time of initial presentation was the only predictor of poor outcome, whereas the presence of abdominal pain correlated with a positive outcome. Loening-Baucke reported a 43% success rate at 12 months in 97 children with chronic constipation, with and without encopresis, treated with magnesium hydroxide.[128] Factors predictive of a poor outcome in this group were the presence of an abdominal fecal mass or soiling at the time of initial presentation. Notice that the common denominator in all three studies reporting greater than a 70% success rate was the use of mineral oil and close monitoring. This supports our opinion that in retentive constipation (which is the majority of chronic idiopathic constipation in children), mineral oil is quite effective and, with close monitoring, improves constipation in many patients. This view is, in fact, supported by a study in which Sondheimer and Gervaise randomized a group of children with chronic constipation, with and without encopresis, to receive either mineral oil or senna.[129] After 6 months, 89% of the mineral oil group was having daily bowel movements compared with 50% of the senna group. Soiling was reported by 6% of the mineral oil group compared with 44% of the senna group. Fifty-five percent of the mineral oil group had successfully discontinued regular medication compared with 22% of the senna group. Our 1994 survey of 274 patients seen in our clinic in 1992 showed a 75% success rate based on a 73% response rate. More recently, Staiano and colleagues, who treated patients with lactulose after initial disimpaction, reported persistence of constipation in 52% of 62 children after 5 years of follow-up.[130] Early onset of constipation (< 1 year) and a family history of constipation seemed to predict poor outcome. Sutphen and colleagues reported resolution of constipation in 70% of 43 children followed for an average of 6.8 years.[131] The patients were not treated with a single laxative. Some were treated with magnesium salts, some with mineral oil, and others with senna; no mention is made of how the type of laxative used was determined. An enema was given if the child went a day without a bowel movement. The main predictor of outcome was the duration of symptoms prior to referral to a gastroenterologist. The longer the period prior to referral, the poorer was the outcome. We await long-term follow-up studies of patients treated with electrolyte-free PEG 3350 to see how it impacts the overall success rates in the treatment of children with chronic constipation.

CONCLUSIONS

Constipation is a very common problem during childhood. In its acute form, it is easily corrected by increasing fluid and dietary fiber intake. If not properly treated in the acute stage, a cycle begins when painful defecation leads to deliberate withholding of feces, which may result in an acquired megacolon and, often, overflow incontinence. Once a withholding pattern has evolved, many patients will do well on a program that includes careful explanation of the pathophysiology of the disorder to all involved, evacuation of any impaction, the use of a laxative in sufficient doses to overcome withholding, a program to regularize the bowel habit, and, above all, close follow-up. In those not responding to this mode of treatment, an unprepared barium enema and anorectal manometry will identify those needing a rectal biopsy to exclude Hirschsprung disease. Anorectal manometry will also identify a subgroup with abnormal defecatory dynamics who may benefit from biofeedback treatment. Magnetic resonance imaging of the lumbosacral spine and colonic manometry may be necessary to identify occult spine abnormalities and occult myopathy or neuropathy of the gastrointestinal tract. A role for colonic transit studies has not been well defined in pediatric patients with chronic constipation. It is conceivable that in school-age children and teenagers with chronic constipation in whom a history of withholding is denied, this study will be obtained to differentiate those with slow-transit constipation from those with a functional outlet obstruction so that appropriate therapy can be initiated. Studies are needed to determine what percentage of children with chronic idiopathic constipation suffer with constipation as adults.

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2. Dysmotilities

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The motility of the small and large intestine is a function of the intestinal smooth muscle, which is controlled by the enteric nervous system (ENS) and the interstitial cells of Cajal (ICC). The autonomic and central nervous systems, gastrointestinal hormones, and immune system modulate gastrointestinal motility. Any disturbances of one or more of these systems may lead to dysmotility, which may be restricted to a particular bowel segment or may involve the entire gut, with or without the esophagus and stomach. This chapter is a systematic review of intestinal dysmotility disorders. Many of the diseases may finally result in chronic intestinal pseudo-obstruction, which represents the extreme end of the clinical manifestation and is discussed in Chapter 46.4, "Chronic Intestinal Pseudo-obstruction Syndrome."

Usually, dysmotility diseases are classified into primary visceral myopathies, primary visceral neuropathies, and secondary to toxic, metabolic, infectious, or other systemic disorders affecting the smooth muscle or the enteric or extrinsic nervous system (secondary myopathies and neuropathies; see also Chapter 46.4, Table 46.4-1).[1] Some diseases do not fit into this rough classification because both the muscle layer and the enteric nervous plexuses are involved either primarily, as in some of the mitochondrial disorders, or secondarily, as in fibrosis or ischemia. The term "idiopathic" is applied if the disorder does not fulfill the criteria of a recognizable genetic syndrome, if there is no apparent underlying disease, and if affected tissue is unavailable for investigation or appears normal under histologic examination.

Further classification may change, especially within the primary disorders, which are mostly based on histopathologic findings.[2] Many techniques have been applied, including different types of fixation, cutting and staining of the tissue, light and electron microscopy, and several enzyme histochemical and immunohistochemical methods. Interpretation of morphologic features for histologic diagnosis is a subjective integrating process that is influenced by an individual's expertise and subject to marked interobserver variation.[3] Comparisons of morphologic findings between single case reports or series of patients are therefore difficult to interpret. Few data on the normal postnatal maturation and appearance of the ENS are available. Postmortem studies have limitations owing to autolytic processes and prefinal conditions of the child. The available data indicate that not all enteric nerve cells are morphologically mature at birth, and maturation continues for at least the first 2 to 4 years of life.[4,5] For example, the absence of silver-positive enteric nerve cells, which is considered abnormal in adults, is consistently found in preterm infants and is a normal variant in children up to the age of 1 year.[6] Secondary phenomena owing to severe chronic bowel dilatation or therapeutic interventions (ie, drugs or surgery interrupting the flow of the chyme) need to be distinguished from primary pathology. In clinical practice, histologic classification of neuromuscular disorders of the gut is also hampered by the fact that full-thickness biopsies are usually required for the pathomorphologic diagnosis. Exceptions are Hirschsprung disease and a few other neuropathies in which a definite histologic diagnosis can be made from rectal suction or deep forceps biopsies. After exclusion of aganglionosis, the knowledge of the exact neuromuscular classification hardly alters therapeutic management in most affected children with primary dysmotility disorders. Therefore, full-thickness bowel biopsies are usually not justified unless an operation is necessary for other clinical indications (ie, ileostomy, resection).

Classification has also been attempted based on clinical phenotypes such as age at onset, bowel and bladder involvement, or mode of inheritance. This approach can be very misleading, but it is helpful in a few cases with specific (distinguished) concurrent extraintestinal features. For example, the megacystis-microcolon-intestinal hypoperistalsis syndrome (MMIHS) describes a certain phenotype, but histopathologic investigations reveal myopathic changes in most children, myopathic and neuropathic changes in other children, or both. In some children, no histologic abnormalities are found. Progress in molecular genetics should help to define additional familial forms of gastrointestinal motility disorders.

Clinical Manifestations

Clinical symptoms are variable and often nonspecific. The location of the affected bowel (diffuse or segmental) seems a more important determinant of clinical manifestation than the underlying cause. Within the same family with a defined genetic disorder, a spectrum of symptoms may occur, ranging from none to chronic intestinal pseudo-obstruction.[7] Primary and secondary neuromuscular disorders of the intestine may present at any age, but most children become symptomatic during the neonatal period or early infancy.[8–10] In congenital disorders, antenatal ultrasonography may show dilated bladder and/or bowel loops in a mother with or without polyhydramnios.[8,9,11–13] After birth, the main symptoms are bilious vomiting, failure to pass meconium, severe constipation, and a distended abdomen. Differentiation from mechanical obstruction is often very difficult, and many of

TABLE 46.2-1 NEUROMUSCULAR DISORDERS PRESENTING IN INFANCY, CHILDHOOD, OR ADOLESCENCE THAT MAY CAUSE SYMPTOMS OF INTESTINAL DYSMOTILITY INCLUDING PSEUDO-OBSTRUCTION

DISORDERS OF THE INTESTINAL SMOOTH MUSCLE
Primary visceral myopathies
Familial visceral myopathies
Autosomal dominant with megaduodenum
Autosomal recessive (mitochondrial-gastrointestinal encephalomyopathy)
Autosomal recessive without extraintestinal manifestations
Autosomal recessive with gastroparesis and tubular, narrow small intestine
X-linked with abnormal muscle layering
Nonfamilial or sporadic visceral myopathies
Infantile or childhood visceral myopathy
African degenerative leiomyopathy
Megacystis-microcolon-intestinal hypoperistalsis syndrome
Secondary visceral myopathy
Progressive systemic sclerosis and other connective tissue diseases
Enteric myositis (autoimmune myopathy)
Muscular dystrophy
DISORDERS OF THE ENTERIC NERVOUS SYSTEM (MYENTERIC PLEXUS)
Primary visceral neuropathies
Familial visceral neuropathies
Autosomal dominant without extragastrointestinal manifestations
Autosomal dominant with neuronal intranuclear inclusions
Autosomal recessive, autosomal dominant, or X-linked with short bowel, malrotation, and pyloric hypertrophy
With neurologic involvement
Associated with multiple endocrine neoplasia type IIB
Associated with neurofibromatosis
Nonfamilial or sporadic neuropathies
Hypoganglionosis
Hyperganglionosis
Qualitative abnormalities of ganglion cells
Secondary or acquired visceral neuropathies or intestinal motility disorders
Infections
Toxic agents and drugs
Radiation
Inflammation (post–necrotizing enterocolitis, Crohn disease, autoimmune ganglionitis, celiac disease)
Autonomic neuropathy (familial, secondary, idiopathic)
Endocrine disease
Metabolic disease
Eating disorders (anorexia, bulimia)

Familial or sporadic forms of aganglionosis are excluded; see Chapter 46.3.

these infants undergo exploratory laparotomy. If the bladder is involved, failure to void and urinary infection owing to incomplete emptying may be the initial symptoms. Common symptoms reported in children with an acquired dysmotility disorder include abdominal distention, constipation, vomiting, failure to thrive, dyspepsia, and abdominal pain. Intermittent diarrhea occurs in some children as a result of bacterial overgrowth and malabsorption.

Chronic intestinal pseudo-obstruction is a term that describes the most severe symptoms associated with dysmotility (see Chapter 46.4). Pseudo-obstruction can occur in most intestinal neuromuscular diseases (Table 46.2-1).[14,15] It was defined by pediatric and adult gastroenterologists in a consensus report as "a rare, severe, disabling disorder characterized by repetitive episodes or continuous symptoms and signs of bowel obstruction, including radiographic documentation of dilated bowel with air-fluid levels, in the absence of a fixed, lumen-occluding lesion."[14] The rather strict definition that makes radiologic signs obligatory has been criticized because in some patients with severe disabling symptoms, bowel dilatation and air-fluid levels are found only intermittently. Dilated bowel loops with air-fluid levels can be observed in newborns with severe diarrhea owing to congenital transport defects, that is, chloride diarrhea, imitating a severe gastrointestinal motility disease. Chronic intestinal pseudo-obstruction, which is a phenotype, not a disease entity, is discussed in Chapter 46.4.

DISORDERS OF THE INTESTINAL SMOOTH MUSCLE

Intestinal dysmotilities are less frequently caused by smooth muscle than by neural disorders. In general, symptoms are more severe, and the prognosis is worse in primary myopathies compared with primary neuropathies. Most affected children develop symptoms at birth or shortly thereafter. Definite diagnosis requires careful study of the full thickness of the gastrointestinal wall with sectioning across muscle fibers. A characteristic myopathic pattern on antroduodenal manometry may support the diagnosis in cases in which no tissue is available for investigation. The term "hollow visceral myopathy" is used when smooth muscle of both the gastrointestinal and the urinary tract is affected.

Primary Visceral Myopathies

Primary disorders of the intestinal smooth muscle represent abnormalities in morphogenesis, resulting in alterations in intestinal muscle layering (additional muscle coat or an absence of a muscle layer) or intrinsic myocyte defects comprising varying degrees of fibrosis, myocyte atrophy, vacuolation, or an altered contractile protein. Smith and Milla identified five histologic phenotypes of smooth muscle disease in full-thickness biopsies from children with functional obstruction using routine microscopy, histochemistry, immunohistochemistry with monoclonal antibodies against different neural and smooth muscle markers, and electron microscopy.[9] Routine microscopy on paraffin sections of the smooth muscle was abnormal in only 15 of 27 patients. In the remaining patients, myopathic changes would have been missed without application of other techniques. Twenty-five of the children had a primary myopathy, and two had an acquired enteric myopathic disorder.

Clinically, primary visceral myopathies occur as familial genetic diseases with a defined mode of inheritance or as sporadic cases. Four types of familial visceral myopathies (FVMs) have been described. They may begin at any age, but particularly in the second decade; therefore, they should be considered when symptoms begin in school-age children. A few families with probable hereditary forms of infantile or childhood visceral myopathies have been reported; all affected children presented at birth, during infancy, or during early childhood.[16]

FVM Type 1. Familial occurrence of megaduodenum resembling type 1 FVM was first described in 1938 in a German family.[7] Since then, several families have been reported and confirmed that type 1 FVM is transmitted by an autosomal dominant inheritance, although there is female predominance in frequency and severity.[16,17] The disease is characterized by esophageal dilatation, megaduodenum, and an elongated, redundant, usually dilated colon.[1,18] Barium studies show an aperistaltic esophagus and often normal gastric emptying but a flaccid and dilated duodenum with prolonged retention of barium (up to 19 days in one case).[19] The small intestinal caliber is normal, except the proximal jejunum, which may be dilated. The bladder is affected in about half of the cases, but megacystis is often asymptomatic.

Most patients, especially females, become symptomatic around puberty with recurrent abdominal pain, nausea, vomiting, heartburn, and severe constipation or, occasionally, diarrhea. Some affected family members remain asymptomatic despite abnormal barium studies. About 10% of all patients develop pseudo-obstruction.[1] Mydriasis is common in type 1 FVM.

Schuffler and Pope investigated full-thickness intestinal biopsies of a 15-year-old girl with type 1 FVM by using different staining methods, including Masson trichrome in addition to electron microscopy. They observed marked thinning and degeneration and vacuolation of smooth muscle with replacement by fibrous tissue.[16]

Treatment that depends on the severity of symptoms includes dietary modification with a low-fat, low-fiber, low-lactose diet and intermittent use of antibiotics to treat bacterial overgrowth. Neostigmine was helpful in one patient to improve severe constipation, although systemic side effects limit the use of the drug.[18] Surgical treatment is indicated in patients refractory to dietary and medical management and in cases with pseudo-obstruction. Side-to-side duodenojejunostomy to drain the duodenum or partial duodenal resection below the papilla of Vater and anastomosis with the jejunum have benefited carefully selected patients.[19,20]

FVM Type 2. This disease, first reported as oculogastrointestinal muscular dystrophy, was recently renamed mitochondrial neurogastrointestinal encephalomyopathy.[1,21] The mode of inheritance is autosomal recessive. Phenotypic features include external ophthalmoplegia with ptosis and diplopia, a cardiac conduction defect, mild muscular atrophy, and dilatation of the entire gastrointestinal tract with scattered small bowel diverticula.[22] Gastrointestinal symptoms may develop during teenage years with dyspepsia, heartburn, abdominal pain, and weight loss. Skeletal muscle biopsy specimens show the "ragged" red fibers that are typical of mitochondrial myopathies. A deficiency of cytochrome-*c* oxidase in the muscle fibers was demonstrated,[21] and lactate levels are often elevated. The histologic features of the gastrointestinal smooth muscle are similar to those of type I FVM. The prognosis is poor. Most patients require total parenteral nutrition because neither medical nor surgical treatment is effective.

FVM Type 3. Anuras and colleagues reported four siblings with dilatation of the entire gastrointestinal tract but no extraintestinal manifestations.[23] One of the siblings died at age 7 years with intestinal obstruction; the others became symptomatic during adulthood. The prognosis of this autosomal recessive disorder is poor.[1]

FVM Type 4. Two families have been described with this type 4 FVM, which is characterized by gastroparesis, a tubular narrow small intestine, and a normal esophagus and colon.[24,25] Symptoms, including vomiting, abdominal pain and distention, and diarrhea, occurred as early as 2 years of age. The hypertrophy of circular muscle layer produces the tubular narrowing of the small intestine. An autosomal recessive trait is assumed because in one family two siblings and in the other family three siblings were affected.

Familial Childhood Visceral Myopathy with Diffuse Abnormal Muscle Layering. Three related males have been described with this disorder: the index case, his half-brother from the same mother, and his maternal uncle, suggesting an X-linked inheritance.[9] All presented during the neonatal period with vomiting and abdominal distention. All had short gut and malrotation. The most striking histologic finding was an extracircular muscle coat between the outer and inner layer of the muscularis propria; the myenteric plexus appeared to be embedded within them.

Sporadic Infantile or Childhood Visceral Myopathy. Most cases occur sporadically, but new dominant mutations may be responsible for some.[26] In families with several members affected, a dominant gene with variable expressivity and incomplete penetrance, or an autosomal recessive inheritance, is possible.[26,27] Most affected children develop symptoms at birth or within the first year, including severe constipation, bowel dilatation, and abdominal distention. Failure to thrive and malnutrition are common. When the disease progresses to chronic intestinal pseudo-obstruction, most patients depend on long-term parenteral nutrition or require bowel transplant. Distal to the esophagus, the entire gastrointestinal tract is affected and markedly dilated. Almost all patients have involvement of the urinary tract system with megaureters and megacystis (hollow visceral myopathy).[26,28] Microscopic findings from the small and large bowel reveal gross fibrosis of the muscularis propria identified with the Masson trichrome staining and profound atrophy of the smooth muscle cells,[29] similar to those from adults with familial and sporadic forms of visceral myopathy.[30] α_2-Actin deficiency could be shown in some sporadic cases.[29] Smith and Milla related these changes to an intrinsic myocyte defect and/or changes in the extracellular matrix. Using different staining techniques, they distinguished two subtypes: a myopathy with autophagic activity and a pink blush myopathy with nuclear crowding.[9] The prognosis is poor in patients with diffuse disease; many die during childhood owing to complications of mal-

nutrition or long-term total parenteral nutrition.[12,31] Some patients tolerate oral feeding and survive into adulthood.

African Degenerative Leiomyopathy. This disease is now recognized as a distinctive, nonfamilial form of degenerative visceral myopathy of uncertain etiology that occurs largely in Africa (particularly southern, eastern, and central Africa). Histologic features include atrophy of myocytes with vacuolated cytoplasm, extracellular edema, and gross fibrous replacement in the circular muscle layer.[29,32] Neuronal loss was absent, but hyperplasia of the myenteric plexus was observed in some cases.[30] Bowel dilatation with severe constipation and pseudo-obstruction developed after the age of 6 months, with a mean age of presentation of 9.5 years.[29] The bladder was affected in about 10% of the patients. Therapy included neostigmine, a low-residue diet, laxatives, enemas, and surgical intervention. Steroids or another immunosuppressive treatment has not been tried.

Megacystis-Microcolon-Intestinal Hypoperistalsis Syndrome. This most severe form of pseudo-obstruction was first described in 1976 by Berdon and colleagues,[33] and more than 90 cases have been reported to date. MMIHS affects female infants four times more often than males.[34] In some cases, the syndrome can be detected by prenatal ultrasound scans showing megacystis, hydronephrosis with hydroureter, and/or bowel abnormalities or gastric distention.[34] The syndrome is identified by a marked, nonobstructive bladder enlargement, a dilated aperistaltic proximal small bowel, a narrowed distal small bowel, and a malrotated microcolon located entirely on the left side of the abdomen.[33] Occasionally, a megaesophagus is associated.[35] The intestinal length is shortened to up to one-third of normal. MMIHS is usually caused by a myopathy with vacuolar degenerative changes of the smooth muscle cells.[36] However, the phenotype can also be a manifestation of a neuropathy with hypo- or hyperganglionosis and giant ganglia, indicating genetic heterogeneity. Based on findings from affected siblings, an autosomal recessive pattern of inheritance has been suggested in some families.[37,38] However, most cases are sporadic.

There is now some indication that the absence of the α3 subunit containing nicotinic acetylcholine receptor plays a role in this congenital disorder. The neuronal nicotinic acetylcholine receptor subunits are widely expressed throughout the central, peripheral, and autonomous nervous systems. They are pentamers made up by at least eight α subunits (α2–α9) and three β subunits (β2–β4) encoded by 11 distinct genes. Transgenic mice lacking the α3 or both β2 and β4 subunits show some of the phenotypic features of MMIHS. Richardson and colleagues used in situ hybridization and immunocytochemistry to study the expression of this subunit in tissue from 10 patients with MMIHS and 12 control children. They found a wide distribution of α3 messenger ribonucleic acid in the enteric ganglion cells, smooth muscle, and epithelium of normal small bowel but not in MMIHS patients.[39] However, in affected individuals with MMIHS and/or their parents, no loss of function mutations have been identified to date within the genes encoding for α3 or β3 subunit.[40]

After birth, the children develop massive abdominal distention, which is often relieved when a huge bladder, which may contain several hundred milliliters of urine, is catheterized. Most patients require several operations on the gastrointestinal and urinary tract and continue to remain dependent on parenteral nutrition. The prognosis is poor because there is no effective medical treatment. Most children die within the first year of life owing to renal insufficiency, postoperative complications, or sepsis.[37] No patient has reached adulthood. Bowel transplant may be an option for some children.

The MMIHS must be differentiated from the prune-belly sequence owing to early intrauterine urethral obstruction. This disorder affects predominantly male infants, who present with a dilated abdomen, constipation, hydronephrosis, megacystis, and often intestinal malrotation but no intestinal hypoperistalsis or microcolon.

Systemic Disorders Involving the Intestinal Smooth Muscle (Secondary Myopathies)

Most secondary myopathies have been described in adults because it may take several years of a disease process such as a connective tissue disorder until the intestinal smooth muscle layer is damaged. With modern histologic techniques and the availability of intestinal manometry, secondary myopathies are recognized even in young children. It is important to identify these entities because sometimes medical treatment can achieve symptomatic improvement and prevent secondary complications or progression of the disease process.

Connective Tissue Disorders. Scleroderma or progressive systemic sclerosis is a systemic disease characterized by excessive deposition of collagen and other matrix elements by fibroblasts in the skin and, in the systemic form, in multiple internal organs. It is associated with prominent and often severe alterations of the microvasculature, the autonomic nervous system, and the immune system. Gastrointestinal involvement with symptoms of clinical relevance occurs in approximately 50% of patients with progressive systemic sclerosis.[41] It affects most patients with the diffuse cutaneous systemic sclerosis, but less often and later, it affects patients with the limited cutaneous systemic sclerosis, the CREST variant (calcinosis, Raynaud phenomenon, esophageal dysmotility, sclerodactyly, telangiectasias). The esophagus is the most commonly affected organ, with 75 to 90% of all patients showing abnormalities on esophageal motility testing. Involvement of the anorectum is the next most frequent manifestation (50–70%), followed by small bowel hypomotility in 40%. In spite of severe dysmotilities, clinical symptoms may be mild or absent owing to a concurrent visceral sensory neuropathy.

The lesions of scleroderma are similar throughout the gastrointestinal tract, with atrophy and fragmentation of the muscularis propria, collagen infiltration, and fibrosis in the late stage of the disease. The findings are more marked

in the circular than in the longitudinal layer. The same histologic findings have been reported in adults and even in a child with intestinal myopathy, who did not have any cutaneous manifestations of scleroderma.[42]

Symptoms of small bowel involvement of scleroderma include nausea, vomiting, distention, abdominal cramps, diarrhea with malabsorption, or severe slow-transit constipation if the colon is markedly affected. Fecal incontinence is the most common colonic presentation of systemic sclerosis. Episodes of pseudo-obstruction occur only in patients with advanced systemic sclerosis. Manometric studies in patients with gastric and intestinal involvement of scleroderma may show a complete absence of the migrating motor complex (MMC), which predisposes the patient to bacterial overgrowth and bezoar formation. In the early stage of the disease with predominant neuropathic impairment, MMCs can be generated by the long-acting somatostatin analog octreotide, the cholinergic agonist cisapride, or the motilin agonist erythromycin.[43] Treatment of intestinal dysmotilities in scleroderma is symptomatic and supportive, with dietary modifications, prokinetic drugs (cisapride, octreotide, and erythromycin), antibiotics against bacterial overgrowth, and loperamide in cases of diarrhea.[41]

Gastrointestinal dysmotilities, including chronic intestinal pseudo-obstruction owing to progressive atrophy and fibrosis of the intestinal smooth muscle, have occasionally been reported in patients with Ehlers-Danlos syndrome, dermatomyositis, polymyositis, or mixed connective tissue disease.[44] Smooth muscle dysfunction with bowel dilatation can also result from systemic lupus erythematosus–induced vasculitis.[45]

Chronic Enteric Myositis and Autoimmune Myopathy. Infiltration of intestinal smooth muscle layer by lymphocytes is found in Crohn disease and other forms of chronic intestinal inflammation associated with altered motility. Four young women have been reported with diffuse polyclonal lymphoid infiltration of the intestinal muscularis propria by McDonald and others.[46] These patients became symptomatic between 18 and 35 years of age with diarrhea, vomiting, and abdominal distention and later developed episodes of intestinal pseudo-obstruction. Only one of the four patients was treated with immunosuppressive drugs (cyclophosphamide and prednisone) and responded with some improvement of her symptoms.

Acquired myositis of the muscularis propria was reported in two children who presented with functional intestinal obstruction at 1 and 2.5 years of age.[9] Dense lymphocytic infiltrates, mainly of T cells, were found along the large and small intestine. Antibodies against smooth muscle were detected in one of the patients, indicating an autoimmune process.

Muscular Dystrophy. Myotonic muscular dystrophy is a progressive systemic disease characterized by myotonia and wasting of skeletal muscle. The major features of the congenital form of the disease are reduced fetal movements and polyhydramnios, severe muscular hypotonia, facial diplegia, respiratory insufficiency, difficulties in sucking and swallowing, and skeletal anomalies such as talipes equinovarus. Cataracts and endocrine disturbances, which are typical for the adult form of the disease, are not found in infants and young children. Involvement of the smooth muscle of various organs, including the gastrointestinal tract and the bladder, is well documented. Histologic studies of small intestinal and colonic smooth muscle have shown similar changes to those described in dystrophic skeletal muscle, with swollen, partially destroyed smooth muscle cells that are progressively replaced by fat. Motor abnormalities are found in the entire gastrointestinal tract, from the esophagus to the anal sphincter. Gastrointestinal symptoms with dysphagia, abdominal cramping, diarrhea, malabsorption, or severe constipation are frequent and may precede other clinical manifestations of the disease for years. Lenard and colleagues reported on two brothers whose mother and maternal grandmother were affected by the adult form of the disease.[47] The younger brother, who presented with typical features of congenital myotonic dystrophy at birth, developed severe constipation and megacolon during the second year of life. The presenting symptoms of the older brother were repeated episodes of pseudo-obstruction in the neonatal period and diarrhea, vomiting, and abdominal distention during the first 2 years of life. Thereafter, he suffered from severe constipation with encopresis owing to megacolon. Repeated electromyographic examinations between 2 and 6 years of age were normal, and the first myotonic tracing was obtained when he was 8 years of age.

Duchenne muscular dystrophy is an X-linked disorder that causes skeletal and cardiac muscle degeneration leading to progressive weakness and death, usually from respiratory failure. Histologic studies of skeletal muscle show myofiber degeneration accompanied by necrosis and accumulation of fat and connective tissue and hypertrophy of the remaining muscle fibers. The cause of the disease is a deficiency of dystrophin, which has also been identified in the heart and in smooth muscle. Visceral smooth muscle involvement of the gastrointestinal tract can be detected by manometry with a typical myopathic pattern in many children, even in those who have only minimal skeletal muscle symptoms and an absence of gastrointestinal dysfunction. Symptoms such as diarrhea, constipation, and abdominal cramping may be related to dysmotility of the small and large intestine. Chronic intestinal pseudo-obstruction as a complication of Duchenne muscular dystrophy is rare.[48]

DISORDERS OF THE ENTERIC AND AUTONOMOUS NERVOUS SYSTEM

The ENS is a collection of neurons in the gastrointestinal tract, which has been referred to as the "brain of the gut." It can function independently of the central nervous system (CNS). The cell bodies of the ENS are grouped into small ganglia that are connected by bundles of nerve processes forming two major plexuses. The myenteric (or Auerbach) plexus is located between the longitudinal and circular muscle layers and primarily provides them with

motor innervation. The submucous (or Meissner) plexus, which lies in the submucosa between the circular muscle layer and the muscularis mucosae, is important in regulating secretory control. The ganglia of the plexus consist of tightly packed nerve cell bodies, terminal bundles of nerve fibers, and glial cells, which even outnumber enteric neurons. There are numerous projections between the two plexuses, and both are connected to the central autonomic neural network in the CNS by parasympathetic and sympathetic nerves.[49]

The ENS controls the segmental and forward propagating contractions, the exocrine and endocrine secretions, the microcirculation, and the immune and inflammatory processes in the gastrointestinal tract. Both quantitative (eg, hypo- or hyperganglionosis or aganglionosis) and qualitative abnormalities (ie, degeneration, immaturity) of the ENS have been identified using different staining methods, including silver stains and immunohistochemistry.[11,50] However, the results should be interpreted with respect to age because there are notable postnatal alterations in the myenteric plexus with a steep decline of nerve cell density.[5] To date, these specific abnormalities have not been linked to specific symptoms; most patients have disturbances in gastrointestinal transit and functional obstruction from infancy. Excessive intestinal secretion or other features of ENS dysfunction often remain clinically unrecognized. Visceral neuropathies are called diffuse if the entire small and large intestine is affected or segmental with only certain parts of the bowel being involved. Apart from aganglionosis (see Chapter 46. 3, "Hirschsprung Disease"), only a few visceral neuropathies (ie, ganglioneuromatosis[51] or neuronal intranuclear inclusion neuropathy[52] can be classified by investigating only the submucous plexus in specimens obtained from rectal suction or deep forceps biopsies.[11]

Intestinal Neuronal Dysplasia

Intestinal neuronal dysplasia (IND or IND type B) is a term that has been used over the last three decades to describe different quantitative (ie, hypo- and hyperganglionosis) and qualitative (ie, immature or heterotopic ganglion cells) abnormalities of the myenteric or submucous plexus or both. The term has raised confusion and controversy among clinicians and pathologists. IND has been observed in an isolated form and proximal to an aganglionic segment. It has been reported in all age groups, mostly infants, but also in adults with chronic constipation not dating back into childhood.[53] It was first considered to be a developmental defect of the submucous plexus,[54] but others suggested that some of the histologic features may be secondary to functional or mechanical obstruction.[55] Other intra- and extraintestinal anomalies are found in a high percentage of children diagnosed to have IND.[56] Diagnosed often in Switzerland and Germany,[3,57] IND was rarely reported in British or North American series.[58–60] One of the main criteria of IND, the presence of giant ganglia in the submucous plexus defined as more than seven ganglion cells per ganglion,[61] has been considered a normal finding of the enteric plexuses in patients without any gut dysmotility.[62,63] The confusion was complete when IND was used not only as a histologic description but also as a clinical diagnosis. Although neither retrospective nor prospective studies showed a correlation between the morphologic features of IND and symptoms or long-term outcome, surgical procedures were recommended. Sphinctermyetomies and bowel resections have been performed in both children and adults with uncomplicated constipation who were found to have IND on intestinal biopsies. The histologic definitions of IND have changed considerably over time, and this may be responsible for some of the discrepancies.[60,64] However, even when the same criteria for the diagnosis of IND were applied to a prospective study of rectal biopsies, the interobserver variation was enormous and close to chance agreement.[3] In this study, 377 coded specimens from 108 children, aged 4 days to 15 years with intestinal dysmotilities, were judged for 20 histologic features and a final diagnosis by three pathologists, who had previously participated in a consensus meeting on diagnostic guidelines. There was complete agreement for the diagnosis of Hirschsprung disease, but in only 14% of the remaining children was there a concordant diagnosis by the pathologists. The criteria were significantly more often fulfilled in infants compared with older children. Furthermore, the diagnosis of IND had no prognostic value for the outcome in constipated children, as assessed by clinical symptoms 1 year after the biopsies.[3]

Genetic studies in children with histologic features of IND could not identify any mutations in the coding regions of the *RET*, *GDNF*, *EDNRB*, and *EDN3* genes.[65,66] Mice with mutations of *Hox11L1*, a homeobox gene involved in nervous system development, show a megacolon with an ENS pathology with increased neuron numbers in the colon.[66] The human homolog of this gene has been found on chromosome 2p13.1,[67] but, to date, no patients with gastrointestinal motility disorder and proposed histologic features of IND have been found to have a mutation of this possible candidate gene.

Because the term IND describes neither a specific histologic nor a clinical entity, it should be used with caution or not at all until better defined. Therefore, IND has not been included in Table 46.2-1 and is not further discussed in this chapter.

Primary Visceral Neuropathies

Primary visceral neuropathies can be subdivided into familial visceral neuropathies, which follow a certain trait of inheritance, and sporadic cases characterized by distinct morphologic and clinical findings. In clinical practice, many neuropathic motility disorders remain unclassified (idiopathic).

Familial Visceral Neuropathy. *Familial Visceral Neuropathy without Extraintestinal Manifestations.* This is an autosomal dominant disorder that affects mainly the large and distal part of the small bowel.[68] Age at onset differs within the same family, but symptoms develop after infancy. Severe slow-transit constipation, with or without intermittent diarrhea, nausea, abdominal distention, and cramping, is the main complaint. Pseudo-obstructive

episodes with severe intestinal dilatation occur in approximately half of the patients and may require colonoscopic decompression. There is no evidence of extrinsic autonomic dysfunction. Histologic findings include a markedly reduced number and degeneration of argyrophilic neurons and nerve fibers of the myenteric plexus, with hypertrophy of the smooth muscle. No Schwann cell proliferation, intranuclear inclusions, or inflammatory cells are observed. Subtotal colectomy may give transient relief but may accelerate small bowel disease; therefore, it is contraindicated.

Familial Visceral Neuropathy with Neuronal Intranuclear Inclusions. This neuropathy is probably an autosomal dominant disorder because it was described in three siblings (two female, one male) and their father.[52] Most patients develop symptoms during childhood. Gastrointestinal symptoms included dysphagia, diarrhea, constipation, and intestinal pseudo-obstruction. Autonomic dysfunction, ataxia, dysarthria, mental retardation, and dementia are part of this neuropathic disease. Characteristic eosinophilic intranuclear inclusions have been identified in the neurons of the degenerated myenteric plexus all along the gastrointestinal tract.[11] The same inclusions are found postmortem in nerve cells of the central and peripheral nervous systems.[69] In some patients, the intranuclear inclusions could be detected within the ganglion cells of the plexus mucosus on rectal biopsies.[52,70] Electron microscopy and histochemistry identified the inclusions as nonviral proteins. Manometric studies showed a typical neuropathic pattern with lack of phase III of the MMC and irregular, nonpropagated contractions in the small intestine. There is no treatment; some patients survive into mid- or late adulthood.

Familial Visceral Neuropathy with Short Bowel, Malrotation, and Pyloric Hypertrophy. This sequence has been described in several families.[71] Symptoms of functional obstruction occur in the neonatal period, and most patients die during the first year of life. Morphologic studies of the bowel showed abnormalities of the myenteric plexus, with shrunken, degenerated neurons, clumped chromatin within the nuclei, and lack of argyrophilic neurons. This syndrome may be inherited in an autosomal recessive pattern because it occurred in siblings of both genders and in families with consanguineous marriages.[71,72] An autosomal dominant pattern was likely in one family with an infected 2-year-old girl, her mother, and her maternal grandfather (personal observation, 2003). In addition to malrotation, short bowel, and pyloric hypertrophy, an abnormal ileocecal connection with an absent appendix was noted during laparoscopy in all three. Recently, Auricchio and colleagues performed linkage analysis in a family in which, over four generations, only the male members related through female members were affected. The authors assigned the defect to a gene locus on the chromosome Xq28 region but suggested that the same clinical entity may be associated with different loci.[73]

Familial Visceral Neuropathy with Neurologic Involvement. Several families with at least two affected siblings have been reported. Symptoms occurred during early childhood with neuropathic gastrointestinal dysmotilities and involvement of the peripheral or central nervous system. The trait of inheritance remains unclear. Faber and colleagues reported two Jewish families of Iranian origin with a progressive sensory and motor peripheral neuropathy, ophthalmoplegia, and hearing loss but no evidence of CNS involvement.[74] The description and combination of the pathologic findings make a mitochondrial disorder likely. Cockel and colleagues described a familial disorder defined by megaduodenum, dilatation of the small bowel, steatorrhea, mental retardation, and calcification in the subcortical white matter of the brain and basal ganglia.[75] The age at onset was during childhood. The myenteric plexus appeared normal on conventional light microscopy, but silver staining revealed a degeneration of argyrophobic cells. A similar case was reported by Navarro and colleagues.[11]

A de novo mutation of the *SOX10* gene with a heterozygous deletion of 1 bp (795delG) in the last coding exon with chronic intestinal pseudo-obstruction, peripheral neuropathy with unusual multiplication of nerve fascicles, and deafness since early infancy has been described in an 8-year-old girl.[76]

Familial Visceral Neuropathy (Ganglioneuromatosis) Associated with Multiple Endocrine Neoplasia Type IIB. Of the three different multiple endocrine neoplasia (MEN) type II syndromes (MEN IIA, MEN IIB, and isolated medullary thyroid carcinoma), MEN IIB is the only form that involves the gastrointestinal tract. The susceptibility gene for these autosomal dominant inherited disorders is the *RET* proto-oncogene located on chromosome 10, which encodes a receptor tyrosine kinase. The *RET* plays an important role in the development, function, and migration of cells of neural crest origin. In MEN IIB, 94% of cases are due to a single identical germline point mutation at codon 918 in exon 16. In the remaining patients, a mutation at codon 883 in exon 15 or no *RET* mutation was found. Approximately 50% of diagnosed cases seem to be due to de novo mutations. In most affected patients, gastrointestinal dysmotility is the first manifestation of the disease. It is important to be aware of possible other symptoms in combination with the typical features of MEN IIB (Table 46.2-2).

The characteristic pathologic finding of intestinal ganglioneuromatosis is an increased density of ganglion cells in the submucous and myenteric plexus, with penetration of hyperplastic nerves into the mucosal zone. Hyperplasia of the nervous tissue is characterized by large ganglionic nodes with numerous glial cells. Acetylcholinesterase staining reveals increased activity along the hypertrophic myenteric plexus and within the lamina propria, but less than that observed in Hirschsprung disease. These abnormalities are present along the entire gastrointestinal tract, from the mouth to the anus, including the appendix. Ganglioneuromatosis may be confused with isolated, diffuse, or segmental hyperganglionosis, characterized by an excess of intestinal neurons only or, preferentially, in the myenteric plexus (see below).

Colonic dysfunction with chronic constipation and bowel dilation are the prominent clinical features. If it presents at birth, this disorder may be confused with Hirschsprung disease. Serious complications of ganglioneu-

TABLE 46.2-2 FEATURES OF MULTIPLE ENDOCRINE NEOPLASIA TYPE IIB

GASTROINTESTINAL MANIFESTATIONS
Abdominal distention
Feeding problems
Dysphagia
Vomiting
Chronic constipation
Paradoxal diarrhea
Megacolon
Intestinal pseudo-obstruction
Ganglioneuromatosis
FACIAL ABNORMALITIES
Thickened and everted upper eyelids
Prominent eyebrows
Neuromas on eyelids and conjunctiva
Thickened corneal nerves
Elongated face
Thickened lips
Neuromas of buccal mucosa, tongue, and palate
MARFANOID HABITUS/MUSCULOSKELETAL MANIFESTATIONS
Tall stature
High-arched palate
Long extremities
Kyphoscoliosis or lordosis
Joint laxity
Pes cavus
Pectus excavatum
Slipped capital femoral epiphysis
DENTAL ABNORMALITIES
TUMORS
C-cell hyperplasia/medullary thyroid carcinoma
Pheochromocytoma
Parathyroid hyperplasia

Adapted from de Krijger RR et al.[76]

romatosis are failure to thrive, chronic intestinal pseudo-obstruction, severe colonic diverticulosis, and bowel perforation. Defunctioning ileostomy or elective subtotal colectomy for chronic constipation may be required. Gastrointestinal symptoms often develop years before other features of the syndrome are recognized and MEN IIB is diagnosed.[77] Searching for a *RET* mutation is recommended in patients with apparently isolated intestinal ganglioneuromatosis or hyperganglionosis proven by rectal suction biopsy or a resected bowel segment.[51] Early diagnosis of MEN IIB is essential to perform prophylactic thyroidectomy before medullary thyroid carcinoma develops and to screen for development of pheochromocytoma.[78]

Visceral Neuropathy Associated with Neurofibromatosis (von Recklinghausen Disease). Single or multiple neurofibromas in the small intestine occur in about 10% of patients with this autosomal dominant disorder. Intestinal neuromas may cause both functional and mechanical obstruction.

Nonfamilial or Sporadic Visceral Neuropathies. *Hypoganglionosis.* Hypoganglionosis is defined by a reduced number of ganglion cells in the myenteric plexus and in the submucous plexus in some cases. The location of the sample within the bowel is important for comparison with age-matched controls, and the same methods of tissue preparation (cutting, staining, counting) need to be applied to both. Whereas aganglionosis is easily recognized on rectal suction biopsies, hypoganglionosis of the myenteric plexus requires a full-thickness sample of adequate size (> 1 cm length). Because the subjective impression of hypoganglionosis can be very misleading, morphometric measurements are required using standardized neuron counts in a sufficient number of sections. The few published studies on neuronal density in control subjects gave very conflicting results, perhaps because of the different methods applied or the different selection criteria used for controls. Neuronal density is affected by the age of the patient, tissue freshness, different diseases, and intestinal dilatation, especially when the specimen is cut transversely to the long axis of the bowel. Sections should be at least 30 µm apart to avoid counting each neuron more than once. In postmortem specimens from non–gut-diseased children of different ages, Smith reported a mean neuronal density of 3.6 per mm for the jejunum, 4.3 per mm for the ileum, and 7.7 per mm for the colon, with no major differences between transversal and longitudinal sections.[79] Depending on the section thickness and various techniques used, neuronal density may differ by a factor up to 200.[5]

Hypoganglionosis can always be detected in a short segment, called the transitional zone, proximal to the aganglionic bowel in Hirschsprung disease. An extensively long hypoganglionic segment may cause symptoms of dysmotility after the pull-through operation. Non–Hirschsprung disease–related hypoganglionosis, with a clinical picture similar to the histology of the transition zone, has been reported in many children with severe chronic constipation. It was the most frequent diagnosis in two large series reported on children with chronic intestinal pseudo-obstruction owing to visceral neuropathy.[11,50] Navarro and colleagues reported hypoganglionosis of the myenteric plexus in 13 of their 26 patients.[11] Ganglions were smaller than normal and often infiltrated with collagen fibers. Ganglion cells were sparse and sometimes difficult to identify with conventional staining techniques. Immunostaining with different neuronal markers confirmed the paucity of the nervous tissue. Between the ganglion nodes, there were numerous and thickened Schwann nerve fibers that stained strongly with acetylcholinesterase. In the majority of their patients, hypoganglionosis was confined to the distal segment of the colon. Diffuse disease was reported in only two patients, one of whom presented with intestinal obstruction on the second day of life, had a severe course of the disease, and died of sepsis 1 year later. The second patient with diffuse hypoganglionosis presented at the age of 1 month with pseudo-obstructive episodes. She was the only patient in this series who developed extraintestinal symptoms with severe progressive peripheral neuropathy, absent deep tendon reflexes, ataxia, and tetraplegia. In addition to hypoganglionosis and Schwann cell hypertrophy, she had intranuclear inclusions within the enteric neurons and in peripheral nerve cells. Her disease fit the pattern of familial visceral neuropathy with intranuclear inclusions described above. This case illustrated that visceral hypoganglionosis (like hyperganglionosis; see below) is not confined to a certain disease entity. The second series

of children with intestinal pseudo-obstruction owing to visceral neuropathies was reported by Krishnamurthy and colleagues.[50] They performed hematoxylin and eosin and silver staining on biopsies from 26 children aged 2 months to 10 years and 14 control infants who died of several non–gut-related causes. They distinguished three groups of patients by different morphologic criteria. Group 1 consisted of three children with absence of the myenteric plexus by hematoxylin and eosin and silver staining but the presence of submucosal neurons. Group 2 included four patients with hypoganglionosis of the myenteric plexus by hematoxylin and eosin staining but no nerve tracts and meshlike structures of the myenteric plexus by silver staining. Group 3 consisted of 19 patients with normal or slightly abnormal neuron density but deficiency of argyrophilic neurons by silver staining. The latter finding has to be interpreted with caution in infants because argyrophilic neurons were found to be absent in preterm babies and could be detected in only some non–gut-diseased infants until the age of 1 year.[6] Many neurons in these patients were abnormal, with scant cytoplasm, enlarged nuclei, and prominent chromatin. Argyrophilic neurons, when present, were small and had few processes. The authors speculated that a maturational arrest at different stages of development could be responsible for the findings. In the first two groups, migration of the neurons may have been arrested or hampered, whereas in the third group, the maturation process of normally migrated neurons was disturbed. Maturation of enteric neuronal structures continues after birth. This postnatal maturation process may explain why some of the patients with pseudo-obstructive symptoms at birth show improvement or complete resolution of their gastrointestinal dysmotility during the first years of life.

The clinical course of patients with hypoganglionosis varies markedly. Most cases present in the newborn period with symptoms of Hirschsprung disease, but some become symptomatic as late as 4 years of age. Most undergo operation (ileostomy, colonic resection, and pull-through procedure), which relieves symptoms in only a few; many children remain dependent on parenteral nutrition or die. This diverse outcome emphasizes that clinical decisions should not be based on the morphologic finding of enteric hypoganglionosis.

Hyperganglionosis. Hyperganglionosis is characterized by an excess of intestinal neurons in the myenteric plexus with or without involvement of the submucous plexus. Knowledge of normal neuronal density is a conditio sine qua non for this diagnosis. Because hyperplasia of the submucosal plexus with large ganglia containing more than seven ganglion cells (giant ganglia) was one of the main criteria of IND, the age dependency of neuronal density is relevant to the accuracy of diagnosis. Several authors reported a significantly higher proportion of infants, especially newborn babies, fulfilling these criteria compared with older children.[3,58,80] In non–gut-diseased children, a higher neuronal density in the submucosal plexus is found in infants than in older children.[81] Using whole-mount preparations stained with reduced nicotinamide adenine dinucleotide phosphate diaphorase histochemistry (identical to nitric oxide synthetase) and cuprolinic blue (a general neuronal marker), Wester and colleagues described an exponential decrease of ganglion cells within the myenteric plexus meshwork during the first 4 years of life.[5] Therefore, the finding of hyperganglionosis in a young infant with dysmotility needs to be interpreted with caution.

Severe hyperganglionosis is the hallmark of the visceral neuropathy (ganglioneuromatosis) occurring in patients with MEN IIB. In fact, many of the young children reported to have severe hyperganglionosis later developed medullary thyroid carcinoma; some of them were initially labeled as having intestinal neuronal dysplasia.[51,82] Severe myenteric and submucous plexus hyperplasia with signs of penetration of hyperplastic nerves into the mucosal were typical findings in children with MEN IIB, reported by Navarro and others.[11] In contrast, four patients with no signs of MEN IIB on follow-up showed only mild to moderate hyperganglionosis. Their findings were much more pronounced in the myenteric than in the submucosal plexus. Three patients presented with obstructive symptoms and one with enterocolitis during the neonatal period. Two of these children became asymptomatic after ileostomy, and the other two recovered without surgical treatment.

These observations underline the quantitative and qualitative differences between isolated hyperganglionosis and ganglioneuromatosis associated with MEN IIB. Infants and children with severe hyperganglionosis, especially when the submucous plexus is involved, should be investigated for a mutation within the *RET* gene, regardless of a negative family history for MEN IIB. In contrast, mild hyperganglionosis in a young infant seems to be have a much better prognosis and may be an age-related variation of the normal enteric maturation process.[5] Further work is needed, using more sophisticated techniques, to clarify whether isolated mild or moderate segmental hyperganglionosis is a distinct entity that causes long-term motility problems.

Hyperplasia of the myenteric and submucous plexus proximal to the transition zone has been reported in patients with Hirschsprung disease.[3] Most of them had increased acetylcholinesterase activity in the lamina propria of the affected bowel segment. The significance of these findings for motility problems after the pull-through procedure remains controversial. Nevertheless, patients with disabling motility disturbances after a pull-through procedure or after colostomy, who have proven hyperganglionosis in both plexuses, may benefit from resection of the affected part of the colon. Interestingly, mucosal neuromas or extraintestinal features of MEN IIB have not been reported in patients with Hirschsprung disease–associated hyperganglionosis. The *RET* mutation found in aganglionosis results in a loss of function effect, whereas in MEN IIB mutations, a common gain of function effect has been shown. It is unknown whether patients with Hirschsprung disease–associated hyperganglionosis have more mutations in the *RET* gene than patients without these morphologic findings in the bowel proximal to the aganglionic segment.

Qualitative Abnormalities of Ganglion Cells. These have been reported in children of all ages presenting with primary intestinal motility disorders. These morphologic

findings may be an expression of immaturity or degeneration and are almost always seen in hypo- or hyperganglionosis. However, qualitative abnormalities may be detected in patients with no changes of neuronal density and normal-looking enteric nerve plexuses on conventional light microscopy,[11,50] even when more sophisticated techniques, including Smith silver stain and immunohistochemistry for different neural markers and excitatory and inhibitory neurotransmitters, are applied.[80,83] The qualitative pathologic changes may occur as an isolated visceral neuropathy but also in association with malrotation of the bowel, gastroschisis, omphalocele, or other gut malformations or in combination with extraintestinal abnormalities.[84] Clinical symptoms are nonspecific and indistinguishable from other enteric neuropathies.

Secondary or Acquired Visceral Neuropathies

In secondary or acquired neuropathies, the damage of the ENS is due to a known agent (ie, toxic, infectious) or secondary to a systemic disease (ie, endocrine or metabolic disorder, chronic inflammation). Secondary visceral neuropathies may manifest at any age, even after birth if the damaging agent was present in utero. They occur less often in children compared with adults because it may take many years to cause neuronal damage severe enough to cause dysmotility.

Infectious Agents. Infectious etiologies are proposed for several functional bowel disorders. Most symptoms, such as abdominal pain, fullness, or nausea after enteritis, are transient and resolve over weeks or months. Chagas disease caused by infection with *Trypanosoma cruzi* is a well-known example of ENS destruction; neuronal degeneration is not caused by the parasite itself but is due to an autoimmune response elicited by the infection. The most common clinical presentation is achalasia, followed by dysfunction of the intestine, including episodes of chronic intestinal pseudo-obstruction. Obstructive episodes have also been observed during acute Lyme disease. A viral etiology was suspected in patients infected with neurotropic viruses from the herpes virus family, including cytomegalovirus, varicella-zoster virus, Epstein-Barr virus, herpes simplex virus type 1, and human immunodeficiency virus (HIV).[85,86] Debinski and colleagues provided the first proof for a viral etiology by identifying Epstein-Barr virus in the myenteric plexus of a patient with chronic pseudo-obstruction.[87] Full-thickness biopsies of the patient showed myenteric inflammatory infiltrates but no evidence for a myopathy. Because the authors did not identify virus material in the tissue of 12 other patients with chronic pseudo-obstruction, they concluded that viral infection is a rare cause of severe intestinal dysmotility. Besnard and colleagues reported a 13-year-old boy with intestinal pseudo-obstrurction, acquired hypoganglionosis, and severe acute dysautonomia related to Epstein-Barr virus reactivation.[88]

Toxic Agents. Abnormal antroduodenal manometry suggestive of enteric neuropathy was reported in five children with fetal alcohol syndrome.[89] All patients presented during infancy with disabling symptoms of gastrointestinal dysmotility, including vomiting, abdominal pain and distention, constipation, and intermittent diarrhea, resulting in failure to thrive. However, prior to referral between the ages of 20 months and 9 years, all patients had undergone multiple operations. Nissan fundoplication, with pyloric emptying procedures, was performed in four children and bowel resection in one. Because no tissue was available from these patients, it remains undetermined whether the observed manometric motility pattern was the direct result of in utero alcohol neurotoxicity on the enteric nerves. In a rat model, ethanol ingestion from the sixth to the twelfth day of pregnancy showed histologic and structural changes in the offspring's colon and bladder wall compared with those of control rats.[90]

Drugs. Drugs may affect gastrointestinal motility as a main target organ by intent (eg, various prokinetics or loperamide) or as unwarranted side effects (ie, opiate analgesics, macrolides, or anticonvulsive drugs). Symptoms such as diarrhea, abdominal cramping, or constipation resolve as soon as the drugs are stopped. Two infants with hypoperistalsis and idiopathic intestinal pseudo-obstruction have been reported who were treated during the perinatal period with zidovudine.[91] In both, symptoms resolved when the drug was discontinued. Nonreversible visceral neuropathy owing to long-term intake of cathartics or chemotherapeutic agents has been reported in adults.

Radiation. Radiation damages all structures of the small and large bowel, including the mucosa, blood vessels, connective tissue, nerves, and smooth muscle. Acute manifestations usually subside within weeks, whereas late complications may appear months to decades after radiotherapy. Most affected patients are adults. Symptoms of the late radiation enteropathy include diarrhea, urge defecation, abdominal pain, nausea, vomiting, bloating, and pseudo-obstructive episodes. Late radiation injury is characterized by vascular degeneration and intestinal wall fibrosis evident in all layers of the gut wall. Both neuronal and muscular structures of the bowel are affected and contribute to the motility disorder. Contrast studies may show dilated loops, hypoperistalsis, and a thickened wall. Antroduodenal manometry showed a wide variety of abnormalities, preferentially in patients with more severe symptoms and malnutrition.[92] The major features were an attenuated postprandial motor response and a reduced intensity of the MMC during the night.

Chronic Inflammation and Autoimmune Disease. Gut dysmotility has been reported in patients with different noninfectious inflammatory diseases. Chronic and acute transmural inflammation as in Crohn disease or post–necrotizing enterocolitis causes fibrosis and ischemia and may damage both enteric nerves and muscular structures of the bowel. Dysmotility in untreated celiac disease is probably due to mucosal atrophy, resulting in disturbed secretion of several gastrointestinal hormones. Neuropathic changes have not been reported. Common complaints are symp-

toms of delayed gastric emptying, abdominal distention, and bloating. Hypoperistalsis of the small and large bowel results in constipation in about 10% of untreated celiac patients in spite of malabsorption. A few celiac patients with intestinal pseudo-obstruction have been described. Symptoms of dysmotility resolve on a strict gluten-free diet. Two patients have been reported with an autoimmune visceral neuropathy (ganglionitis) and acquired progressive aganglionosis as a result of a severe T cell–mediated inflammatory ganglionitis of both enteric plexuses.[93] Both patients became symptomatic during childhood, with severe constipation, anorexia, abdominal distention, and abdominal pain, and later developed pseudo-obstructive episodes. No extraintestinal involvement or autonomic dysfunction was observed. High titers of an immunoglobulin G class autoantibody directed against enteric neurons were found in both patients. There was no evidence of a paraneoplastic syndrome because no tumor was found during a period of up to 8 years. The autoimmune inflammatory nature of this acquired severe neuropathy with secondary aganglionosis is supported by the improvement of symptoms during treatment with prednisone and relapse after withdrawal of steroids.

Autonomic Neuropathy. The autonomic nervous system constitutes one of three levels of control of gastrointestinal motor function and interacts with the ENS and the excitable smooth muscle cells. Therefore, it is not surprising that disorders affecting the extrinsic nervous system of the gut cause intestinal dysmotility, even in the absence of morphologic abnormalities of the ENS. About half of the children with the autosomal recessive inherited familial dysautonomia (Riley-Day syndrome) develop gastrointestinal problems.[94] Attacks of severe vomiting affect children after the age of 3 years and are one of the most disturbing afflictions of the disorder. The crises usually last less than 24 to 72 hours and are associated with hypertension, sweating, and erythematous blotching of the skin. Severe gastric distention and abdominal pain may accompany the attacks. The children are at high risk for profound dehydration and aspiration. Children with triple A syndrome (achalasia-addisonian-alacrimia syndrome or Allgrove syndrome), an autosomal recessive disorder, often suffer from a variety of neurologic features with autonomic dysfunction, including gastrointestinal dysmotility with vomiting, retching, diarrhea, or constipation.[95] The *AAAS* gene has been mapped on chromosome 12q13 and encodes a protein, which is involved in signal transduction, ribonucleic acid processing, and transcription.[96]

An acquired idiopathic autonomic neuropathy was described in 27 patients aged 7 to 75 years from the Mayo Clinic, most of whom had an acute or subacute onset of symptoms.[97] In 16 cases, a presumed viral infection preceded symptoms. Gastrointestinal symptoms, reported in 19 patients, consisted of various combinations of nausea, vomiting, diarrhea, and severe constipation. Most of the patients had a partial recovery without relapse. An immune-mediated mechanism comparable to Guillain-Barré syndrome was considered a possible cause by the authors.

Autonomic neuropathy owing to poorly controlled diabetes mellitus is common in adults but rarely reaches clinical significance in childhood. Gastroparesis and diarrhea are the main manifestations. Celiac disease needs to be excluded because it affects 3 to 5% of children with insulin-dependent diabetes mellitus (see Chapter 44.1, "Celiac Disease").

Endocrine Disorders. Several endocrine disorders with decreased or excessive hormone release may manifest with symptoms of gastrointestinal dysmotility. Severe slow-transit constipation or even pseudo-obstructive episodes may be the presenting symptom of hypothyroidism, whereas diarrhea with or without steatorrhea owing to rapid intestinal transit occurs in hyperthyroidism. Impaired gut contractile activity with small bowel dysmotility and steatorrhea has been observed in children with hypoparathyroidism, especially when it is part of a polyendocrine deficiency syndrome. Tumors producing catecholamines or vasoactive peptides, that is, neuroblastoma, pheochromocytoma, carcinoid, ganglioneuroblastoma, VIPoma, and gastrinoma, may present with dilated small bowel loops and dysmotility in addition to watery diarrhea.[98]

Metabolic Disorders. Several metabolic disorders, with or without electrolyte disturbances, may result in acute or chronic gastrointestinal dysmotility (eg, organic aciduria, end-stage liver disease, or renal disease). In some disorders, material stored within the bowel wall may result in permanent damage of the neuromuscular structures (ie, Fabry disease, amyloidosis).

Anorexia Nervosa and Bulimia. Patients with eating disorders frequently complain of bloating, early satiety, fullness, and constipation. Delayed gastric emptying and slow bowel transit have been documented. Symptoms improve or resolve when the behavioral problem is successfully treated.

Acute Colonic Pseudo-obstruction (Ogilvie Syndrome). This is a severe form of adynamic ileus with massive dilatation of the colon in the absence of mechanical obstruction. It occurs in hospitalized patients with a wide variety of medical and surgical conditions. Most cases respond to conservative management. Colonoscopic decompression is often performed to prevent ischemia and perforation, but it is not without risk. Intravenous neostigmine rapidly decompresses the colon in adult patients and may be tried in children with this condition who do not respond to conservative treatment.[99]

DISORDERS OF THE INTERSTITIAL CELLS OF CAJAL

Throughout the gastrointestinal tract, there are non-neural cells derived from mesenchymal precursors, the ICC, that generate and propagate slow waves. These cells are very important modulators of communication between nerves and muscle, and loss or defects of ICC significantly com-

promise neuronal regulation of gastrointestinal motility.[99,100] ICC can be identified in normal tissue by their immunoreactivity for the tyrosine kinase receptor Kit and by ultrastructural criteria in transmission electron microscopy. However, some limitations and pitfalls are reported with respect to identification of ICC in disease states: The ultrastructural criteria for ICC may not apply to pathologic conditions, and absence of detectable Kit-positive cells may not only indicate loss of ICC but may also be due to artefactual loss of Kit positivity by ICC. For example, Kit positivity is impaired by paraffin embedding, and certain antigen retrieval procedures are mandatory to detect Kit positivity on such material.[99] In addition, long-standing dilatation of the intestine or hypertrophy makes it difficult to quantify the number of both neuronal cells and ICC and may result in an underestimation or overestimation of the cell density. Most important, any changes with respect to ICC observed in tissue from patients with advanced intestinal motility problems may be the cause of the disease or be secondary to the disease process. In animal models, it could be demonstrated that chronic bowel obstruction or chronic inflammation results in defects in ICC networks.[101] Therefore, at this point, we can only summarize the recent knowledge of the role of ICC in human gastrointestinal motility disorders.

Delayed Maturation of ICC

Two infants, one of whom was born prematurely, with neonatal intestinal pseudo-obstruction, have been reported who showed lack of ICC in the intestine at the side of ileostomy.[102] The intestinal motility normalized over the first months of life, and at the time of closure of the stoma, a normal distribution of Kit-positive ICC was documented. The authors speculated that a developmental delay of Kit-positive ICC may have been the underlying mechanism. Their finding was confirmed in six other children with meconium ileus who required ileostomy during the neonatal period. No Kit-positive ICC were detected in the colonic biopsies of two children, and there was a scanty distribution in the remaining four compared with controls. All children showed a normal pattern of ICC when the ileostomies were closed between 39 and 104 days of age.[103]

Reduced Density or an Abnormal Distribution of Kit-Positve ICC

This has been described in patients with children with hypoganglionosis,[104] in a woman with intestinal pseudo-obstruction and megaduodenum owing to myopathy,[105] and in the sigmoid colon of adults with slow-transit constipation.[106] However, the cause/consequence relationship in these cases remains unresolved.

For the diagnostic and therapeutic approaches to motility disorders, the reader is referred to Chapter 46.4 and Chapter 76.3, "Motility."

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3. Hirschsprung Disease

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Hirschsprung disease is the congenital absence of the enteric nervous system (ENS) extending continuously for a variable distance to the internal anal sphincter. The ENS consists of ganglion cells in the myenteric and submucosal plexuses and associated connecting nerves within the wall of the intestine. Short-segment Hirschsprung disease is restricted to the rectum and sigmoid colon and accounts for nearly 90% of cases. Long-segment Hirschsprung disease generally describes disease that begins proximal to the sigmoid colon. Long-segment disease accounts for approximately 10% of cases. This includes disease that involves the entire colon (total colonic aganglionosis; approximately 5% of cases) and portions of the small bowel.[1] Rarely, aganglionosis includes most or all of the small intestine.[1–4] The existence (and definition) of an "ultrashort" form of the disease is debated.

In recent years, great progress has been made in our understanding of the molecular genetics of this disorder, which appear to be quite complicated. Hirschsprung disease has become a paradigm for multigene disorders because the same basic phenotype is associated with mutations in at least seven distinct genes. This chapter discusses the history, diagnosis, and treatment of the disorder as well as the current understanding of the pathogenesis and genetics of Hirschsprung disease and the potential practical applications of this information.

HISTORY

Harald Hirschsprung provided the first detailed description of the disease that bears his name in 1886 when he reported the autopsy of two infants, age 8 months and 11 months, who presented with constipation and megacolon.[5] In the two centuries prior to Hirschsprung's classic description, more than 13 individuals described a clinical entity suggestive of congenital distal intestinal aganglionosis. The earliest was Frederick Ruysch, a Dutch anatomist, who described a 5 year old with "enormis intestini coli dilatatio" (enormous dilatation of the colon) on autopsy in 1691.

After Hirschsprung's description, great debate ensued regarding the etiology of this disease. It was not until 1948, when Whitehouse and Kernohan and Zuelzer and Wilson independently confirmed an absence of ganglion cells in affected individuals, that this abnormality was widely accepted as the cause of Hirschsprung disease.[6,7] At roughly the same time, Orvar Swenson performed the first successful operation for the disease, involving resection of the aganglionic bowel with anastomosis of ganglionic bowel near the internal anal sphincter.[8]

EPIDEMIOLOGY

The general incidence of Hirschsprung disease is approximately 1 in 5,000 live births.[9–11] Recent studies suggest that incidence rates may vary among different ethnicities, with incidence rates in whites, blacks, Asians, and Pacific Islanders of 1 in 7,000, 1 in 5,000, 1.4 in 5,000, and 3.8 in 5,000, respectively.[12,13] The male-to-female ratio for Hirschsprung disease is 4:1 for short-segment disease and approaches 1:1 as the length of involved segment increases. Overall, 7% of cases have a family history of the disorder. However, the familial prevalence is 21% in individuals with total colonic disease.[14]

Approximately 15% of individuals with Hirschsprung disease present with at least one other congenital anomaly.[15,16] Excluding individuals with Down syndrome, cardiac anomalies are found in 4.5%, central nervous system anomalies are found in 3.9%, genitourinary anomalies are found in 5.6%, and other gastrointestinal anomalies are found in 3.9% of individuals with Hirschsprung disease.[15] In some instances, these anomalies may constitute part of a known syndromic disorder (Table 46.3-1).

Chromosomal anomalies are seen in 12% of individuals with Hirschsprung disease. Trisomy 21 is the most commonly associated chromosomal abnormality and is found in 2 to 8% of individuals with Hirschsprung disease.[1,10,15,17]

PATHOPHYSIOLOGY AND MOLECULAR GENETICS

ENS precursors are derived from the neural crest and colonize the gut in a cranial-to-caudal progression between weeks 5 and 12 of embryogenesis. Hirschsprung disease is caused by failure of the ENS precursors to colonize the distal intestine.[18] Absence of the myenteric and submucosal nerve plexus in the affected bowel results in inadequate relaxation of the bowel and bowel wall hypertonicity (see Chapter 4, "Motility," for further details). The hypertonic bowel causes intestinal obstruction, which can lead to bacterial overgrowth and enterocolitis.

The inheritance pattern of Hirschsprung disease can be autosomal dominant or recessive. The genetics of Hirschsprung disease displays three characteristics: (1) the penetrance of mutations is generally low, (2) there is a

TABLE 46.3-1 ANOMALIES ASSOCIATED WITH HIRSCHSPRUNG DISEASE

SYNDROMES	KEY FEATURES
Chromosomal*	
Down	Mental retardation with characteristic features
Syndromes requiring Hirschsprung disease	
Goldberg-Shprintzen†	Cleft palate, hypotonia, mental retardation, facial dysmorphism
Shah-Waardenburg‡	Pigmentary abnormalities (white forelock, depigmentaton of skin, premature graying, heterochromic irides), and sensorineural deafness
Hirschsprung disease with distal limb anomalies (several syndromes)	Polydactyly, brachydactyly, or nail hypoplasia with other assorted anomalies
BRESHEK	Brain abnormalities, mental retardation, ectodermal dysplasia, skeletal malformation, ear/eye anomalies, kidney dysplasia (ie, BRESHEK with Hirschprung disease)
Syndromes with Hirschsprung disease as an occasional finding	
Mowat-Wilson†	Facial dysmorphic features, mental retardation
Congenital central hypoventilation	Abnormal autonomic control of respiration
Bardet-Biedl	Pigmentary retinopathy, obesity, hypogonadism, mild mental retardation, postaxial polydactyly
Multiple endocrine neoplasia IIA	Medullary thyroid carcinoma, pheochromocytoma, parathyroid hyperplasia
Kaufman-McKusick	Hydrometrocolpos, postaxial polydactyly, congenital heart defect
Smith-Lemli-Opitz	Growth retardation, microcephaly, mental retardation, hypospadias, syndactyly of second and third toes, dymorphic features
Cartilage-hair hypoplasia	Short-limb dwarfism, metaphyseal dysplasia, transient macrocytic anemia, immunodeficiency, fine and sparse blond hair
Syndromes with a possible association with Hirschsprung disease§	
Fukuyama congenital muscular dystrophy	Muscular dystrophy, polymicrogyria, hydrocephalus, mental retardation, seizures
Clayton-Smith	Dysmorphic features, hypoplastic toes and nails, deafness, ichthyosis
Kaplan	Agenesis of the corpus callosum, adducted thumbs, ptosis, muscle weakness
Dermotrichic	Alopecia, ichthyosis, mental retardation, seizures
Okamoto	Hydrocephalus, cleft palate, agenesis of the corpus callosum, familial dysautonomia

Adapted from Chakravarti A and Lyonnet S.[46]

*Hirschsprung disease is also seen in association with the following chromosomal disorders: chromosome 2p deletion syndrome, chromosome 22q11.2 deletion, cat-eye syndrome (supernumerary dicentric chromosome 22q), chromosome 20p deletion, and deletions/duplications of chromosome 17q21-q23.

†Goldberg-Shprintzen and Mowat-Wilson syndromes may or may not be related disorders.

‡Other neurocristopathies are reported in association with Hirschsprung disease that likely share a molecular basis with Shah-Waardenburg syndrome. These include Yemenite deaf-blind hypopigmentation (Online Mendelian Inheritance in Man database [OMIM] #601706), ABCD syndrome (OMIM #600501), familial piebaldism (OMIM #172800), and congenital deafness.

§Hirschsprung disease is also reported in association with the following syndromes: Pallister-Hall, Fryns, Jeune asphyxiating thoracic, frontonasal dysplasia, osteopetrosis, Goldenhar, Lesch-Nyhan, Rubinstein-Taybi, Toriello-Carey, and spondyloepimetaphyseal dysplasia with joint laxity.[46]

sex difference in the penetrance and expression of mutations, and (3) the penetrance of a gene mutation depends on the extent of aganglionosis in affected family members. Most identified gene mutations associated with Hirschsprung disease are best thought of as susceptibility factors. That is, the mutation increases the individual's odds of having Hirschsprung disease but is not predictive of the abnormality.

Multiple Hirschsprung disease susceptibility genes are identified, primarily through studies of animal models (Table 46.3-2). Among the identified gene mutations are two signaling pathways, one involving the RET receptor and the other involving the endothelin B receptor.

The *RET* gene encodes a transmembrane receptor, tyrosine kinase. Mutations of *RET* were the first gene mutations identified in Hirschsprung disease.[19,20] Inactivation of the tyrosine kinase domain of RET is associated with Hirschsprung disease and accounts for up to 50% of familial cases of Hirschsprung disease.[19–22] Mutations in *RET* are more common in long-segment Hirschsprung disease, where they can be identified in up to 75% of individuals.[23] Mutations in the gene encoding the RET ligand, glial cell line–derived neurotrophic factor, are also associated with Hirschsprung disease, and loss of function mutations in its coreceptor, glial cell line–derived neurotrophic factor family receptor α-1 (GFRα-1), cause a similar disease in mice.[24–26]

RET mutations are dominant, with generally low penetrance. In families with low-penetrance *RET* mutations, the *RET* mutation likely represents a weakly expressing allele, and other gene mutations are necessary to express Hirschsprung disease. One possible modifying gene resides at chromosome 9q31.[27] Germline *RET* mutations also cause multiple endocrine neoplasia (MEN) syndrome IIA and IIB, as well as familial and sporadic cases of medullary thyroid carcinoma.[28–32] These mutations are generally activating mutations. Interestingly, some of the same *RET* mutations that cause Hirschsprung disease cause MEN IIA.[33] The pathophysiologic basis for this overlap is unclear, but it suggests that some individuals with Hirschsprung disease and their family members may be at risk for neuroendocrine tumors.[26,34]

RET and GFRα-1 are expressed in ENS precursors as they migrate through and colonize the gut during embryogenesis.[24, 35] Glial cell line–derived neurotrophic factor is expressed by the mesenchyme of the developing gut.[36,37] Mice deficient in ret signaling display an absence of ENS

TABLE 46.3-2 GENE MUTATIONS ASSOCIATED WITH HIRSCHSPRUNG DISEASE

GENE	GENE PRODUCT	HUMAN LOCUS	% OF HIRSCHSPRUNG DISEASE	HOMOZYGOTE PHENOTYPE	HETEROZYGOTE PHENOTYPE	PENETRANCE (%)	ANIMAL MODEL
RET	Receptor tyrosine kinase	10q11.2	Familial, 50; sporadic, 15–35[21]	Long-segment Hirschsprung disease (1 case reported)[4,23]	Hirschsprung disease	51–72[21]	Mouse
GDNF	Glial cell line–derived neurotrophic factor (RET ligand)	5p12–13.1	< 1[26,116]	None reported	Hirschsprung disease	Unknown	Mouse
GFRA1	GDNF family receptor α_1	10q26	None reported	None reported	None reported	Unknown	Mouse
EDNRB	Endothelin B receptor	13q22	5[50,117]	Shah-Waardenburg syndrome (often with total intestinal aganglionosis)[21,43,118,119]	Hirschsprung disease (with or without features of Waardenburg syndrome)[43,120]	30–85[121]	Mouse, rat, horse
EDN3	Endothelin 3	20q13.2-13.3	< 5[122,123]	Long-segment Shah-Waardenburg syndrome[44,45]	Shah-Waardenburg syndrome (1 case reported)[124]	Unknown	Mouse
ECE1	Endothelin converting enzyme 1	1p36.1	< 1	None noted	Hirschsprung disease with cardiac defects, craniofacial defects, and autonomic dysfunction (1 case reported)[51]	Unknown	Mouse
SOX10	Sex-determining region on the Y chromosome–related high-mobility group box gene 10, transcription factor	22q13.1	< 1	None noted	Shah-Waardenburg syndrome often with other neurologic deficits.[62–65,125]	> 80[121]	Mouse
ZFHX1B (SIP1)	Zinc finger homeobox 1B (SMAD interacting protein 1), transcription factor	2q22	< 1	Hirschsprung disease with microcephaly, mental retardation, epilepsy, and characteristic facial features	Hirschsprung disease with microcephaly, mental retardation, epilepsy, and characteristic facial features	Unknown	None

development distal to the stomach along with renal anomalies.[24,25,38–40] Ret-expressing cells are multipotent progenitors of the mammalian ENS.[41] In mice, ret-deficient ENS precursors fail to colonize the gut beyond the gastric cardia and a reduced population of neurons and glia survives in the esophagus.[42]

Endothelins are 21–amino acid peptides that interact with G protein–coupled receptors and play a number of roles in mammalian physiology and development. Mutations in the endothelin B receptor and its ligand, endothelin 3, cause Hirschprung disease that may be associated with pigmentary abnormalities (regional hypopigmentation, white forelock, bicolored irides) and sensorineural deafness.[43–45] This combination of defects is known as Waardenburg syndrome type 4, or Shah-Waardenburg syndrome. Patients homozygous for mutations in the endothelin B or endothelin 3 gene express the complete Shah-Waardenburg syndrome, whereas patients heterozygous for mutations in these genes may have isolated Hirschsprung disease or Waardenburg syndrome.[46] Mutations in endothelin B are found in approximately 5% of individuals with isolated Hirschsprung disease, as are mutations in endothelin 3.[44,45,47–49] In one large, inbred kindred, the penetrance of an endothelin B mutation was greater in males than in females: whereas 85% of males homozygous for the mutation exhibited Hirschsprung disease, only 60% of females homozygous for the mutation exhibited Hirschsprung disease.[43] Unlike *RET* mutations, endothelin B mutations are often associated with short-segment Hirschsprung disease. Hirschsprung disease is reported in one patient with a large deletion near, but not including, the endothelin B gene. This suggests that nearby genomic rearrangements may affect endothelin B expression and may explain why mutations within the gene are not found more commonly in individuals with Hirschsprung disease.[50]

Mutations in the gene encoding endothelin-converting enzyme 1, a protease responsible for the biologic activation of endothelin 3, may also be associated with Hirschsprung disease. An endothelin-converting enzyme 1 mutation is reported in a single individual with Hirschsprung disease associated with cardiac, craniofacial, and other abnormalities.[51]

Endothelin B is expressed by early ENS precursors and endothelin 3 is expressed by the surrounding mesenchyme.[52] Studies in the mouse demonstrate that, in the absence of endothelin B activation, ENS precursors show abnormalities in gut colonization beginning in the region of the cecum. Several lines of evidence suggest that endothelin B activation during this phase of intestinal colonization is critical for complete colonization of the distal gut.[53–56] In vitro evidence indicates that endothelin 3/B signaling inhibits neuronal differentiation.[57,58] It is hypothesized that, by inhibiting differentiation, endothelin B signaling maintains the ENS precursors in a colonization-competent state. Mice and rats with mutations affecting endothelin B signaling are reported to exhibit abnormalities in the ganglia of ENS-containing bowel. These abnormalities are hypothesized to play a role in the prolonged dysmotility observed in some Hirschsprung disease patients after surgical resection of aganglionic bowel.[59–61]

Mutations in *SOX10* also cause Shah-Waardenburg syndrome.[62] Most *SOX10* mutations are dominant and frequently occur de novo. Although the penetrance of the identified mutations is high, some individuals with *SOX10* mutations may have only some of the features of Shah-Waardenburg syndrome.[63] They tend to have short-segment aganglionosis or even hypoganglionosis of the colon. Some of these individuals show other abnormalities involving the central or peripheral nervous system.[63–65] Not all patients with Shah-Waardenburg syndrome have identified mutations in the genes encoding endothelin B, endothelin 3, or SOX10.

SOX10 is a transcription factor that is expressed in early ENS precursors and appears to support their survival. In mice homozygous for *sox10* mutations, ENS precursors undergo apoptosis before they reach the foregut. Mice heterozygous for *sox10* mutations exhibit early delays in ENS precursor migration, although final gut colonization may be complete. This observation has led to the hypothesis that haploinsufficiency for sox10 selectively affects an early subset of ENS precursors.[66,67]

Inactivating mutations in the gene encoding the zinc finger homeobox 1B gene *ZFHX1B* (formerly *SIP1*) cause Mowat-Wilson syndrome (characteristic facial features, microcephaly, mental retardation) with or without Hirschsprung disease.[68] Individuals with Mowat-Wilson syndrome carry dominant de novo mutations in this gene.[68,69] ZFHX1B is believed to be a transcriptional repressor that directly binds promoters and interacts with Smad proteins.[68–70]

Identified gene mutations currently account for only about half of all cases of Hirschsprung disease. Our current understanding of the molecular genetics of Hirschsprung disease comes largely from studies of syndromic or long-segment disease. Much less is known regarding molecular genetics of the most common form of Hirschsprung disease, nonsyndromic, short-segment disease. Recently, three loci were identified as both necessary and sufficient to explain the incidence and recurrence risk of Hirschsprung disease in 49 nuclear families ascertained through short-segment Hirschsprung disease probands. One of these loci appears to be the *RET* gene, although the majority of the significant mutations are apparently in noncoding sequence. The other two loci involve as yet unidentified genes at chromosomes 3q21 and 19q12. Interestingly, this analysis suggests a significant difference in the parent of origin of the abnormal *RET* allele, with 78% of affected individuals carrying the maternally derived *RET* mutation.[71]

Finally, several factors are thought to contribute to the pathophysiology of Hirschsprung disease–associated enterocolitis. The major problem is likely bowel stasis secondary to the aganglionosis, although enterocolitis often occurs after resection of aganglionic bowel. Stasis is thought to lead to bacterial overgrowth and translocation of bacteria into the intestinal wall. Histologically, as the disease progresses, the epithelium becomes ulcerated, and perforation may occur. In addition, defects in intestinal epithelial defense mecha-

nisms are identified in Hirschsprung disease. These include luminal immunoglobulin A deficiency and altered mucin composition.[72,73] How these defects relate to the identified gene mutations in Hirschsprung disease is unclear.

CLINICAL MANIFESTATIONS

The clinical manifestations of Hirschsprung disease depend on the age of presentation and the length of aganglionic bowel. Kleinhaus and colleagues reported in 1979 that 40% of individuals with Hirschsprung disease are diagnosed in the first 3 months of life and 60% are diagnosed in the first year of life.[14] Over the past few decades, the mean age at diagnosis has decreased so that the majority of patients are now diagnosed in the first 3 months of life. Individuals with total colonic disease tend to be diagnosed earlier than individuals with short-segment disease.[74]

The neonate with Hirschsprung disease is usually full term, with normal birth weight. Failure to pass meconium on the first day of life should raise the suspicion of Hirschsprung disease. Approximately 94% of neonates with Hirschsprung disease fail to pass meconium in the first 24 hours of life (as opposed to 10% of neonates without Hirschsprung disease), and 57% of infants with Hirschsprung disease fail to pass meconium in the first 48 hours of life.[75] Abdominal distention, vomiting, constipation, and poor feeding are also commonly seen in neonates with Hirschsprung disease. Diarrhea is seen in approximately one-third of neonates and often indicates the presence of enterocolitis, the most common cause of morbidity and mortality in cases of Hirschsprung disease.

The infant or child with Hirschsprung disease usually presents with constipation that begins in infancy and responds poorly to medical management. Fecal urgency, stool-withholding behaviors, and fecal soiling are usually not noted. Poor weight gain, anemia, hypoalbuminemia, and bouts of diarrhea (associated with enterocolitis) are common. Urinary retention with enlarged bladder, ureter, and hydronephrosis may occur owing to ureteral compression.[76]

Physical examination may reveal a tympanitic distended abdomen with palpable fecal masses, particularly in the older infant and child. A rectal examination is essential to evaluate for imperforate anus, an anteriorly displaced anus, and anal stenosis. The digital rectal examination in Hirschsprung disease will demonstrate a normally placed anus that will easily admit the finger but may remain somewhat tight. Stool is generally not found in the distal rectum, and the rectal mucosa remains snug to the examiner's finger (the "finger in glove" sensation). Often there is an explosive release of air and liquid stool as the examiner's finger is removed. The patient with enterocolitis will have diarrhea and appear ill, usually with fever and lethargy.

DIFFERENTIAL DIAGNOSIS

The clinical history and physical findings are very valuable in differentiating Hirschsprung disease from other disorders. Hirschsprung disease should be considered in any child who has a history of constipation dating back to the newborn period. Suspicion should increase in any child with associated abdominal distention, vomiting, and/or poor growth. Suspicion should also increase in a child with a family history of Hirschsprung disease.

It is important to differentiate Hirschsprung disease from functional constipation (Table 46.3-3). The patient with Hirschsprung disease often has problems that begin in the neonatal period with delayed passage of meconium and poor growth. Stool caliber in Hirschsprung disease is often small and ribbon-like, whereas children with constipation occasionally pass large stools. Rectal examination in Hirschsprung disease generally fails to detect stool in the rectal vault.

In addition to functional constipation, the clinical findings associated with Hirschsprung disease can mimic several other clinical entities. Anatomic causes of intestinal obstruction, endocrine disorders, electrolyte imbalances, sepsis, and drug side effects should all be included in the differential (see Chapter 46.1, "Idiopathic Constipation").

INVESTIGATIONS

Radiographic Studies

Abdominal radiographs frequently show loops of distended intestine and may show a paucity of air in the rectum. Prone films are particularly helpful as this position facilitates the movement of air into the rectum of individuals without an obstruction. The presence of free air indicates a perforation and is more frequently seen in infants.[77]

TABLE 46.3-3 CLINICAL FEATURES DIFFERENTIATING HIRSCHSPRUNG DISEASE AND FUNCTIONAL CONSTIPATION

CLINICAL FEATURE	HIRSCHSPRUNG DISEASE	FUNCTIONAL CONSTIPATION
Age at onset	Under 1 yr of age	Over 1 yr of age
Passage of meconium	Delayed	Normal
Encopresis	Absent	Present
Growth	Poor	Normal
Abdominal pain	Rare	Frequent and colicky
Stool size	Small ribbon-like or pebble-like	Large
Stool-withholding behavior	Absent	Present
Abdominal examination	Distended	Not distended
Rectum	Empty	Filled with stool
Rectal examination	Explosive passage of stool	Stool in rectum

Because the proximal, ganglionic intestine may not be significantly dilated in the first few weeks of life, an unprepared contrast enema is most likely to aid in the diagnosis of Hirschsprung disease in children older than 1 month of age. Similar difficulties arise in interpreting contrast studies in individuals with total colonic aganglionosis (Figure 46.3-1A). Classic findings include a contracted distal colon with an abrupt transition to a widely dilated proximal colon (Figure 46.3-1B). In the absence of this finding, it is important to compare the diameter of the rectum with that of the sigmoid colon. A rectal diameter that is the same as or smaller than the diameter of the sigmoid colon is suggestive of Hirschsprung disease (Figure 46.3-1C). A radiograph taken 24 hours after the study may also be helpful in showing retained contrast in individuals with Hirschsprung disease.[78]

The transition zone demonstrated by the contrast study (and by gross inspection of the intestine) does not necessarily provide reliable evidence regarding the length of aganglionosis.[79] Only histologic analysis can determine how much bowel will need to be resected. Finally, a contrast enema should not be undertaken in a patient with clinical enterocolitis or recent rectal biopsy because the risk of perforation is increased.

Anorectal Manometry

Anorectal manometry is a measure of ENS function. Distention of a proximal colonic segment normally leads to relaxation of the neighboring distal segment. The technique looks for relaxation of the internal anal sphincter in response to distention of the rectum with a balloon (Figure 46.3-2). The reported sensitivity and specificity of the test vary widely.[80–82] In experienced hands, the technique can be quite sensitive.[83,84] However, the procedure generally requires some patient cooperation and can be difficult to perform on young children in the absence of sedation. In addition, the anorectal reflex may not be completely developed in premature or full-term infants less than 12 days of age,[85] and the technique can be technically difficult in very young infants. Anorectal manometery is best done by a very experienced operator.

Rectal Biopsy

Rectal biopsy is the gold standard for diagnosing Hirschsprung disease. The diagnosis is usually established by a rectal suction biopsy, which can be performed at the bedside or in an outpatient setting. Two difficulties most commonly arise with the technique. The first is in obtaining an adequate amount of submucosa to adequately evaluate the ganglion cells. The other difficulty is in getting biopsies from the appropriate location. A segment of hypoganglionosis exists normally in the submucosal plexus just above the dentate line. This ranges from 3 to 17 mm in length.[86] To avoid taking biopsies in this area, the general practice is to obtain suction rectal biopsies no closer than 2 cm above the dentate line. If an adequate specimen is obtained from the correct location, the test is highly accurate. The technique is also quite safe, although perforations and significant hemorrhages are reported.[87] Because the length of normal hypoganglionosis above the dentate line is shorter in the myenteric plexus, full-thickness biopsies can be obtained more distally than rectal suction biopsies. They are generally reserved for difficult cases in which rectal suction biopsies have failed to resolve the clinical question.

In experienced hands, examination of hematoxylin and eosin–stained sections from an adequate biopsy is sufficient to establish a diagnosis of Hirschsprung disease (Figure 46.3-3). Acetylcholinesterase staining on unfixed specimens can aid the diagnosis. Hypertrophied extrinsic nerve fibers in the lamina propria and muscularis mucosa are often, but not always, identified in the aganglionic bowel in Hirschsprung disease (Figure 46.3-4). Acetyl-

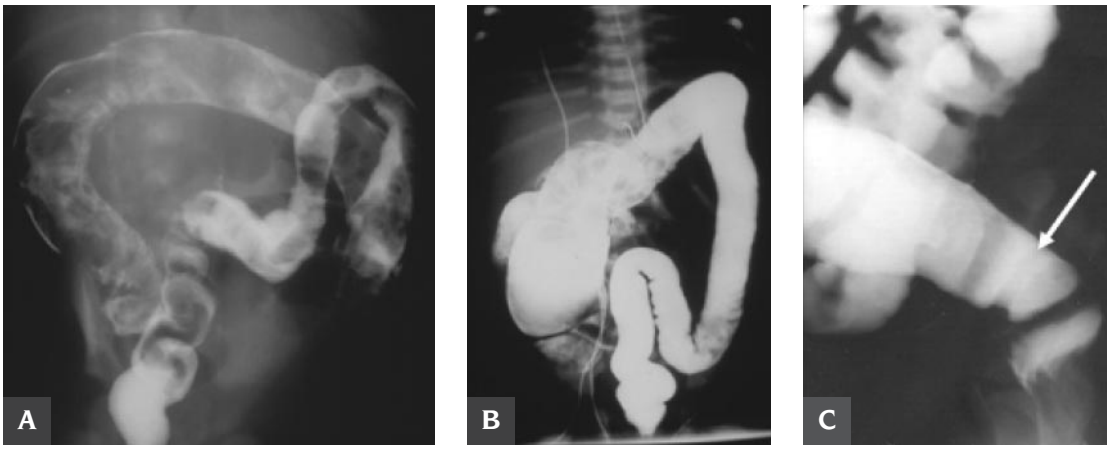

FIGURE 46.3-1 *A*, Contrast enema in an infant with total colonic aganglionosis. Note the consistent small caliber of the colon and the lack of an identifiable transition zone. Reproduced with permission from Teitelbaum D, et al.[89] *B*, Typical contrast enema appearance in long-segment Hirschsprung disease. The transition zone is in the ascending colon. Courtesy of Daniel Teitelbaum, Department of Pediatric Surgery, University of Michigan, Ann Arbor. *C*, Contrast enema of an infant with short-segment Hirschsprung disease. *Arrow* indicates rectum. Note that although the distal sigmoid is not dilated, the rectal diameter is smaller than that of the sigmoid colon. Courtesy of Peter Strouse, Department of Pediatric Radiology, University of Michigan, Ann Arbor.

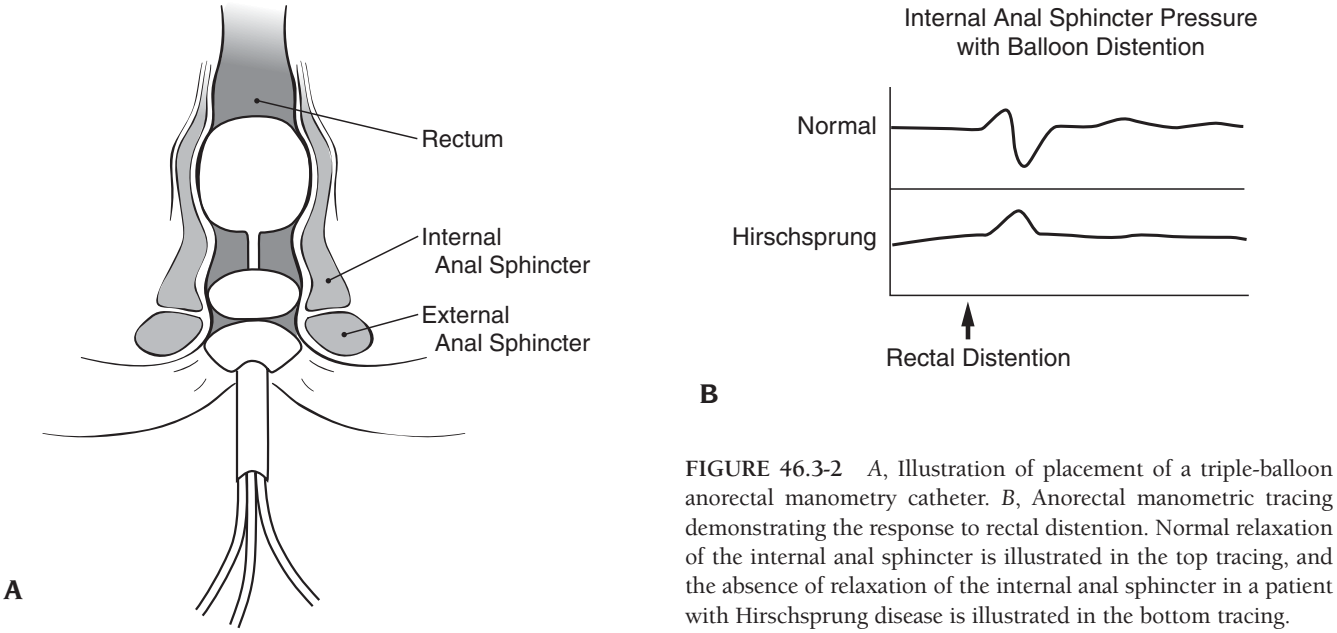

FIGURE 46.3-2 *A*, Illustration of placement of a triple-balloon anorectal manometry catheter. *B*, Anorectal manometric tracing demonstrating the response to rectal distention. Normal relaxation of the internal anal sphincter is illustrated in the top tracing, and the absence of relaxation of the internal anal sphincter in a patient with Hirschsprung disease is illustrated in the bottom tracing.

cholinesterase staining is most useful if the biopsy contains limited submucosa and no ganglion cells are identified. In this case, acetylcholinesterase staining typical for Hirschsprung disease can be diagnostic.[88]

Ultrashort-segment Hirschsprung disease is also referred to as anorectal achalasia and may represent a different pathophysiologic process. The major controversy with this disease entity is that the gold standard for diagnosing Hirschsprung disease (a rectal biopsy 3–5 cm above the dentate line) is normal. In addition, the clinical history is generally consistent with functional constipation (often with encopresis). Only anorectal manometry demonstrates the characteristic abnormality of Hirschsprung disease: failure of reflex relaxation of the internal anal sphincter on distention of the rectum. This disorder may go undiagnosed because many centers do not routinely perform anorectal manometry on patients with this presentation.

TREATMENT

Definitive treatment for Hirschsprung disease is surgical. Medical management is important in stabilizing the patient, especially if the patient has enterocolitis, and to adequately prepare the bowel for surgery.

The treatment of Hirschsprung disease–associated enterocolitis involves the placement of a soft rectal tube to decompress the colon and to allow saline washes. Patients generally need intravenous antibiotics. Rectal tube decompression and washes may not be effective in long-

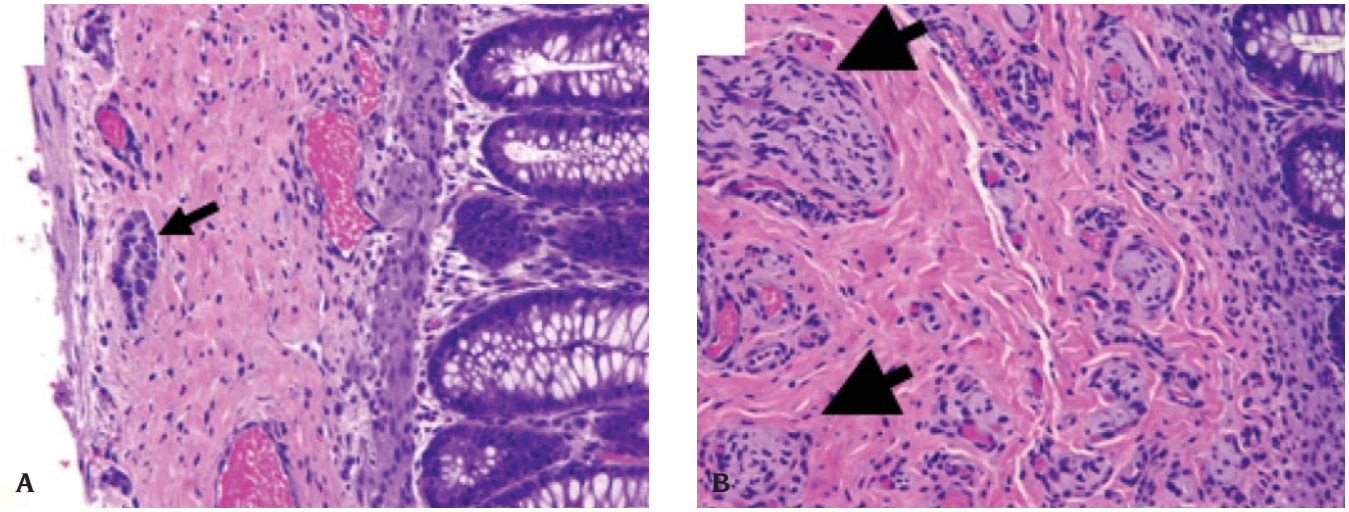

FIGURE 46-3-3 Hematoxylin and eosin–stained sections of a surgically resected specimen from a patient with Hirschsprung disease (×200 original magnification). *A*, Proximal section demonstrating normal myenteric ganglia (*small arrow*). *B*, Distal (rectal) section showing numerous hypertrophic peripheral nerves (*large arrows*). Ganglion cells are not identified. Photograph courtesy of Robert Ruiz, Department of Pathology, University of Michigan, Ann Arbor.

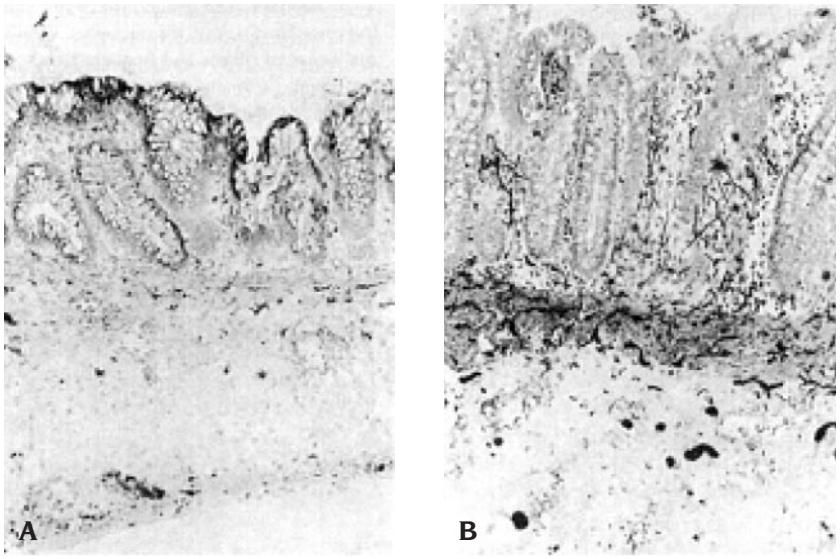

FIGURE 46-3-4 Acetylcholinesterase staining of rectal suction biopsy frozen sections. *A*, In an individual without Hirschsprung disease, staining is limited to a few nerve twigs in the muscularis mucosa. *B*, In Hirschsprung disease, staining reveals more and thicker nerve fibers in the muscularis mucosa and the lamina propria than in normal tissue. Reproduced with permission from Dahms B. The gastrointestinal tract. In: Stocker J, Dehner L, editors. Pediatric pathology. 2nd ed. Lippincott Williams and Wilkins; 2001. p. 631–704.

segment disease, in which case, a decompressing ostomy may be required.

The bowel should be prepared for surgery with warm saline enemas (10 mL/kg) through a rectal tube. In addition, the patient should be given a clear liquid diet for 2 to 3 days or a polyethylene glycol electrolyte solution (25 mL/kg/h) for 8 hours on the day prior to the procedure.[89] Hypertonic phosphate enemas should never be given to a patient with proven or suspected Hirschsprung disease. Retention of these enemas leads to hyperphosphatemia, hypocalcemia, hypokalemia, and metabolic acidosis. These abnormalities may result in tetany, dehydration, acute renal failure, cardiac arrest, and death.[90]

Four surgical pull-through approaches are commonly used in the treatment of Hirschsprung disease: the Rehbein, Swenson, Soave, and Duhamel procedures. Historically, obstruction was relieved in all infants with the creation of a stoma proximal to the aganglionic segment. Definitive surgery was delayed until the child weighed 9 to 10 kg (20 lbs) or until the dilated proximal segment regained a normal caliber. Currently, many infants undergo a primary pull-through procedure, which is often done laparoscopically. The results with the primary pull-through appear comparable to those with the staged procedure.[91–94]

In the Rehbein procedure (which is more frequently used in Europe than the United States), an anterior approach is used to remove the defective aganglionic tissue. The procedure leaves 3 to 5 cm of aganglionic colon above the dentate line (Figure 46.3-5A).

The Swenson procedure consists of dissection of the rectal wall distally to the level of the internal anal sphincter. The ganglion-containing bowel is pulled through the pelvis and anastomosed end to end to the rectum within 2 cm of the dentate line. Both a muscular and a mucosal anastomosis is created, and no muscular cuff is left in place (Figure 46.3-5B).

In the Soave procedure (endorectal pull-through), the aganglionic intestine is dissected circumferentially in the seromuscular layer distally to within 1.5 cm of the anus in older children and less than 1 cm in newborns. The ganglionic intestine is incised and pulled through the muscular cuff, and an anastomosis is created to the submucosal-mucosal tube close to the anus (Figure 46.3-5C).[89] The Soave procedure has been associated with a higher incidence of postoperative enterocolitis and constipation. This may be related to the length of the muscular cuff. Because of this concern, a muscular cuff that extends no more than 1 to 2 cm above the levator muscle complex is advocated.[89]

The Duhamel procedure involves positioning the proximal ganglionic intestine posterior to the rectum. The ganglionic bowel is anastomosed side to side to the posterior wall of the aganglionic rectum through an incision 1 to 2 cm proximal to the dentate line (Figure 46.3-5D). The Duhamel procedure has been modified by Martin. The Martin-Duhamel procedure involves an extended side-to-side anastomosis of the small intestine to the aganglionic left colon.

LONG-TERM COMPLICATIONS AND RESULTS

Hirschsprung disease–associated enterocolitis remains the major cause of morbidity and mortality in the disease. Enterocolitis may be a presenting complaint in Hirschsprung disease, but it also frequently occurs after pull-through. The published mortality rates range from 6 to 30%[14,95,96] and appear to be decreasing.[97] Individuals with Down syndrome are at increased risk for enterocolitis.[94,98] Patients with repeated episodes of enterocolitis after pull-through may require further evaluation and surgical intervention. Repeat resections and pull-through procedures are not uncommon.[99]

Anorectal stenosis is a frequent occurrence after pull-through and is associated with recurrent enterocolitis. Some patients can be managed by anal dilatations. Twenty to 30% of patients may require anal myectomy or myotomy for relief of constipation.[100]

After resection of aganglionic bowel, some patients are unable to absorb adequate nutrients and are dependent on parenteral nutrition. This includes patients with extensive

aganglionosis involving the small bowel and patients with dysmotility in the remaining bowel. Dysmotility may improve with time, and often no histologic abnormality in the remaining bowel can be identified. Rarely, individuals with Hirschsprung disease also exhibit intestinal neuronal dysplasia (IND B), which is characterized by hyperplastic submucosal ganglia (see Chapter 46.4, "Chronic Intestinal Pseudo-obstruction Syndrome").[101] In addition, Hirschsprung disease is identified in up to 20% of individuals diagnosed with IND B in the proximal intestine.[102] Although specific histologic features of IND B are defined, a recent study demonstrated high interobserver variation in making the diagnosis.[103] No genetic link is established between Hirschsprung disease and IND.[104] Finally, abnormalities in the network of interstitial cells of Cajal in the ganglion-containing bowel of patients with Hirschsprung disease are reported and may contribute to dysmotility.[105]

Fecalomas develop in a small subset of patients. Fecalomas in the rectum are frequently associated with the retention of a septum between aganglionic rectum and the pulled-through intestine in patients who have undergone the Duhamel procedure.

Long-term follow-up with individuals surgically treated for Hirschsprung disease reveals that ongoing difficulties with fecal incontinence and weight gain are not uncommon. Ludman and colleagues found that 7 to 17 years after definitive operation, 60% of patients treated for rectosigmoid aganglionosis were incontinent, whereas 86% of patients treated for total colonic aganglionosis were incontinent.[106] Similarly, Tsuji and colleagues found that, in patients with total colonic disease, the fecal incontinence rates were 82% at 5 years, 57% at 10 years, and 33% at 15 years. [107] With time, most individuals have improved fecal continence, but this may not occur until late adolescence. Yanchar and Soucy surveyed teenage patients who underwent definitive surgical treatment in the first year of life and their parents. They concluded that incontinence significantly impacted the patients' social and family lives. Despite this, most parents were satisfied with their child's outcome.[108] Individuals with Down syndrome appear to have prolonged difficulties with continence, particularly at night.

Few data exist regarding possible damage to pelvic neuronal structures in the process of the pull-through procedure. Most reports fail to mention the subject. Sherman and colleagues found no abnormalities in urinary or sexual function in their review of a large number of adult postoperative patients.[77]

GENETIC COUNSELING

Hirschsprung disease is a sex-modified multifactorial disorder. The generalized risk to siblings is 4% and increases as the length of involved segment increases.[1,17] In Hirschsprung disease associated with known syndromes, genetic counseling may focus more on the prognosis related to the syndrome than on the recurrence risk of Hirschsprung disease. In isolated Hirschsprung disease, a more precise risk table can now be created (Table 46.3-4). Prenatal diagnosis is possible if the mutation within the family is known. However, because the penetrance of single gene mutations is low (except for *SOX10* mutations), the clinical usefulness of genetic testing is limited. Genetic testing in Hirschsprung disease is currently performed as a research tool and is not used in clinical assessments of recurrence risk.

In addition to prenatal evaluation and assessments of recurrence risks, genetic testing in Hirschsprung disease

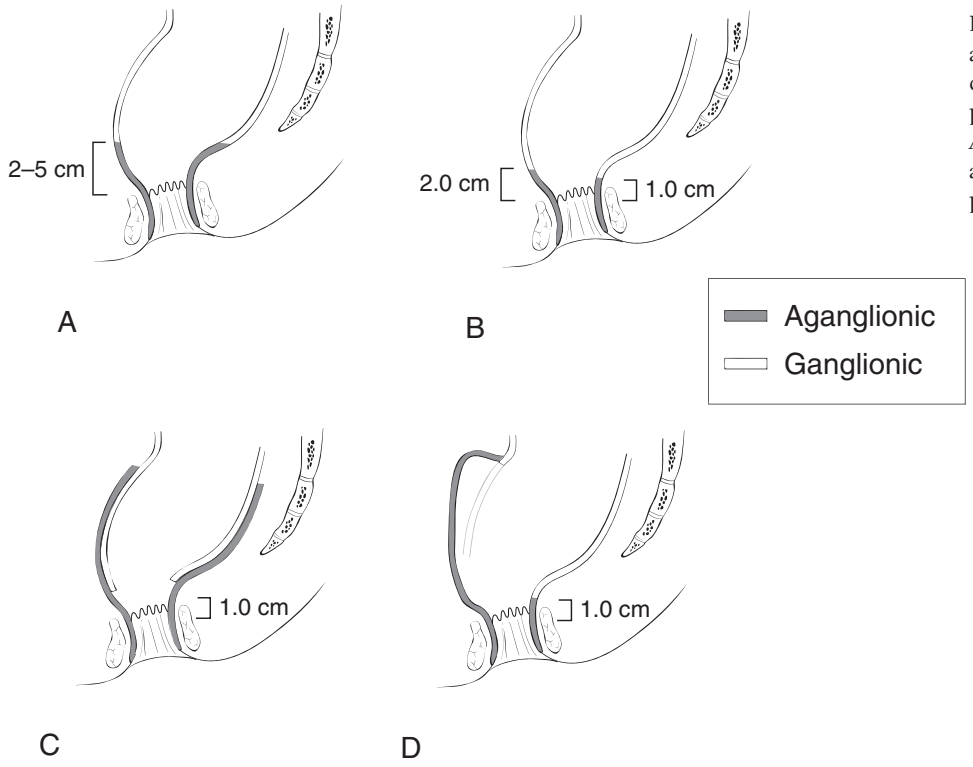

FIGURE 46-3-5 Summary of anorectal anatomy following the more commonly performed pull-through procedures for Hirschsprung disease: *A*, Rehbein; *B*, Swenson; *C*, Souve; and *D*, Duhamel. Reproduced with permission from Teitelbaum D et al.[89]

TABLE 46.3-4 PERCENT RECURRENCE RISK OF HIRSCHSPRUNG DISEASE BY PROBAND AND CONSULTAND GENDER

	AGANGLIONOSIS PROXIMAL TO SPLENIC FLEXURE		AGANGLIONOSIS BEGINNING IN THE DESCENDING COLON		AGANGLIONOSIS OF RECTUM AND/OR SIGMOID ONLY	
CONSULTAND	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE
Sibling of affected male	11	8	10	7	4	1
Sibling of affected female	23	18	13	10	6	2
Offspring of affected male	18	13	11	9	~0	~0
Offspring of affected female	28	22	15	11	~0	~0

Adapted with permission from Chakravarti A and Lyonnet S.[46]

has the potential to identify significant risks for other diseases in individuals with Hirschsprung disease or their family members. Currently, this is most clearly illustrated in Hirschsprung disease patients with *RET* mutations identical to those observed in individuals with MEN IIA. Mutations of *RET* codons 609, 618, and 620 (within exon 10) are rarely associated with MEN IIA and Hirschsprung disease. In addition, there have been rare cases of Hirschsprung disease with exon 10 mutations identical to those found in hereditary medullary thyroid carcinoma.[109] A family history of thyroid, parathyroid, or adrenal cancer should be sought in all patients with Hirschsprung disease. A recent consensus statement from an international group of endocrinologists recommends *RET* exon 10 mutation analysis in all children with Hirschsprung disease.[110] Although the vast majority of these tests will be negative, the significance of identifying a MEN IIA mutation carrier to that individual and family justifies such testing. If a MEN IIA mutation is identified, first-degree relatives should be screened. Prophylactic thyroidectomy in carriers should be considered before the age of 5 years as well as regular screening for pheochromocytoma and hyperparathyroidism.[110]

Recently, Newby and colleagues assessed the in vivo vasomotor responses to endothelin B stimulation in a group of healthy adults with a history of nonsyndromic Hirschsprung disease, without regard to the length of aganglionosis or genetic mutation. They reported that the individuals with a history of Hirschsprung disease exhibited an abnormal vascular response to the injection of the endothelin B selective agonist and that the pattern of abnormality suggested a defect in endothelin B signaling.[111] This result raises the possibility that children treated for Hirschsprung disease may be at increased risk for cardiovascular disease in adulthood. Endothelin B is normally expressed on the vascular endothelium and smooth muscle, where it appears to be involved in the maintenance of basal vascular tone. Rats genetically deficient in endothelin B exhibit salt-sensitive hypertension.[112] However, no association has yet been made between Hirschsprung disease and noncongenital cardiovascular disease.

FUTURE DIRECTIONS

As our understanding of the molecular genetics of ENS development has improved, so has our ability to identify and isolate ENS stem cells. This allows the pursuit of a new treatment option for Hirschsprung disease: transplant of ENS precursors into the aganglionic gut. Sandgren and colleagues, using the endothelin 3–deficient mouse model of Hirschsprung disease, transplanted myenteric ganglia and smooth muscle from the proximal to the distal intestine with promising results.[113] Recently, Bixby and colleagues and Kruger and colleagues successfully isolated enteric neural crest stem cells (capable of self-renewal and differentiation into neuronal, glial, and myofibroblast phenotypes) from both embryonic and postnatal rats by flow cytometry.[114,115] One day it may be possible to "seed" the aganglionic gut of patients with Hirschsprung disease with these stem cells, creating a distal ENS and minimizing the need for surgical resection.

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4. Chronic Intestinal Pseudo-obstruction Syndrome

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Chronic intestinal pseudo-obstruction (CIP) is a rare disorder in which impaired intestinal and/or colonic motility lead to signs and symptoms of bowel obstruction in the absence of a mechanical obstructive lesion. CIP can result from a variety of disorders of the enteric neuromusculature. Its classification is based on histopathologic data into myopathic, neuropathic, or unclassified forms, with each category occurring as a primary disorder or secondary to a nongastrointestinal disease. The diagnosis of CIP has been controversial and has recently been defined in children as a clinical syndrome "characterized by repetitive episodes or continuous symptoms and signs of bowel obstruction, including radiographic documentation of dilated bowel (except in a few congenital cases), in the absence of a fixed lumen occluding lesion."[1] It is clear that pediatric CIP is distinct from adult forms: children are affected predominantly by primary disorders of enteric neuromusculature, whereas adults present most often with CIP secondary to systemic diseases such as diabetic neuropathy, systemic sclerosis, or paraneoplastic syndromes.[2]

Although knowledge and prognosis of this heterogeneous syndrome have improved in recent years, CIP is one of the most severe intractable problems in pediatric gastroenterology: almost half of pediatric patients never tolerate enteral feeding.[3–5]

PATHOPHYSIOLOGY AND CLASSIFICATION

Gastrointestinal motility is controlled by the intrinsic enteric nervous system, which is modulated by its sympathetic and parasympathetic supply. Transmitters released from the enteric neurons modulate the peristaltic reflex. The intrinsic nervous system includes neurons, glial cells, and the interstitial cells of Cajal (ICC). The latter act as intestinal pacemakers and as modulators of cellular communication between enteric neurons and smooth muscle cells in the delivery of gastrointestinal motility (see Chapter 4, "Motility").[6] Any congenital or acquired abnormality of the enteric neuromuscular system may result in intestinal pseudo-obstruction. The clinical presentation of these disorders is dependent on the localization of the anomaly of the enteric neuromuscular network within the gastrointestinal tract.

The comprehensive histopathologic classification of enteric neuromuscular disorders is available in Chapter 46.2, "Dysmotilities."

Neuropathic Pseudo-obstruction

A neuropathic disorder affecting gut motility may theoretically be confined to either extrinsic innervation, the intrinsic enteric nervous system, or to both levels of neural control. In pediatric patients, most neuropathic forms of CIP involve the intrinsic innervation of the gut. One or more components of the enteric nervous system (ie, neurons, glial cells, and ICC) may be affected. The neuropathy is considered primary if no etiology known to induce such disorders is documented (Table 46.4-1). Most childhood cases of CIP are a result of an alteration in the number of enteric neurons and glial cells: both insufficient (hypoganglionosis)[7,8] and excessive numbers of these elements (hyperganglionosis, multiple endocrine neoplasia [MEN] syndrome type IIB,[7] intestinal neuronal dysplasia[9]) result in severe dysmotility. ICC, which are of mesodermal embryonic origin,[10] can also be affected transiently[11] or permanently.[12]

Most often, the exact mechanisms responsible for the developmental anomalies are unknown. However, recent advances in knowledge on enteric nervous system embryogenesis have provided new tools to understand these disorders.[13] A number of genes and regulatory molecules are involved in the development of the enteric nervous system. In rodents, transcription factors (Sox 10, Pax 3, Phox2a, Phox2b, Hand 2, Mash-1) and growth factors (Gdnf-Gfrα1/Ret, endothelin 3-ednrb, neurotrophin 3 [NT3]-TrkC, BMP2-4-BMPRI, BMPRII-SIP1) have been shown to be involved at various stages in normal enteric nervous system development.[13] All of these factors may be theoretically involved in the pathophysiology of primary enteric neuropathies.[14] In fact, derangement of function of *SOX10* and of the RET-GDNF, ET3-EDNRB, and BMP2-4-BMPRI pathways accounts for some cases of Hirschsprung disease in humans (see Chapter 46.3, "Hirschsprung Disease"). The etiology of other forms of visceral neuropathies is less clear. Germline mutations of the *RET* gene, causing a gain of function, have been reported in MEN type IIB.[15] There are also three reports of *SOX10* mutations in patients with CIP without aganglionosis: one girl with CIP, deafness, and peripheral demyelinating neuropathy[16] and two individuals with CIP, deafness, agenesis of the semicircular canals, developmental delay, white matter changes on magnetic resonance imaging, a white forelock, and blue eyes.[17]

TABLE 46.4-1 CAUSES OF SECONDARY INTESTINAL PSEUDO-OBSTRUCTION IN CHILDREN

TOXIC
Ketamine
Carbamazepine
Clonidine
Atropine, anticholinergics
Theophylline
Fludarabine
Vinblastine and other vinca alkaloids
Neuroleptics
Antidepressants
Phenothiazine
Opiates
Calcium channel blockers
Fetal alcohol syndrome[118]
METABOLIC
Electrolyte imbalance (K^+, ↑ Mg^{++}[119], Ca^{++})
Hypothyroidism
Hypoparathyroidism
Carnitine deficiency[87]
Vitamin E deficiency ("brown bowel syndrome")[88,89]
INFECTIOUS
Viral: CMV,[74] EBV,[52,76] herpes zoster,[71] rotavirus[78]
Trypanosoma cruzei (young adults)
Lyme disease[120]
IMMUNE
Celiac disease
Systemic sclerosis[85]
Lupus (myopathy)[86]
Autoimmune leiomyositis[94,95]
Autoimmune enteric ganglionitis (with antienteric neurons antibodies, anti-PCNA antibodies)[96,97]
Guillain-Barré syndrome[121]
TUMORAL
Neural crest cell tumor: neuroblastoma, ganglioneuroblastoma[91]
Pheochromocytoma[122]
Thymoma (with antiacetylcholine receptor antibodies)[123]
STRIATED MYOPATHY
Myotonic dystrophy[27,29]
Duchenne muscular dystrophy[28]
Desmin myopathy[34]
Mitochondrial myopathy[90,124]
CENTRAL OR PERIPHERAL GENERALIZED NEUROPATHY
Degenerative process: diabetes, amyloidosis (not reported in children)
Mitochondrial neurogastrointestinal encephalopathy[93]
Familial dysautonomia[83]
Acquired cholinergic dysautonomia or acquired pandysautonomia[81]
MISCELLANEOUS
Angioedema[125]
Postradiation enteropathy[126]
Kawasaki disease[127]

CMV = cytomegalovirus; EBV = Epstein-Barr virus; PCNA = proliferation cell nuclear antigen.

Myopathic Pseudo-obstruction

Myopathic forms of CIP result from smooth muscle cell injury.[18–20] Visceral myopathies may be primary or secondary to a systemic disease (see Table 46.4-1). Attempts to characterize primary visceral myopathies at the molecular level have been difficult because differential expression of proteins critical for contractile function and cytoskeletal integrity of smooth muscle may be secondary to intestinal distention.[21] Further, the histopathology of affected smooth muscle may be normal, even in the presence of a molecular defect. In one individual with CIP and visceral myopathy, complete absence of smooth muscle α-actin was documented in the presence of normal histopathology.[22]

Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) is an autosomal recessive disorder involving the enteric neuromuscular system associated with multiple deletions and depletion of mitochondrial deoxyribonucleic acid (mtDNA). MNGIE is characterized by a myopathy of striated muscle with ragged red fibers and mixed enteric neuromyopathy.[23] The pathogenic mechanism is related to aberrant thymidine metabolism, which results in impaired replication and/or maintenance of mtDNA.[24] Several mutations have been reported of the gene coding for thymidine phosphorylase, located on chromosome 22q13.32-qter.[25] Duchenne muscular dystrophy (DMD) and myotonic dystrophy are the most frequent skeletal myopathies that may present with severe digestive motor disorders (gastroparesis[26,27] and intestinal pseudo-obstruction[28,29]) owing to smooth muscle involvement. Indeed, dystrophin, the protein product of the DMD locus, has been localized to enteric muscle,[30] and digestive motor abnormalities have been demonstrated in patients with both DMD[31] and myotonic dystrophy.[32] In DMD, the digestive symptoms may occur late in the course, often after orthopedic procedures. In myotonic dystrophy, digestive motor symptoms may herald the disease: neonates with the disorder may present with CIP.[27] CIP with atrophic smooth muscle fibers has also been reported in cases of desmin-related myopathy (characterized by intracytoplasmic accumulation of desmin in skeletal muscle).[33,34]

CLINICAL FEATURES

Prevalence

CIP is a rare condition, and epidemiologic data in children are lacking. According to the American Pseudo-obstruction and Hirschsprung's Disease Society, an estimated 100 new cases of childhood CIP are reported every year in the United States.[35] One recent study reported 105 cases collected over a 19-year period in France and Belgium.[3] Another series of 44 cases collected over a 18-year period was also reported in Great Britain.[4]

Sex

The male-to-female ratio in CIP is thought to be 1:1; however, girls have been found to be more frequently affected in the neonatal forms.[3,4,36,37]

Family History

Familial cases of CIP account for less than 5% of cases in the large pediatric series.[3,5]A variety of myopathic forms and neuropathic forms have been reported in infants and children (see Chapter 46.2).

Prenatal Symptoms

Prenatal signs may be detected in about 20% of cases.[3,4] Megacystis is the most frequently reported sign, whereas dilated bowel is quite rare. This has been noted in megacystis-microcolon–intestinal hypoperistalsis syndrome, in which an antenatally enlarged bladder is seen by ultrasonography

in 88% of cases, hydronephrosis in 53%, increased volume of amniotic fluid in 34%, and gastric distention in only 10%.[37] Although some reports have described the detection of these signs by ultrasonography as early as 16 weeks, more often the abnormalities are noted very late in gestation.[38] Antenatally diagnosed nonobstructive megacystis, with neonatal urologic symptoms, may precede gastrointestinal symptoms of pseudo-obstruction for several months.

Age at Onset

Two-thirds of patients present within the first month of life and 80% by 1 year of age. The remainder are detected sporadically throughout the first two decades of life.[3–5, 39]

Clinical Presentation

Prenatal- or Neonatal-Onset Form. In the neonatal form, CIP presents as severe abdominal distention with bilious vomiting (Table 46.4-2). The abdominal radiograph shows dilated bowel loops with air-fluid levels suggestive of an organic intestinal obstruction. In megacystis-microcolon–intestinal hypoperistalsis syndrome, an obstructed urinary system leading to an abdominal distention may be the presenting feature, with symptoms of intestinal obstruction that may appear within days to 12 months later. To avoid unnecessary surgery, an exploratory laparotomy should be deferred in a neonate with antenatal diagnosis of megacystis. Some affected infants may present with abdominal distention and diarrhea secondary to bacterial overgrowth.

CIP may be mimicked by immaturity of intestinal motility in preterm infants; thus, this diagnosis should be made with caution in this group. For instance, the migrating motor complex does not appear in its mature form until a gestational age of 34 to 35 weeks.[40]

Infantile- or Late-Onset Form. ***Major Forms with Acute Presentation.*** Some patients present with subacute and/or recurrent episodes of gastric, intestinal, and/or colonic obstruction leading to frequent drainage and fluid replacement. The symptoms depend on the regions of the gastrointestinal tract involved. They may be acute or insidious and chronic. They may be persistent but are most often intermittent. Exacerbations may be precipitated by various causes, including intercurrent infections, fever, vaccines, general anesthesia, and stress. Diarrhea owing to bacterial overgrowth is frequent and may alternate with constipation or episodes of partial obstruction. Abdominal pain is often severe enough to lead to feeding difficulties, resulting in malnutrition. Notwithstanding frequently detected esophageal involvement by manometry, dysphagia is rarely reported.[41]

Recurrent episodes of functional partial bowel obstruction may be very difficult to differentiate from true mechanical obstruction in the child who has undergone a prior laporotomy and who may have adhesions.

Urinary tract involvement occurs in 33 to 92% of cases, independent of the type of CIP.[3,42–44] Megacystis with a hypocontractile detrusor, increased bladder capacity, and compliance is the most frequent pattern of urologic abnormality (bladder adynamia). Ureterohydronephrosis is seen in 56 to 68% of cases, but vesicoureteral reflux occurs in less than 10%.[44] Urinary tract infection is frequent but may be asymptomatic. In myopathic forms of CIP without megacystis, urinary tract infection remains a frequent problem.[4] The renal prognosis is generally good provided that careful, active evaluation and management of the adynamic bladder are performed to ensure adequate bladder emptying and to prevent urinary tract infection.[44]

Bladder biopsies show nonspecific fibrotic changes in neuropathic and myopathic forms of CIP and are thus not useful for subtype classification of CIP.

Moderate Forms with Subacute Presentation. Seventy percent of these patients present with progressively severe constipation (ie, less than one stool per 7 to 15 days), abdominal distention, bilious vomiting, and failure to thrive. Following exclusion of Hirschsprung disease and other causes of mechanical obstruction, CIP should be considered, and urinary symptoms should be carefully checked. The colonic form of CIP (colonic inertia) may result in severe intractable constipation without upper digestive tract involvement.[45]

DIAGNOSIS

The diagnosis of CIP is difficult because of the variable clinical presentation and the lack of a specific diagnostic test. The diagnosis should be suspected in children presenting with symptoms of intestinal obstruction without an occluding lesion. The diagnosis of CIP should be also considered when there is persistent vomiting after a Ladd procedure for malrotation,[46] when intestinal obstruction is

TABLE 46.4-2 CLINICAL SYMPTOMS IN CHILDREN WITH CHRONIC INTESTINAL PSEUDO-OBSTRUCTION

STUDY	NUMBER OF PATIENTS	ABDOMINAL DISTENTION	VOMITING	CONSTIPATION	FAILURE TO THRIVE	ABDOMINAL PAIN	DIARRHEA	DYSPHAGIA
Faure et al[3]	105	100	94	70	64	46	29	9
Vargas et al[39]	87	73	50	51	23	NA	21	2
Granata and Puri[37]	59	59	31	27	NA	NA	26	NA
Krishnamurthy et al,[8] Schuffler et al[18]	30	23	19	20	15	NA	16	NA
Heneyke et al[4]	44	31	40	31	NA	NA	—	NA
Total	325	286 (88%)	234 (72%)	199 (61%)	102 (31%)	—	92 (28%)	11 (3%)

NA = not available.

associated with bladder dysmotility, or when, in a full-term neonate, there is persistent or recurrent obstruction after exclusion of Hirschsprung disease and hypothyroidism. The differential diagnosis should be carefully considered (Table 46.4-3) because establishing a diagnosis of CIP may be invasive, and the psychological consequences in children and their families are significant.

The diagnosis is made after careful analysis of the clinical course, radiographs, motility testing, and, if available, pathologic data. Diagnosis tests should then be performed in three steps to (1) definitely rule out any fixed, occluding lesion; (2) confirm the abnormal motility of the gastrointestinal tract; and (3) find a treatable systemic cause of secondary CIP.

Definitively Rule Out Any Fixed Obstructive Lesion

The definitive exclusion of intestinal obstruction by a fixed occluding lesion is the first step to affirm the "pseudo" obstruction. Although aganglionosis results in a real "pseudo" obstruction, establishing a diagnosis of Hirschsprung disease is essential because the treatment and prognosis of this condition are well established and different from CIP (see Chapter 46.3).

Specific manometric features such as nonpropagated, prolonged contractions in small bowel differentiate CIP from a partial mechanical obstruction in adults.[47] These manometric features should be interpreted with caution, and, at present, antroduodenal manometry should not be used for differentiating "true" obstruction from "pseudo" obstruction.

Radiographic Studies. Plain abdominal films show signs typical of intestinal obstruction with air-fluid levels, a dilated stomach, small intestine, and/or colon. In neonates, the dilatation may be absent because the digestive tract has never been used.[48] Megacystis may be the main radiographic sign with a gray aspect over the lower half of the abdomen.

Contrast studies should be performed to exclude any intraluminal or extrinsic lesion that could explain the symptoms.[49,50] They should be performed with a nontoxic, water-soluble medium to prevent intraluminal solidification, leading to a true obstruction. Enteroclysis may be necessary to rule out a partial mechanical obstruction. Progression of the contrast medium may be slow with stasis in the stomach and small bowel. Impaired and retrograde peristalsis may occur. Small bowel and/or colonic dilatation are the most frequent signs, present in more than 60% of cases. Contrast enema results may be normal when the process of the disease involves only the upper digestive tract. Sometimes the dilatation may occur only in a segmental part of the small bowel or colon. A microcolon is found in neonates. Diverticulosis of the small bowel or the colon has been reported in some cases, reflecting severe dysmotility.[51–53] Pneumatosis cystoides intestinalis has been reported in children with CIP.[54]

Malrotation is frequent, especially in neonates (up to 40% of cases),[3–5] and has been reported in cases of myopathy, neuropathy, and familial syndromes associating CIP, malrotation, congenital short bowel, and pyloric nonhypertrophic stenosis.[55–57]

TABLE 46.4-3 DIFFERENTIAL DIAGNOSIS OF CHRONIC INTESTINAL PSEUDO-OBSTRUCTION IN CHILDREN

Aerophagia
Gastroparesis
Constipation
Cyclic vomiting syndrome
Severe irritable bowel syndrome
Bacterial overgrowth of various origin (lactase deficiency, disaccharidase deficiency, intestinal duplication)
Aerodigestive fistula
Munchausen syndrome by proxy

Surgery. One of the aims of an accurate diagnosis is to avoid an unnecessary exploratory laparotomy, among other reasons because surgery may cause adhesions rendering difficult the interpretation of subsequent obstructive episodes. However, some patients with an acute presentation undergo emergent exploratory laparotomy. The prevalence of surgery at diagnosis of pediatric CIP approaches 50%.[3,4,8,18,37,39] Laparotomy should be reserved to exclude an organic obstructing lesion in cases in which radiographs, manometry, or the clinical course of the illness suggests that one is likely.

Confirm Abnormal Motility of the Gastrointestinal Tract

Functional Motility Testing. Manometric studies are the most sensitive tools to evaluate the strength and the organization of the smooth muscle contractions at different levels of the gastrointestinal tract and, therefore, its functional motor capacity. Recall that manometry of any part of the gastrointestinal tract should be performed on nondilated segments because tracings from dilated bowel are of poor quality and often noninterpretable. The manometric studies should be performed in cooperative patients in a stable condition, far removed from any acute episode of obstruction or surgery.

Schematically, visceral neuropathies result in contraction of normal amplitude with abnormal propagation and compromised organization.[41] In myopathic forms, the amplitude of contractions is weak but correctly propagated and organized.[58,59]

Esophageal manometry has been found abnormal in 50 to 90% of patients with both forms of CIP, independent of esophageal symptoms.[60] In visceral neuropathies, in addition to the neuropathic manometric signs, the lower esophageal sphincter may fail to relax normally.[41]

The rectoanal inhibitory reflex is found in all patients with CIP and is absent only in Hirschsprung disease.[41] Although there are reports of absence of this inhibitory reflex in some patients with the neuropathic form of CIP, this is likely due to chronic rectal distention rather than to a primary absence of this reflex.

Antroduodenal manometry provides important information in confirming the dysmotility of the upper digestive tract, which is almost always involved in children with CIP.[58,61] Studies are performed in the fasted state and after a

meal if the patient is able to tolerate food. It should be emphasized that, especially after several years of clinical course, the manometric anomalies may be difficult to interpret because chronic distention of the gut may itself induce severe motor disturbances, particularly in postsurgical patients.[62] Stimulation with erythromycin[63] or octreotide,[64] which induces phase III motor contractions arising from the antrum or duodenum, is also used: the lack of response to these powerful prokinetic agents indicates a severe alteration of the enteric neuromusculature. Conversely, normal antroduodenal manometry essentially rules out the diagnosis of CIP.[65] If the clinical symptoms support the possibility of intestinal dysmotility, normal antroduodenal manometry is a strong argument against a motility disorder: this situation should alert the physician to the possibility of psychological or behavioral disorders, as well as Munchausen syndrome by proxy.[66] Antroduodenal manometry allows the characterization of myopathic and neuropathic forms of CIP.[58] In the group of the neuropathies, the abnormalities reported are absence of phase III motor complexes, abnormal configuration or propagation of phase III, bursts of uncoordinated phasic activity, and sustained periods of prolonged phasic activity during fasting. After a meal, the inability to induce a fed motility pattern is also reported.[41,58,67] In some patients with very severe disease, no contractile activity may be seen despite prolonged recording under excellent technical conditions. Nonpropagating prolonged contractions after a meal suggest a partial mechanical obstruction.[47] There are no specific manometric signs that correlate with respect to pathologic types of visceral neuropathies.[41] The prognostic value of antroduodenal manometry has been reported by several groups. The ability to tolerate enteral feeding is more likely when phase III or organized and propagated clusters of contractions in the duodenum-jejunum are present.[41,67,68]

Colonic manometry provides information on the motor activity of the colon during fasting and after stimulation with a meal or a laxative (bisacodyl). Weak basal activity with an absence of gastrocolic response is found in visceral neuropathies involving the colon.[69]

Electrogastrography is noninvasive and may be used in pediatric patients. Unfortunately, the results of this technique are often nonspecific and, at present, do not differentiate CIP from other forms of intestinal dysmotility. Further validation of this technique is needed.[70,71]

Nuclear medicine technetium 99m studies generally show delayed gastric emptying of solids and liquids in affected children. Normal values for scintigraphic transit time have not been established in children for the small intestine and colon.

Histopathology. Histopathologic data may confirm the abnormal aspect of the enteric neuromusculature. Description and classification of the various types of visceral myopathies and neuropathies are provided in Chapter 46.2. Although pathologic features may have a great importance in establishing the diagnosis of CIP, interpretation is often difficult and requires highly experienced pathologists.

Full-thickness intestinal wall specimens should be taken during surgery when an ostomy is needed and performed. When such a surgery is planned, pathologists should be alerted to organize optimal procedures for fixation and staining of the specimens. It is important to obtain large samples (> 1 cm) and to preserve them to permit routine light microscopy, ultrastructural examination (glutaraldehyde fixation), and immunohistochemistry (snap-freezing). Owing to the severe consequences of laparotomy in CIP patients, it is not recommended to perform surgery only to obtain intestinal specimens for histopathologic analysis. A full-thickness rectal biopsy may often be informative and may reflect the neuromuscular pathologic pattern of the colonic or intestinal wall.[7,72] It thus may be performed in patients for whom a pathologic investigation is requested, such as when an inflammatory process is suspected.[52,72]

Find a Treatable Systemic Cause of Secondary CIP

Although much rarer than in adults, finding a secondary cause of CIP is of great importance because the specific treatment of the condition may lead to the total cure of the disease. The causes of secondary pseudo-obstruction that have been described in children are listed in Table 46.4-1. Some of these causes are treatable and should be carefully excluded. Others are related to intractable or degenerative conditions but are important to be characterized for adaptation of symptomatic treatment and for giving a prognosis.

The family history should be explored to identify hereditary neurologic, myopathic, and autoimmune diseases. One should ascertain whether the patient has previously used or is currently using drugs such as anticholinergics, phenothiazines, antihypertensives, tricyclic antidepressants, serotoninergic agents, dopaminergic drugs, opiates, and calcium channel blockers or whether there is any prior exposure to radiation.[73] In atypical cases, with normal manometry, Munchausen syndrome by proxy should be considered.[66]

Any history of travels should be elicited, although Chagas disease develops exclusively in young adults after several years of evolution. The possibility of a viral infection at the onset of disease should be noted, although the demonstration of an active or past infection does not prove causation. Infections with cytomegalovirus[74,75] and Epstein-Barr virus[52,76] have been implicated as causes of CIP because these viruses have been found in enteric neurons[52,77] or involving extrinsic vagal innervation[76] of affected patients. Rotavirus has been described as a cause of both severe and prolonged gastroparesis.[78]

The physical examination should encompass a thorough neuromuscular assessment, including testing for pupillary reactions to light and accommodation and external ocular movements to help identify conditions associated with autonomic neuropathy or mitochondrial diseases. Testing for orthostatic stability should be performed in children because postural dizziness, visual disturbances, and sweating abnormalities suggest the presence of an underlying autonomic neuropathy.[79] Dysautonomia may be familial[80] or acquired.[76,81] In familial cases, reported in Ashkenazi Jewish families, the clinical diagnosis is based on the presence of five signs: lack of axon flare after intradermal injection of histamine, absence of fungiform papil-

lae on the tongue, myosis of the pupil after conjunctival instillation of methacholine, absent deep tendon reflexes, and diminished tear flow.[82] In these cases, chronic vomiting is the main digestive symptom,[83] although small bowel motility disorders have also been reported.[84]

The dermatologic examination should note signs of connective tissue disease (ie, systemic sclerosis, lupus), including Raynaud phenomenon, skin eruption, palmar erythema, telangiectasia, nodules, and scleroderma of the hands, feet, face, and forearms. Digestive symptoms may precede the skin involvement in these disorders.[85,86]

Laboratory investigations should include electrolytes, thyroid-stimulating hormone, free thyroxine, antitransglutaminase antibodies, urinary catecholamines, plasma vasoactive intestinal peptide, erythrocyte sedimentation rate, fraction of complements 3 and 4, and antinuclear antibodies. Carnitine and vitamin E levels should be checked in cases with possible fat malabsorption or maldigestion because their deficiency has been reported to be involved in enteric myopathic disorders with deposition of lipofuscin in smooth muscle cells.[87–89] Metabolic screening for mitochondrial diseases—that is, plasma lactic acid and pyruvate—should be performed when the physical examination shows external ophthalmoplegia.[90]

Neural crest–derived tumors and pheochromocytoma should be suspected and ruled out in children and infants with CIP: appropriate computed tomographic and ultrasonographic studies should be considered to exclude the presence of thoracic or abdominal tumors.[91]

Audiologic assessment is important to rule out deafness, seen in patients with a *SOX10* gene mutation.[16,17] External ophthalmoplegia associated with deafness suggests a MNGIE defect. The onset of symptoms (gastrointestinal, ocular, or both) generally occurs during adolescence, although very-early-onset disease has been reported (5 months of age).[92] Peripheral neuropathy and diffuse muscle weakness are the predominant manifestations, although almost all patients have indices of leukoencephalopathy on magnetic resonance imaging of the brain.[93] Thymidine phosphorylase activity and plasma thymidine should be measured when suspecting such a diagnosis.[24,92]

Cardiac function should be evaluated by echocardiography because involvement should lead one to suspect muscular diseases such as desmin myopathies.[33]

Full-thickness biopsies of the rectal wall or of other parts of the digestive tract may be of interest in seeking an inflammatory process of the smooth muscle cells[94,95] or of the enteric ganglia,[96] which may be amenable to anti-inflammatory or immunomodulatory therapy. Indeed, autoimmunity may directly affect either muscular cells or enteric neurons via a cytotoxic effect[96] or via circulating antienteric neuronal antibodies.[97] Inflammation may also occur in connective tissue disorders.

TREATMENT

Treatment of CIP is always supportive and, when possible, curative. Steroids have been successfully used in cases of immune-mediated myositis[95] and ganglionitis,[97] although full recovery may not be achievable in the setting of advanced neuroenteric or myopathic injury. Supportive treatment is a major objective in pediatric CIP because recovery may be protracted even in secondary forms and because curative therapy is not available for the primary forms of CIP. Supportive care is provided to bridge the gap until intestinal adaptation occurs or, if indicated, to intestinal transplant. The aims of supportive care include maintaining an adequate nutritional status, preserving growth and development, avoiding life-threatening episodes of sepsis originating from intestinal bacterial translocation or from infection of a central venous catheter, and maintaining an acceptable quality of life.[98]

Symptomatic Treatment

Acute episodes of bowel pseudo-obstruction and exacerbations in children with chronic bowel distention may benefit from bowel decompression using nasogastric suction. In children with frequent exacerbations, the placement of a venting gastrostomy may be of great benefit to avoid the recurrent placement of nasogastric tubes.[99] Normal electrolyte and acid-base balance should be restored by intravenous fluid replacement. Fever suggests the possibility of a urinary tract infection, bacterial overgrowth, bacterial translocation from the gut, or catheter sepsis, necessitating treatment with intravenous antibiotics.

Nutritional Support

Artificial nutritional support should be considered as soon as malnutrition occurs in the course of the disease because failure to thrive is reported in about 60% of untreated CIP patients.[3] Normalization of the nutritional status may also lead to improvement of intestinal dysmotility.

Enteral feeding should always be preferred to using parenteral nutrition. When possible, the intragastric route is preferred over the jejunal route because of the technical difficulties found in the placement and maintenance of the gastrojejunal tube position because the tubes may migrate if the child vomits. In the case of severely impaired gastric emptying and intractable vomiting, continuous intragastric feeds should be tried, and on failure, a surgical jejunostomy should be placed.

When enteral feeding is not possible or when malnutrition is too severe, parenteral nutrition should be employed. About two-thirds of pediatric patients with CIP require parenteral nutrition during the course of their disease. Total parenteral nutrition (TPN) is one of the main therapeutic modalities in children with CIP. Unfortunately, TPN is also the major cause of morbidity and mortality owing to infectious, thrombotic, and TPN-associated hepatic disorders.[3–5] It appears that when TPN is required, every effort should be made to establish some tolerance of enteral feeding and, ultimately, weaning off TPN.

Prokinetics and Antibiotics

Bacterial overgrowth can be treated with nonabsorbable antibiotics to prevent bacterial translocation and hepatic complications of TPN. However, the use of antibiotics leads to the development of resistant bacterial strains, ren-

dering the treatment of bacteremia owing to translocation more difficult.

Almost all prokinetic drugs have been tried in CIP, usually without any proven benefit in terms of improvement of clinical symptoms. Cisapride,[100,101] erythromycin,[63,102] octreotide,[64] and trimebutine[103] have been shown manometrically to induce and to enhance duodenal motility in children with CIP but are often less effective in providing clinical benefit.

Erythromycin (3 mg/kg intravenously every 8 hours), a motilin receptor agonist that induces strong and powerful phase III–like antral contractions, has been used in combination with octreotide, which induces duodenal phase III–like contractions. This combination has been tested with success in adults with systemic sclerosis and pseudo-obstruction.[104]

Bethanechol, a muscarinic agonist acting on both the digestive and the urinary tract, may be useful in some patients with megacystis. Metoclopramide, domperidone, misoprostol, trimebutine, naloxone, and leuprolide have been tested sporadically and may be useful in selected cases.

Several new agents are being tested in adults and could potentially help children with CIP. Tegaserod is a 5-hydroxytryptamine$_4$ receptor partial agonist shown to enhance gastric and colonic motility.[105] Two neurotrophins (NT3 and brain-derived neurotrophic factor) have been reported to accelerate the colonic transit time in constipated patients. They may act as neurotransmitters as well as on the neuronal plasticity of the enteric nervous system.[106]

Surgery

As previously stated, gastrostomy and/or jejunostomy are often required, representing the most effective intervention in many cases of CIP children. The Malone procedure can also help patients, improving bowel movements.[107]

When attacks of intestinal obstruction are frequent or life threatening or when tube feeding is not possible, enterostomy (ileostomy or colostomy) should be performed to bypass the functional obstruction and obtain digestive decompression. Bowel decompression may prevent or cure severe dilatation, which itself impairs and reduces effective motility of the gut. Intestinal motility may improve as the bowel dilatation is reduced. Symptom relief, sometimes dramatic, may occur in 50% of cases in some series, making possible withdrawal of parenteral nutrition.[3,4] Closure of the stoma may be considered when a clear improvement is observed, with weaning off TPN, and prolonged (for at least 2 years) effectiveness and tolerance of enteral feeding are obtained, without exacerbations. Ileorectal anastomosis with the Duhamel procedure has been suggested as effective in some cases.[4] Patients for whom surgery is ineffective in terms of dependence on artificial feeding methods may nevertheless benefit from venting ostomies, which may decrease bacterial overgrowth, translocation, chronic distention, abdominal pain, and vomiting.

Surgery has been reported to result in dense adhesions in these patients, and any further laparotomy performed to exclude mechanical obstruction may add to them. This is reflected in the small number of patients in the published series who underwent a single laparotomy during their illness, suggesting that others underwent further surgery because occlusive episodes recurred, raising the possibility of adhesions.[3–5,8] Laparotomy should always be avoided at diagnosis (see above). If necessary, laparotomy should be planned for bowel decompression and not undertaken only for obtaining biopsies. During the course of the disease, exploratory laparotomies for obstruction should be performed only when a clear mechanical obstruction has been demonstrated or when focal signs suggest a likely obstruction.

Intestinal transplant is the only definitive curative treatment for patients with CIP. Isolated small bowel or multivisceral transplant in such patients is challenging, however, because of the possible associated esophageal (achalasia) and gastric dysmotilities that may be unmasked after the transplant. Also, previous surgeries that patients have undergone prior to the transplant make procedures more difficult.[108–110]

Sympathetic plexus neurolysis, by interrupting sympathetic efferent (and inhibitory) activity on the upper digestive tract, has been shown to improve symptoms in two CIP patients.[92,111]

Urinary Tract

In children with urinary tract involvement, the management of megacystis and prevention of long-term renal complications are of great importance. Intermittent urethral catheterization to aid in bladder emptying and to decrease the frequency of urinary tract infections is the mainstay of treatment. Surgery to construct an alternative catheterizable channel or vesicostomy may be required in intolerant patients. Prophylaxis of urinary tract infections should be undertaken.[44]

Overall Strategy

The overall strategy is summarized in Figure 46.4-1.

OUTCOME AND PROGNOSIS

Complications

Stoma prolapse, recurrent pancreatitis,[112] diversion colitis,[113] and excessive fluid losses with high ileostomy output[114] have been reported in CIP patients. In patients with gastric and upper digestive tract motor involvement, gastric perforation and gastric bezoars may occur.[3] Medullary thyroid carcinoma associated with MEN type IIB and neurogangliomatosis should be searched for by measuring serum calcitonin levels, and early prophylactic thyroidectomy may be considered.[7]

Outcome

In secondary and acquired forms of CIP, outcome is dependent on the underlying disease responsible for the dysmotility. In cases of destruction of enteric innervation or musculature, deterioration may occur rapidly without specific treatment.[97]

Most often, viral infections resolve spontaneously,[52,74] but some chronic cases have been reported.[77,115]

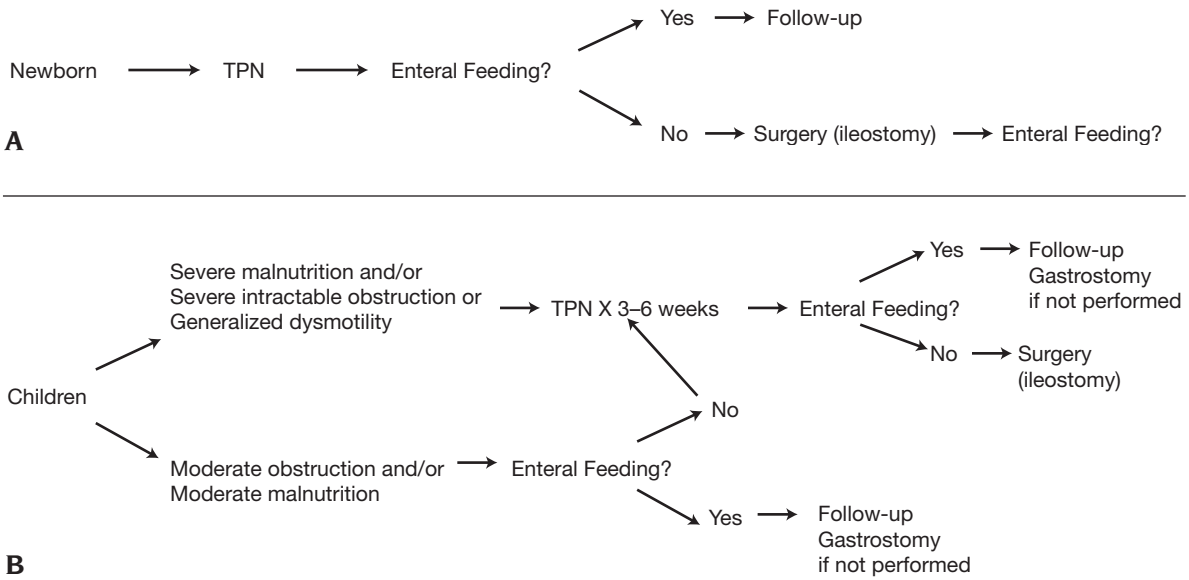

FIGURE 46.4-1 Overall therapeutic strategy for *A*, newborns and *B*, children. Enteral feeding: When possible, the intragastric route is preferred over the jejunal route. In the case of intractable vomiting, continuous gastric feeds should be tried, and on failure, a jejunostomy should be placed. TPN = total parenteral nutrition.

In primary forms of CIP, the prognosis is poor. In one series of 105 patients, two-thirds required parenteral nutrition, and 41% could not be enterally fed. More than half of the patients were TPN dependent for periods ranging from 2 months to 16 years. Eleven patients received TPN for more than 10 years. Twenty-four of the 58 patients who underwent bypass surgery were able to eat normally, and 20 of those eventually had their stoma closed.[3] Heneyke and colleagues reported that if TPN is required for more than 6 months, the child will probably be TPN dependent for at least 4 years.[4]

Mortality

Progress in the management of parenteral nutrition and the use of bowel decompression have modified the high mortality rate reported in historical series in neonates, for whom up to 90% of patients died before 1 year of age.[36,37] In series published more recently, mortality varied from 10 of 105 patients[3] to 14 of 44[4] and 22 of 85 patients.[5]

Underlying CIP is rarely the cause of death except in cases with MEN type IIB and medullary carcinoma. In pediatric series reported to date, the high mortality rate is almost always due to iatrogenic complications. Long-term TPN-related complications, including central venous catheter–associated sepsis and liver failure, and post-transplant complications are the major contributing factors to mortality and morbidity in CIP patients.[3–5] Sudden cardiac arrest has been reported in two patients with CIP.[116]

Prognostic Factors

In the large pediatric series published to date, comparison between patients requiring and those no longer requiring artificial feeding shows significant clinical differences in terms of the likelihood of neonatal onset, urinary tract involvement, requirement for surgery during the course of the disease, and myopathic disorders, all features that are more frequent in cases with a poor prognosis.[3–5] The presence of phase III of the migrating motor complex on antroduodenal manometry has been reported by several groups to be a good prognostic indicator for tolerance of enteral feeding,[41,68] response to cisapride,[117] and mortality.[67]

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CHAPTER 47

SECRETORY TUMORS

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Amine precursor uptake and decarboxylation cells are postulated to be of neural crest origin and to have migrated during embryogenesis to a variety of organs, including the pituitary gland, thyroid, parathyroid, adrenal medulla, lung, pancreas, and gut. These cells, which are capable of making a variety of hormones and neurotransmitters, have been divided into enterochromaffin and nonenterochromaffin cells according to the products they secrete. Enterochromaffin cells secrete one or more neurotransmitters, including serotonin, substance P, histamine, kallikrein, motilin, prostaglandins, and kinins. Nonenterochromaffin cells secrete one or more of the peptide hormones, including gastrin, glucagon, insulin, somatostatin, vasoactive intestinal polypeptide (VIP), and pancreatic polypeptide. Sporadic tumors of these cells arise, secreting one or more and, frequently, several of these hormones or neurotransmitters in pharmacologic quantities.[1] All tumors of the enterochromaffin cells used to be known as carcinoids. The term carcinoid tumors is now reserved for classic midgut tumors secreting serotonin. Other types of carcinoid tumors are called neuroendocrine tumors of the lung, thymus, and so on. The release of transmitters from large carcinoids produces what is referred to as "malignant carcinoid syndrome." This syndrome is extremely rare in childhood, and carcinoids are discussed here only briefly.[2] Islet cell tumors in adults and ganglioneuromas and ganglioneuroblastomas in children are among the most common tumors to secrete peptide hormones affecting the gut. Figure 47-1 shows the computed tomographic (CT) scan of the abdomen, gross pathology of the surgical resection, and immunohistochemistry with neuron-specific enolase and VIP of a child who presented with explosive watery diarrhea and was found to have elevated levels of gastropancreatic hormones. Although tumors may secrete several hormones, the clinical syndrome produced generally reflects the action of only one of the hormones. Syndromes that have been identified include gastrinoma (Zollinger-Ellison) syndrome, VIPoma (Verner-Morrison) syndrome, somatostatinoma syndrome, and glucagonoma syndrome. The first two of these syndromes have been described in children

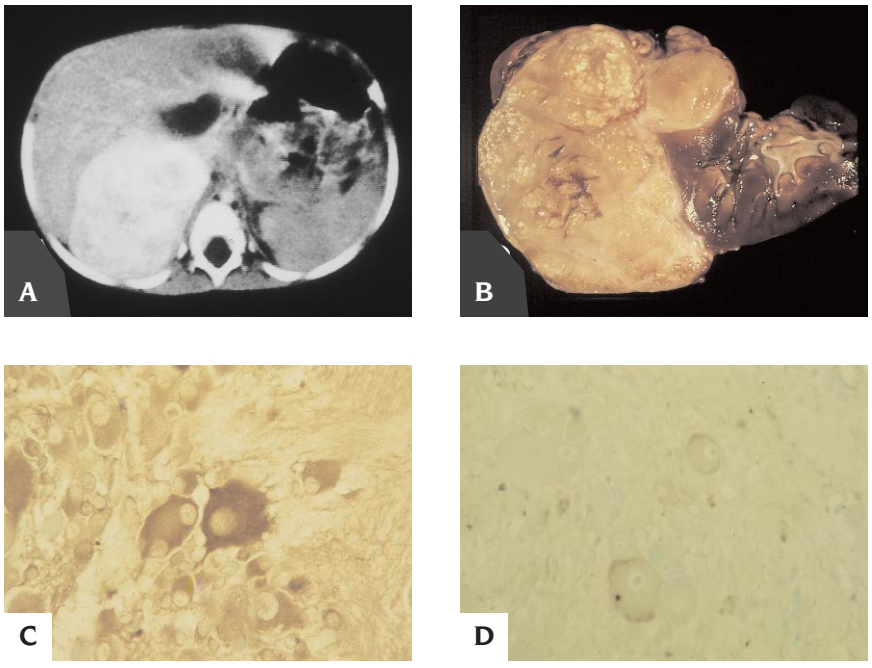

FIGURE 47-1 Child with vasoactive intestinal polypeptide (VIP)-secreting ganglioneuroblastoma. A 20-month-old child presented with a 10-month history of watery, explosive diarrhea, and abdominal distention. She was found to have elevated serum levels of norepinephrine, epinephrine, VIP, dopamine, vanillylmandelic acid, and homovanillic acid. Evaluation by computed tomography demonstrated a suprarenal mass (*A*) consistent with the tumor found at resection (*B*). Staining of the tumor revealed neuron-specific enolase, a neuroendocrine enzyme marker (*C*), and VIP (*D*) immunoreactivity. Courtesy of Dr. Philip M. Sherman, FRCP, Departments of Pediatrics and Microbiology, and Dr. Ernest Cutz, Department of Pathology, Hospital for Sick Children, University of Toronto, ON.

and are discussed here in some detail. Somatostatinoma syndrome and glucagonoma syndrome have been reported only in adults; they are discussed briefly on the assumption that they will be recognized in childhood eventually.

GENETIC INSIGHTS

Knudson initially proposed that cancers in affected individuals require two gene mutations, and this model has subsequently been confirmed and expanded.[3–5] This "two-hit" hypothesis suggests that such susceptible individuals are born with a mutation that predisposes them to acquire the cancer and that a second mutation allows the cancer to appear (the presence of both mutations in utero would be expected to be lethal). This model has been used to explain why certain families are predisposed to endocrine tumors, and three distinct types of multiple endocrine neoplasia (MEN) have been identified: I, IIA, and IIB. There is now clear evidence that "two hits" occur in MEN I[4] and II.[6] In MEN I, mutations occur in the putative tumor suppressor gene, menin, on chromosome 11q13.[7,8] Menin is a nuclear protein that interacts with several proteins involved in transcription and cell growth regulation.[9] Menin inactivation also antagonizes transforming growth factor-β–mediated cell growth inhibition.[10] The *MENI* gene is also frequently mutated in many sporadic tumors, including parathyroid adenomas, gastrinomas, insulinomas, and bronchial carcinoids.

Both patients with MEN IIA (which is associated with medullary carcinoma of the thyroid, parathyroid hyperplasia, and pheochromocytoma) and patients with MEN IIB (multiple mucosal and alimentary tract neuromas [Figure 47-2], medullary carcinoma of the thyroid, and pheochromocytoma) have a mutation in the region of chromosome 10 coding for the *RET* proto-oncogene.[11] The mutation in MEN IIA involves the extracellular portion of the molecule, whereas the mutation in MEN IIB involves the intracellular portion of the molecule. Mutations in the same gene are associated with the development of Hirschsprung disease.[12] It appears that loss of function mutations of the *RET*-encoded tyrosine kinase result in Hirschsprung disease,[13] whereas overexpression (gain of function) mutations result in MEN IIA and MEN IIB.[14] Rarely, overexpression mutations of *RET* have been associated with both MEN IIA and Hirschsprung disease.[15] Clinical aspects of the MEN tumors are discussed in more detail under the specific syndromes described below.

Genetic changes have also been described in sporadic pancreatic neuroendocrine tumors. Mutations in the *DPC4/SMAD4* gene, a tumor suppressor gene located in pancreatic cancer locus 4, have been seen in nonhormone-secreting pancreatic neuroendocrine tumors but not in

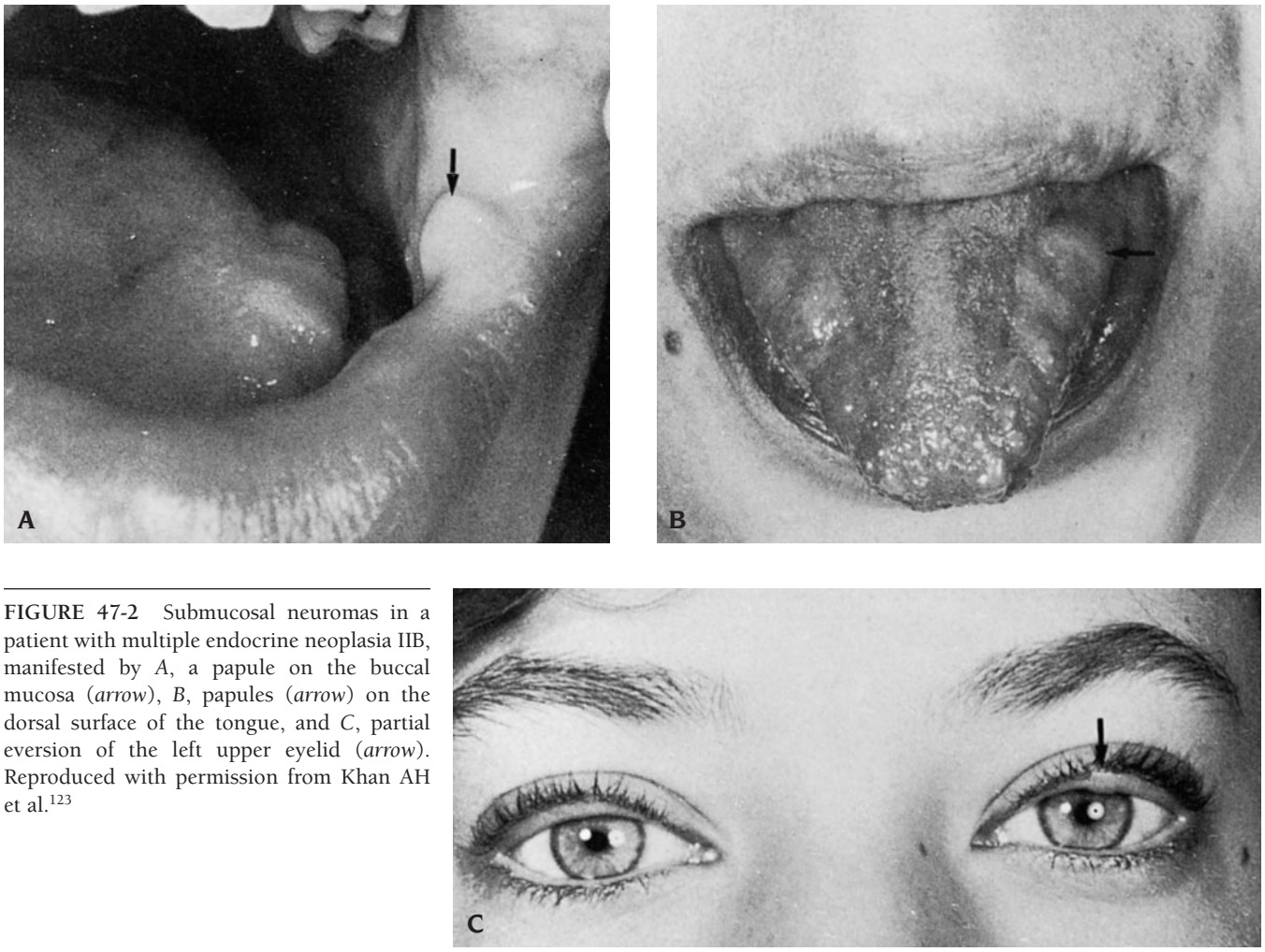

FIGURE 47-2 Submucosal neuromas in a patient with multiple endocrine neoplasia IIB, manifested by *A*, a papule on the buccal mucosa (*arrow*), *B*, papules (*arrow*) on the dorsal surface of the tongue, and *C*, partial eversion of the left upper eyelid (*arrow*). Reproduced with permission from Khan AH et al.[123]

their hormone-secreting counterparts.[16] Inactivation of the *p16/MTS1* tumor suppressor gene has been described in both secreting and nonsecreting tumors.[17] Anomalous overexpression of *p27(Kip1)*, a tumor suppressor, has been described in pancreatic endocrine tumors.[18] Mutations of *Reg*, a gene that encodes a secretory protein proposed to regulate islet beta cell and gastric mucous cell growth, have been associated with enterochromaffin-like (ECL) cell carcinoids in rats.[19]

GENERAL DIAGNOSTIC CONSIDERATIONS

Neuroendocrine tumors affecting function of the gastrointestinal tract are uncommon and present many clinical, diagnostic, localization, and therapeutic challenges. The clinical syndromes caused by these neoplasms are due to the systemic effects of their secretory products. Their diagnosis rests on the identification of humoral factors rather than on tissue biopsy. Their small size—often less than 1 cm—and frequent occult location can make diagnosis and localization difficult. These tumors, with the exception of insulinomas, often possess high concentrations of the somatostatin receptor *SSTR2*, which has a very high affinity for octreotide.[20] This receptor lends itself to imaging with the labeled somatostatin analog octreotide ([^{111}In-diethylenetriamine pentaacetic acid–DPhe^{1}] octreotide). Thus, octreotide can be used in the diagnosis and treatment of tumors that express the *SSTR2* receptor, and somatostatin receptor scintigraphy (SRS) is helpful in tumor detection.[21] Surgical resection of localized neuroendocrine tumors leads to a complete cure. Where complete resection is not possible, debulking helps by reducing humoral levels of the offending hormone and facilitating medical management. In addition, medical therapies can help control symptoms in patients with unresectable tumors.

GASTRINOMA SYNDROME (ZOLLINGER-ELLISON SYNDROME)

In 1955, Zollinger and Ellison described a syndrome of peptic ulcerations in the jejunum, associated with islet cell tumors of the pancreas.[22] They postulated that this represented secretion by the islet cells of a substance that stimulated gastric acid secretion. This factor was subsequently demonstrated to be gastrin.[23] The major features of the syndrome (gastric acid hypersecretion, peptic ulceration, diarrhea, and steatorrhea[24]) are understandable as responses to excessive levels of gastrin.

Pathogenesis

Under physiologic conditions, G cells in the gastric antrum secrete gastrin,[25] a peptide that mediates 90% of meal-related acid release. The release of gastrin is affected by elevation of gastric pH, the presence of peptides in the stomach, mechanical distention, and a direct effect of the vagus nerve. A negative feedback exists between the H^+ concentration and the secretion of gastrin. In addition to stimulating gastric acid secretion, gastrin stimulates ECL cell growth. Indeed, high circulating gastrin levels in some disease states are associated with ECL carcinoid-like tumors.[26] Gastrin also appears to stimulate growth of acid-producing tissue and appears to do this independently of histamine. Vagal tone also supports both ECL cell mass and parietal cell mass independently of gastrin.[27]

Normally, a number of systemic and local mechanisms operate to limit or decrease gastric acid secretion. In addition to gastric acid, secretin, released in response to acid delivery to the duodenum, inhibits gastrin secretion.[28] Somatostatin reduces gastrin release by G cells and exerts suppressive effects on both histamine release by ECL cells and acid secretion by parietal cells.[29] Histamine exerts negative feedback on its own secretion via the H_3 receptor on ECL cells.[30] Prostaglandins also suppress the effect of histamine on parietal cells and inhibit histamine release by ECL cells.[31,32] This carefully balanced regulation is important for normal homeostasis. Zollinger-Ellison syndrome (ZES) results from the unregulated secretion of gastrin from an ectopic source, and excessive acid secretion causes most of the features of the disease. Peptic ulcers in ZES resolve when acid secretion is reduced below 10 mEq/h.[33] The diarrhea and steatorrhea seen in patients with this syndrome are also acid related. The mechanisms of diarrhea include the delivery of large volumes of acid into the duodenum, resulting in jejunitis owing to acidification and hypermotility of the intestine secondary to high gastrin levels. Steatorrhea may result from inactivation of pancreatic enzymes or from precipitation of bile salts.

Clinical Features

The age at onset of reported cases of ZES has been from 7 to 90 years of age, with a peak incidence between 30 and 50 years of age.[23] Approximately one-third of adult patients with ZES have the familial MEN I, in which are found (in decreasing order of frequency) tumors of the parathyroids, pancreatic islets, and pituitary gland.[34] Only 5% of patients with MEN I and 2.5% of patients with sporadic disease had the onset of ZES below 20 years.[35] Most patients present with signs of recurrent peptic ulcer disease or unexplained diarrhea.[35] Three-quarters of the patients present with abdominal pain or diarrhea. Heartburn is seen in 42%, whereas vomiting and gastrointestinal bleeding are each seen in roughly one-quarter of patients. Approximately 18% of patients present with weight loss. A sign that is observed in 94% of ZES patients is prominent gastric folds on endoscopy. Uncommonly, patients have esophageal strictures (3%) or pyloric/duodenal scarring (10%). Nearly 75% of the patients have confirmed peptic ulcer disease.[35] Although multiple ulcers in unusual locations are considered pathognomonic of ZES, nearly 75% of the ulcers in ZES patients are solitary and located in the duodenal bulb.[36]

Clear-cut criteria for undertaking detailed diagnostic studies for ZES in children have not been established. Most children present with abdominal pain; symptoms of typical ulcer disease, such as hematemesis, vomiting, and melena, are less frequent. Patients with unusual presentations of peptic ulcer disease, patients with a family history of ZES, or symptoms in a patient with a family history of MEN I

and other conditions listed in Table 47-1[37] should stimulate an evaluation. Persistent diarrhea with an abnormal small bowel biopsy, steatorrhea, and a negative workup for infection, celiac disease, or allergy should lead to an evaluation for gastrinoma. Patients with ulcer disease and kidney stones should also be evaluated for ZES because excessive acid secretion interferes with bile salt action, causing calcium to combine with fats to form soaps in the gut lumen; oxalate is then free to go into solution and form oxalate calculi in the kidney.

Diagnosis

The sign of gastrinoma is the unregulated and excessive secretion of gastrin. Figure 47-3 shows an algorithmic approach to the diagnosis of a gastrinoma. The best screening test is a fasting serum gastrin level, which, in children with at least an 8-hour fast, should be less than 125 pg/mL.[38] Often patients with a gastrinoma have levels more than 5- to 10-fold higher. An elevated serum gastrin may be seen in other conditions (listed in Table 47-2)[39] and should be further investigated with a provocative test, the easiest and most specific of which is the secretin stimulation test.[40,41] For this test, 2 U/kg of pure secretin are injected intravenously over 30 seconds. Blood samples are taken for gastrin measurement 5 minutes before injection, just before injection, and at 5-minute intervals for 30 minutes after injection. Secretin has no effect on the gastrin level in normal patients and patients with benign G-cell hyperplasia but results in at least a doubling of the gastrin level in patients with gastrinoma (it usually increases by more than 200 pg/mL). A negative result in a patient with recurrent ulcer disease should be investigated with a study of gastric acid secretion, using pentagastrin. Normal basal acid secretion in children has been estimated at 0.04 ± 0.01 mEq/kg/h.[42] Some important diagnostic criteria for ZES in adults with reference to a pentagastrin-stimulated gastric acid secretion test are as follows: (1) basal acid output ≥ 15 mEq/h (highest sensitivity and specificity); (2) basal acid H^+ concentration/maximal acid H^+ concentration ≥ 0.6; and (3) in a patient with hypergastrinemia, gastric pH > 2 rules out ZES.[43] A positive secretin stimulation test or, where secretin is unavailable, an elevated fasting gastrin level in association with an elevated basal acid secretion (if available) should trigger an intensive search for a gastrinoma.[41]

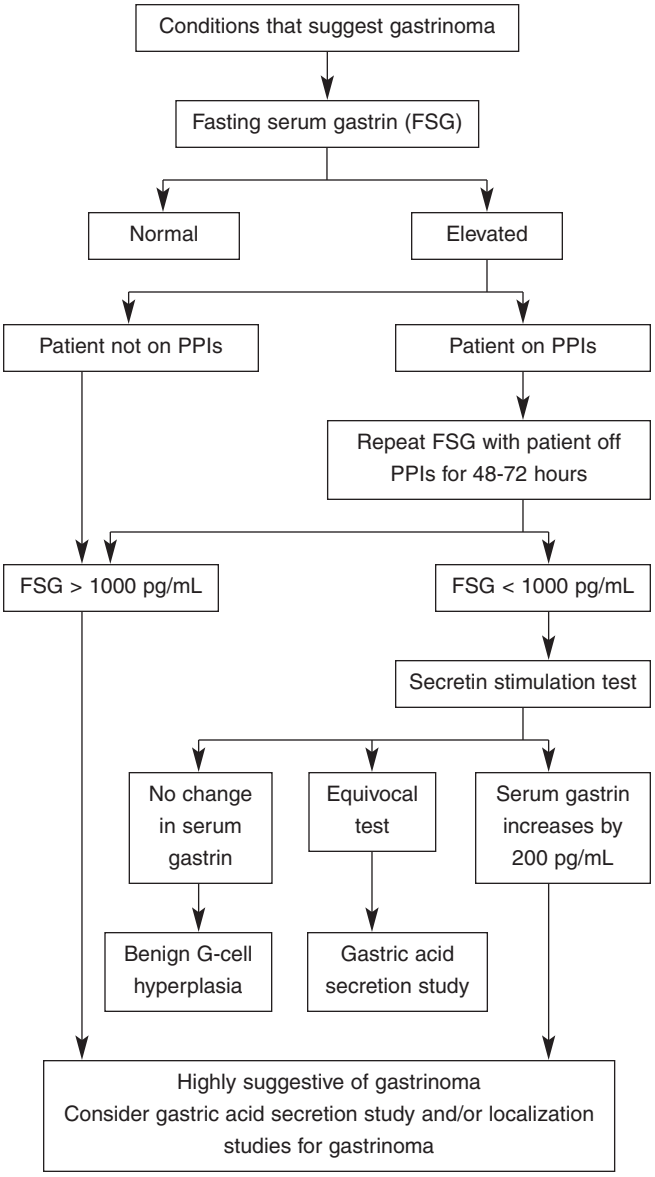

FIGURE 47-3 Elevated fasting gastrin level or positive secretin stimulation test in the setting of elevated basal acid secretion strongly suggests the presence of a gastrinoma. PPI = proton pump inhibitor.

TABLE 47-1 CONDITIONS IN CHILDREN THAT SUGGEST TESTING FOR GASTRINOMA

SYMPTOMS
Peptic ulcer disease and weight loss
Long history of peptic ulcer disease (> 5 yr)
Peptic ulcer disease unresponsive to standard medical therapy
Peptic ulcer and diarrhea
Strong family history of peptic ulcer
Personal or family history of MEN I
Peptic ulcer disease and urinary tract calculi
Recurrent peptic ulcer disease negative for *Helicobacter pylori* and without a history of NSAID use
Unexplained secretory diarrhea
SIGNS
Prominent gastric folds
Multiple upper gastrointestinal tract ulcers
Ulcers distal to the first portion of the duodenum
Gastric acid hypersecretion, hypergastrinemia, or both
COMPLICATIONS OF PUD
Bleeding, perforation, esophageal stricture, gastric outlet obstruction

Adapted from McGuigan JE[37] and Roy PK et al.[35]
MEN = multiple endocrine neoplasia; NSAID = nonsteroidal anti-inflammatory drug; PUD = precursor uptake and decarboxylation.

Localization

Gastrinomas are frequently very small (3 cm or less in size), and multiple tumors are common.[41] Typically, they are found in the "gastrinoma triangle," which lies between the cystic duct, the third portion of the duodenum, and the neck of the pancreas and includes the head of the pancreas, the duodenum, and the porta hepatic.[44] In children, gastrinomas

TABLE 47-2 CAUSES OF HYPERGASTRINEMIA

HYPERGASTRINEMIA WITHOUT GASTRIC HYPERSECRETION
Achlorhydria or hypochlorhydria associated with
Pernicious anemia
Atrophic gastritis
Gastric ulcer
Prior vagotomy
Helicobacter pylori infection
Therapy with proton pump inhibitors
HYPERGASTRINEMIA WITH GASTRIC HYPERSECRETION
Gastrinoma
Renal failure
Retained antrum
Antral G-cell hyperplasia
Pheochromocytoma
Hypercalcemia
Duodenal ulcer
Pyloric obstruction

Adapted from Spindel E et al.[39]

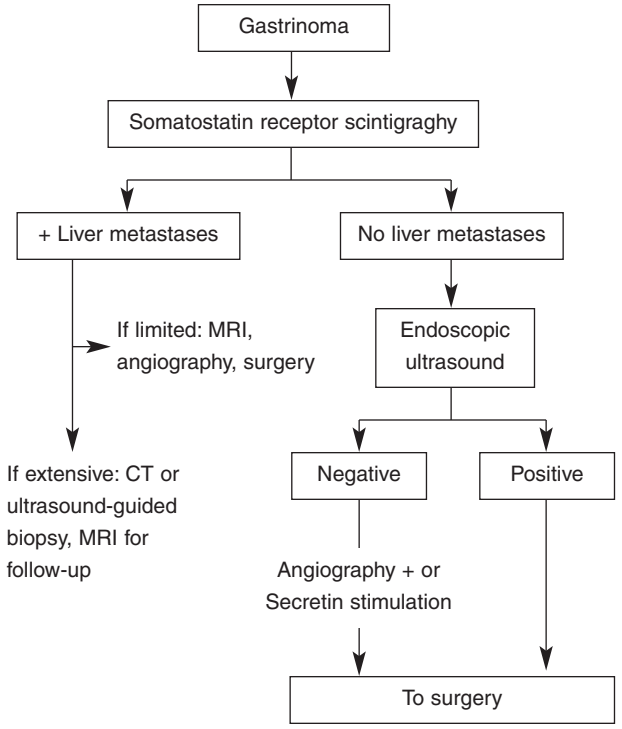

FIGURE 47-4 Approach to localization of gastrinomas. The same algorithm can be used to localize VIPomas. CT = computed tomography; MRI = magnetic resonance imaging. Reproduced with permission from Jensen RT et al.[47]

have been reported in the liver and kidney.[45,46] At least 60% of gastrinomas are malignant, and many have already metastasized by the time they are found. The most common sites of metastases are local lymph nodes and the liver.

The algorithm delineated in Figure 47-4 presents a useful approach to the localization of a gastrinoma.[47] Various studies have shown that the sensitivity of SRS is better than any single conventional imaging study, whether ultrasonography (US), computed tomography (CT), magnetic resonance imaging (MRI), or selective angiography, and is at least equal to or better than all other imaging modalities combined.[48,49] The specificity of SRS appears generally excellent. This and the ability to image the entire body make SRS the initial imaging modality of choice in gastrinomas and other neuroendocrine tumors (with the exception of insulinomas). If liver metastases are seen on SRS and are extensive, as is the case with 80 to 90% of liver metastases, then surgery is generally not a consideration. The diagnosis of gastrinoma can then be confirmed using CT- or US-guided biopsy. In the case of extensive metastases, an MRI study can be used to estimate tumor size and facilitate ongoing assessment of tumor growth. If metastases are limited and cytoreductive surgery is considered, then selective angiography and MRI can be performed to delineate the exact location and size of the metastases and their relationship to surrounding vessels. Hepatic arterial injection of secretin followed by hepatic venous sampling changed the clinical decision-making process in 22% of patients with limited hepatic metastases.[50] As SRS detects more than 90% of all hepatic metastases, and other studies add very little to this figure, additional investigations are unnecessary to rule out hepatic metastases if SRS is negative. However, 40% of extrapancreatic primary tumors may be missed by SRS; thus, the primary lesion may need to be localized using endoscopic ultrasonography (EUS). EUS localizes almost all pancreatic endocrine tumors, although it may miss 50% of duodenal neuroendocrine tumors.[51,52] Combining EUS and SRS provides 93% sensitivity in preoperative localization of gastrinomas.[53] If EUS and SRS are negative, selective intra-arterial injection of secretin followed by hepatic venous sampling should be performed.[54] At surgery, intraoperative US is essential, and duodenal exploration with a duodenotomy should be performed. Intraoperative gastrin levels with and without an intraoperative venous secretin stimulation test have been used to demonstrate completeness of resection.[55] This test depends on a very rapid turnaround time for gastrin measurement, a feature not available in most centers.

Surgical Management

The only potential cure for gastrinoma is complete surgical resection (Table 47-3). Because the tumors are small, preoperative efforts to localize them with radiographic and selective sampling techniques should be aggressive. Careful surgical exploration with the assistance of duodenal transillumination and intraoperative US of the pancreas is most likely to localize a tumor.[56] Aggressive resection of local lymph nodes and resectable metastases is recommended. On rare occasions, pancreaticoduodenectomy is required. Reported short-term cures in patients with sporadic gastrinomas without metastases on imaging studies approach 80%. Aggressive surgical intervention in MEN I patients is controversial because the disease has virtually a 100% recurrence rate. In unresectable cases and in MEN I, a highly selective vagotomy at the time of surgery may make subsequent medical management easier.[57] Proton pump inhibitors have practically obviated the need for gastrectomy.

If it is possible to safely resect the primary tumor and the liver metastases, surgery should be performed. Even if complete resection is not possible, selected patients may still benefit from debulking.[58] When liver debulking is not

TABLE 47-3 TREATMENT OPTIONS IN GASTRINOMA

CURE: Surgical resection
PALLIATION OF ACID-RELATED SYMPTOMS
Proton pump inhibitor therapy
Octreotide
Parathyroidectomy (MEN I patients)
Palliation of tumor
Streptozocin and 5-fluorouracil
Octreotide
Interferon-α

MEN = multiple endocrine neoplasia.

possible, selective hepatic embolization is another consideration to control symptoms related to excess hormone secretion or pain.[59]

MEDICAL MANAGEMENT OF ACID SECRETION

Acid secretion can be controlled with high doses of proton pump inhibitors given twice daily (adults need 60 to 120 mg/d of omeprazole, although higher doses may be used).[60] Therefore, proton pump inhibitors are the cornerstone of medical therapy in ZES. Intravenous pantoprazole (160–240 mg/d) effectively controls acid production in > 90% of patients with ZES.[61,62] The object of therapy is to keep acid secretion rates below 10 mEq/h by using an adequate dose and monitoring its effect on gastric pH. Acid secretion can be medically controlled in virtually all patients with ZES.

CHEMOTHERAPY

In patients with extensive metastatic disease, significant tumor regression has been achieved with streptozocin, used either alone or in combination with 5-fluorouracil. The morbidity of these agents has been reduced by new antiemetics. The addition of α-interferon and octreotide to chemotherapy produces a more pronounced antiproliferative effect, overcomes resistance developing to chemotherapeutic agents,[63] and has extended the median survival of patients with liver metastases to 50 months.[64] Chemotherapy may be used for palliation when ablative techniques have failed or when significant extrahepatic disease is present. Because of the modest success of current chemotherapeutic regimens, patients with advanced disease in need of treatment may be encouraged to enrol in clinical trials testing newer agents or treatment strategies.[65] The results have generally been dismal when liver transplant has been performed in disease metastatic to the liver.[65]

PROGNOSIS

With an aggressive diagnostic and surgical approach, about 30% of patients can be cured of sporadic gastrinomas by tumor resection.[66] Even when they cannot be cured, patients have a 15-year survival rate of 83% for ZES-related deaths.[67] The correlates of a poor prognosis are primary pancreatic (not duodenal) lesions; metastases to lymph nodes, liver, and bone; ectopic Cushing syndrome; and a highly exaggerated gastrin level.[67] Patients with MEN I who have apparently localized gastrinomas can rarely be cured of the disease. Only 5% of patients with MEN I and ZES were biochemically cured at 5 years after diagnosis. In a significant subset of patients with MEN I and ZES, aggressive tumor growth occurs and can lead to decreased survival. The identification of prognostic factors that identify this group will be important clinically.[68] Although only a small proportion of these patients can be cured of their disease, long-term survival is usual.

All patients with potentially resectable ZES who do not have MEN I should undergo exploration because surgical outcome is not related to preoperative testing.[69] A normal fasting serum gastrin or secretin stimulation test immediately postoperatively predicts a long-term cure. Limited data in children indicate that 25- to 30-year survival rates are possible.[70]

VIPOMA SYNDROME (VERNER-MORRISON SYNDROME; WATERY DIARRHEA, HYPOKALEMIA, ACHLORHYDRIA SYNDROME; PANCREATIC CHOLERA)

In 1958, Verner and Morrison described a syndrome of refractory watery diarrhea and hypokalemia associated with islet cell tumors of the pancreas.[71] In 1970, Said and Mutt described a polypeptide, isolated from the small intestine, that caused systemic vasodilatation, hypotension, and hyperglycemia.[72] Bloom and colleagues in 1973 demonstrated that patients with watery diarrhea, hypokalemia, achlorhydria (WDHA) syndrome have markedly elevated levels of this VIP.[73] It was subsequently shown that the major features of WDHA syndrome resulted from the actions of VIP.[74]

PATHOGENESIS

VIP is widely distributed through the gut and the central nervous system. It stimulates adenylate cyclase activity, raises intracellular levels of cyclic adenosine monophosphate, and initiates a signal transduction cascade that results in intestinal secretion.[75] Intravenous infusion of VIP, both in humans and in animals, stimulates cyclic adenosine monophosphate production by enterocytes, producing effects very similar to those of cholera toxin. The combination of VIP-induced active secretion of chloride (water and sodium follow passively) by crypt cells and decreased passive absorption of water and salt by villous cells results in copious watery diarrhea of an isotonic solution of electrolytes. Passive potassium absorption in the small bowel is reduced when bulk absorption of water and sodium is reduced. Studies in patients with WDHA syndrome show that most potassium losses in this disease are colonic.[76] The VIP stimulation of adenylate cyclase activity in the colon causes potassium secretion. It has been suggested (but not proven) that aldosterone released in response to vascular volume contraction also stimulates potassium secretion in the colon. The results of this increased potassium secretion are hypokalemia and acidosis.

Vasodilatation, manifested by facial flushing, was one of the earliest reported effects of VIP. This symptom has been reproduced in humans by the infusion of VIP, producing

blood levels seen in WDHA syndrome.[72] Hyperglycemia, which has been reported in animals, has been seen in some patients with WDHA syndrome but has not been reproduced by VIP infusion.[74,77] Hypercalcemia has been reported in patients with VIPoma, but the mechanism is not known.[77]

Clinical Features

WDHA syndrome has been reported in all age groups. Virtually all patients have presented with watery diarrhea and hypokalemia, and several have been incontinent. Fecal losses while fasting have been at least 20 mL/kg/d and in most cases exceed 50 mL/kg/d. Fecal osmolality is entirely accounted for as twice the sum of the concentrations of sodium and potassium. Notably, steatorrhea is not a feature of the syndrome. Hypochlorhydria occurs in about 70% of patients, hyperglycemia in 20 to 50%, and hypercalcemia in 20 to 50%. Flushing is present in about 20% and is frequently intermittent, indicating that hormone release by the tumor is also sporadic.[77,78]

In children, most reported VIPomas are ganglioneuromas or ganglioneuroblastomas found in the neck, thorax, or suprarenal or pelvic areas.[78] Neuroblastomas may also uncommonly secrete VIP and may mature to the ganglioneuroblastoma or ganglioneuroma phenotype. There are two reports of VIP-secreting pancreatic tumors (the most common VIPomas in adults) in children.[79,80] There has been a case report of islet cell hyperplasia associated with VIP hypersecretion and villous atrophy and another report of villous atrophy with VIP hypersecretion with no evidence of tumor or hyperplasia.[81,82] The relationship between VIP and villous atrophy in these cases is not clear. As opposed to pancreatic tumors, ganglioneuromas and ganglioneuroblastomas tend to metastasize less frequently (30% versus 56%) and exhibit characteristics of malignancy less often (one-third versus two-thirds). Almost 90% of the neurogenic tumors can be resected as opposed to ~ 70% of pancreatic VIPomas.[83]

Extrapancreatic lesions have been located in the usual areas of neurogenic tumor occurrence, including the adrenal gland and the paravertebral sympathetic tissues in the abdomen, chest, and neck. These tumors have been associated with hypercalcemia, hypophosphatemia, hyperglycemia, and increased urinary catecholamines. The symptoms of WDHA syndrome precede the recognition of a neural crest tumor in most cases.[84–86] They usually cause hypochlorhydria as opposed to the traditional achlorhydria.[82] In contrast to individuals with VIP-secreting pancreatic tumors, patients with neurogenic lesions have normal levels of pancreatic polypeptide, gastrin, insulin, and somatostatin.

Diagnosis

Diarrhea that persists during fasting in amounts greater than 20 mL/kg/d should suggest VIPoma as a possible diagnosis.[87,88] Table 47-4 lists a variety of causes of secretory diarrhea. Common causes include infection and laxative administration. Stool electrolytes and osmolality should be measured in initial investigations. If twice the sum of the sodium and potassium concentrations is significantly less than the osmolality, a search should be initiated for an osmotic agent (eg, magnesium or lactulose). Stool should be screened for phenolphthalein, senna, and other stimulant laxatives. If true secretory diarrhea is found, cultures should be obtained for toxigenic organisms. These include *Clostridium difficile* and toxigenic *Escherichia coli*, which are not recognized on routine stool cultures. These toxigenic strains can be identified by reference laboratories. In the absence of a positive stool culture, a small bowel biopsy should be examined for adherent *E. coli* organisms. If vomiting is present, a screen for emetine (which does not appear on routine toxic screens) should be obtained because secretory diarrhea has been seen with ipecac intoxication. If all of these steps do not yield a cause for secretory diarrhea, hormone-secreting tumors should be considered. Secretory diarrhea is seen with VIPoma, tumors secreting prostaglandin E_2,[77,89] ZES, and carcinoid syndrome. If the level of suspicion is high, gastrin, 5-hydroxyindoleacetic acid, prostaglandin E_2, and serotonin levels should be checked. Chromogranin A is a standard probe for immunohistochemical analyses of some neuroendocrine tumors, including gastrinomas and carcinoid tumors, and elevated serum chromogranin A levels are diagnostic of these neoplasms. Measurement of chromogranin A levels in blood can also be used to monitor the progression or regression of neuroendocrine tumors during treatment.[90]

Hormone levels should be measured in an experienced reference laboratory, and abnormal values should be repeated. Although VIP levels are usually considerably elevated in this condition, VIPomas should be considered in patients with values just above the upper limit of normal.[91] "Falsely" elevated VIP levels may be seen in neurofibromatosis, small bowel resection, chronic renal failure, and prolonged fasting.[91] The incidence of false-negative tests is not known. If blood tests support the diagnosis of VIPoma, a vigorous search for the tumor must begin, remembering that in childhood, most of the tumors are ganglioneuromas or ganglioneuroblastomas and may be found anywhere from the pelvis to the neck. They are frequently identified on chest radiography, US, EUS,[92] CT, MRI, or preoperative angiography. However, SRS is again the procedure of choice.[93] Because these tumors may secrete a variety of hormones, preoperative screening with a broad panel of

TABLE 47-4 DIFFERENTIAL DIAGNOSIS OF SECRETORY DIARRHEA

Infection with toxigenic organisms (eg, *Escherichia coli*, *Vibrio cholerae*, *Salmonella* spp, *Clostridium difficile*)
Mucosal necrosis or atrophy
Surreptitious drug administration
Laxatives
Ipecac
Bile acid malabsorption
Congenital electrolyte transport defects (eg, chloride-losing diarrhea)
Structural enterocyte disorders (eg, microvillus inclusion disease, tufting enteropathy/epithelial dysplasia)
Hormone-secreting tumors
Vasoactive intestinal polypeptide
Prostaglandin E_2
5-Hydroxyindoleacetic acid
Serotonin

hormones, including somatostatin, pancreatic polypeptide, insulin, and adrenocorticotropic hormone, is often performed in adults because metastases may eventually secrete only one of them.

Therapy and Prognosis

Operative tumor resection offers the only possible cure for VIPoma. In childhood, in which 70 to 80% of VIPomas are resectable, the outlook is good.[78,94] The overall survival in children with neuroblastomas has been relatively poor (35–40%). However, children with neuroblastomas that secrete VIP do better than other children with non–VIP-secreting tumors.[84]

Preoperative stabilization of fluid and electrolyte balance is critical. Intraoperative US may provide additional help in locating tumors not seen on preoperative imaging. It has been shown that with successful resection, VIP levels fall to normal within hours postoperatively.[95] When the tumor is not resectable, a long-term symptomatic remission may be achieved with medication, although management has been very difficult. Supportive therapy includes intravenous fluid and oral glucose electrolyte solutions. A variety of agents have been used to reduce the diarrhea. Prednisone has been effective in reducing diarrhea temporarily in many patients, but it has no effect on the circulating level of VIP.[77,78] Somatostatin inhibits the release of VIP from pancreatic tumors, and a long-acting somatostatin analog octreotide has been very useful in controlling diarrhea from pancreatic VIPomas.[96–98] Long-acting preparations of octreotide and lanreotide have been effective in adults, but the role of these somatostatin analogs in the treatment of unresectable tumors causing VIPoma syndrome in childhood, in which most of the tumors are ganglioneuromas, has not yet been fully explored. Somatostatin has been used in one patient with a VIP-secreting neuroblastoma, with reduction in VIP levels but without cessation of diarrhea.[99] Good control was obtained in an infant with an enlarged pancreas and elevated VIP levels.[100] The dose of octreotide should be titrated to achieve normal stooling with improved fluid and electrolyte balance without necessarily normalizing VIP levels.[101] Streptozocin, 5-fluorouracil, α-interferon, and octreotide have been used in isolation and in combination[102] with success in the treatment of pancreatic tumors, but their effectiveness in treating ganglioneuroblastoma has not been reported.

SOMATOSTATINOMA

Somatostatin is a potent inhibitor of the release of a variety of hormones, including gastrin, cholecystokinin, insulin, and secretin.[103] The symptoms of somatostatinoma, therefore, are diabetes, gallstones, steatorrhea, and hypochlorhydria.[104] Somatostatinoma has not yet been recognized in a patient younger than 18 years of age.

GLUCAGONOMA

Excessive secretion of glucagon induces a catabolic state, glucose intolerance, hypoaminoacidemia, and a characteristic dermatitis. The most striking clinical features of glucagonoma syndrome are weight loss, rash, and diabetes.[105–107] The rash begins as red scaly lesions on the extremities, in the creases, and about the mouth. These become bullous and, finally, crusty and confluent. Dystrophy of the nails and alopecia are also seen. All of these findings are probably manifestations of the severely catabolic state. Glucagonoma has not yet been described in a patient younger than 18 years of age. Diagnosis is suggested by diabetes and a characteristic rash; it is confirmed by finding an elevated level of circulating glucagon.

MEN Syndromes

Several familial groupings of multiple endocrine tumors have been classified according to the pattern of organ involvement as MEN I (hyperparathyroidism, islet cell tumors of the pancreas, and nonfunctional adenomas of the pituitary), MEN IIA (medullary carcinoma of the thyroid, parathyroid hyperplasia, and pheochromocytoma), and MEN IIB (multiple mucosal and alimentary tract neuromas, medullary carcinoma of the thyroid, and pheochromocytoma).[108] A patient may be considered to have MEN I if two of the three principal MEN I–related tumors affecting the parathyroids, pancreatic islets, or anterior pituitary gland have occurred.[109] Patients with MEN I comprise approximately one-sixth of all patients with hyperparathyroidism, one-third of all patients with ZES, 4% of all patients with insulinoma, and a small fraction of patients with VIPoma, somatostatinoma, and glucagonoma syndromes.[110] The extent of organ involvement tends to increase with the patient's age, and the parathyroid glands are most frequently involved. In one extensively studied kindred, the frequency of parathyroid involvement was essentially 0% at 15 years of age and 100% at 40 years of age.[111] In that kindred, hyperparathyroidism always preceded hypergastrinemia. In another kindred, ZES in a child was the first manifestation.

Screening recommendations for first-degree relatives of patients with MEN I are shown in Table 47-5. To test for MEN I, molecular genetic testing using direct deoxyribonucleic acid (DNA) screening strategies is the preferred method of screening. Whereas screening family members in a MEN I family with a known mutation can be fast and inexpensive, screening for a mutation in a new MEN I family or proband is an expensive and laborious task.[112] Neuroendocrine tumors in these patients are frequently extrapancreatic (duodenum and lymph nodes) and multiple and are therefore rarely resectable. However, they are late to metastasize, and long survival is the rule.[110] The surgical management of ZES in MEN I is controversial. Some suggest annual screening of gastrinomas with SRS and resection when tumors attain 3 cm in size because metastases rarely occur when tumors are < 3 cm.[113] However, once the primary tumor is > 3 cm in MEN I, surgery does not prevent the development of metachronous liver metastases.[114] Therefore, most groups do not require such substantial tumor burdens, and many recommend operation if the imaged lesion is 1 cm.[115,116] Several believe that the goal in MEN I is cancer prevention and that surgery

TABLE 47-5 TEST SCHEDULES TO SCREEN FOR TUMOR EXPRESSION IN A HIGHLY LIKELY CARRIER OF MEN I MUTATION

TUMOR	AGE TO BEGIN (YR)	ANNUAL BIOCHEMICAL TESTS	IMAGING TESTS EVERY 3 YR
Parathyroid adenoma	8	Calcium (Ca^{++}), Parathyroid hormone	None
Gastrinoma	20	Gastrin*	None
Insulinoma	5	Fasting glucose, insulin	None
Other enteropancreatic	20	Chromogranin A, glucagon, proinsulin	SRS, CT scan or MRI
Anterior pituitary	5	PRL, IGF-I	MRI
Foregut carcinoid	20	None	CT scan

Adapted from Brandi ML et al.[15]
CT = computed tomography; IGF = insulin-like growth factor; MEN = multiple endocrine neoplasia; MRI = magnetic resonance imaging; PRL = prolactin; SRS = somatostatin receptor scintigraphy.
*Gastric acid output measured if gastrin is high; secretin-stimulated gastrin measured if gastrin is high or if gastric acid output is high.

should be performed if the biochemical diagnosis is unequivocal, even without other signs.[117] There may also be other benefits to more global treatment of MEN I; there is evidence that hypergastrinemia is significantly ameliorated by parathyroidectomy.[118]

Major morbidity from MEN I is rare in children. The earliest reported major morbidity in MEN I has been a pituitary macroadenoma in a 5 year old.[119] Clinically evident hyperparathyroidism in MEN I has been reported between the ages of 5 and 8 years. MEN I–associated prolactinomas have been reported in children ages 10 to 13 years. Gastrinoma, the other defining feature of MEN I, has not been seen earlier than age 12 years in MEN I.[35] MEN I–associated insulinoma has been described as early as age 6 years.[119] WDHA syndrome has not been reported in a patient with MEN I who is younger than 18 years of age. All or most of these prior reports of early-onset endocrinopathy have been anecdotal.

Patients with MEN II develop tumors of the thyroid (medullary carcinoma of the thyroid), adrenal medulla (pheochromocytoma), and, sometimes, parathyroid glands. The MEN II group has been divided into two subcategories, depending on the presence (B) or absence (A) of mucosal and intestinal neuromas. MEN IIA accounts for over 75% of MEN II.[120] The MEN IIB subcategory, also known as Sipple syndrome and on occasion referred to as MEN III, is particularly important to pediatric gastroenterologists because children with this disorder present with gastrointestinal symptoms and have a very high risk of developing malignant thyroid tumors. Early identification by regular monitoring of serum levels of calcitonin or thyroidectomy permits early intervention, the only hope of curing medullary carcinoma of the thyroid.

Infants with MEN IIB have feeding problems, including poor suck, colic, and constipation.[121] Severe constipation in the first year of life is common, and mistaken diagnoses of Hirschsprung disease or segmental dilatation of the colon have been made. Rectal or colonic biopsies show ganglioneuromatosis of the colon, which should suggest the diagnosis[122] and may also be seen in Cowden disease. Less common gastrointestinal manifestations have included vomiting and diarrhea; the diarrhea is felt to be the result of calcitonin secreted by the thyroid tumor. Patients usually do not develop hyperparathyroidism. As MEN IIB patients get older, they develop overt mucosal neuromas that appear as nodules on the buccal mucosa, lips, tongue, and eyelids, giving an everted appearance to the eyelids.[123] Photographs of these lesions are shown in Figure 47-2. A decreased upper-to-lower body ratio and corneal neuromas may also be seen. Older patients generally have a marfanoid habitus, and skeletal abnormalities, including pes cavus, slipped capital femoral epiphysis, scoliosis, and pectus excavatum, are very common. The tumors themselves may stain positive for cytokeratin, carcinoembryonic antigen, calcitonin, bombesin, chromogranin, serotonin, and Leu 7.[124]

All patients affected by MEN IIB develop medullary carcinoma of the thyroid, which responds poorly to therapy. The tumor is frequently multifocal and can develop at a very early age. Medullary carcinoma of the thyroid has been seen in a 2-year-old child with MEN IIA and in a 1-year-old child with MEN IIB.[125] Screening for *RET* mutations must be performed in patients suspected of having MEN IIA and IIB and has been suggested in all patients with Hirschsprung disease (for the rare possibility of identifying a *RET* overexpression mutation). Mutations of *RET* occur predominantly in one of six codons in MEN IIA and can be detected with a high likelihood of success.[126] Ninety-nine percent of MEN IIB patients have one of two mutations that can be detected by polymerase chain reaction and single-strand conformational polymorphism.[122,127] Children with MEN IIB should have a prophylactic thyroidectomy certainly by 4 years of age,[128,129] and some have proposed thyroidectomy within the first 6 months and preferably within the first month of life.[15] For all other conditions associated with *RET*-activating mutations, the precise mutation determines the age at which thyroidectomy must be performed.[15]

CARCINOID SYNDROME

The symptoms of carcinoid syndrome include episodic flushing, facial swelling, palpitations, abdominal pain, and explosive diarrhea.[130] Late accompaniments of the syndrome may include mesenteric fibrosis and endocardiac and cardiac valvular fibrosis. The syndrome is caused by tumors of enterochromaffin cells, and symptoms do not appear until the tumors are metastatic. The symptoms appear to be mediated primarily by serotonin, bradykinin, and histamine secreted by the tumor. The fibrosis appears

to be induced by serotonin. Mesenteric fibrosis can cause severe abdominal pain. The secretory diarrhea is thought to be mediated via various neurotransmitters, including serotonin and histamine.

Carcinoid tumors in children are usually seen as an incidental finding at appendectomy,[131] and malignant carcinoid syndrome in a child has only rarely been reported.[132,133] Appendiceal carcinoids less than 1 cm in diameter are treated by simple excision and are thought to have little or no risk of metastasis. Tumors larger than 2 cm in diameter are at risk of metastasis.[134] Right hemicolectomy is recommended for tumors > 2 cm to remove the regional lymph nodes to which these tumors metastasize.[134] Once the tumors are metastatic, some patients appear to have very indolent courses; others have rapidly progressive courses. Poor prognostic indicators include multiple liver metastases, the presence of carcinoid syndrome, and high levels of tumor markers. Diagnosis is made by measurement of 24-hour urine 5-hydroxyindoleacetic acid.[130] SRS is sensitive and can be used to localize primary and metastatic carcinoid tumors.[135] Positive SRS scans also predict the clinical response to somatostatin analogs.[136] Surgical resection and embolization of liver metastases can offer effective palliation and can prolong survival in selected patients.[137] The treatment of choice in metastatic disease is a somatostatin analog,[138] and octreotide has been particularly helpful in relieving the symptoms of carcinoid syndrome.[139] Depot preparations—octreotide (given every 4 weeks) and lanreotide (given every 2 weeks)—are also effective. These somatostatin analogs cause symptomatic improvement, biochemical improvement, and temporary cessation of tumor growth[140] and may rarely cause complete tumor regression.[141] Chemotherapy for carcinoids is confined to patients with metastatic disease who are symptomatic and when other treatments are ineffective.[63] In these cases, chemotherapy with streptozocin, 5-fluorouracil, doxorubicin (Adriamycin), and α-interferon, among other chemotherapeutic agents, has been useful.[142] Tumor-targeted treatment with radioactive somatostatin analogs has also been developed.[143]

SUMMARY

Neuroendocrine tumors affecting gut function are fascinating but rare; they manifest themselves through the products they secrete. Diagnosis requires an understanding of the special clinical features of these tumors and a focused and timely evaluation. Diagnosis should be followed by accurate localization of the tumor because resection of a localized tumor is usually curative. Where disease is advanced, debulking and a variety of potent drugs facilitate medical management.

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CHAPTER 48

SYSTEMIC ENDOCRINOPATHIES

Jonathan E. Teitelbaum, MD

Systemic endocrinopathies have effects on multiple organ systems, including the gastrointestinal (GI) tract. Effects on the GI system are secondary to shared genetic susceptibilities (eg, diabetes and celiac disease [CD]), as well as altered homeostatic and metabolic functions (eg, hyperthyroidism and diarrhea). The pathophysiologic basis for these alterations is often poorly understood. In part, the effects can be meditated through the effects of the endocrinopathies on other organ systems, such as the enteric nervous system. Finally, because the endocrine system plays a vital role in organ development and growth, endocrinopathies have effects on both the developing gut and the mature intestine.

DIABETES MELLITUS

Previously called juvenile-onset diabetes, type 1 diabetes mellitus (DM) is a common, serious disease of childhood and adolescence. These patients are insulinopenic and require exogenous insulin to prevent ketosis and preserve life. The prevalence of type 1 diabetes in the United States among children and adolescence is between 1.2 and 1.9 cases per 1,000 members of this age group.[1]

Type 2 diabetes is most commonly found among adults and obese persons. Previously called adult-onset diabetes, this type of diabetes is the result of insulin resistance without adequate compensatory insulin secretion. Thus, a relative, not absolute, insulin deficiency occurs. Affected patients are not dependent on insulin for survival but may require exogenous insulin for metabolic control.

The diagnosis of diabetes in a child is rarely subtle. Most children present with classic symptoms of polyuria, polydipsia, polyphagia, weight loss, and lethargy. GI manifestations are well described (Table 48-1), although many adult studies will combine the data obtained from both type 1 and type 2 patients. Thus, one must be cautious in applying these findings to pediatric patients in whom type 1 disease is the most prevalent. Mechanisms leading to these GI disturbances include autonomic neuropathy, microangiopathy, hyperglycemia, electrolyte disturbances, and abnormalities in plasma insulin, glucagon, and other hormones, such as motilin and gastric inhibitory polypeptide.[2]

Infants of Diabetic Mothers

Gestational diabetes is defined as carbohydrate intolerance resulting in hyperglycemia of variable severity with onset during pregnancy irrespective of whether or not insulin is used for the treatment or if the condition persists after pregnancy. The frequency of gestational diabetes is variable and mirrors the frequency of type 2 diabetes in the population. In China and South India, the rate is 0.6%; in Australia, 15%; and in the United States, 4%.[3] A normal glucose tolerance test early in pregnancy does not exclude the development of this condition later in gestation. There are significant morbidities and mortality associated with children born to diabetic mothers. Indeed, an early description of these parents read, "They gave birth astride a grave, with a grave awaiting both the mother and fetus."[3] The mother is also at risk for preterm labor, pylonephritis, and hypertension.

Infants of diabetic mothers have been documented to have a large number of associated anomalies. The fetus carries an increased risk of abortion, congenital malformations, macrosomia, intrauterine growth retardation, trauma, asphyxia, respiratory distress syndrome, hypoglycemia, hypocalcemia, jaundice, and cardiomyopathy. Most important among these is the marked increase in perinatal mortality, with an incidence of up to 30% in those who have poorly controlled diabetes. This can be decreased to 2 to 4% with strict glycemic control throughout the pregnancy.[4] Thirty to 40% of these deaths are associated with congenital malformations. Overall, the incidence of such malformations is 6 to 13%, which is 2 to 4 times that seen in the general population.[4] In the Diabetes Control and Complication Trial, 1,441 type 1 diabetics had 270 births. If there was excellent glucose control (hemoglobin [Hb]A_{1c} 4.7 SD above the mean) during pregnancy, then the rate of congenital malformations and abortions was similar to that of the general population (0.7%).[3] Also in this group, the incidence of small for gestational age babies was 20% versus 11% in healthy controls.[5] However, conventional controls (HbA_{1c} 6.3 SD above the mean) had a rate of malformations of 5.9%. These results suggested that the goal should be to maintain a HbA_{1c} less than 5 SD above the mean.[3] The exact teratogen remains unknown, with the major candidates being hyperglycemia, hypoglycemia, increased ketones, effects of increased glycosylation products, changes in amino acid or prostaglandin profiles, and increased free radical production.

The children of diabetic mothers can demonstrate an increase in organ size, specifically the heart and liver, whereas the brain and kidney do not demonstrate visceromegaly. The etiology of hepatomegaly is based on the

TABLE 48-1 EFFECTS OF DIABETES MELLITUS ON THE GASTROINTESTINAL SYSTEM

ABNORMALITY/ASSOCIATION	GASTROINTESTINAL MANIFESTATION
Diabetic ketoacidosis	Nausea, anorexia, vomiting
Esophageal dysmotility	Dysphagia, reflux esophagitis
Esophageal candidiasis	Odynophagia, dysphagia
Gastroparesis/gastritis	Nausea, vomiting, gastric outlet obstruction
Small intestine dysmotility	Malabsorption, diarrhea, bacterial overgrowth
Impaired intestinal fluid reabsorption	Diabetic diarrhea
Celiac disease	Diarrhea, steatorrhea
Steatohepatitis	Abnormal transaminases, hepatic fibrosis
Hepatocellular carcinoma	Twofold increased risk
Cholelithiasis	Biliary sepsis

Adapted from Weber JR and Ryan JC.[86]

increase in maternal blood glucose. This increase causes a similar rise in fetal blood glucose. The fetus, therefore, has an appropriate increase in insulin production to obtain euglycemia. Insulin is the primary anabolic hormone in the growing fetus and results in visceromegaly. Macrosomia is the result of increased adipose tissue, and birth weight correlates with blood insulin levels.[6] Ultrasound monitoring of the fetus can be helpful in predicting fetal well-being. Measurements of fetal abdominal circumference (AC) between 29 and 33 weeks gestation can identify those infants with an AC greater than the 75th percentile. If these women are then treated with insulin, the rate of macrosomia is decreased from 45 to 13%. Those children who have a greater than 1.1 cm increase in their AC per week appear to be at risk for macrosomia. Vohr and colleagues reported that previously macrosomic infants who were followed up at the ages of 4 and 7 years had increased body size and adiposity compared with controls and those infants born to gestational diabetics who were born at the appropriate weight.[7]

Infants of diabetics are also noted to have increased levels of bilirubin. Whereas some suggest that prematurity, ABO incompatibility, red blood cell life span, or osmotic fragility accounted for this increase, other studies do not support a difference in these parameters between infants of diabetic mothers and normal infants.[4] Stevenson suggested that the delayed clearance of bilirubin was a factor.[8] However, polycythemia is felt to be the most important factor accounting for elevated levels of indirect bilirubin in this population.[4]

Neonatal small left colon was first described by Davis and colleagues in 1974. It results in a low obstruction of the large bowel, which clinically is manifest by failure to pass meconium in a timely fashion, tympanic abdominal distention, or bilious vomiting. Barium enema reveals smooth narrowing of the sigmoid and descending colon with proximal dilatation (Figure 48-1). An early series of 20 patients revealed that 17 did well after diagnostic or therapeutic enema.[9] Eight of the 20 (40%) had diabetic mothers. Perforation, typically of the cecum, also has been reported. The etiology is still unclear; theories include disorders of fetal colonic motility perhaps secondary to temporary ganglionic dysfunction.[10] Treatment is typically conservative, with initial parenteral nutrition and nasogastric decompression. At times, the barium enema has been therapeutic and allowed for a graded approach to feeding. The condition commonly resolves within the first 1 to 2 weeks of life.

Diabetic Ketoacidosis and GI Tract

Diabetic ketoacidosis (DKA) is commonly associated with GI symptoms such as nausea, anorexia, and vomiting. At times, the abdominal presentations are severe and raise concerns about coexistent appendicitis or other causes of an acute abdomen. Clinicians should recognize that the symptoms can be solely due to the associated metabolic derangements and will resolve with correction of the acidosis. The patient should be closely observed and correction of the metabolic disturbances undertaken prior to any invasive interventions.

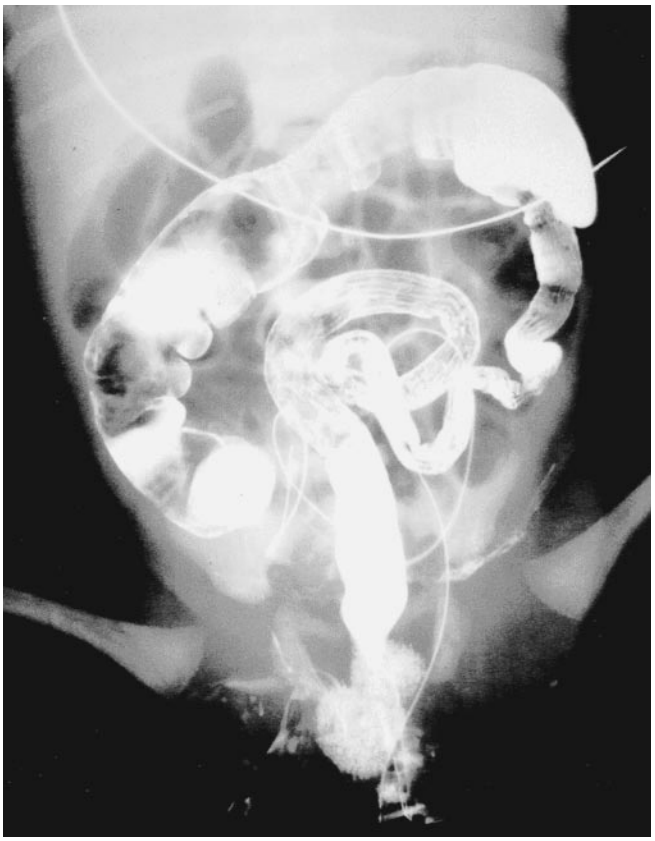

FIGURE 48-1 Barium enema of a newborn with small left colon syndrome. Courtesy of Thomas J. Kelly, MD, Monmouth Medical Center, NJ.

Elevations of amylase may be present and raise concerns about pancreatitis. However, isoenzyme analysis often shows that the amylase is of salivary origin.[11] Marked dilatation of the stomach during DKA has been reported and resolves with nasogastric tube decompression.[12] Acute hypermagnesemia can also contribute to the dysmotility seen with DKA.[12] Upper GI hemorrhage has also been reported, typically secondary to erosive esophagitis,[13] and responds to acid blockade therapy.

Diabetes and GI Neuropathy

Because the motility of the GI tract is dependent on the enteric nervous system, neuropathic changes can adversely affect function. Neuropathic changes within the GI tract of diabetics are typically thought to occur over prolonged periods of time with poor diabetic control. Accordingly, diabetic neuropathy among diabetic children is a rare event. However, studies of neuropathic changes, as documented by alteration in nerve conduction and parasympathetic nerve function (R-R variations), have shown that 25% of children have evidence of low sensory nerve conduction and autonomic dysfunction at the time of diagnosis prior to achieving remission.[14] After 2 years of disease, deterioration in function was common, with a correlation between nerve conduction and glycemic control.[14]

Studies in adults suggest that over 75% of diabetics have GI symptoms related to neuropathy. Among this population, many have documented motility abnormalities, although such abnormalities correlate poorly with symptoms. Many explain this discordance as a possible underreporting of complaints, such as dysphagia or fullness.

The neuropathy associated with DM appears to be the result of altered sympathetic function and cholinergic denervation. Although there is damage to the vagus nerve resulting in vagal nerve dysfunction, most alterations are thought to be in the postganglionic nerves, sympathetic ganglia, and intramural adrenergic plexus. The sympathetic nerve dysfunction is particularly implicated in anal sphincter dysfunction. Others suggest that neurotransmitters (ie, an increase in vasoactive intestinal polypeptide) are the etiology of such dysfunction.[12] One must also consider the effects of polypharmacy and electrolyte disturbances on nerve function.

Histologically, nerves in patients with diabetic neuropathy can appear swollen, with irregular processes and vacuolization, and there can be fragmentation of dendrites or Schwann cells.[15]

Diabetes and Nonspecific Abdominal Pain

Owing to the altered intestinal motility associated with the autonomic neuropathy of long-standing diabetes, there has been an assumption that nonspecific abdominal pain is more common among patients with diabetes. Studies evaluating the prevalence of chronic dyspepsia and chronic constipation among diabetics are somewhat conflicting. Among children with diabetes, one uncontrolled study indicated that the prevalence of gastroesophageal reflux disease (GERD) was 7%.[16] Those affected had poor linear growth and more frequent hospital admissions. In contrast, a larger study of 118 children and adolescents with type 1 diabetes was unable to demonstrate a significant difference in the frequency of recurrent abdominal pain, chronic constipation, or chronic dyspepsia between those with diabetes and controls.[17]

A study of adult diabetics found no significant difference in the presence of upper and lower GI tract symptoms among 75 patients with type 1 diabetes compared with controls.[18] However, among 68 patients with type 2 diabetes, there is more constipation (22.1% vs 10.3%; $p < .05$) and nausea (11.8% vs. 2.9%; $p < .5$). The difference among those with type 2 diabetes is not likely to be due to autonomic neuropathy because diabetes duration and glycemic control did not influence the frequency of symptoms. It seems more likely that these changes are due to an altered pathophysiology in obese patients because obesity has been reported to result in increased amounts of GERD[19,20] and constipation.[21] A larger study by Xia and colleagues surveyed 429 diabetic patients (both type 1 [$n = 49$] and type 2 [$n = 380$]) and compared them with 170 controls. There was no difference in the percentage of patients with GI complaints such as epigastic pain, bloating, distention, early satiety, heartburn, nausea, or vomiting between diabetics and the controls (51% vs 44.7%).[22]

It is unclear if pancreatic transplant can reverse preexisting intestinal damage. However, in a study of 32 patients who underwent transplant, 24 of whom had GI symptoms, almost all (96%) had fewer GI complaints after transplant. One must be aware that with the transplant, the patients had correction of multiple metabolic derangements, so the underlying etiology of the improvement is unclear.[23]

Diabetes and the Esophagus

Alterations in esophageal function in children with diabetes are rare. The majority of reported abnormalities described are limited to case reports, including that of one child with a 4-year history of DM who presented with dysphagia and was found to have manometric features of a nutcracker esophagus.[24]

Although many adult patients with peripheral neuropathy secondary to DM have documented alterations in esophageal motility or gastric emptying, only 30% have clinical symptoms of chest pain, heartburn, or dysphagia. Motility disturbances documented by esophageal manometry reveal few alterations in upper esophageal sphincter function. However, the length of the esophagus can demonstrate a decrease in the amplitude of contractions, aperistaltic contractions, and prolonged contractions, as well as decreased lower esophageal sphincter tone.[12] Such peristaltic alterations can result in a prolonged esophageal transit time. This, along with possible alterations in the immune system,[25]can result in *Candida* esophagitis in this population, with resulting odynophagia.[26]

Diabetes and the Stomach

Gastroparesis in DM was first described in 1945.[27] Alterations in gastric emptying among pediatric diabetics are uncommon and mainly limited to case reports and small case series. Reid and colleagues described three children 1

to 7 years after the onset of diabetes with delayed gastric emptying and postprandial antral hypomotility.[28] Oduwole and colleagues described an adolescent with severe gastroparesis that developed despite good glycemic control and a HbA_{1c} of 7.4%, which normalized after 2 months of treatment with metoclopromide.[29]

Studies have documented delays in gastric emptying in up to 58% of adult diabetics. The onset of symptoms is typically insidious, with early satiety, decreased weight, anorexia, postprandial nausea and vomiting, and epigastric distress. A sense of fullness or bloating correlates with a delay in gastric emptying, whereas nausea and vomiting do not.[30] Physical examination may reveal a succession splash and gastric distention.

The gold standard of diagnosis is scintigraphy. The study should be done during a period of euglycemia, preferably using solids. The development of scintigraphic breath tests likely will be a useful screening tool in the future.[30] Alterations can be documented with a gastric emptying scan, and endoscopy or contrast radiography can rule out pathologic narrowing or obstruction.

The etiology of delayed gastric emptying appears to be due to vagal dysfunction. This dysfunction can be documented by a rise in serum gastrin and a fall in acid secretion during sham feeding.[31] Nerve dysfunction may be related to the direct effect of hyperglycemia on nerves. For instance, experimentally, neurons in the small intestine of rats are responsive to glucose concentration.[32] In diabetics, as well as normal subjects who experience acute hyperglycemia with serum glucose concentrations above 200 mg/dL, one can observe a marked delay in gastric emptying.[33,34] Motility studies reveal a decrease in the frequency of migrating motor complexes in patients with gastroparesis. This may account for the observed increase in motilin in these patients, perhaps as a compensatory change. These changes are reversed with the use of prokinetics.[35] In diabetic mice, interstitial cells of Cajal, which provide pacemaker activity for the stomach, are greatly depleted in the antrum. Accordingly, these mice have delayed gastric emptying.[36] However, such histologic changes have not been reported in humans.

Alteration in the fasting and fed pattern of these patients reveals that there are postcibal alterations in antral rhythm and poor receptive relaxation. Electrogastrography reveals alterations in the normal 3 counts per minute (cpm) electrical pace of the stomach such that one sees bradyrhythmias (1–2.5 cpm) and tachyrhythmias (3.7–10 cpm). Also, the perception of normal patients with nausea, fullness, and distention is more intense with increased levels of serum glucose.[30] Delaying emptying of a solid in the fasting state can contribute to bezoar formation.[12] In the postprandial state, antral contractions are decreased in number and amplitude. In addition, the pylorus exhibits prolonged high-amplitude contractions.[12]

Treatment of these motor disorders begins with better glycemic control. Dietary changes, with the introduction of smaller, more frequent meals and liquid supplements, can be beneficial. In addition, patients should be advised to consume diets with low amounts of residue to avoid bezoar formation.[12] Alternative treatments include gastric pacemakers and *Clostridium botulinum* toxin injection into the pylorus to improve gastric emptying.[37]

Pharmacotherapy is also helpful as adjunctive therapy. Antiemetics do not appear to help to any great degree. Prokinetics coordinate pyloric relaxation and duodenal peristalsis with smooth muscle contraction in the stomach, thus promoting improved gastric emptying.[38,39] Erythromycin, which stimulates the motilin receptor,[40] has had some effect, although some patients develop tachyphylaxis to the drug. A study in DM children with dyspepsia and delayed gastric emptying revealed that domperidone is superior to cisapride in reversing delayed gastric emptying and relieving clinical symptoms.[41] Rarely, recalcitrant patients require parenteral nutrition or transpyloric enteral tube feeding.

Other gastric alterations that have been described in DM include hemorrhagic gastritis, acute and chronic gastritis, atrophic gastritis, and pernicious anemia. The incidence of pernicious anemia among diabetics is 10.5 in 1,000 versus 2 in 1,000 in the general population.[12] Peptic ulcers among diabetics appear to occur at a lower rate compared with those of the general population. It is hypothesized that this is due to the increase in glucagon in response to the hyperglycemia, which subsequently decreases the production of gastric acid.[12]

Diabetes and Helicobacter pylori

Simon and colleagues were the first to suggest that *Helicobacter pylori* infection is more prevalent among patients with DM.[42] Some have hypothesized that the immune dysregulation associated with DM, coupled with the autonomic neuropathy that can lead to gastroparesis, places diabetics at increased risk for *H. pylori* infection. However, a large study by Xia and colleagues, including 429 patients with DM, found no difference in the incidence of *H. pylori* infection between the diabetic patients and controls (32.9% vs 31.7%).[22] Smaller studies of children with diabetes also found no difference in the overall rate of infection between the diabetics and controls.[43]

A study by Jones and colleagues comparing diabetic adults with *H. pylori* infection and those without found no difference in the number with GI symptoms, rate of gastric emptying, glycemic control, or autonomic function.[44] Indeed, the overall rate of infection with *H. pylori* was identical to that of the population without diabetes, indicating that DM is not a risk factor for infection. A study in Bangladesh of 520 DM patients came to the same conclusion.[45] This is in contrast to other studies, which report that DM patients with *H. pylori* have slower gastric emptying[46] or increased numbers of upper GI symptoms and subsequent improvement after eradication of the infection.[47,48]

It has been suggested that eradication of *H. pylori* among those with diabetes can result in better glycemic control; however, the evidence for such a change is weak. The proposed mechanism of this improvement is based on the ability of *H. pylori* to cause an increase in the production of various cytokines, including tumor necrosis factor-α, interferon-γ, and interleukins 1, 6, and 8.[49] In such studies, Begue and colleagues found no change in insulin require-

ments over the 2 years after eradication of *H. pylori*, but there was a modest decrease (2%) in their HbA_{1c} levels.[50] This is similar to another study, which found no difference in glycemic control after *H. pylori* eradication.[17]

Diabetes and Autoimmune Gastritis

Fifteen to 20% of DM patients have parietal cell antibodies (PCAs) compared with 2 to 10% of nondiabetic patients.[51,52] Thus, diabetic patients account for 20 to 40% of all PCA-positive patients. Such antibodies target the gastric proton pump (H/K adenosine triphosphatase) and are a serologic marker for autoimmune atrophic gastritis. This, in turn, is associated with iron deficiency anemia and pernicious anemia and may predispose the patient to gastric cancer and carcinoid tumors. A study of 229 diabetic patients found 69 to be PCA positive.[53] The presence of PCA was associated with human leukocyte antigen (HLA)-DQA1*0501-B1*0301. In those patients with PCA, there is a higher prevalence of iron deficiency anemia, pernicious anemia, autoimmune gastritis, and hypochlorhydria. Signs of preatrophic gastritis are also more common and documented by histologic features, including pronounced lymphocytic infiltration in the corpus mucosa and parietal atrophy of oxyntic glands. The presence of concomitant *H. pylori* infection does not represent a separate risk factor for these findings.[53] Similar studies in pediatric patients with DM are lacking.

Diabetes and Small Bowel Motility

Like other portions of the gut, small intestinal motility can be affected by the neuropathic changes of long-standing DM. Delayed intestinal transit can be seen in up to 33% of diabetics, as demonstrated by the breath hydrogen test.[54] This is due to decreases in the amplitude of contractions and alterations in migrating motor complexes.

Diabetes and Celiac Disease

In 1969, Walker-Smith and Grigor described an 8-year-old with DM who developed CD.[55] CD is associated with HLA-DQ2 and -DQ8; diabetes is also linked to the same DQ2 molecule.[56] In diabetes, it is the DQ molecule that influences the selection and binding to autoantigenic peptides.[56] The prevalence of CD in DM is 10 to 30 times that of the general population, with an incidence of 2 to 8.5%. The wide variation is based on how CD is defined, where using clinical symptoms and histology, the incidence is at the lower end, 2%. If, however, seropositivity is the defining characteristic, then the incidence is 8 to 9%.[57] Among children of type 1 diabetics, 3.5% have CD.[58] CD does not appear to be increased among patients with type 2 diabetes.[58]

A meta-analysis of 20 studies evaluating the incidence of a dual diagnosis of CD and DM found rates in children between 1 in 6 (16.4%) and 1 in 103 (0.97%).[57] If one excludes the study from Algeria, which accounted for the highest prevalence, the remainder of the studies reported a prevalence between 0.97 and 6.2%. In adults, a meta-analysis of 10 studies revealed rates ranging from 1 in 16 (6.4%) to 1 in 76 (1.3%).[57]

A large study by Pocecco and Ventura evaluated 4,500 type 1 diabetics and found two subgroups of patients.[56] The first group included those with type 1 DM who were later found to have "silent CD"; this represented 88% of the dual-diagnosis cases. The second group was those with prediagnosed CD who were later found to have type 1 DM, representing 12% of the cases. Group 1 had minimal GI symptoms and was diagnosed with CD between 11 and 17 years of age. Group 2, however, experienced numerous GI symptoms and was diagnosed with CD at younger ages (between 6 and 10 years). It is thought that the later age at which CD was diagnosed among the group 1 patients was due to the minimal GI symptoms they exhibited. Indeed, in some studies, short stature is the only sign, accounting for up to 33% of the patients.[57] Maki and colleagues screened 238 DM patients with immunoglobulin A antireticulin antibodies (ARAs), and 16 were positive.[59] However, of these, 11 were negative at first screening. CD was confirmed in 9, with typical changes on small bowel histology. Of note, 2 children had a negative ARA and normal small intestinal biopsy at the time of DM diagnosis. One then developed rising ARA titers and after 2 years had flat villi on biopsy. The other child had a positive ARA, but after 8 years, villi were still normal even though there were increased numbers of intraepithelial lymphocytes. The authors recommend that DM patients undergo yearly CD screening.[59]

If the patient had clinical symptoms of CD, then the gluten-free diet typically results in improved diabetic control.[57] However, in those patients who were diagnosed by routine screening alone, studies are varied, with some revealing no improvement in glycemic control, whereas others show better control or fewer hypoglycemic events.[57]

Similarities in peptide sequences can result in a cross-reaction of epitopes at the T-cell level. However, it is unclear whether such similarities exist between gliadin and tissue transglutaminase and glutamic acid decarboxylase or insulin. If such cross-reactivity does exist, then the coexistence of DM and CD can be explained by "molecular mimicry."[56] A study of nonobese diabetic rats provided regular chow versus a gluten-free diet for 320 days decreased the incidence of type 1 diabetes from 64 to 15%.[60]

Diabetes and the Liver

The most common cause of elevated transaminase levels or hepatomegaly among patients with diabetes is steatosis. This is the result of deposition of large, macronodular fat droplets within the parenchyma of the liver. When the fat deposition is associated with an inflammatory infiltrate, one can make a histologic diagnosis of nonalcoholic steatohepatitis, which, in some instances, can progress to fibrosis and cirrhosis.[61] The predominant lipid deposited is the triglyceride, but fatty acids also contribute to the toxic effects. The accumulation is the result of fatty acids brought to the liver at a rate greater than the liver can either metabolize the fatty acid or secrete very-low-density lipoprotein. Furthermore, the increase in liver size can be secondary to increased glycogen stores.[62] Overall, fatty liver is diagnosed in 4.5 to 17% of type 1 diabetics. It typically reflects poor glycemic control and resolves with better control of the diabetes. In type 2 diabetics, the incidence of fatty liver is 45%. This suggests that the increase in inci-

dence among type 2 diabetics is likely secondary to obesity rather than the diabetes itself. However, some studies suggest that histologic evidence of steatosis is even more common than appreciated by transaminase elevation or imaging alone. For example, one study of 68 insulin-treated children found evidence of increased glycogen in the cytoplasm and nucleus of hepatocytes in 58%.[63]

Imaging of the liver with computed tomography or ultrasonography can be helpful in identifying the respective enhancement or echogenicity associated with fatty liver. The degree of transaminase elevation does not correlate to the degree of liver injury. Treatments include weight loss, better glycemic control, and, possibly, vitamin E supplementation.[64] Rapid weight loss can be detrimental, and it is therefore suggested that the loss be no faster than 1.6 kg/wk.

Mauriac syndrome refers to hepatomegaly associated with increased glycogen stores, hypoglycemia, dwarfism, and a cushingoid appearance. Previously, it was described in patients classified as brittle diabetics.[65] This is less frequently seen owing to advances in insulin preparations and glucose monitoring. The disease results from increased serum glucose leading to moderate hepatic glycogen accumulation, which subsequently inactivates glycogen phosphorylase. This inactivation leads to an inhibition of glycogenolysis and increased glycogen synthase and a subsequent increase in glycogen stores. Treatment with insulin results in continued activation of glycogen synthase and further glycogen accumulation. Hypercortisolism is thought to be responsible for the growth retardation and delayed puberty. Better glycemic control results in resolution of the syndrome.

Among diabetics receiving peritoneal dialysis with added insulin, there is a risk of developing subcapsular fatty changes.[62] Also, hepatocellular carcinoma is twice as prevalent in diabetic patients.[12]

Diabetes and Biliary Function

An increase in the frequency of cholecystitis and cholelithiasis is seen in adult diabetics, with the risk of cholelithiasis being twice that of the general population.[12] These increases in prevalence are thought to be secondary to decreased motility and a lithogenic bile composition.[12] Despite these increases in risk, the routine practice of cholecystectomy among diabetics has fallen out of favor.

A study of 20 diabetic children found that the fasting gallbladder volume in these children was greater than that in controls. However, there was no difference in ejection fraction or maximal contraction between the two groups.[66] It is questioned whether the dilation of the gallbladder heralds future autonomic neuropathy and an increased risk for gallstone formation. In addition, there is an increased risk of ascending cholangitis. Reports of organisms, such as *Yersinia enterocolitica*,[67] have rarely been found to be causative.

Diabetes and the Exocrine Pancreas

Thirty percent of adult diabetics have decreased exocrine pancreatic secretion, although this is typically not associated with clinical symptoms. The decrease is likely due to glucagon excess, malnutrition, vagal nerve dysfunction, and a decrease in insulin effects. Among those diabetics who consume alcohol, there is a two- to fourfold risk of adenocarcinoma of the pancreas compared with that of the general adult population.[12]

Diabetic Diarrhea

Diabetic-associated diarrhea can occur in up to 20% of patients with DM. It is typically worse at night and more common among males. The etiology is likely multifactorial and can be associated with rapid intestinal transit or a defect in adrenergic stimulation of colonic water reabsorption. One should consider confounding causes such as drugs, including sorbitol found in sugar-free foods,[68] and small bowel bacterial overgrowth.[69] Some studies suggest that clonidine can aid in increasing water reabsorption. Octreotide has also been used to decrease the diarrhea.[15] Among those with diarrhea, 40% suffer from fecal incontinence. This is hypothesized to be secondary to decreased anal sphincter tone and decreased sensation to rectal distention. Although diarrhea is a relatively common complaint among young diabetics, the disabling form of autonomic diabetic diarrhea rarely begins before middle age. One should thus consider other causes, including those confounders mentioned above, routine enteric infections, and CD in DM children with persistent diarrhea.

AUTOIMMUNE POLYGLANDULAR SYNDROME

Autoimmune polyglandular syndrome type I (Online Mendelian Inheritance in Man [OMIM] #240300), also known as autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy, is typically an autosomal recessive disease. The disease is characterized by a combination of the following:

1. Failure of parathyroid, adrenal cortex, gonads, beta islet cells, parietal cells, and thyroid and/or hepatitis
2. Chronic mucocutaneous candidiasis
3. Dystrophy of dental enamel, nails, alopecia, vitiligo, and/or keratopathy[70]

Patients are typically of Iranian, Jewish, Finnish, or Scandinavian descent.[71] A multicenter review of patients with chronic mucocutaneous candidiasis demonstrated that 50% later developed disease components typical of autoimmune polyglandular syndrome.[72] Finland has published the largest cohort of these patients,[70]encompassing a group of 68 patients, with a mean follow-up of 11.2 years between 1910 and 1988. Owing to a founder effect, one major mutation accounts for approximately 90% of these cases. Linkage studies identified a gene at chromosome 21q22.3. This gene, likely a transcription regulator, has been cloned and named autoimmune regulator 1.[71]

Within the Finnish group, the number of endocrine organs involved varied between 1 and 8, with most having 4.[70] Hypoparathyroidism is the most common (79%) and was diagnosed between 19 months and 44 years of age. Adrenocortical failure is second at 72%, being diagnosed between 4.2 and 41 years. Cortisol and aldosterone deficiency occur at the same time in 38 of 49 patients. Gonadal failure occurs in 60% of females and 14% of males, all by

age 30 years. Diabetes is diagnosed in 12% and vitamin B_{12} deficiency in 13%. Rarely, patients have been documented to have coexistent CD[73] or intestinal lymphangiectasia.[74]

The majority of patients (78%) have documented nonendocrine manifestations prior to the first endocrinopathy. In 60% of cases, this includes oral candidiasis, 9% malabsorption, 4% keratopathy, and 1 case of hepatitis. Endocrine problems arose at mean ages of 8.7 years for the first endocrinopathy, 13.3 years for the second, and 16 years for the third. In those patients with adrenal failure as their first endocrinopathy, they had fewer additional organs involved. However, those patients with malabsorption or keratopathy as their first manifestation often had five subsequent organs involved.

All patients have oral candidiasis, the onset of which varies between 1 month and 21 years. Nail involvement is seen in 71% and dermal involvement, typically the hands and face, in 9%. Four patients have had documented esophageal candidiasis, one with a stricture. However, an additional 11 patients had retrosternal pain that resolved with antifungal treatment. Malabsorption was documented in 18% at 4 months to 21 years from onset of disease. All but one of these patients had decreased parathyroid function, and decreased serum calcium appears to correlate with the malabsorption. Of the 43 patients evaluated, 33 had evidence of dental enamel hypoplasia unrelated to hypoparathyroid status. Patients also appear to be at a greater risk for developing carcinoma of the oral mucosa, likely owing to the chronic candidiasis.

Overall, autoimmune hepatitis appears to be a component of the disease in 10 to 18% of patients.[70] Indeed, there are reports in which a sudden onset of hepatitis resulted in death. The first hepatic autoantigen in these patients was identified as cytochrome P-450 1A2.[75] A second autoantigen was identified as aromatic-L-amino acid decarboxylase (AADC).[76] This enzyme, active in the biosynthesis of neurotransmitters, is expressed in the cytosol and was originally described as a beta-cell autoantigen. Among the Finnish cohort, 50% of those tested had AADC autoantibodies. However, up to 92% of autoimmune polyglandular syndrome type I patients with vitiligo and autoimmune hepatitis had such antibodies.[71]

HYPERPARATHYROIDISM

Primary hyperparathyroidism (PHPT) is a rare condition in childhood and often goes undiagnosed. The disease is identified in those patients with hypercalcemia, low-normal phosphate level, and elevated parathyroid hormone. Since its initial description in 1939, approximately 100 cases have been reported among children and adolescents less than 16 years.[77] The etiology of the disease is similar to that of adults, with sporadic adenomas being causative. Rarely, the hyperparathyroidism is a manifestation of a systemic disease such as multiple endocrine neoplasia type I or II. Fewer than 40 neonatal cases have been reported. Here the etiology involves hyperplasia of the parathyroid chief cells. The clinical manifestations of hypercalcemia include muscle weakness, paralysis, and hyporeflexia. GI symptoms (Table 48-2) may be absent or nonspecific, including fatigue, poor appetite, weight loss, abdominal pain, nausea, constipation, peptic ulcer disease, and vomiting.[77] A case report of an adolescent with hyperparathyroidism revealed that hypercalcemia was the etiology of his acute pancreatitis.[78] Abdominal symptoms appear to be seen more frequently among children affected by PHPT than adults, 8% versus 1%, respectively.[79] Nephrocalcinosis has been described in 30 to 70% of PHPT children. Severe pancreatitis immediately following parathyroidectomy for PHPT may occur in as many as 3% of patients.[80]

HYPOPARATHYROIDISM

Hypoparathyroidism among children is rare, although it can be part of other systemic syndromes such as autoimmune polyglandular syndrome, Pearson marrow pancreas syndrome, and DiGeorge syndrome. Biochemically, there is hypocalcemia, hyperphosphatemia, and decreased parathyroid hormone levels. The clinical manifestations of hypocalcemia predominate and result in neuromuscular instability with seizure, tetany, paresthesias, laryngospasm, bronchospasm, or prolonged Q–Tc. Idiopathic hypoparathyroidism is also associated with malabsorption, pernicious anemia, and Addison disease. Indeed, approximately 11% of those with decreased parathyroid function have chronic diarrhea or steatorrhea.[81]

The mechanisms by which parathormone is involved in intestinal absorption are unknown, but symptoms of malabsorption may be the earliest sign of hypoparathyroidism. The diarrhea typically ceases with vitamin D therapy.[81] It has been noted that magnesium deficiency must be ruled out in patients who present with malabsorption and findings of hypoparathyroidism because functional hypoparathyroidism occurs in patients with severe and prolonged hypomagnesemia.[82] Intestinal lymphangiectasia with protein-losing enteropathy also has been reported in association with malabsorption and hypoparathyroidism.[83]

TABLE 48-2 EFFECTS OF HYPERPARATHYROIDISM ON THE GASTROINTESTINAL SYSTEM

ABNORMALITY/ASSOCIATION	GASTROINTESTINAL MANIFESTATION
Increased serum calcium	Constipation, nausea, vomiting
Peptic ulceration	Bleeding, abdominal pain, perforation
Pancreatitis	Acute pancreatitis
Multiple endocrine neoplasia type I	Gastrinoma, VIPoma

Adapted from Weber JR and Ryan JC.[86]
VIPoma = vasoactive intestinal peptide–secreting tumor.

TABLE 48-3 EFFECTS OF HYPOTHYROIDISM ON THE GASTROINTESTINAL SYSTEM

ABNORMALITY/ASSOCIATION	GASTROINTESTINAL MANIFESTATION
Altered colonic function/transit	Constipation, pseudo-obstruction
Impaired esophageal motility	Reflux esophagitis
Liver test abnormalities	Normal histology, prolonged neonatal jaundice
Celiac disease	Diarrhea, steatorrhea

Adapted from Weber JR and Ryan JC.[86]

GI problems similar to but less severe than those found in hypoparathyroidism are found in pseudohypoparathyroidism, but plasma parathyroid hormone levels are high.

HYPOTHYROIDISM

Hypothyroidism is defined as a state in which the thyroid gland fails to secrete sufficient quantities of thyroid hormone. Congenital hypothyroidism has been associated with GI manifestations (Table 48-3), including constipation, feeding difficulties, and prolonged neonatal jaundice. The frequency of CD in patients with autoimmune thyroid disease is 4.3% compared with 0.4% for nonautoimmune hypothyroid controls.[56] The association is likely due to a common genetic predisposition, namely the DQ2 allele.

Among hypothyroid adults, studies show that affected patients have an average of three bowel movements a week (range of 1–7/wk).[84] Studies to determine the pathophysiologic basis of the constipation include anorectal manometry studies, which reveal that hypothyroid individuals have normal maximal resting and squeeze pressures. However, sensation threshold for impending evacuation is greater than that for controls. There is no change in whole-gut transit time.[84] Although there is evidence of increased vasoactive intestinal peptide expression from the anterior pituitary of hypothyroid rats, it is unlikely that these changes play a pathophysiologic role in GI disorders seen with hypothyroidism.[85]

Myxedema has also been associated with decreased esophageal peristalsis and impaired lower esophageal sphincter function, resulting in reflux esophagitis. Hypothyroidism can also cause severe gastric hypomotility and secondary pseudo-obstruction.[86]

Studies of hypothyroid murine models reveal that hypothyroidism decreases the deoxyribonucleic acid (DNA) and protein content of the intestinal mucosa. Villi in the jejunum appear shorter, as does crypt depth. In addition, hypothyroidism decreases the rates of glucose and glutamine use by epithelial cells of the small intestine and colon.[87]

Liver transaminases are mildly abnormal in 50% of hypothyroid patients; however, liver histology is typically normal.[88] Hypothyroidism can cause exudative ascites in the absence of overt liver disease.[86] Rarely, one can have associated chronic active hepatitis, diabetes mellitus, or Cronkhite-Canada syndrome. Hashimoto thyroiditis has also been associated with ulcerative colitis.[86]

HYPERTHYROIDISM

Hyperthyroidism occurs when excessive amounts of circulating thyroid hormone are present. Hyperthyroidism (Table 48-4) has been associated with diarrhea, with some studies showing that such individuals pass an average of 14 bowel movements per week (range 7–21/wk).[24] Attempts at discerning the etiology of the diarrhea include anorectal manometry studies, which reveal that hyperthyroid individuals have a lower maximal resting pressure and maximal squeeze pressure compared with those of controls.[84] In addition, they have a lower threshold sensation for impending evacuation.[84]

Studies of intestinal motility reveal no difference in gastric emptying between controls and hyperthyroid adults. However, hyperthyroid individuals have accelerated small bowel and colonic transit. Small bowel transit time appears to be inversely related to triiodothyronine (T_3) concentrations.[89] Indeed, increased small intestinal myoelectric activity has been reported.[90] This presumably accounts for the GI symptoms and diarrhea exhibited by hyperthyroid individuals. However, there is also evidence for a secretory component to the diarrhea because thyroid hormone can cause increased intestinal secretion via an increase in intracellular levels of cyclic adenosine monophosphate.[86] Changes in intestinal transit appear to normalize once patients are rendered euthyroid.[91]

Hyperthyroidism may cause myopathy, resulting in dysfunction of the striated muscles of the pharynx and proximal esophagus. Decreased propulsive force of the muscles and abnormal closure of the upper esophageal sphincter can result in dysphagia and aspiration. Esophageal peristalsis is increased in thyrotoxic patients.[86]

Appreciation of the GI disturbances in thyroid disease is often overshadowed by other organ dysfunction, including that of the cardiovascular, neuromuscular, and ocular systems. Of note, Graves disease is associated with ulcerative colitis.[86] Also, hyperthyroidism is associated with minor histologic changes in the liver. One can detect mildly ele-

TABLE 48-4 EFFECTS OF HYPERTHYROIDISM ON THE GASTROINTESTINAL SYSTEM

ABNORMALITY/ASSOCIATION	GASTROINTESTINAL MANIFESTATION
Accelerated intestinal transit	Diarrhea
Myopathy of the upper esophagus	Dysphagia
Liver test abnormalities	Minor histologic changes
Ulcerative colitis	Bloody diarrhea

Adapted from Weber JR and Ryan JC.[86]

vated transaminases in approximately 33% of patients, and 5% have an unconjugated hyperbilirubinemia.[86]

PITUITARY HORMONES

Pituitary hormones have important effects on the gut. Adrenocorticotropic hormone stimulates cortisol secretion and enhances brush border enzyme activity, and thyrotropin-stimulating hormone causes thyroxine secretion, with major effects on gut motility.[92] Growth hormone causes intestinal villus growth and enhances absorption.[93] Infants with intrauterine hypopituitarism may present at birth with hypoglycemia, prolonged jaundice,[94] and, in males, micropenis and undescended testes. Excessive secretion of pituitary growth hormone results in acromegaly, which is associated with an increased incidence of adenomatous colonic polyps and cancers of the colon and stomach.[86]

HYPOADRENOCORTICISM

The adrenal cortex secretes glucocorticoids and mineralocorticoids. Glucocorticoids bind to specific cytoplasmic receptors on the enterocyte, and the activated receptor-steroid complex is translocated to the nucleus, where it triggers the synthesis of messenger ribonucleic acid. The net effect is to increase the absorptive capacity of the small intestine and enhance brush border membrane digestive capacity without increasing the number of cells.[95] The dominant GI effect of adrenal gland disorders is related to abnormalities of aldosterone metabolism. Although the effect of increased aldosterone production in increasing sodium absorption in the colon is overshadowed by its effect on the kidney, lack of aldosterone production and end-organ failure to respond to aldosterone are often associated with marked intestinal salt wasting in addition to urinary losses.[96] Thus, in salt-losing states associated with hyperadrenocorticism, the intestinal losses contribute to dehydration.

Some patients with Addison disease have steatorrhea with normal jejunal histology,[97] which resolves with hormone replacement. GI symptoms can include anorexia, weight loss, vomiting, and abdominal pain. There is a high incidence of associated anomalies in children with Addison disease, including autoimmune polyglandular syndrome, adrenoleukodystrophy, and AAA syndrome (Addison disease, achalasia, and alacrimation).

DIENCEPHALIC SYNDROME

Diencephalic syndrome is a complex of signs and symptoms related to hypothalamic dysfunction. The association between brain tumors of the anterior hypothalamus and severe failure to thrive is almost exclusively seen in infants and young children, with 85% occurring at ages less than 2 years.[98] Most patients have space-occupying lesions in the region of the optic chiasm, typically a low-grade, slow-growing glioma or a juvenile pilocytic astrocytoma.

Three major features include failure to thrive in spite of normal energy intake, motor hyperactivity, and apparent euphoria. Autonomic disturbances include skin pallor, profuse sweating, and erratic temperature control. Rotatory nystagmus may be the only neurologic sign.[98]

At the time of diagnosis, length is typically maintained and head circumference is normal, except in the 33 to 58% with hydrocephalus. Vomiting occurs in 68% of affected patients.[98] The etiology of the extreme loss of subcutaneous fatty tissue is unclear. Some suspect increased lipolysis secondary to elevated growth hormone excretion, whereas others feel that it is the effect of the tumor on the satiety center.[98] There is some evidence that these patients have a 30 to 50% increase in resting energy expenditure.[99] Optimal treatment involves complete tumor resection, although this is not always possible. Chemotherapy and radiation have also proven effective.

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CHAPTER 49

APPROACH TO NEONATAL CHOLESTASIS

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Cholestasis, defined physiologically as a reduction in canalicular bile flow, is primarily manifested as conjugated hyperbilirubinemia. The major clinical consequences, however, are presumably related to retention of other substances, such as bile acids, which are dependent on bile flow for excretion. The attendant histopathologic features often reflect the nature and degree of the physiologic disturbance and imply the pathophysiologic basis.

There are multiple causes of cholestasis in early life, related either to the response of the neonatal liver to exogenous agents or to specific congenital pathologic conditions. Immature hepatic excretory function creates a milieu wherein infants are susceptible to further impairment of biliary excretion owing to infectious or metabolic insults. Although recognized disorders associated with neonatal cholestasis are numerous, the majority of cases fall into a few discrete and overlapping categories, one of the more frequent ones being the generic "neonatal hepatitis."

Efforts are being made to alert generalists and specialists worldwide to recognize the neonate with cholestasis at the earliest opportunity. Nevertheless, evaluation of the infant with cholestasis remains a difficult task owing to the diversity of cholestatic syndromes, to their obscure pathogenesis, and to the often nonspecific clinical and pathologic presentation. Prompt identification and diagnostic assessment of the infant with cholestasis are imperative to recognize disorders amenable either to specific medical therapy (eg, galactosemia, sepsis) or to early surgical intervention (eg, biliary atresia) and to institute effective nutritional and medical support to allow optimal growth and development. Although the advent of pediatric liver transplant has saved many, early intervention may avoid the need for organ replacement in some of these patients. For example, in tyrosinemia, a nontransplant option is now readily available and efficacious.

DEVELOPMENTAL PHYSIOLOGY OF HEPATOBILIARY FUNCTION

Although comprehension of liver and biliary development is still at the embryonal stage, it is known that the extrahepatic biliary tree develops from an outgrowth of the ventral foregut, whereas the intrahepatic tree differentiates from the multipotent hepatoblast in a centrifugal fashion.[1] Furthermore, the physiology of bile flow in the adult is well described, and its understanding may assist in the approach of the cholestatic infant and the interpretation of laboratory tests. However, it is paramount to remember that the liver of the term infant is "immature" both in its metabolic and excretory functions. With the increased survival of very premature infants, pediatricians, neonatologists, and gastroenterologists are more likely to be confronted by cholestasis and abnormal liver tests. Thus, a basic understanding of physiology, together with prompt recognition and management, should help improve the outcome of these patients. Our goal is to discuss the expeditious and cost-effective approach to the infant with conjugated hyperbilirubinemia, allowing recognition of those who need specialized care.

Bile flow has traditionally been divided into two components: (1) bile acid–dependent flow, which involves active canalicular transport of bile acids, accompanied by osmotic water flow and diffusion of other solutes, and (2) bile acid–independent flow, which is thought to be mediated by active transport of other anions and cations.[2] The primary motive force in the generation of bile flow in early life is the hepatocytic secretion of bile acids; there is little contribution of the bile acid–independent component during the neonatal period.[3] The hepatobiliary excretory system is both functionally and anatomically underdeveloped at birth, leaving the neonate with a unique propensity toward cholestasis.[4–6]

Substantial evidence supports the existence of a period of "physiologic cholestasis" associated with immature or altered metabolism and transport of bile acids at birth (Table 49-1). Serum bile acid concentrations, which reflect the net efficiency of intestinal absorption and hepatobiliary function, are maintained at low levels in the fetus by carrier-mediated transplacental transport to the mother.[7–9] Postnatally, in the normal infant, both fasting and postprandial serum bile acid concentrations are significantly higher than those found in older children. These levels are similar to those attained in adults with cholestatic disease[10,11] and persist through the first several months of life. Factors contributing to decreased bile flow and inefficient enterohepatic cycling of bile acids in the neonate include (1) inefficient intestinal and hepatic bile acid uptake owing to the pace of ontogenic expression of bile acid transport proteins, (2) qualitative and quantitative deficiencies of bile acid synthesis, (3) immature hepatic bile acid metabolism, and (4) inefficient hepatocellular secretion.[12]

The suckling rat model has been used extensively in studies of the developing hepatobiliary system.[13,14] Lower rates of hepatic uptake of bile acids have been demonstrated in experimental systems such as isolated hepatocytes[15] and purified basolateral (sinusoidal) membrane vesicles of developing rats,[16,17] reflecting immaturity of sodium-coupled bile acid transport. This appears to be secondary to reduced expression of specific transport proteins.[18] In the adult rat, avid extraction of bile acids by periportal hepatocytes results in a decreasing periportal to central lobular gradient for bile acid uptake.[19,20] Using similar radioautographic techniques, no acinar gradient could be demonstrated in the 14-day-old rat liver,[21] further supporting the concept of inefficient uptake of bile acids. There is enhanced efflux of taurocholate from suckling rat hepatocytes, which may represent back-diffusion across the sinusoidal membrane; this also contributes to the inefficient hepatic bile acid transport.[22] In the ileum, a similar developmental pattern for the transport of bile acids can be demonstrated, with decreased active bile acid uptake during the suckling period.[23,24] There is significant passive absorption of bile acids in the jejunum of suckling rats, which may combine with decreased hepatic uptake to lead to decreased intraluminal concentrations of some bile acids.[25] A recent study in rats looked at the correlation between intestinal resection length and expression of the apical sodium bile acid transporter. The authors reasoned that there may be an intestinal length "threshold" that determines whether the apical sodium bile acid transporter is up- or down-regulated in response to ileal resection. In parallel, hepatic synthesis of bile acids increases to compensate for decreased absorption up to a certain level, later decreasing when the bile acid pool is severely reduced. These findings are important for two reasons: they illustrate the plasticity of infant bile acid physiology and they offer a hypothesis for the pathogenesis of cholestasis in patients with short bowel.[26]

TABLE 49-1 MANIFESTATIONS OF UNDERDEVELOPED BILE ACID TRANSPORT AND METABOLISM IN EARLY LIFE

Increased serum bile acid levels (physiologic cholestasis)
Decreased hepatic uptake of bile acids from portal blood
Absent lobular gradient
Qualitative and quantitative differences in bile acid synthesis
Decreased conjugation, sulfation, and glucuronidation of bile acids
Enhanced bile acid efflux from hepatocyte
Decreased bile acid secretion rate
Decreased bile acid pool size
Low intraluminal concentrations of bile acids
Decreased ileal active transport of bile acids

Quantitative and qualitative differences in bile acid synthetic pathways are also apparent during early life. Bile acid synthesis begins on day 11 of the 21-day gestation in the rat[27] and near week 12 in the human fetus.[28] A decreased cholate-to-chenodeoxycholate ratio has been observed in the human fetus compared with that of the adult, indicating immaturity of hepatic α-hydroxylation.[29–31] It is believed that a "threshold"' concentration of cholic acid, the primary bile acid, is needed to initiate and maintain bile flow. Cholic acid may be trophic to the developing hepatic excretory system. In the absence of sufficient quantities of cholic acid, there is decreased bile flow.

The immaturity of bile acid synthetic function is also reflected in the presence of "atypical" bile acids found in the fetus and normal neonate.[30,31] Certain of these atypical bile acids, such as the monohydroxylated compound 3-β-hydroxy-5-Δ-cholenoic acid, which has been detected in amniotic fluid[32] and meconium,[33,34] are thought to directly impair bile acid excretion. Significant amounts of nonsulfated tetrahydroxylated bile acids have been identified in the urine of healthy neonates[35] and in the urine of older children and adults with cholestatic liver disease.[33] This polyhydroxylation may increase bile acid solubility, providing a potential alternative pathway for excretion of "toxic" bile acids at a time when transformation and biliary secretion are not fully developed.

Although the mechanisms of intracellular biotransformation of bile acids are not well defined, there is evidence that both the conjugation and sulfation of these organic anions are underdeveloped in early life.[36,37] Conjugation of bile acids with the amino acids taurine and glycine provides a potential mechanism for detoxification and allows efficient intestinal fat digestion and absorption. In isolated hepatocytes obtained from fetal and suckling rats, the rate of conjugation of a radiolabeled bile acid was shown to increase with postnatal age.[37]

The development of effective bile acid secretion from the hepatocyte appears to lag behind the onset of bile acid synthesis, as would be expected if cholic acid truly plays a trophic role. This is suggested by studies of the distribution of the bile acid (taurocholate) pool in fetal and newborn rats.[38] In the fetus, more than 85% of the bile acid pool is localized in the liver, with only 10% found in the intestinal lumen. By postnatal day 5, this distribution is reversed, with more than 85% of the bile acid pool localized in the intestine. Canalicular excretion of bile acids appears to be the rate-limiting step. Reduced canalicular excretion of bile acids in the fetus appears to be related to an immaturity of the canalicular membrane transport sys-

tems for bile acids. The potential-dependent transport protein is not detected in rat liver until postnatal day 7, and transport does not occur until day 14.[39] The adenosine triphosphate–dependent portion of the transport system, however, appears to be functional in the neonatal period and may play a role in bile acid secretion.[40] It has been recognized recently that the regulation of bile acid synthesis occurs by a feedback mechanism involving the nuclear receptor farnesoid X.[41] One can speculate that as these feedback loops mature, they may participate in the imbalance between the hepatocellular bile acid pool and canalicular excretory function. Furthermore, the same ontogenic principles apply to other metabolic pathways and transporters localized on hepatocytes and biliary epithelial cells. Thus, metabolism and excretion of xenobiotics (eg, bacterial toxins, maternal drugs) into bile are likely to be both modified by and potentially exacerbate cholestasis by imposing further demand on the immature liver, especially in the sick newborn. Thus, when investigating an infant with cholestasis, especially a preterm infant, one must look beyond the liver because cholestasis is a nonspecific response to a wide variety of insults in the infant.[42]

During fetal development, canaliculi differentiate from simple intracellular invaginations of two adjacent cell membranes into well-defined structural lumina filled with microvilli.[43] Specific changes in the pericanalicular cytoskeleton, which has been implicated in promotion of bile formation, are also noted during development. Compared with adult cells, cultured fetal hepatocytes have a decreased frequency and force of canalicular contractions, which appear to be related to a lack of pericanalicular cytoplasmic actin.[44] Structural immaturity of both the canaliculi and the pericanalicular cytoskeleton may be significant factors in impaired bile acid secretion during development. Furthermore, studies in both preterm humans and newborn piglets suggest that gallbladder contractility and response to cholecystokinin are also slow to mature, adding an extrahepatic factor to the long list of intrahepatic mechanisms responsible for the increased susceptibility to cholestasis in the infant, in particular the patient dependent on total parenteral nutrition.[45,46]

Despite abundant data suggesting structural and functional immaturity of hepatic excretory function, the clinical and physiologic implications of "physiologic cholestasis" are unclear. However, a reasonable hypothesis could be advanced: in the presence of lower rates of bile flow, compounds destined for biliary excretion would accumulate in the hepatocyte.[11] Certain of these compounds, such as atypical bile acids, are damaging to the membrane or organelle, making hepatic injury likely. Exogenous factors, such as infusion of parenteral nutrition solutions, prolonged fasting, sepsis, or hypoxia, will perturb this already precarious situation and result in the anatomic and clinical manifestations of cholestasis.

DIFFERENTIAL DIAGNOSIS OF CHOLESTASIS

The causes of neonatal cholestasis are diverse (Table 49-2). These include structural anomalies of the biliary tract, both intrahepatic and extrahepatic, which result in obstruction of bile flow, and infectious, metabolic, hemodynamic, or toxic insults, which cause functional impairment of the hepatic excretory process and bile secretion.

Although the differential diagnosis of cholestasis in the neonate is varied, the clinical presentation is similar, reflecting the underlying decrease in bile flow. Specifically, infants with cholestasis present with variable degrees of jaundice, dark urine, light stools, and hepatomegaly. Synthetic dysfunction and hepatocellular necrosis may be present. In certain patients with rapid progression of hepatocellular disease, fibrosis occurs, with signs of decompensation, such as ascites, appearing early in life. Failure to thrive is not always manifest early in the course; normal development may be falsely reassuring and should not detract the clinician from initiating a workup. Similarly, although premature infants are at increased risk for cholestasis, gestational age and side effects of neonatal intensive care should remain a "default" diagnosis once surgical and medical emergencies have been ruled out. The diagnosis of "transient neonatal cholestasis," the most frequent form, may be a more limited subset of the generic "idiopathic" neonatal hepatitis.

Jacquemin and colleagues used the term "transient neonatal cholestasis" to describe a group of 92 patients with early-onset neonatal cholestasis, identifiable perinatal complications incriminated in cholestasis, and a spontaneously favorable outcome.[47] In 85% of the patients, there was a history consistent with acute or chronic perinatal distress. Mean gestational age was 37 weeks, and birth weight was 2,705 g, with one-third of the patients being small for gestational age. Histology was consistent with the previous description of "neonatal hepatitis." The authors did not identify a correlation between histologic findings and perinatal events. Jaundice resolved in all patients, together with normalization of liver biochemical markers and, importantly, growth. The mean duration of jaundice was 3.5 months, and hepatomegaly resolved at a mean age of 13 months. Most had a biphasic progression of their cholestatic markers, with γ-glutamyl transpeptidase reaching its peak as conjugated bilirubin levels normalized. The importance of this study lies in its description of a subset of patients with early-onset neonatal cholestasis and hepatomegaly, perinatal distress, and a characteristic pattern of biochemical markers, in whom it is appropriate to defer liver biopsy and offer supportive care only. It is all the more important that the population of premature babies is increasing, with numerous perinatal hypoxic and toxic insults. Special attention should be paid to those infants who do not have a clearly identifiable cause of prematurity and develop cholestasis or intrauterine growth retarded infants with cholestasis; together, these problems may be indicative of primary liver disease or of an underlying metabolic defect.

DIAGNOSTIC APPROACH

Because of the severity of many of the conditions leading to neonatal cholestasis, early recognition of cholestasis in an infant and prompt diagnosis of the underlying disorder are imperative to identify disorders that will respond to a specific treatment and to institute general supportive care that

TABLE 49-2 CLASSIFICATION OF DISORDERS ASSOCIATED WITH CHOLESTASIS IN THE NEWBORN

EXTRAHEPATIC DISORDERS
- Biliary atresia
- Bile duct stricture/neonatal sclerosing cholangitis
- Choledochal cyst
- Anomalies of the pancreaticoduodenal junction
- Spontaneous perforation of the bile duct
- Inspissated bile
- Mass
  - Intraductular: stone, rhabdomyosarcoma
  - Extraductular: hepatoblastoma, neuroblastoma

INTRAHEPATIC DISORDERS
- Idiopathic
  - "Idiopathic" neonatal hepatitis
  - Intrahepatic cholestasis, *persistent*
    - Severe intrahepatic cholestasis with progressive hepatocellular disease (see Chapter 55.6, "Biliary Transport")
    - Alagille syndrome (syndromic paucity of the intrahepatic bile ducts, arteriohepatic dysplasia)
    - Nonsyndromic paucity of the intrahepatic bile ducts
  - Intrahepatic cholestasis, *recurrent*
    - Benign recurrent intrahepatic cholestasis
    - Hereditary cholestasis with lymphedema (Aagenaes syndrome)
- Anatomic
  - Congenital hepatic fibrosis or infantile polycystic disease (liver and kidney)
  - Caroli disease
- Metabolic or endocrine disorders
  - Disorders of amino acid metabolism
    - Tyrosinemia
  - Disorders of lipid metabolism
    - Cholesterol ester storage disease (Wolman)
    - Niemann-Pick disease
    - Gaucher disease
  - Disorders of carbohydrate metabolism
    - Galactosemia
    - Fructosemia
    - Glycogen storage disease type IV
  - Disorders of bile acid metabolism, primary
    - 3β-Hydroxysteroid Δ^5-C_{27} steroid dehydrogenase/isomerase
    - Δ^5–3-Oxosteroid 5β-reductase (multiple mutations)
  - Disorders of bile acid metabolism, secondary
    - Zellweger syndrome (cerebrohepatorenal syndrome)
    - Peroxisomal enzymopathies
  - Disorders of bile acid transport
    - Rotor syndrome
    - Dubin-Johnson syndrome
  - Mitochondrial hepatopathies
  - Other metabolic defects
    - α_1-Antitrypsin deficiency
    - Cystic fibrosis
    - Hypopituitarism
    - Hypothyroidism
    - Neonatal iron storage disease
    - Infantile copper overload (Menkes syndrome)
    - Hemophagocytic lymphohistiocytosis
    - Arginase deficiency
  - Toxic
    - Total parenteral nutrition–associated cholestasis
    - Fetal alcohol syndrome
    - Other drugs (maternal or used in neonatal intensive care)
- Cholestasis associated with infection
  - Sepsis with possible endotoxemia (urinary tract infection, gastroenteritis)
  - Syphilis
  - Toxoplasmosis
  - Listeriosis
  - Congenital viral infections
    - Cytomegalovirus
    - Herpesvirus (herpes simplex and human herpesvirus 6)
    - Cocksackievirus
    - Echoviruses
    - Rubella virus
    - Hepatitis B virus
    - Other hepatitis viruses: C? nonAnonB?
    - Human immunodeficiency virus (HIV)
    - Parvovirus B19
- Chromosomal
  - Trisomy 18
  - Trisomy 21 (Down syndrome)
  - Donohue syndrome (leprechaunism)
- Vascular disorders
  - Budd-Chiari syndrome
  - Perinatal asphyxia
  - Multiple hemangiomata
  - Cardiac insufficiency
- Miscellaneous
  - Congenital disorders of glycosylation
  - Shock, hypoperfusion
  - Intestinal obstruction
  - Neonatal lupus
  - ARC syndrome (arthrogryposis, renal tubular dysfunction, and cholestasis)

may ameliorate the clinical course. The majority of infants with prolonged cholestasis will be found to fall into the diagnostic category of either biliary atresia or "neonatal hepatitis" (Table 49-3); the latter is a "default diagnosis." As research progresses in pediatric liver disease, the number of cases falling into the "default" category are decreasing. However, at the present time, because of the preponderance of these disorders and the clinical importance of differentiating between them, this chapter focuses on neonatal hepatitis as we know it today; biliary atresia is covered elsewhere in this text. Other specific disorders associated with neonatal cholestasis are discussed in subsequent chapters.

IDIOPATHIC NEONATAL HEPATITIS VERSUS BILIARY ATRESIA

Extensive evaluation of the infant with cholestasis leads to a diagnosis of either idiopathic neonatal hepatitis or biliary atresia in approximately 40% of infants (see Table 49-3). These terms are descriptive and imply a clinical phenotype rather than an etiology. The precise etiology and mechanism of injury in the majority of cases of neonatal hepatitis and biliary atresia remain obscure. The term "idiopathic obstructive or obliterative cholangiopathy" has been used to include disorders that manifest a range of pathology from predominantly hepatocellular injury to predominantly extrahepatic biliary tract injury.

Several overlapping hypotheses attempt to conceptually unify the pathogenesis of these disorders:

1. The ductal plate malformation theory, proposed initially by Jorgensen,[48] suggests that altered embryogenesis may be partially responsible for clinically apparent disorders of cholestasis in the neonate. During normal embryogenesis, the earliest form of the bile duct is a cylindric ductal plate, which is remodeled through an

TABLE 49-3 ESTIMATED FREQUENCY OF VARIOUS CLINICAL FORMS OF NEONATAL CHOLESTASIS

CLINICAL FORM	CUMULATIVE PERCENTAGE
"Idiopathic" neonatal hepatitis	15
Extrahepatic biliary atresia	25–30
α_1-Antitrypsin deficiency	7–10
Intrahepatic cholestasis syndromes (eg, Alagille, PFIC type I)	20
Bacterial sepsis	2
Hepatitis	
Cytomegalovirus	3–5
Rubella, herpes	1
Endocrine (hypothyroidism, panhypopituitarism)	1
Galactosemia	1
Inborn errors of bile acid biosynthesis	2–5

PFIC = progressive familial intrahepatic cholestasis.

interaction between the ingrowing mesenchyme and disappearing ductal plate. Defective remodeling or incomplete dissolution, with failure of recanalization, has been postulated to lead to malformation of the ductal plate and subsequent anatomic abnormalities such as biliary atresia or cystic diseases of the hepatobiliary system. Desmet has suggested that ductal plate malformation is a basic morphologic lesion that occurs at different levels of the biliary tree and may be seen in a variety of disorders in addition to biliary atresia, including congenital hepatic fibrosis.[1]

On a molecular level, it was recently proposed by Clotman and colleagues that intra- and extrahepatic biliary tract development is regulated in part by a cascade involving hepatocyte nuclear factor (HNF) 6 and HNF1 and that ductal plate malformations are visible in knockout models of these transcription factors.[49] Similarly, McCright and colleagues created a mouse model of Alagille syndrome, which suggests that Notch-Jagged interactions are necessary for normal intrahepatic biliary development.[50]

2. Landing set forth the concept of infantile obstructive cholangiopathy, suggesting that these cholestatic disorders represent the pathophysiologic continuum of a single, underlying, obliterative process.[51] According to this hypothesis, an initial insult leads to inflammation at various levels of the hepatobiliary tract. The clinical sequelae represent a static or a progressive inflammatory process at the specific site of injury. If the site of injury is predominantly the bile duct epithelium, the resulting cholangitis could lead to progressive sclerosis and obliteration of the bile duct, clinically manifest as biliary atresia. If, on the other hand, the inflammation is primarily hepatocellular, the clinical picture may be one of neonatal hepatitis. The interrelation between these two processes is further supported by evidence of intrahepatic ductal injury in patients with biliary atresia.[52,53]

Although no specific virus has been consistently identified in patients with "obstructive cholangiopathies," there has been much interest in several specific potential pathogens in these disorders. The majority of studies dealing with viral etiologies in these conditions are related to biliary atresia and thus are discussed in that section. Inborn errors of bile acid synthesis associated with the clinical picture of neonatal hepatitis have also been identified.[6,54,55] As our understanding of immune dysregulation and autoimmunity evolves, it appears that in some cases, the primary insult directed against the hepatocyte or cholangiocyte may be (auto)immune,[56] not unlike diseases found in older subjects (autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis). Immunohistochemical analysis of the "giant cell" reveals that these cells likely result from hepatocellular fusion. However, these observations have not shed light on the underlying insult or trigger for this idiosyncratic cellular response.[57] These studies and others support the contention that the neonatal liver is uniquely susceptible to injury, which, in turn, is manifest in a unique fashion. The initial stereotypic histologic reaction and perpetual injury in infantile obstructive cholangiopathy may result from a wide variety of insults at any level of the hepatobiliary system or beyond in another organ system.

Distinguishing between the intrahepatic, hepatocellular process of neonatal hepatitis and the extrahepatic or mixed injury in biliary atresia is achieved through cholangiography and biopsy and is discussed below.

IDIOPATHIC NEONATAL HEPATITIS

Idiopathic neonatal hepatitis represents the third most common diagnosis in infants with neonatal cholestasis, accounting for 15% overall.[11,58,59] This relative percentage has steadily decreased since the initial description by Stokes and colleagues.[60] This shift is attributable to identification of specific disorders (such as α_1-antitrypsin deficiency and bile acid transport and synthesis defects, which present with a clinical picture of neonatal hepatitis) that were previously included in this category. This diagnosis should be restricted to cases of prolonged neonatal cholestasis in which the classic histologic changes described by Craig and Landing[61] are present on liver biopsy and known infectious or metabolic causes of neonatal hepatocellular disease have been excluded (see Table 49-2). Based on epidemiologic data, two categories of neonatal hepatitis have been proposed: sporadic and familial.[11] The increased incidence within certain families suggests that, at least in these cases, hereditary or metabolic factors are operant. In fact, recent studies have suggested that specific forms of intrahepatic cholestasis, previously included in the "idiopathic neonatal hepatitis" category, can be further subdivided based on the observed pathology and presumed pathophysiology (Table 49-4).[11] It is from this latter group that future discoveries related to the genetic and molecular basis of bile acid synthesis and transport are likely to be made. As research continues to make progress in these areas, the number of identifiable diseases will increase, and the category of idiopathic neonatal hepatitis will proportionately decrease.

TABLE 49-4 PROPOSED SUBTYPES OF INTRAHEPATIC CHOLESTASIS

Bile duct paucity
Syndromic (Alagille)
Nonsyndromic
Progressive (familial) intrahepatic cholestasis
Disorders of canalicular transport
Bile acid transport
Phopholipid transport
Disorders of bile acid biosynthesis
Undefined

Clinical Presentation

"Idiopathic" neonatal hepatitis appears to be associated with low birth weight, but a cause and effect relationship is unclear. The clinical course is highly variable: more than 50% develop jaundice, to a varying degree, within the first week of life. In our experience, the majority appear well; however, as much as one-third have evidence of chronic disease, such as failure to thrive. Acholic stools are uncommon with this disorder but may be present if the cholestasis is severe. The liver (and occasionally the spleen) is firm and enlarged. Biochemical evaluation reveals bilirubin and aminotransferase levels, which are mildly to moderately elevated (2 to 10 times the upper limit of normal). Alkaline phosphatase and γ-glutamyl transpeptidase levels are variably increased (see below). Serum bile acid levels are markedly elevated. A bleeding diathesis, resulting from vitamin K deficiency and/or decreased synthesis of clotting factors, may be present in those with a more fulminant course. Other signs or associated abnormalities such as microcephaly, chorioretinitis, or vascular or skeletal anomalies are unusual and should suggest alternative diagnoses. However, if these signs are absent, the child appears well, and there is a clear history of perinatal distress, "transient" neonatal cholestasis is likely, and the child should be biopsied at the earliest worrisome sign (failure to thrive, acholic stools).

Pathology

Although several histologic features such as giant cell transformation and extramedullary hematopoiesis are nonspecific and represent a stereotypic response of the neonatal liver to injury, the biopsy can be helpful in excluding other causes of neonatal hepatitis. In biopsy tissue obtained early (ie, within the first 2 months of life), there is disarray of the lobular architecture with hepatocellular swelling (ballooning), focal hepatic necrosis, and multinucleated giant cells (more than four nuclei per cell), representing fusion of adjacent hepatocytes (Figure 49-1). Portal triads may be expanded with inflammatory infiltrate of lymphocytes, neutrophils, and occasional eosinophils. There is extramedullary hematopoiesis, as well as varying degrees of portal fibrosis. Although hepatocellular/canalicular bile stasis in the lobule may be prominent, bile duct proliferation and bile duct plugging in portal triads are usually absent. Interlobular bile ducts/ductules are few in number in certain cases, suggesting paucity. The severity of hepatocellular injury usually correlates with the degree of cholestasis.[60,62]

Management

Neonatal hepatitis represents a heterogeneous disorder with no specifically delineated causative or perpetuating factors by definition. Management, therefore, is usually directed at nutritional support, vitamin supplementation, and general medical management of the clinical complications of cholestasis, such as pruritus. General medical management of chronic cholestasis is discussed in detail below.

Prognosis

The overall prognosis in idiopathic neonatal hepatitis is difficult to estimate owing to the variability of the clinical course and the generally ill-defined pathogenesis. The factors that allow perpetuation of the cholestatic process and hepatocyte injury are not fully understood. No specific biochemical or histologic correlates with clinical outcome have been identified. A composite of several large series reviewing outcome of patients with idiopathic neonatal hepatitis is presented in Table 49-5.[63–67] From these data, it is clear that sporadic cases (classic giant cell hepatitis) have a more favorable outcome than familial cases. The poor prognosis in a number of familial cases presumably relates to the presence of underlying inborn errors, specifically defects in bile acid metabolism or transport, as have been described in familial cases of clinically defined neonatal hepatitis (eg, progressive familial intrahepatic cholestasis).[6,54,55] As the underlying causes and pathogenesis of neonatal hepatitis are further defined, more precise prognoses can be established. It is therefore paramount to follow these patients well beyond the normalization of their biochemical markers because neonatal cholestasis may be the harbinger of a metabolic or immune defect manifesting itself later in life. Repeating a liver biopsy for histology, electron microscopy, and metabolic studies (respiratory chain enzymes) is crucial for any child who does not follow a "normal" course, for example, by having a prolonged cholestasis (> 3.5 months), by developing other symptoms such as fasting hypoglycemia, or by presenting with a recurrence of cholestasis.

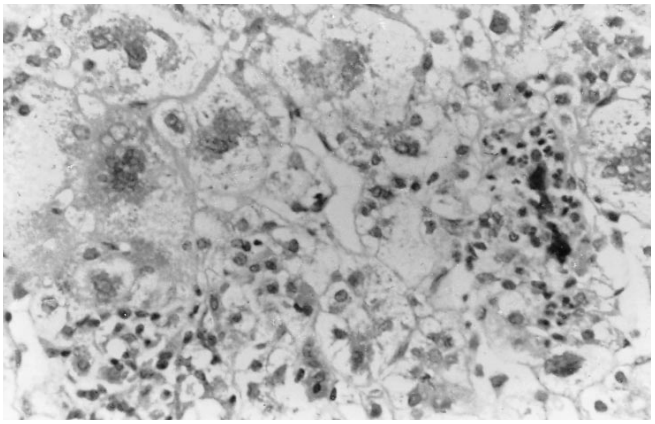

FIGURE 49-1 Liver histology in neonatal hepatitis. This biopsy specimen demonstrates disruption of hepatic lobular architecture with multinucleated giant cells. There are also inflammatory cells within the portal area (hematoxylin and eosin; ×400 original magnification).

TABLE 49-5 STAGED EVALUATION OF NEONATAL CHOLESTASIS

Differentiate cholestasis from physiologic breast milk jaundice and determine severity of disease
Clinical evaluation (history, physical examination, stool color)
Fractionated serum bilirubin (+ serum bile acids)
Tests of hepatocellular and biliary disease (ALT, AST, alkaline phosphatase, GGT)
Tests of hepatic function (serum albumin, prothrombin time, blood glucose, ammonia)
Exclude treatable and other specific disorders
Bacterial cultures (blood, urine)
VDRL test and viral serology as indicated (think HSV)
α_1-Antitrypsin phenotype
T_4 and TSH (rule out hypothyroidism)
Metabolic screen: urine-reducing substances (drugs may cause false positives), urine bile acids, serum amino acids, ferritin, urine organic acids
Sweat chloride/mutation analysis
Differentiate extrahepatic biliary obstruction from intrahepatic disorders
Ultrasonography
Hepatobiliary scintigraphy (not always essential)
Liver biopsy

ALT = alanine transaminase; AST = aspartate transaminase; GGT = γ-glutamyl transpeptidase; HSV = herpes simplex virus; T_4 = thyroxine; TSH = thyroid stimulating hormone; VDRL = Venereal Disease Research Laboratory.

EVALUATION OF THE INFANT WITH CHOLESTASIS

Conjugated hyperbilirubinemia in the newborn period always requires further evaluation, which must be prompt and decisive. Fractionation of the bilirubin, which allows identification of patients with cholestatic (as opposed to physiologic or breast milk) jaundice, should be obtained in any infant with prolonged (ie, more than 14 days) hyperbilirubinemia. Cholestasis traditionally is defined as the presence of a conjugated (or direct-acting) fraction of more than 2 mg/dL (35 μmol/L) or more than 20% of the total bilirubin[11]; however, we prefer to seriously regard any elevation of conjugated bilirubin. Cost-effectiveness should be considered, and a staged approach should be taken in the evaluation of neonatal cholestasis (see Table 49-5). First, treatable disorders such as sepsis, galactosemia, endocrinopathies, and inborn errors of bile acid synthesis must be identified to initiate appropriate therapy that may prevent further damage to the liver and/or reverse the existing injury. Next, biliary obstruction must be differentiated promptly from intrahepatic cholestatic disorders because early surgical intervention is associated with a better prognosis. Finally, the clinical complications of cholestasis, including coagulopathy owing to hypoprothrombinemia or vitamin K deficiency and the nutritional consequences of fat malabsorption, must be addressed because therapy may improve the ultimate outcome and the general quality of life.

HISTORY AND PHYSICAL EXAMINATION

During the evaluation of the infant with cholestasis, the family history, prenatal and postnatal clinical course, and physical examination on presentation may provide important clues. Irritability, poor feeding, and vomiting may indicate a generalized infection or a metabolic disorder such as galactosemia or tyrosinemia or suggest encephalopathy, which is particularly difficult to identify in this age group. Vertebral arch anomalies, posterior embryotoxon, and the murmur of peripheral pulmonic stenosis suggest the diagnosis of Alagille syndrome.[68] Hepatomegaly is the norm. Splenomegaly should lead to the consideration of a systemic disease, infectious or other. Dysmorphic signs suggest chromosomal abnormalities.

It is important to consider that many cholestatic infants appear well at the onset of their disease: normal weight gain and development do not preclude a condition as severe as biliary atresia. In fact, the hallmark of these children is that, initially, many appear to be prospering in spite of their cholestasis.

In differentiating biliary obstruction from intrahepatic cholestasis, the presence of persistently acholic stools is suggestive but not diagnostic of biliary atresia because they may also be associated with severe intrahepatic cholestatic disease. Conversely, the presence of pigmented stools suggests patency of the biliary system and generally excludes the diagnosis of biliary atresia. Alagille identified four clinical features that, although nonspecific, supported the correct diagnosis of intrahepatic or extrahepatic cholestasis in 82% of the cases.[69] These clinical variables included stool color within 10 days of admission, birth weight, age at onset of acholic stools, and the features of hepatic involvement, specifically the presence of hepatomegaly and consistency of the liver on palpation. In this study, addition of liver histology to the evaluation increased the diagnostic accuracy by only 3%. In other studies, despite the use of this scoring system, 10% could not be differentiated,[70–72] also suggesting that further evaluation is sometimes necessary.

Recently, the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN) published the Neonatal Cholestasis Clinical Practice Guidelines; these are available on-line(<www.naspgn/sub/positionpapers.asp>). These guidelines represent the current practice in most tertiary centers in North America and are thought to be the most efficient and cost-effective way to approach the complex problem of neonatal cholestasis.

LABORATORY EVALUATION

There is no pathognomonic or prognostic biochemical feature of neonatal cholestasis. There is no single test consistently reliable in differentiating neonatal hepatitis from biliary atresia. It is not possible to predict either clinically or based on the result of a neonatal screen which infant will develop cholestasis. Nevertheless, several tests may help identify specific causes of cholestasis and assess and monitor the degree of hepatobiliary dysfunction.[11,70] The laboratory data (see Table 49-5) must be analyzed in the context of the clinical setting. For example, urine-reducing substances may be falsely negative if the infant is not receiving a galactose-containing formula or is vomiting. In these situations, the diagnosis of galactosemia may be

made by measuring the red blood cell galactose-1-phosphate uridyl transferase activity, provided that the infant has received no recent blood transfusions. Elevated serum methionine and tyrosine levels, detected during a metabolic screen, may reflect severe liver disease but not necessarily be diagnostic of an underlying metabolic defect. The diagnosis of tyrosinemia should be confirmed by identification of specific metabolites (succinylacetone, succinylacetoacetate). A phenotype is preferred in the evaluation for α_1-antitrypsin deficiency because neonates may have low levels of α_1-antitrypsin despite normal phenotypes, and heterozygotes may have elevated levels in the presence of inflammation. The traditionally requested TORCH (toxoplasmosis, other agents, rubella, cytomegalovirus, herpes simplex) titers have a low diagnostic yield and should be replaced by a request for specific viral titers or cultures only if there are suspicious features. For example, cytomegalovirus serologies should only be obtained based on maternal history and the clinical setting. It is sometimes difficult to obtain an adequate amount of sweat for a sweat chloride test in a neonate, but this test or more specific testing should be performed if the diagnosis of cystic fibrosis remains in question.

γ-Glutamyl transpeptidase, an enzyme located in the epithelial lining of the biliary tree and canaliculi, is elevated in most cholestatic disorders,[73] including biliary atresia, Alagille syndrome, α_1-antitrypsin deficiency, and idiopathic neonatal hepatitis. Normal levels, however, are seen in progressive familial intrahepatic cholestasis and disorders of bile acid synthesis,[74–76] where there is an abnormality of bile acid export into the canaliculus—hence, no bile acid–mediated injury of the canalicular membrane.[77]

As recommended in the NASPGHAN consensus guidelines, part of the stepwise workup always involves assessment of synthetic function by obtaining coagulation studies and metabolic function by measuring glucose and ammonia in serum. Although not used routinely in all centers, quantification of serum bile acids can help orient the diagnosis in neonatal cholestasis syndromes. Elevated levels are found in most forms of cholestasis; low serum bile acid concentration in the face of persistently elevated conjugated bilirubin levels should suggest an inborn error of bile acid synthesis. Other third-line investigations are warranted according to the clinical context: transferrin immunoelectrophoresis should be considered when the constellation of signs is consistent with a congenital defect in glycosylation. Serum α-fetoprotein should be measured especially when considering tyrosinemia because of the associated risk of malignancy. This test can be diagnostic and serve as a baseline for subsequent follow-up. In the presence of splenomegaly, Niemann-Pick disease should always be considered and appropriately addressed by performing a bone marrow aspirate. Similarly, neurologic findings should raise the question of mitochondriopathies and fatty acid oxidation defects. Finally, dysmorphic features, as always in pediatrics, should warrant evalutation. Unfortunately, at the present time, there is no reliable antenatal screening method for most of the conditions leading to neonatal cholestasis.

RADIOLOGIC EVALUATION

Ultrasonography

Real-time ultrasonography is an important adjunct in the diagnosis of neonatal cholestasis.[78] The study is most helpful in ascertaining the presence of a choledochal cyst, or, rarely, a tumor, which can have a clinical presentation similar to that of biliary atresia. The absence of a gallbladder on a fasting study is suggestive but not diagnostic of biliary atresia. Similarly, the presence of a gallbladder does not exclude this diagnosis. Dilated ducts are usually not present in biliary atresia, reflecting the fibro-obliterative or sclerotic nature of the coincident intrahepatic duct lesion.

In recent years, emphasis has been on trying to find a reliable, noninvasive method of diagnosing biliary atresia. The triangular cord sign, when performed by an experienced sonographer, is a potentially helpful diagnostic tool. In one small study, the characteristic cone-shaped finding cranial to the portal vein bifurcation demonstrated a positive predictive value of 100% when visualized together with an abnormal gallbladder and 88% when the gallbladder was normal.[79] The main disadvantage is that this ultrasonographic finding is operator dependent.

Recently, pediatric surgeons have also looked at the value of antenatal ultrasonography in the diagnosis and management of hepatobiliary lesions. Indeed, routine ultrasonography at 20 weeks gestation has led obstetricians and radiologists on occasion to find cystic lesions at the hepatic hilum.[80] Little is known about the natural history of these findings. As more experience is gained in imaging the hepatobiliary system antenatally, this tool may become part of the diagnostic algorithm of neonatal cholestasis.

Finally, a plain chest radiograph should be performed in investigating neonatal cholestasis to look for situs abnormalities, as well as butterfly vertebrae, or rickets, which may help orient the diagnosis.

Radionuclide Imaging

Hepatobiliary scintigraphy, using technetium-labeled iminodiacetic acid analogs, may be used to differentiate biliary atresia from nonobstructive causes of cholestasis. The hepatic uptake and secretion into bile of these derivatives of iminodiacetic acid occur by a carrier-mediated organic anion pathway and depend on the structure of the specific analog, the integrity of hepatocellular function, and biliary tract patency.[81,82] In patients with biliary atresia, particularly early in the disorder, parenchymal function is not compromised; therefore, uptake of the radioisotope is unimpaired, although subsequent excretion into the intestine is absent (Figure 49-2A). Conversely, uptake is usually delayed in infants with neonatal hepatitis owing to hepatocellular dysfunction, but eventually excretion into the bile and intestine occurs (Figure 49-2B). Pretreatment with oral phenobarbital (5 mg/kg/d for 5 days) enhances biliary excretion of the isotope and can increase sensitivity to 94%.[81,82] There are limitations to this study, however; therefore, the diagnosis should not be made solely on the results of this test. Nonexcretion may be related to severe

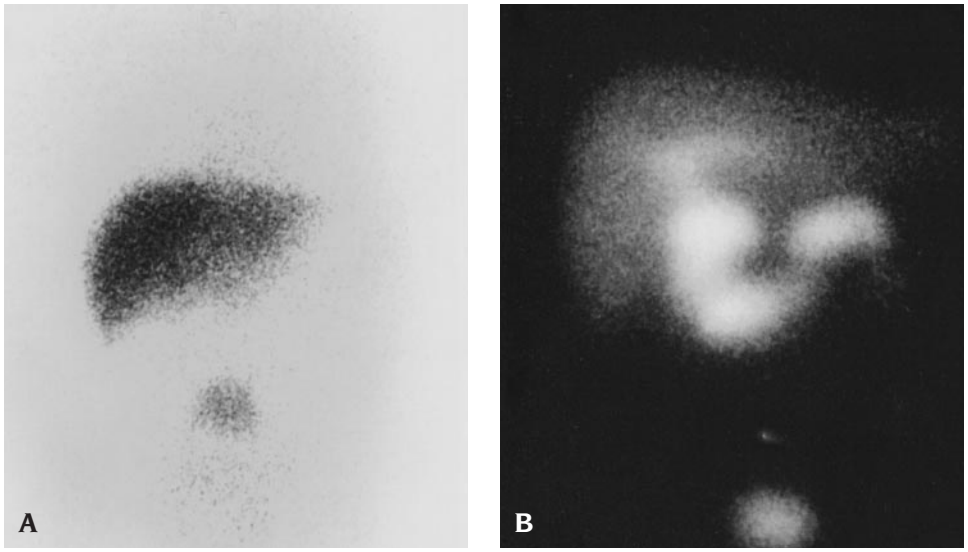

FIGURE 49-2 *A*, Radioisotope scan in biliary atresia. On a delayed scan, there is good uptake of the isotope by the liver, but there is no evidence of intestinal excretion. *B*, Radioisotope scan in neonatal hepatitis. Uptake of the isotope by the liver is delayed and decreased; however, excretion into the intestine is noted.

intrahepatic cholestasis rather than extrahepatic obstruction. In a retrospective study, 12 of 21 infants with intrahepatic causes of cholestasis had no excretion in their first study, despite the use of phenobarbital.[83] In our experience, one patient with isotopic demonstration of a "patent" biliary system was subsequently diagnosed with biliary atresia. In addition, the 5 days required for phenobarbital administration to optimize diagnostic yield may ultimately affect outcome by delaying surgical intervention. Whereas the passage of the tracer into the gastrointestinal tract is 100% sensitive in excluding biliary atresia, a nonexcreting result is only 60% specific for biliary atresia. This poor specificity, together with the time required to prepare for the study, is progressively excluding hepatobiliary scanning from the diagnostic algorithm.[77]

Although there has been much hope that magnetic resonance imaging would provide a means of exploring the biliary tract, there is no evidence at present to suggest that this method is of benefit in the diagnostic armamentarium when studying small infants.[84] Other radiographic studies, such as percutaneous transhepatic cholangiography or endoscopic retrograde cholangiopancreatography, are not only difficult to perform, but experience has been limited in these infants.[85–87]

Liver Biopsy

In our experience, the liver biopsy remains the most reliable and definitive procedure in the evaluation of the neonate with persistent conjugated hyperbilirubinemia. Tissue may be obtained, in most cases, using a percutaneous technique with local anesthesia.[11,70,88,89] Careful interpretation by an experienced pathologist yields the correct diagnosis in 90 to 95% of cases. Prompt diagnosis may expedite surgery for biliary atresia and preclude unnecessary surgical exploration. The typical findings in neonatal hepatitis are discussed above. Like the biochemical evaluation, the biopsy needs to be interpreted in the clinical context because the histology of neonatal hepatitis is nonspecific: giant cell transformation and hepatocyte ballooning with lobular disarray represent a nonspecific response of the newborn liver to an insult. Many of the characteristic histologic findings stem from observational studies in term infants in their first few months of life. Less is known about the histology of the early course of some neonatal cholestasis syndromes or of preterm infants with cholestasis. These conditions are evolving processes, and more information may be gained by performing a repeat biopsy to look for more characteristic findings and to appreciate the evolution.

Conclusions regarding Evaluation

In evaluating a neonate with cholestasis, both surgical and medical emergencies have to be excluded and attended to in a timely fashion. If galactosemia is suspected, appropriate dietary measures should be taken immediately. If sepsis is the likely cause of the cholestasis, this must be managed urgently. If biliary atresia is suggested, an exploratory laparotomy, often with an intraoperative cholangiogram, is performed to verify the nature and site of the obstruction prior to hepatoportoenterostomy. Finally, if no specific etiology is determined but extrahepatic obstruction is unlikely, the infant is followed and re-evaluated frequently. Empiric therapy may also be instituted to optimize growth and development and ameliorate the consequences of chronic cholestasis (discussed below).

The need to correctly differentiate biliary atresia from intrahepatic disorders is illustrated by a report from Markowitz and colleagues in which four patients who underwent hepatoportoenterostomies on the basis of hepatobiliary scans and intraoperative cholangiograms were subsequently found to have Alagille syndrome on histologic and clinical criteria.[90] None had adequate drainage postoperatively, two progressed to cirrhosis, and one died from hepatic failure, indicating that the intervening surgery had adversely altered the course of a usually benign disorder. If careful consideration is given to the history, physical examination, and these selected diagnostic tests (see Table 49-5), institution of appropriate surgery may be expedited, unnecessary surgery avoided, and, in many cases, the precise etiology determined.

MEDICAL MANAGEMENT OF CHRONIC CHOLESTASIS

In infants with intrahepatic cholestasis or those with biliary atresia in whom surgical attempts at establishing adequate biliary drainage are unsuccessful, the presence of the clinical consequences of persistent cholestasis directs medical therapy. These complications are related, either directly or indirectly, to diminished bile flow and reflect (1) retention of substances dependent on bile secretion, such as bile acids, bilirubin, and cholesterol; (2) decreased bile acid delivery to the intestine with resultant fat and fat-soluble vitamin malabsorption; and (3) progressive hepatocellular damage leading to portal hypertension and eventual liver failure (Figure 49-3). Currently, no specific therapy either reverses existing cholestasis or prevents ongoing damage; therefore, therapy is empiric and aimed at improving nutritional status, maximizing growth potential, and minimizing discomfort.[91] The success of this therapeutic intervention is limited by the residual capacity of the liver and by the rate of progression of the underlying disorder. The success is enhanced by introducing these measures in a timely fashion, namely as soon as abnormal weight gain is anticipated.

PRURITUS

Significant clinical morbidity may result from pruritus, and its management is a difficult and sometimes frustrating clinical problem. In some patients, the impairment in quality of life is so severe that liver transplant is indicated. The cause of cholestatic pruritus is not clear.[92,93] It has been reported that skin and serum levels of bile acids did not differentiate between patients with or without pruritus,[93] arguing against bile acids as direct pruritogens. Endogenous opioids have been implicated as mediators of pruritus, specifically cholestasis-induced pruritus.[94] Similarly, the serotonin neurotransmitter system has also been identified as one potential factor.[95] With these recent findings, new approaches to the management of cholestasis-induced pruritus are emerging.

Therapy directed at decreasing the concentrations of bile acids may be efficacious in some patients because of the nonspecific action of these agents. The anion exchange resion cholestyramine has been used historically to interrupt enterohepatic circulation. Because its use entails a complicated regimen, it is seldom used in pediatrics.

Phenobarbital, in therapeutic doses of 5 to 10 mg/kg/d, stimulates bile acid–independent flow and decreases the bile acid pool size.[96] The drug has not been consistently efficacious in relieving pruritus in intrahepatic cholestasis. The sedative side effects of phenobarbital may be a limiting factor in its usefulness. As a rule, its use in cholestasis is becoming more infrequent. The use of rifampin (10 mg/kg/d), which inhibits hepatic uptake of bile acids, has also been tried with variable success in relieving pruritus.[97,98] Like phenobarbital, it is a microsomal enzyme inducer, with the advantage of not having a sedative effect. Related side effects are minimal, but worsening biochemical liver markers can be suggestive of rifampin-induced hepatitis.[99]

Ursodeoxycholic acid (UDCA), which alters bile acid composition, has been shown to be beneficial in the relief of pruritus in studies of adults with primary biliary cirrhosis.[100] Preliminary studies, using 15 to 30 mg/kg/d, suggest that it may be of benefit in ameliorating pruritus in childhood cholestasis as well.[101,102] In our center, UDCA is routinely prescribed to all children with cholestasis for its potential cytoprotective effect on hepatocytes, as well as its

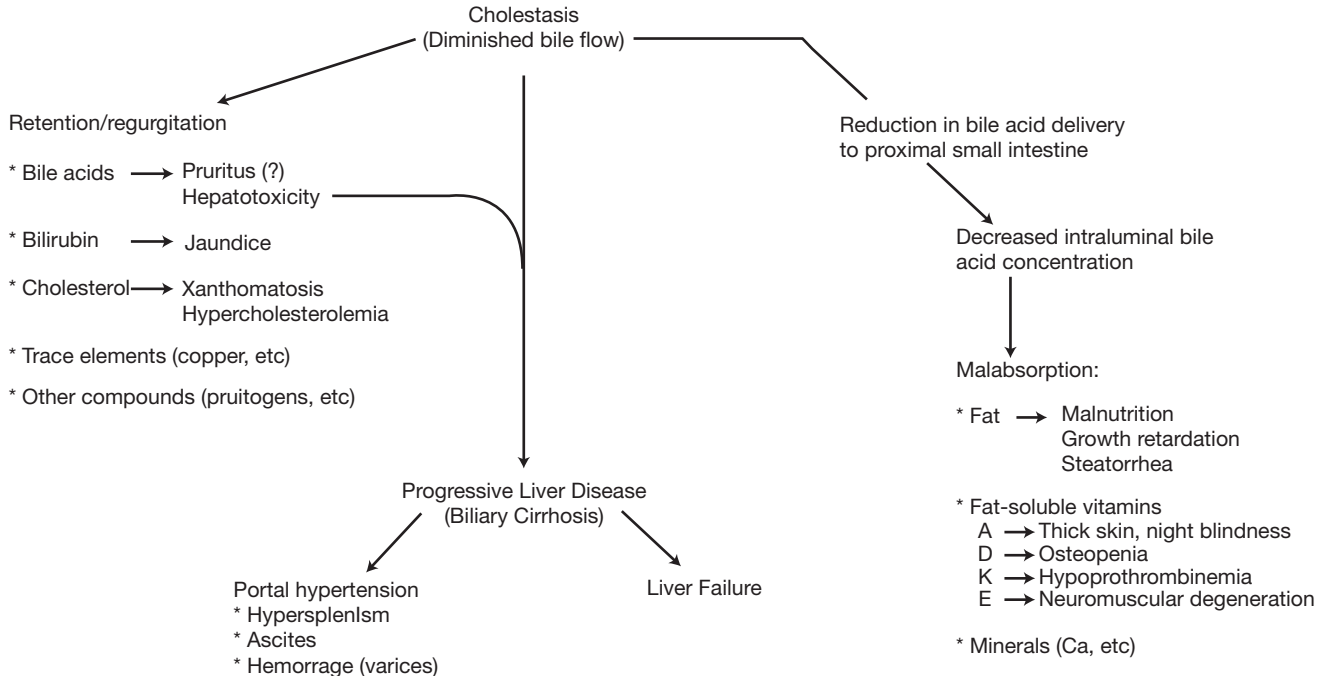

FIGURE 49-3 Clinical sequelae of chronic cholestasis. Numerous consequences of cholestasis become clinically manifest and result from retention of substances excreted in bile, reduction of intestinal bile acids, and progressive damage to the liver. See text for relationship between bile acids and pruritus.

role in relieving pruritus. The current trend is toward the higher dose of 30 mg/kg/d, although there is no documented evidence that the effect is dose dependent.

As mentioned above, interest in the role of the opiate receptor system in pruritus of cholestasis was prompted by the results of studies in which opioid antagonists relieved pruritus.[98,102] The presumed mechanism of action is that they prevent the binding of endogenous opioid agonists, which have been shown to be elevated in cholestasis. The three known opioid antagonists (naloxone, nalmefene, and naltrexone) have been studied and have demonstrated an aleviating effect, although none have completely abolished pruritus. There are problems associated with the use of opioid antagonists: they precipitate a withdrawal-like effect, and a "breakthrough" phenomenon has been described, which consists in an exacerbation of the pruritic symptoms after an initial improvement. Thus, determining the appropriate dose and management can be difficult, and consultation with anesthesia may be appropriate.[99] As mentioned earlier, there have been some studies implicating serotonin in the pathophysiology of pruritus. As such, the use of ondansetron in the relief of pruritus has been studied with some promising results, but more studies are required to confirm these findings.[99]

For those children with intrahepatic cholestasis and intractable pruritus unresponsive to therapy, partial external biliary diversion has been performed.[103] Patients with progressive intrahepatic cholestasis had a good response, with relief from itching and concomitant improvement in their biochemical tests of liver function and histology. In a retrospective review performed at our center, patients and parents reported a marked improvement in quality of life, as defined by school attendance and interactions with peers.[104] Finally, there have been anecdotal reports on the efficacy of a variety of other therapies, including phototherapy and plasmapheresis.

Malabsorption and Malnutrition

Lipids. One of the major and more immediate complications of chronic cholestasis is fat malabsorption related to decreased intraluminal bile acids, which leads to malnutrition and fat-soluble vitamin deficiency. Decreased excretion of bile acids leads to a low intraluminal micellar concentration; therefore, long-chain triglyceride lipolysis and absorption are ineffective. Medium-chain triglycerides (MCTs) are more water soluble than their long-chain counterparts and are therefore readily absorbed by the gastric and intestinal mucosa in the face of low intraluminal concentrations of bile acids, making them a more adapted source of fat calories; MCTs can best be administered as MCT-containing formulas. MCT oil alone is insufficient because it does not contain essential fatty acids. In those children who are unable to take in sufficient calories orally, nocturnal enteral feeding has been shown to improve nutritional indices in many patients with chronic liver disease.[105]

Liposoluble Vitamins. Intestinal absorption of fat-soluble vitamins (A, D, E, and K) that require solubilization by bile acids into mixed micelles is also compromised, and supplementation of at least two to four times the recommended daily allowance is often necessary (Table 49-6). Serum vitamin levels and laboratory tests such as serum calcium and phosphate levels and prothrombin time are useful indices of adequate supplementation. Chronic vitamin E (α-tocopherol) deficiency has been associated with a progressive neuromuscular syndrome characterized by areflexia, cerebellar ataxia, posterior column dysfunction, and peripheral neuropathy.[106,107] The most reliable index of vitamin E status is the ratio of serum vitamin E (mg/dL) to total serum lipids (g/dL) because elevated lipids, as seen in chronic cholestasis, allow vitamin E to partition into the nonpolar phase (plasma lipoprotein fraction), artificially raising the serum vitamin E concentration. In infants and children less than 12 years of age, a ratio of less than 0.6 mg/g indicates vitamin E deficiency.[108] In those children who do not respond to supplementation of vitamin E by traditional methods, oral administration of a water-soluble form of vitamin E, *d*-α-tocopherol polyethylene glycol 1000 succinate (TPGS), has been found to correct biochemical vitamin E deficiency in doses of 15 to 25 IU/kg/d.[109] In truly refractory cases, an admixture of all fat-soluble vitamins with TPGS may be more beneficial than administration of the supplement alone.[110] Although there are no data to support its use in neonatal cholestasis, vitamin E is part of the antioxidant armamentarium prescribed in many forms of acute and chronic liver disease.[111] Careful consideration should be given to fat-soluble vitamin replacement in the nutritional management of these patients because intracranial bleeding from vitamin K deficiency is still a frequent cause of death in infants and toddlers with cholestasis.

Carbohydrates. In animal models of biliary atresia, as much as a 20 to 30% decrease in brush border enzyme activity has been observed. Thus, although there is no published evidence to this effect in human subjects, one should consider changing the carbohydrate composition of the formula if the infant is demonstrating signs consistent with lactose intolerance. This is particularly important because the infant with cholestasis requires as much as 130% of the caloric intake of age-matched controls (as much as 150 kcal/kg/d), 60% of which should be in carbohydrate form.

TABLE 49-6 RECOMMENDED ORAL VITAMIN SUPPLEMENTATION

VITAMIN	PREPARATION, DOSE
Fat soluble	
Vitamin A	Aquasol A: 3,000–25,000 IU/d
Vitamin D	Cholecalciferol: 500–5,000 IU/d or 25-Hydroxycholecalciferol: 3–5 μg/kg/d
Vitamin K	Phytonadione (K1): 2.5–5 mg every other day
Vitamin E	Aquasol E: 50–400 IU/d or TPGS 15–25 IU/kg/d
Water soluble	Twice the recommended daily allowance

TPGS = *d*-α-tocopherol polyethylene glycol 1000 succinate.

Protein. Infants with cholestasis require a normal proportion (20–30%) of their diet in protein form. However, unless malnutrition is so severe, there is no evidence to suggest that protein hydrolysates are necessary. One study in a rat model suggested that adding branched-chain amino acids to the formula may impact growth and nitrogen retention favorably.[112] There is no such evidence in human subjects.

Other Vitamins and Nutrients.

1. Calcium. In spite of aggressive liposoluble enteral or parenteral vitamin replacement, most children with cholestasis suffer from severe osteopenia, often leading to pathologic fractures.[113,114] Thus, both additional calcium and magnesium[115] supplementation is advisable, ensuring that the child or infant receives at least 1,000 to 1,300 mg/d of calcium and 8 to 16 mg/kg/d of elemental magnesium. The time at which the supplement is taken is critical because it has been suggested that UDCA may affect calcium absorption negatively. Finally, monitoring a patient's calcium-phosphorus status using urinary indices and parathyroid hormone may be indicated because serum levels may not always be accurate because of acid-base abnormalities or hypoalbuminemia.
2. Zinc. Because of increased intestinal losses and fat malabsorption, zinc deficiency is more prevalent in cholestasis than is commonly thought. Because zinc is a common cofactor in numerous enzymatic reactions, including in the liver, zinc deficiency may further exacerbate the underlying liver disease.
3. Iron. Iron deficiency is common in these children. Early on, this may be due to insufficient dietary intake. As the course of the disease progresses, however, this is more often the sign of occult, or overt, gastrointestinal bleeding.

Portal Hypertension

In most patients with biliary atresia, and in certain patients with intrahepatic cholestasis, progressive fibrosis and cirrhosis ultimately lead to the development of portal hypertension, the most clinically significant sequelae being ascites and variceal hemorrhage. The medical management of ascites should be dictated by patient comfort and by the relative risk of peritoneal bacterial infection. The judicious use of sodium restriction and diuretics may be helpful in controlling the accumulation of ascites. Initial steps include restricting dietary sodium intake to 1 to 2 mEq/kg/d and introducing a diuretic such as spironolactone, which inhibits the effects of aldosterone. We usually start with 3 to 5 mg/kg/d divided into 3 to 4 doses and increase the dose as needed up to 10 to 12 mg/kg/d to maintain an increased urinary sodium-to-potassium ratio. Refractory ascites with respiratory compromise may be managed by therapeutic paracentesis with concomitant administration of an intravenous colloid such as albumin.[11] Albumin has been shown in adult studies to play a role in the preservation of renal function in large-volume paracentesis and to have a protective effect against the risk of spontaneous bacterial peritonitis.[116] Used together with furosemide, it participates in the elimination of free water and sodium while avoiding the prompt recurrence of ascites.[116]

Esophageal and gastric varices are a potentially life-threatening complication of portal hypertension. Acute variceal hemorrhage is managed in an intensive care unit with intravenous fluids and blood products, gastric lavage, and intravenous vasopressin infusion (0.3 U/1.73 m^2/min) as indicated. Balloon tamponade, used for severe or prolonged hemorrhage, may be associated with significant complications such as esophageal rupture, airway obstruction, and pulmonary aspiration. Endoscopic sclerotherapy is being used more extensively in infants and children for the acute and ongoing management of esophageal varices and may be superior to surgical alternatives,[117] particularly if eventual liver transplant is anticipated. Although not available for the infant population, banding is also used routinely in older children. However, to date, there is no literature to suggest that prophylactic banding or sclerotherapy is preferable to managing varices on an ad hoc basis following a bleed. This topic is covered in greater depth in Chapter 59, "Treatment of End-Stage Liver Disease." Gastric varices are not amenable to this therapy. There has also been interest in long-term administration of β-blocking agents such as propranolol to reduce portal pressure and prevent recurrent variceal bleeding in adults,[102,103] but the results have been variable, and there is limited experience in children. Furthermore, β-blockade may impede appropriate cardiovascular compensation in the event of an acute bleed and is therefore not used routinely in infants. In refractory hemorrhage, pediatric patients may be stabilized using intravenous octreotide, starting at 1 μg/kg/(min) and increasing as needed. Patients should be closely monitored for hyperglycemic side effects. The use of octreotide can be a convenient tool while the patient awaits emergent liver transplant.

Orthotopic liver transplant has become a viable option for infants and children who progress to end-stage liver disease.[118,119] The ability to determine the optimum time in the clinical course to pursue transplant requires careful monitoring and sequential evaluation of hepatic function. Although no one specific functional measure has been shown to reliably assess hepatocellular reserve, prognostic scores have been developed for predicting outcome without transplant. These scores may be compared with operative survival statistics for a particular patient group and thus aid in decision-making. In infants and children with end-stage liver disease, the deciding factor in timing organ transplant is usually organ availability. Therefore, it is important to carry out evaluation early in the course to develop supportive strategies and to stratify based on clinical criteria.

The major limiting factor for successful transplant in infants has been the supply of appropriately sized organs. This situation has been somewhat alleviated by introduction of the techniques of segmental or volume reduction liver transplant, living donor transplant, and "split" organ donation.[120] More effective means for supporting and monitoring infants with chronic liver disease are needed. Ultimately, a better understanding of the pathophysiology of specific underlying disease processes may

lead to more efficacious treatment of the sequelae of persistent infantile cholestasis and to therapeutic interventions that will prevent or reverse the development of chronic liver disease.

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CHAPTER 50

DISORDERS OF THE BILIARY TRACT

1. *Disorders of the Intrahepatic Ducts*

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EMBRYOLOGY OF THE INTRAHEPATIC DUCTS

The development of the intrahepatic biliary tree begins between the fifth and ninth week postfertilization. The biliary epithelium is believed to arise from precursor bipotential hepatoblasts that can differentiate into either hepatocytes or biliary epithelial cells.[1] Genes and gene products regulating this process have been recently identified. *HNF1*β is required for the development of interlobular bile ducts and arteries in mice, and mutant mice lacking *HNF1*β in the liver have a paucity of intrahepatic bile ducts and absence of hepatic arteries.[2] *HNF6* is believed to regulate the expression of *HNF1*β and is required for the development of the gallbladder and extrahepatic ducts.[3]

Differences in cytokeratin (CR) expression between hepatoblasts and biliary epithelial cells have been used to illustrate the development of intrahepatic bile ducts. Precursor hepatoblasts express CK 7, 8, 18, and 19; mature hepatocytes express CK 8 and 18 but not 19, whereas biliary epithelium expresses CK 8, 18, 19, and 20.[4] Around weeks 6 to 7, a prominent rim of CK19-positive cells forms along the outer boundary of the developing portal tracts, forming the so-called ductal plate.[5,6] During the next few weeks, the rim becomes a continuous double-layered saccular sleeve around each portal tract. Remodeling of the ductal plate, starting around week 12, results in the formation of discrete tubular spaces incorporated more centrally into the portal mesenchyme, with loss of excess epithelial elements (Figure 50.1-1). This process is believed to proceed from the hepatic hilum, extending centrifugally toward the periphery of the liver. It appears to continue into the first month of life ex utero. A delicate balance between cell proliferation and apoptosis appears to be a key element of this process.[7] The most cranial portions of the hepatic ducts, themselves derived from the cephalic portion of the hepatic diverticulum, would appear to be in direct continu-

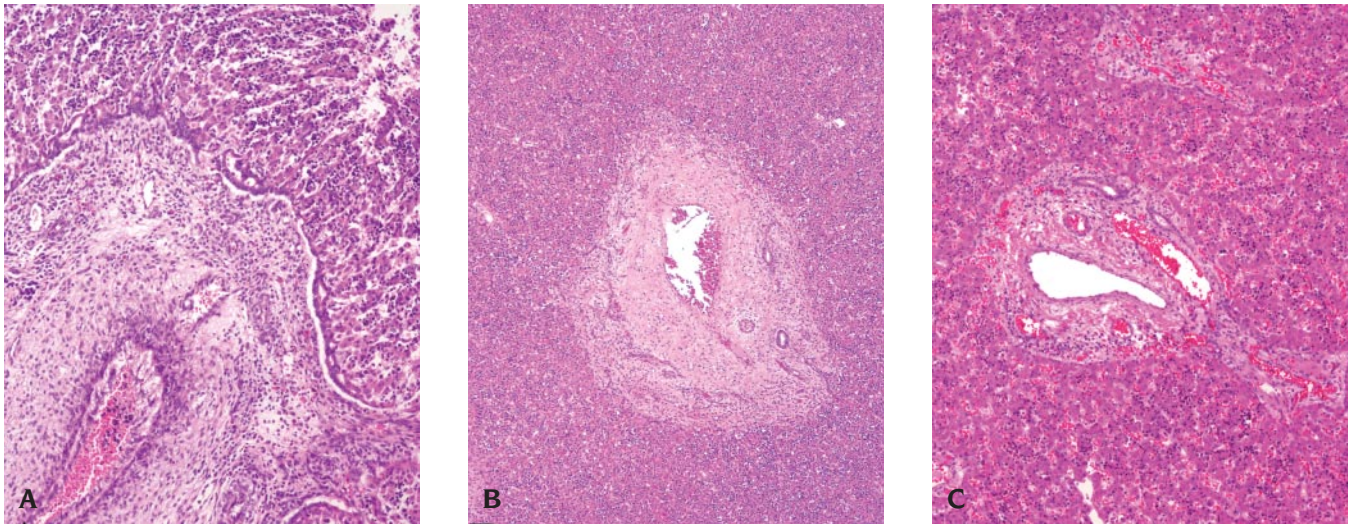


FIGURE 50.1-1 Development of the bile duct as illustrated in fetuses of different ages. *A*, Ten-week-old fetus. The portal vein is in the center of the portal tract. The ductal plate consists of a double-layered saccular sleeve that surrounds the portal tract. *B*, Sixteen weeks. Short segments of the ductal plate become tubules, which are incorporated toward the center of the portal tract. The ductal plate has become discontinuous. *C*, Twenty-five weeks. The ductal plate has largely involuted (hematoxylin and eosin; ×100 original magnification).

ity with converging ductules at the hepatic hilum, which, in turn, are continuous with the ductal plate.[7]

Persistence of the ductal plate in the postnatal liver, appearing as an excessive number of irregular, biliary structures at the site of the original ductal plate and accompanied by an increase in portal tract fibrous tissue, creates a lesion known as the ductal plate malformation (DPM),[8] biliary dysgenesis,[9] or congenital hepatic fibrosis[10] (Figure 50.1-2). The prominent duct elements should not be confused with the proliferating duct elements commonly seen as a response to a variety of hepatic insults, including mechanical obstruction. Jorgenson[8] recognized the similarity between these portal tracts and those seen in fetal life and coined the term "ductal plate malformation" to signify that the lesion represents an arrest in the development of normal portal tract and bile duct structures or, as characterized by Desmet,[11] a disruption of the normal "remodeling" of the embryonic bile duct and portal tract structures into their mature forms. The relevance of this lesion to cystic bile ducts lies in the fact that the abnormal ducts have a propensity to become dilated. This lesion is found in combination with renal abnormalities (usually cysts) in a number of heritable conditions in which there is actual or potential cystic dilatation of the biliary ducts. In addition to these heritable disorders, Desmet has suggested that persistence of the ductal plate can also be associated with extrahepatic biliary atresia,[11] which is not heritable and is not associated with renal disease.

The intrahepatic tree in humans can be divided into large bile ducts (300–800 μm), which include hepatic, segmental, and area ducts, which have associated mucous peribiliary glands, and small bile ducts (< 300 μm), which are not associated with peribiliary glands and include conducting and terminal (or interlobular) bile ducts.[12] Interlobular bile ducts link with intralobular canaliculi via bile ductules or canals of Hering, although the exact anatomy remains unclear. The biliary epithelia of small and large bile ducts express different enzymes and membrane proteins, suggesting that they represent distinct subpopulations.[13] Possible differences in disease susceptibility and responses to injury between epithelia of small and large bile ducts may explain why certain diseases preferentially target smaller bile ducts (primary biliary cirrhosis, graft-versus-host disease), whereas others (sclerosing cholangitis) are primarily directed at larger ducts.[14,15]

INTRAHEPATIC BILE DUCT CYSTIC CONDITIONS

Cystic diseases of the intrahepatic bile ducts present a wide range of disorders. They include both sporadically occurring and heritable conditions and extend from lesions typically discovered incidentally to frank malignancies. A classification scheme is presented in Table 50.1-1. Heritable cystic disease of bile ducts is also referred to as fibrocystic, or fibropolycystic, disease, reflecting the portal fibrosis and DPM that is the histologic hallmark of most of these disorders. The distinction between communicating and noncommunicating cysts is clinically significant because when duct cysts communicate with the biliary tree, they have a greater likelihood of causing clinical disease. Communicating duct cysts can be associated with cholangitis, stone formation, and (relatively uncommonly) neoplasia. Noncommunicating duct cysts are usually asymptomatic but, if sufficiently large, may present as an abdominal mass or biliary obstruction.

SOLITARY CYSTS

Solitary bile duct cysts are generally unilocular cysts lined by a single layer of cuboidal or columnar epithelium (Figure 50.1-3). They tend to present in the fourth to sixth decade with symptoms of fullness or a mass. They are rare in the pediatric age group, 31 having been diagnosed in 63 years at the Boston Children's Hospital.[16] They have been reported in newborns, including a case presenting as a con-

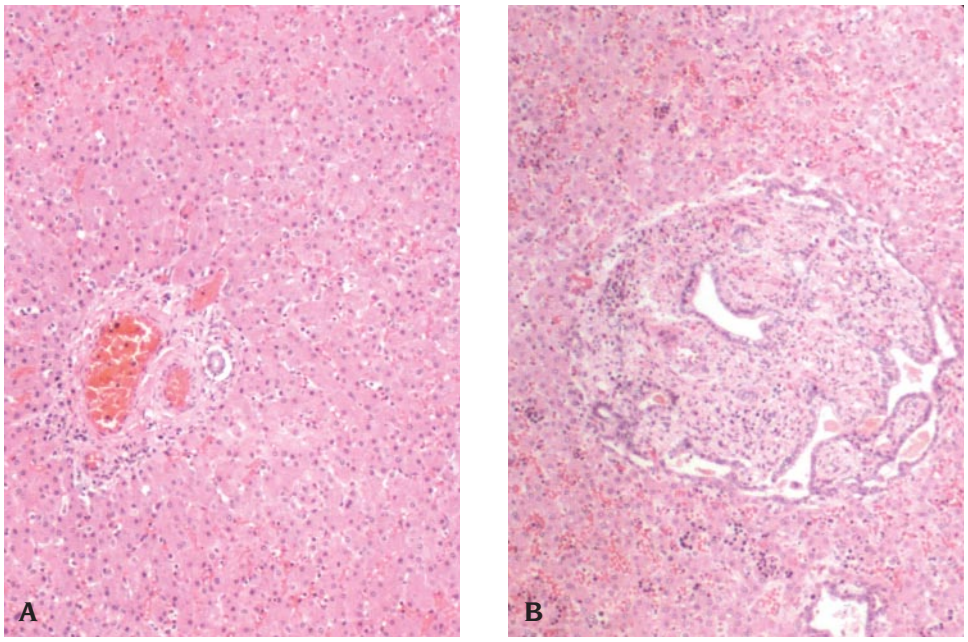


FIGURE 50.1-2 Congenital hepatic fibrosis (CHF). The left panel shows a normal portal tract from a newborn term infant. The right panel, taken at the same magnification, illustrates the hepatic histologic features from a 9-day-old infant with CHF and autosomal recessive polycystic kidney disease. The portal tract is expanded and fibrotic, and there is a proliferation of irregularly dilated biliary structures at the portal tract–lobular interface, a pattern known as "ductal plate malformation" (hematoxylin and eosin; ×100 original magnification).

TABLE 50.1-1 CLASSIFICATION OF HEPATIC CYSTS OF DUCTAL ORIGIN

SOLITARY NONPARASITIC CYSTS
Solitary bile duct cyst
Ciliated hepatic foregut cyst
Peribiliary cyst
Hepatobiliary cystadenoma
HERITABLE HEPATIC CYSTS—FIBROCYSTIC DISEASE
Communicating cysts
Congenital hepatic fibrosis (CHF)
CHF in association with
Autosomal recessive polycystic kidney disease
Nephronophthisis
Malformation syndromes (see Table 50.1-2)
Phosphomannose isomerase deficiency
Caroli syndrome
Noncommunicating cysts
Autosomal dominant polycystic kidney disease
Isolated polycystic liver disease (chromosome 19p13.2-13.1)

genital diaphragmatic hernia.[17] The main differential diagnosis is with autosomal dominant polycystic disease. Imaging studies in the latter would show multiple cysts in the liver and kidney. The cystic structures of Caroli disease are usually part of a more diffuse involvement of the biliary tree (vide infra). In contrast, mesenchymal hamartomas, which are more common in the pediatric age group, have a more complex structure, with multiple cysts and a solid component. Ciliated hepatic foregut cysts, so named because of their alleged origin from the embryonic foregut, are characterized by a lining of ciliated pseudostratified columnar epithelium resting on a basement membrane and surrounded by smooth muscle bundles.[18] They seem to occur more commonly in males in the fourth to fifth decade. Peribiliary cysts are derived from dilatation of the peribiliary glands located in the hilum and large portal areas. They have been reported primarily as findings in autopsy or hepatectomy specimens and have been associated with a variety of liver disorders.[19] They have not been reported in childhood. Hepatobiliary cystadenomas are benign multilocular cystic neoplasms that tend to occur in middle-age women but have been reported in patients from 2 to 87 years of age.[20] They usually occur in the liver but may also arise within the extrahepatic biliary tree and gallbladder. They are lined by a flattened, occasionally papillary, mucin-producing columnar epithelium. In females, the cysts are surrounded by dense mesenchymal tissue resembling ovarian stroma. Its malignant counterpart, the biliary cystadenocarcinoma, has not been reported in children.

HERITABLE INTRAHEPATIC CYSTIC DISEASE

The major heritable conditions characterized by intrahepatic bile duct cysts are congenital hepatic fibrosis (CHF), autosomal recessive polycystic kidney disease (ARPKD), and autosomal dominant polycystic kidney disease (ADPKD). There are also a number of heritable malformation syndromes characterized by potential bile duct cysts and renal disease. The DPM is seen in essentially all of these disorders (least commonly in ADPKD). Whenever the DPM is the basis for the cysts, the cysts communicate proximally and distally with the biliary tree. Renal cysts of tubular origin or other renal developmental lesions are typically present in most of these conditions. It is of note that the renal lesions tend to be dissimilar in the different clinical conditions.

CONGENITAL HEPATIC FIBROSIS

The term "congenital hepatic fibrosis" was coined by Kerr and colleagues[10] and is essentially defined by a characteristic hepatopathology, resulting in portal hypertension and an increased risk of ascending cholangitis. CHF is associated with a wide spectrum of disorders, the most frequent of which is ARPKD, but can also be an isolated condition.

The relationship of ARPKD to CHF is still somewhat controversial. Some investigators maintain that ARPKD and CHF represent a single disorder, whereas others suggest that they are distinct entities with overlapping hepatic histologic features.[5] The hepatic histomorphology in both lesions is essentially similar and is characterized by the DPM. The renal lesions, which also consist of tubular cysts in both, classically differ markedly in both pathology and clinical severity. In newborn patients with ARPKD, the renal lesions are diffuse and prominent clinically, whereas in patients who exhibit the clinical picture of CHF, the renal lesions are often not as evident in early life and are minor. Recently, a gene (*PKHD1*) that maps to the 6p21 locus and encodes a large, receptor-like protein, fibrocystin, has been identified as the site of mutations resulting in ARPKD.[21,22] The identification of this gene will prove to be valuable in the delineation of these two identities.

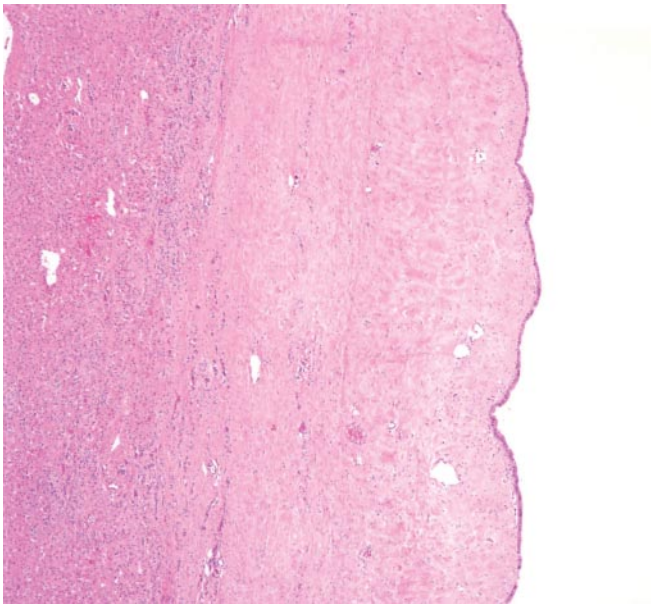


FIGURE 50.1-3 Hepatic cyst. Solitary hepatic cyst from a 9-year-old female. The cyst lining consists of a low cuboidal epithelium resting on a thick fibrous wall. Liver parenchyma can be noted toward the left of the picture (hematoxylin and eosin; ×40 original magnification).

Clinical Findings

A considerable range of clinical presentations is observed in patients with CHF. Most patients present with portal hypertension in the first decades of life. Some patients present with cholangitis, whereas other patients are discovered only incidentally at autopsy (latent CHF).[23] The precise pathogenesis of the portal hypertension is unknown but is thought to be associated with the hepatic fibrosis and/or portal vein abnormalities. Hematemesis or melena is the presenting sign in 30 to 70% of patients from pediatric and mixed population studies.[24,25] In children, the age for presentation of hematemesis may be as early as the first year of life,[26] but it usually ranges from 5 to 13 years. Firm or hard hepatomegaly is present in nearly all patients, often with a prominent left lobe, and this is usually one of the presenting findings. Splenomegaly occurs in the majority, accompanied by hypersplenism with thrombocytopenia. Splenic pressure is elevated, and naturally occurring splenorenal or gastrorenal shunts are occasionally documented. Portal vein abnormalities, characteristically duplication of the intrahepatic branches, are common.[24,27] This is in contrast to histologic descriptions of hypoplastic portal vein branches by others.[5] Occasionally, portal vein thrombosis or cavernous transformation of the portal vein is documented. Renal lesions, when present, appear to be minimal and of little clinical significance. An appearance similar to medullary sponge kidney has been described.[28] Cases of renal insufficiency described in association with CHF may, in fact, represent "late-onset" ARPKD.[29]

Pathology

The cut surface of the liver is speckled with irregular, whitish areas of fibrosis. The characteristic lesion is the DPM, and parenchymal disease is usually absent. The degree of fibrosis is variable, from enlarged portal tracts to broad bands of connective tissue linking portal tracts. It is usually diffuse but occasionally confined to one lobe or even one segment of a lobe.[30,31] Because the lesion may not be equally prominent throughout the liver, needle biopsies may be unreliable in establishing the diagnosis. Although Jorgensen found an increase in the average diameter of bile ducts in patients with CHF compared with those in controls,[8] the exact incidence of gross cysts is not established, nor is it clear whether the incidence of cysts increases with age.[24] Dilatation of bile ducts becomes clinically significant when associated with either cholangitis or malignancy. Cholangitis may be occult, acute, or chronic in nature and contributes significantly to both the morbidity and mortality of CHF. Calculi may form in the dilated ducts, initiating or complicating preexisting cholangitis. Cholangiocarcinoma is an uncommon but serious complication of CHF,[32] and premalignant changes may be observed in the epithelium of cystically dilated ducts. Cholangiocarcinoma may also be found in association with choledochal cysts or dilated intrahepatic ducts in the absence of DPM.

Visceral abnormalities in addition to those in the liver and kidney, described in single or small groups of patients, include congenital heart disease,[33] pulmonary hypertension and arteriovenous fistulae,[34,35] berry aneurysms,[36,37] and osteochondrodysplasia.[38] It is unclear whether these anomalies are intrinsic to the basic disease or coincidental. A syndrome of intestinal lymphangiectasia and protein-losing enteropathy in association with CHF, as described by Pedersen and Tygstrup[39] and by Pelletier and colleagues,[40] has now been demonstrated to be phosphomannose isomerase deficiency[41] (vide infra).

Therapy for CHF

Portosystemic shunting has been the treatment of choice because there is a low incidence of postoperative encephalopathy or hyperammonemia.[24] Prospective trials of other alternative approaches, such as sclerotherapy, banding, or pharmacologic management of varices, are not yet available. Nevertheless, the presence of spontaneous portosystemic shunts in some children suggests that sclerotherapy or banding may be beneficial if either can be shown to hasten the development of hemodynamically significant shunts without surgery. If surgery is selected as the treatment for portal hypertension, the type of shunt should be carefully chosen to prevent the limitation of options for either hepatic or renal transplant in later life.

Prolonged cholangitis is a major complication and has been responsible for hepatic failure and death. Therefore, unexplained fever or serologic evidence of inflammation in the absence of fever warrants a diagnostic liver biopsy and aspirate for culture.[42] Manipulation of the extrahepatic biliary tree carries an increased risk of infection in patients with abnormal ducts or bile stasis.[43] In cases of refractory cholangitis, surgical management and external or internal drainage may be necessary to resolve the hepatobiliary infection. In patients with stasis and refractory cholangitis, a choleretic agent may significantly augment therapy. Ursodeoxycholic acid therapy and prophylactic antibiotic administration have not been adequately studied in CHF-related cholangitis but may have a role in selected patients.

Prognosis for CHF

In general, the prognosis for those older children who present with CHF is good. The limitations are those imposed by complications of the disease, namely portal hypertension, cholangitis, and, occasionally, renal or hepatic failure. Chronic renal failure is most common in patients with a presentation in infancy. As noted, portal hypertension is usually successfully managed and rarely complicated by hepatic encephalopathy. Ascending cholangitis with sepsis and hepatic failure is a major cause of death in most series.[24,25,44] In those patients with chronic cholangitis and/or progressive hepatic dysfunction, liver transplant may prove to be the optimal therapy. Occasionally, patients have received combined renal and hepatic transplants for multiorgan failure.

CHF-Nephronophthisis

In this heritable group of disorders, there is a combination of hepatic lesions with some similarity to CHF with severe tubulointerstitial renal disease.[45–47] Its relation to the previously discussed disorders is not clear because the renal

lesions differ considerably from those seen in the previously discussed disorders, and even the hepatic lesion sometimes does not show a completely typical DPM. These conditions are nonetheless discussed in this chapter given the combination of heredity and the simultaneous presence of renal and liver disease with DPM-like features. The renal lesions are quite different from those of CHF and ARPKD and are characterized by interstitial inflammation and fibrosis with tubular atrophy, cyst formation, and secondary glomerulosclerosis (nephronophthisis). There is usually progressive renal failure with uremia by 20 years of age. The hepatic lesion has features consistent with DPM, although the bile ducts may not feature the characteristic profiles of those in typical DPM, and portal inflammation has been noted in some instances. Morphometric studies by Landing and colleagues on the biliary profiles and portal tracts in some of these diseases suggested heterogeneity in expression and development.[48] These disorders bear some similarities to renal-retinal syndrome (Senior-Loken syndrome), which is characterized by nephronophthisis and retinitis pigmentosa or retinal aplasia. Recent studies have demonstrated significant genetic heterogeneity in Senior-Loken syndrome. The syndrome has been associated with homozygous mutations in the *NPHP1*[49] and *NPHP4*[50] genes. *NPHP1* and *NPHP4* code for nephrocystin and nephroretinin, highly conserved proteins important in cell-cell and cell-matrix recognition.[51] Some patients have also been mapped to a locus on 3q22, overlapping the *NPHP3* locus.[52] Most of these patients do not seem to have liver involvement. It is currently unknown whether the patients with CHF nephronophthisis described in the past, before gene defects in cases of familial nephronophthisis were identified, have similar gene abnormalities.

PHOSPHOMANNOSE ISOMERASE DEFICIENCY

Phosphomannose isomerase deficiency, a disorder of glycosylation, has been associated with DPM and a protein-losing enteropathy.[41,53,54] Kidney disease has not been described in affected patients. Disease appears to result from hypoglycosylation of a number of serum and glycoproteins and suggests a metabolic anomaly leading to the development of the DPM. Phosphomannose isomerase deficiency is particularly interesting in light of the work of Terada and colleagues, who have highlighted cell-matrix interaction and the role of glycoproteins during development of the human intrahepatic biliary system.[55]

CHF AND MALFORMATION SYNDROMES

CHF accompanies a considerable number of malformation syndromes, usually in combination with cystic, dysplastic kidneys (Table 50.1-2). The pathogenetic implications of this coexistence of renal and hepatic cysts in these malformation syndromes and their relationship to ADPKD and ARPKD are not clear, particularly because the renal disease varies considerably in character among the various conditions. Although the literature is replete with descriptions of syndromes with developmental liver lesions, in many reports, the liver findings are poorly defined, often superficially described as "liver fibrosis," "increased bile ducts," or "liver cysts," and lack adequate histologic analysis. Therefore, the syndromes briefly discussed here are those in which there is reasonable evidence that DPM frequently accompanies the other malformations.

Meckel-Gruber syndrome is a recessively inherited lethal condition characterized by a central nervous system malformation, usually an occipital meningoencephalocele, bilaterally large multicystic kidneys, CHF, and polydactyly.[56] There is considerable heterogeneity in clinical findings, and at least three different gene loci have been identified: *MKS1* on 17q, *MKS2* on 11q, and *MKS3* on 8q.[57] Sergi and colleagues reviewed the liver sections of 30 fetuses with Meckel syndrome and found that DPM was a constant anomaly.[58] Two kinds of hepatic lesions were observed: 23 cases showed mainly a cystic dilatation of primitive biliary structures with little portal fibrosis, whereas 7 cases showed mainly rings of interrupted curved lumina around a central fibrovascular axis and pronounced portal fibrosis.

Jeune syndrome (asphyxiating thoracic dystrophy) is a rare autosomal recessive skeletal dysplasia that often leads to respiratory insufficiency because of a severely constricted thoracic cage. Renal disease, pancreatic insufficiency, and abnormalities of the retina, nails, and dental defects are also described.[59] Cystic lesions occur in the kidney and pancreas, whereas the liver changes are mostly characterized by DPM with progressive fibrosis.[60] Clinically significant liver dysfunction may not be apparent in the majority of patients who die in early life, although, among patients who survive beyond infancy, the liver fibrosis seems to be progressive.[61,62] Yerian and colleagues have described abnormalities of intrahepatic portal veins and vascular shunts in the resected liver of an older child who underwent transplant.[60]

Ivemark syndrome (renal-pancreatic-hepatic dysplasia) is one of the two syndromes described by Ivemark. One is usually sporadic and is characterized by asplenia

TABLE 50.1-2 MALFORMATION SYNDROMES WITH DUCTAL PLATE MALFORMATION

DPM AS A FREQUENT OCCURRENCE
Meckel-Gruber syndrome
Jeune syndrome
Ivemark syndrome
Bardet-Biedl syndrome
DPM—OCCASIONAL OR INSUFFICIENTLY DESCRIBED
Tuberous sclerosis
Smith-Lemli-Opitz syndrome
COACH syndrome
Ellis-van Creveld syndrome
Elejalde syndrome
Trisomy 9
Trisomy 13

Adapted from Ruchelli E. Normal and abnormal liver development. In: Russo P, Ruchelli E, Piccoli D, editors. Pathology of pediatric gastrointestinal and liver disease. New York: Springer-Verlag; 2004.
COACH = cerebellar aplasia, oligophrenia, ataxia, coloboma, hepatic fibrosis; DPM = ductal plate malformation.

with visceroatrial heterotaxy but no liver disease.[63] The other syndrome is characterized by dysplastic changes involving the kidneys and liver and pancreatic ducts and, frequently, polysplenia.[64] The liver lesions appear to be consistent with DPM. An autosomal recessive mode of inheritance has been proposed.[64] However, because similar renal, hepatic, and pancreatic abnormalities occur in other syndromes, including trisomy 9, Meckel, Jeune, Saldino-Noonan, and Elejalde types of chondrodysplasia, and glutaricaciduria type II, cases of renal-hepatic-pancreatic dysplasia do not necessarily constitute a homogeneous group.[65,66]

AUTOSOMAL RECESSIVE POLYCYSTIC KIDNEY DISEASE

ARPKD encompasses a spectrum of clinical and pathologic manifestations; however, the two invariant features are DPM and fusiform dilatation of the renal collecting ducts. The renal lesion, when identified in infancy, is characterized by radially arranged tubular cysts occupying most of the large externally smooth renal mass with widely spaced glomeruli (Figure 50.1-4). The longer patients survive, the less characteristic the renal lesions become because the cysts become more rounded, and in some cases with survival beyond the neonatal period, it may be difficult on examination of biopsies to correctly classify the lesion.[67] Using linkage analysis in families with an affected child, prenatal diagnosis became possible.[68]

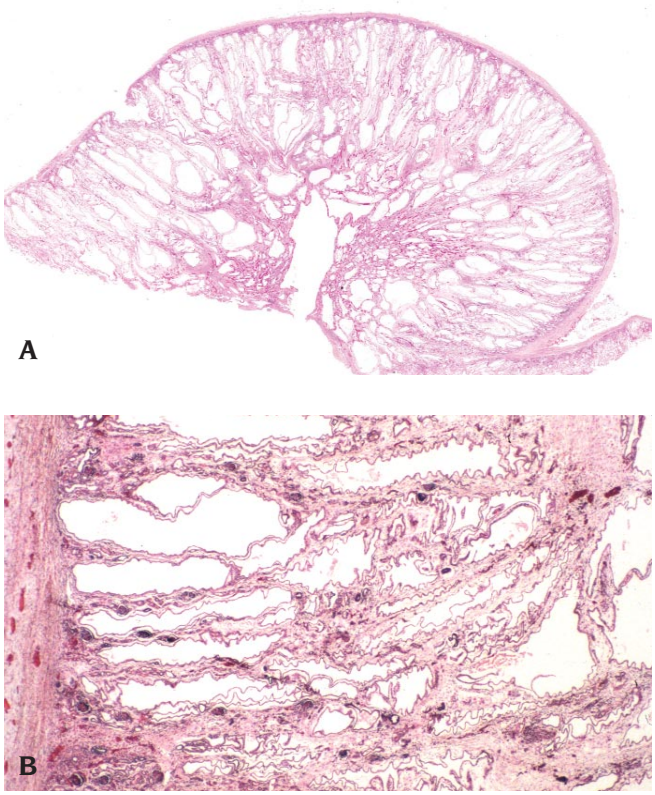


FIGURE 50.1-4 Autosomal recessive polycystic kidney disease. *A*, Low-power microphotograph of the kidney with diffuse fusiform dilatation of tubular segments in a radial arrangement. There is little residual normal parenchyma. *B*, Higher-power view. A few glomerular structures are seen between the dilated tubules and in the subcapsular area to the left of the picture (hematoxylin and eosin; whole mount section).

The renal disease may vary from a lethal perinatal disease to an incidental finding in older children. It has been estimated that 30 to 50% of infants affected with ARPKD will die in the perinatal period,[69] although recent studies suggest a better long-term prognosis.[70] In the infantile form, the kidneys are enlarged and severely dysfunctional. They may be palpable on examination, and an abdominal radiograph will demonstrate bilaterally enlarged kidneys. Excretory urography may only poorly visualize the collecting system. The nephrogram (characteristic of the neonatal presentation) demonstrates a radiolucent mottled parenchyma owing to the cystic changes of the nephrons. Many infants with ARPKD will develop uremia and chronic renal failure. Respiratory distress occurs from compression exerted by the enlarged kidneys, fluid retention, congestive heart failure, concomitant pulmonary hypoplasia, or pneumonia. Progressive renal failure and hypertension may occur over the first few weeks or months of life. Mortality is high in these patients. Patients who survive infancy may develop slowly progressive renal insufficiency and portal hypertension.[29]

The pathogenesis of the renal lesion is at least partially understood. It is tempting to speculate that closely related etiopathogeneses are responsible for both hepatic duct and renal tubular dilatation. Were this so, important insights regarding bile duct development and function might be gained. A variety of animal models have been developed to investigate the mechanisms of cystogenesis in the kidneys. These models have resulted in the speculation that abnormalities in epithelial cell growth, extracellular matrix composition, and fluid secretion are important in cyst formation.[71] Normal renal tubular absorptive epithelia can become cystic if (1) hyperplasia, localized to a distinct nephron segment, requires accommodation of an increased cell mass; (2) secretion, rather than absorption, leads to net accumulation of intratubular fluid; or (3) extracellular matrix abnormalities alter the epithelial microenvironment with resultant abnormal epithelial hyperplasia and secretory activity.[71] Evidence from several studies has suggested the role of the epidermal growth factor-α (EGFR) and transforming growth factor-α receptor axis in promoting epithelial hyperplasia and subsequent renal cyst formation in murine and human ADPKD and ARPKD.[72] Furthermore, similar abnormalities of EGFR expression have been suggested to mediate biliary epithelial hyperplasia and ductal ectasia in a mouse model.[73,74] An inhibitor of tyrosine EGFR tyrosine kinase activity has been shown to markedly reduce collecting tubule cystic lesions, improve renal function, decrease biliary epithelial abnormalities, and improve life span in a mouse model of ARPKD.[75] A murine model of ARPKD, characterized by a mutation in the mouse *cpk* gene, suggests that an inactivating mutation in the *cpk* allele interferes with normal tubular epithelial differentiation in the liver and kidney.[76] This rat model was a critical development in the identification of the *PKHD1* gene.

LIVER DISEASE IN ARPKD

The hepatic lesion is relatively uniform, and grossly visible cysts are uncommon. There is portal tract fibrous enlargement with numerous biliary profiles, as in CHF. Normal interlobular ducts in the center of the tracts are often missing.[5] As in CHF, the bile ducts are in continuity with the rest of the biliary system (communicating cystic disease). The precise incidence of portal hypertension in ARPKD is unclear and may be less than in CHF, although this may reflect the fact that children with ARPKD often die earlier in life. With increasing age of the patients, grossly visible cysts may become more frequent, and an increase in portal fibrosis may be noted.[77] CHF and its attendant complications appear to be a significant cause of mortality and morbidity in older children who have undergone renal transplant.[78] In addition to the renal and hepatic findings, pancreatic fibrosis with duct dilatation or proliferation has been reported occasionally in ARPKD.[79] Interestingly, the *PKHD1* gene has been found to be expressed in the pancreas, as well as in the liver and kidney.[21]

PKHD1 and Fibrocystin/Polyductin in ARPKD

Genetic linkage studies of families with ARPKD allowed the mapping of the disease locus to the 6p21-cen region of chromosome 6.[80] This allowed prenatal diagnosis to be available for siblings of probands.[68] Subsequent studies demonstrated that the clinically diverse presentations of ARPKD, including the severe renal perinatal form, mapped to the same region of chromosome 6,[81] suggesting that mutations in a single gene are responsible for the varied clinical presentations and classifications of fibropolycystic disease. Of the different rodent models of ARPKD, only one rat model with similar manifestations to ARPKD, the Pck rat, mapped to a region syntenic to chromosome 6 in the human, and this model was used to localize and identify the human disease gene.[21] Independently, and using different strategies, Ward and colleagues[21] and Onuchic and colleagues[22] identified the gene *PKHD1*, which encodes a large receptor-like protein. The gene is extremely large, encompassing 67 coding exons spanning more than 469 kb of genomic deoxyribonucleic acid (DNA), and the human transcript is approximately 16 kb. The gene was expressed in the fetal kidney and in the adult kidney and pancreas, with lesser levels seen in the liver.[21,22] The massive protein encoded by *PKHD1*, termed by the authors fibrocystin[21] and polyductin,[22] was predicted to contain 4,074 amino acids with a molecular weight of at least 447 kD.[21,22] This protein has limited homology with other known protein domains but no homology with the polycystins of ADPKD.[22] In 11 of 14 studied kindreds, Ward and colleagues found 6 truncating and 12 missense mutations, with the majority of patients being compound heterozygotes.[21] In a patient group of 25 individuals, Onuchic and colleagues identified potentially pathologic variants in 21 (42%) of the 50 chromosomes, and there was some evidence supporting a genotype-phenotype correlation, with truncating mutations possibly being correlated with more severe manifestations.[22] Bergmann and colleagues[82] and Rosetti and colleagues[83] reported further results of extensive screening for *PKHD1* mutations in ARPKD. In 90 patients, the mutation detection rate was 61%, and 45% of mutations were predicted to truncate the protein. The likelihood of mutation detection was higher for severely affected patients (85%) than for those with moderate ARPKD (41.9%) or for CHF with Caroli disease (32.1%).[83] In both studies, the type of mutation was somewhat correlated with the phenotype, with patients carrying two or one truncating mutation generally having more severe and earlier-onset disease. Except in cases of consanguinity, most patients with two identified mutations were compound heterozygotes.[82,83] The large size of the gene and the widespread location of mutations have, however, limited the applicability of mutation screening for many patients.

AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE

ADPKD is the most common hereditary kidney disorder, with a frequency estimated to be from 1 in 400 to 1 in 1,000 individuals worldwide.[84] In contrast, ARPKD is less common, with an incidence in the range of 1 in 6,000 to 1 in 40,000. Although the disease is clinically manifest primarily in adults, it can be anatomically identified even in fetal life. It is important to recognize for its genetic implications, even though the functional significance of the finding is not apparent until beyond childhood. The hepatic lesions are primarily duct cysts, which are readily demonstrated ultrasonographically. Cysts increase in size from childhood until 40 to 50 years of age (Figure 50.1-5). They are recognized and are perhaps present at an earlier age in women than in men. Commonly, the cysts in this condition are dilated ductal elements, which are not shown to communicate with the distal biliary tree and do not contain bile. However, there may also be portal tract lesions consistent with the (communicating) DPM in a smaller percentage of patients.[85] The

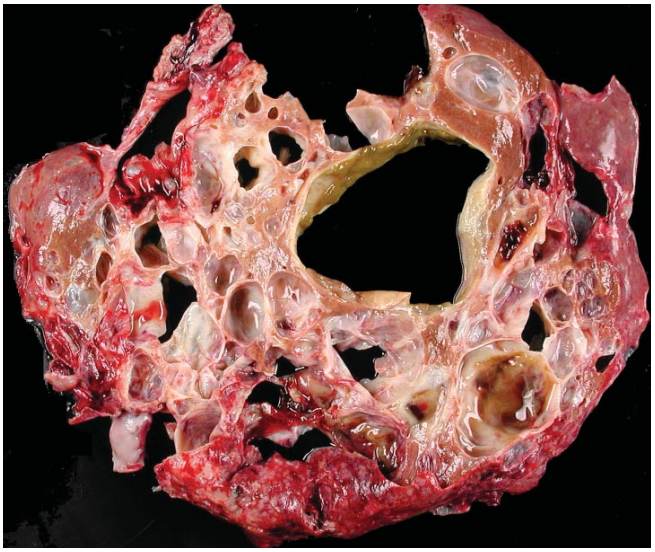


FIGURE 50.1-5 Polycystic liver disease. Hepatectomy specimen at transplant from a 37-year-old male. Photograph courtesy of Dr. Emma Furth, Hospital of the University of Pennsylvania.

significance of the association of communicating (DPM) and noncommunicating lesions for understanding the pathogenesis of the polycystic disease is unclear. Some authors consider the cysts in ADPKD the result of progressive dilatation of von Meyenburg complexes,[86] and Desmet has suggested that the cysts might become separated and noncommunicating as a result of kinks and strictures caused by the strangulating hyaline fibrosis that surrounds them.[5] The hepatic lesions in ADPKD have been thought to be insignificant clinically. However, as more patients with ADPKD survive for longer periods, it has become clear that a significant number of deaths result from hepatic complications.[87] Hepatic complications include infection of the cysts, cholangiocarcinoma, portal hypertension, and pressure effects owing to the cysts.

The renal lesion consists of cysts that appear to arise from multiple areas along the nephron and increase in size with age, eventuating in the kidneys and becoming large cystic reniform masses with inadequate numbers of functioning nephrons.[88]

Cysts may also be found in other organs, including the spleen, pancreas, thyroid, ovary, endometrium, seminal vesicles, and epididymis. Arterial aneurysms are present in up to 30% of cases.[89]

PKD1, PKD2, and the Polycystins

ADPKD results from mutations in one of at least three distinct genetic loci. Mutations in *PKD1*, mapped to chromosome 16p13.3, are responsible for the 85 to 90% of ADPKD in whites,[90,91] and prenatal diagnosis is thus possible. A second ADPKD gene, *PKD2*, has been localized to chromosome 4q13-4q23, and the mutant gene has been identified.[92] The products of *PKD1* and *PKD2* are polycystin 1 and polycystin 2, which have been hypothesized to be part of a common biologic pathway because mutations in these genes produce identical clinical manifestations. However, the onset of renal disease in polycystic kidney disease (PKD) type 2 occurs later in life than in PKD1. The genetic mechanism of disease in ADPKD seems to be a loss of heterozygosity caused by somatic mutations in the wild-type allele in renal epithelial cells. This "two-hit" hypothesis explains the focal nature of ADPKD in that cysts arise from only about 1% of the total nephrons in the kidney.[93] There is also evidence for a third locus for ADPKD, termed PKD3.[94]

ISOLATED HEPATIC POLYCYSTIC DISEASE

A number of reports derived from studies of medicolegal autopsies and occasional families suggested that a polycystic liver disease (PLD) might exist that occurs in the absence of renal disease and is dominantly inherited. Nevertheless, it has been uncertain whether such patients represent a discrete entity or patients with ADPKD, in whom renal cysts were overlooked or were inconspicuous. A study of over 30,000 medicolegal autopsies from Finland identified 22 cases with polycystic disease either of the liver or the kidneys. In only one case were cysts present in both organs, leading the authors to conclude that polycystic disease of the liver and polycystic disease of the kidney were separate entities.[95] Strong genetic evidence that adult PLD is an entity distinct from either PKD1 or PKD2 was presented by Pirson and colleagues, who traced the disorder through three generations of a family and excluded the presence of kidney cysts and linkage of the disease to the genetic markers of PKD1 and PKD2.[96] The genetically distinct nature of isolated PLD is further supported by the finding of Reynolds and colleagues that the causative gene in two large families with PLD mapped to 19p13.2-p13.[97] A Finnish study achieved similar results and also suggested the possibility of a second locus.[98] Drenth and colleagues narrowed the linkage assignment of the PLD locus on 19p and detected a heterozygous mutation at the *PRKCSH* gene.[99] Li and colleagues identified the product of the *PRKCSH* gene as a highly conserved ubiquitous protein that is likely important in signal transduction.[100] Cysts in patients with PLD are believed to arise from dilatation of biliary microhamatomas (von Meyenburg complexes) and from peribiliary glands, as in ADPKD, suggesting that the hepatic cysts in both conditions have a similar pathogenesis.[101] The development of hepatic cysts in PLD appears to be age dependent and rare in childhood, coming to medical attention usually because of symptoms owing to mass effect or from complications such as hemorrhage, infection, or rupture. A relatively higher prevalence of mitral valve abnormalities was also found in patients with PLD.[101]

CAROLI SYNDROME AND CAROLI DISEASE

Caroli disease is a congenital dilatation of the larger intrahepatic bile ducts. Caroli described two variants.[102] One is characterized by pure ductal ectasia without other hepatic pathology, for which the term "Caroli disease" has been proposed. The other variant is a combined type in which Caroli disease is associated with the lesions of CHF, for which the term "Caroli syndrome" has been proposed.[11] Communicating duct cysts occur in both variants. The first variant is very rare and is not known to be heritable. The nature of the second variant, Caroli syndrome, is currently controversial. It is not clear whether Caroli syndrome represents a separate entity from CHF, a variant of it, or a mere radiologic appearance in a variety of biliary disorders. Caroli syndrome has been associated with ADPKD.[103] It has been postulated that Caroli disease represents developmental arrest at the level of the larger intrahepatic bile ducts, whereas Caroli syndrome involves the entire intrahepatic biliary tree such that smaller interlobular ducts are affected (Figure 50.1-6).[6] The mode of inheritance of Caroli syndrome has been reported to be autosomal recessive, although a recent report of a Japanese family suggests an autosomal dominant form of inheritance.[104]

The relationship between Caroli disease and choledochal cyst is also controversial. According to Todani and colleagues, the type V choledochal cyst (intrahepatic cysts) is similar to the appearance of Caroli disease, and they consider the two synonymous.[105] However, those cases described as choledochal cysts in association with

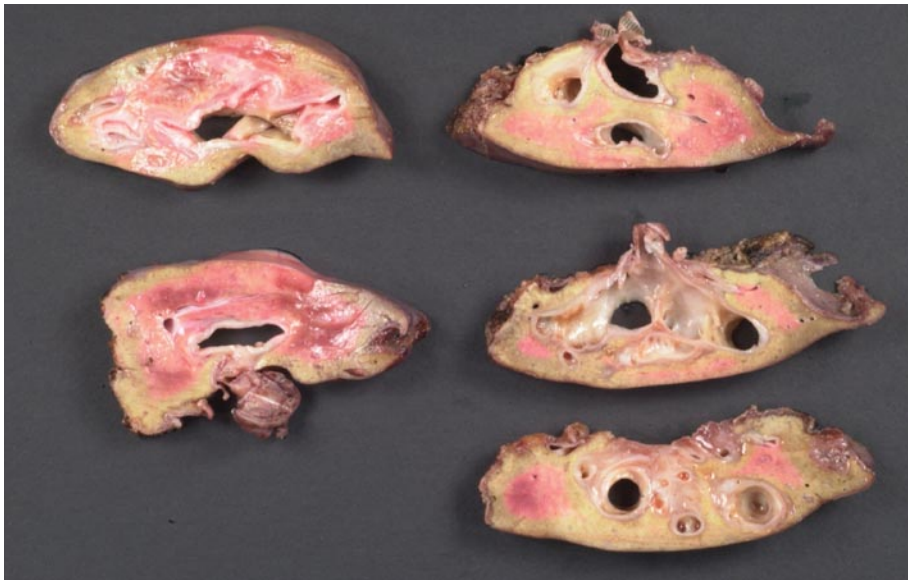
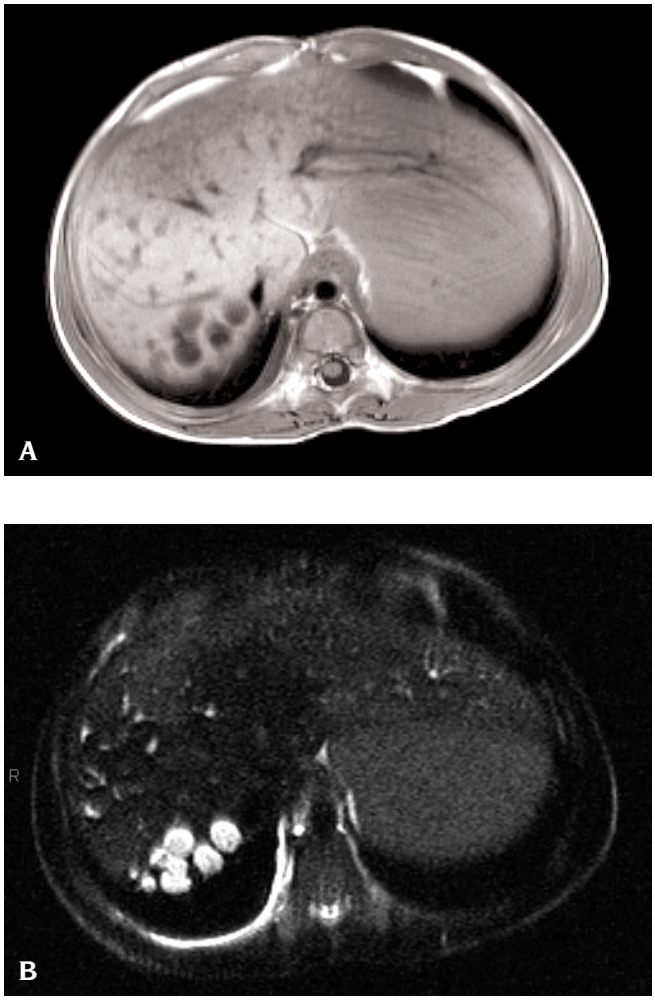


FIGURE 50.1-6 Caroli disease. Extensive intrahepatic bile duct dilatation involving primarily the left lobe of the liver, as illustrated by multiple sections from a partial hepatectomy specimen from a 9-year-old female.

CHF probably have a different pattern of inheritance and, thus, a different etiology and are considered to be a different entity by some authors.[84] Caroli disease typically becomes symptomatic in adults. Patients with Caroli syndrome may present earlier in life owing to the associated liver and renal abnormalities. The duct ectasias in both conditions predispose the patient to bile stasis and repeated attacks of cholangitis and complications such as intrahepatic lithiasis, biliary abscess, sepsis, amyloidosis, and cholangiocarcinoma (Figure 50.1-7).



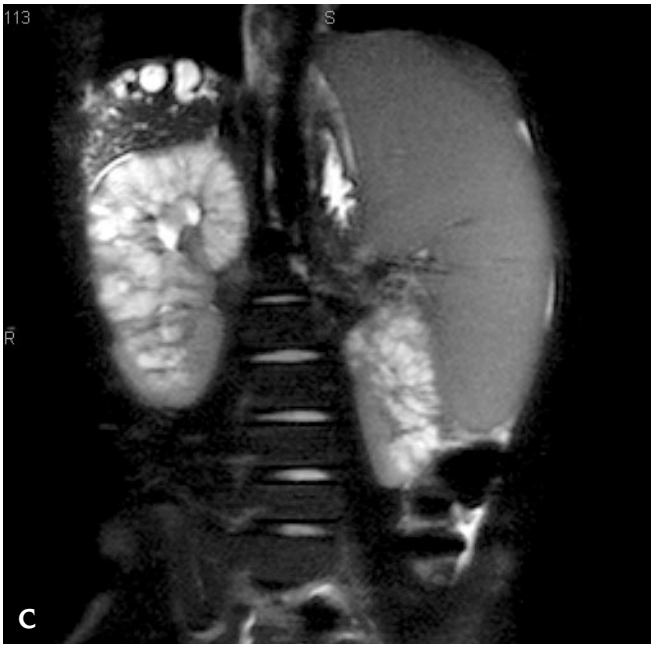


FIGURE 50.1-7 Hepatic magnetic resonance image (*A*) and magnetic resonance cholangiogram (*B*, *C*) demonstrating intrahepatic ductal dilatation in an child with autosomal recessive polycystic kidney disease and congenital hepatic fibrosis and unsuspected Caroli dilatation. Note also massive splenomegaly and nephromegaly with cysts. *A*, Transverse view; *B*, transverse view, enhanced; *C*, coronal view.

BILE DUCT PAUCITY

A decrease in the number of ducts (paucity) is one of the most significant abnormalities of the intralobular bile ducts in children.[106] Bile duct paucity can only be defined histologically. In patients at or beyond 37 weeks of gestational age, paucity is present when histologic examination demonstrates that the ratio of ducts to portal tracts is less than 0.9 (Figure 50.1-8). In determining this ratio, it should be kept in mind that (1) bile ductules should not be included in the counting, (2) counts must involve sufficient portal tracts to be representative of the liver as a whole, and (3) this ratio is not applicable in premature infants.[107] The standard for the number of portal tracts required is 20, although some authors suggest that as few as 5 portal tracts may be sufficient.[53,107] Because 20 portal tracts are obtainable only on an operative wedge biopsy, it is commonly necessary to make, or at least strongly suggest, the diagnosis of paucity with a smaller sample number.

Because there is little precise knowledge of the factors that influence the development, viability, and maintenance of the intrahepatic bile ducts, it is not possible to formulate a genuinely coherent classification of the duct paucity conditions. For example, in some situations, there is an active destruction of previously existing ducts. In others, paucity is associated with a primary disease. For this reason, the disorders outlined in Table 50.1-3 are more a list of conditions than a true classification. Furthermore, for a number of the primary disorders in the table, the incidence of paucity is so low as to perhaps be coincidental.

TABLE 50.1-3 DISORDERS ASSOCIATED WITH BILE DUCT PAUCITY IN CHILDREN

SYNDROMIC BILE DUCT PAUCITY—ALAGILLE SYNDROME
NONSYNDROMIC BILE DUCT PAUCITY
Metabolic and genetic disorders
α_1-Antitrypsin deficiency
Cystic fibrosis (rare)
Peroxisomal disorders (rare)
Progressive familial intrahepatic cholestasis (rare)
Trisomy 21 (rare)
Prune-belly syndrome (rare)
Infection
Congenital cytomegalovirus infection (rare)
Congenital syphilis (rare)
Congenital rubella (rare)
Inflammatory and immune disorders
Graft-versus-host disease
Chronic hepatic allograft rejection
Sclerosing cholangitis
Sarcoidosis (rare)
Other
Drug- or antibiotic-associated vanishing bile duct syndrome
Familial idiopathic adulthood ductopenia
Biliary atresia (late)
Panhypopituitarism (rare)
Idiopathic

SYNDROMIC BILE DUCT PAUCITY (ALAGILLE SYNDROME)

Syndromic bile duct paucity, now referred to as Alagille syndrome (AGS), is defined by dominantly inherited bile duct paucity in conjunction with specific extrahepatic findings. It is a diagnosis that has both genetic and prognostic implications.[108–120] Also known as Watson-Alagille syndrome, arteriohepatic dysplasia, syndromic intrahepatic biliary hypoplasia, intrahepatic biliary atresia, intrahepatic biliary dysgenesis, and syndromic paucity of the interlobular bile ducts, it is recognized as an important and relatively common cause of neonatal jaundice and cholestasis in older children. AGS is caused by mutations in the human gene *Jagged1* (*JAG1*), which is mapped to chromosome 20p12.[121,122] This gene encodes a ligand for the Notch signaling pathway, which is involved in cell fate determination.

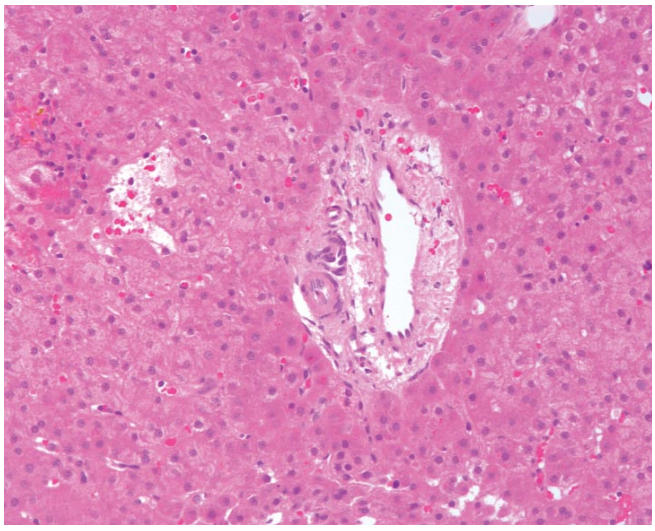


FIGURE 50.1-8 Alagille syndrome. Liver biopsy from a 1-year-old male. Bile ducts are absent from the portal tracts (hematoxylin and eosin; ×200 original magnification).

DEFINITION

AGS is characterized by a marked reduction in the number of interlobular bile ducts and cholestasis, occurring in association with cardiovascular, skeletal, ocular, facial, renal, pancreatic, and neurodevelopmental abnormalities. These occur with variable frequency, and early in infancy, the duct paucity may be absent.

The condition was recognized independently by Watson and Miller[110] and by Alagille and colleagues.[108,109] It is a familial disease with a wide variability in its clinical spectrum, even within individual pedigrees. The list of abnormalities associated with the syndrome has steadily increased since the initial descriptions, but the principal manifestations have remained essentially unchanged (Table 50.1-4).

GENETICS OF AGS

The prevalence of AGS has been reported to be 1 per 100,000 births.[123] This certainly underestimates the true frequency because many symptomatic patients with cardiac disease and mildly affected individuals are not included. There is an equal gender incidence. The family

TABLE 50.1-4 FEATURES OF ALAGILLE SYNDROME

FEATURE	%
Paucity	89
Cholestasis	95
Murmur	94
Vertebral	68
Facies	94
Ocular	81
Renal	44
Growth retardation	68
Developmental delay	7.5
Intracranial bleeding	13

Adapted from references 111, 112, 116–119.

history is positive for related clinical features in at least 15 to 23% of pedigrees,[109,112,117] although this underestimates the number of family members with subclinical forms of the disease or with mutations in *JAG1*. Pedigree analysis in families with multiple affected members demonstrated an autosomal dominant pattern of inheritance, with low penetrance and a great variability of expression.[109,112,117,124–127] For probands with an identified mutation in *JAG1*, mutational analysis of the family members has recently demonstrated that 56 to 70% are new mutations, not present in either parent.[128,129]

A small number of patients have been reported to have cytogenetically visible deletions of chromosome 20. These visible deletions, however, are rare, occurring in 2 to 7% of patients.[128–130] A translocation that segregates concordantly with three affected family members confirmed the location of the Alagille gene region at 20p12.[131] Rare cytologically invisible microdeletions have been identified.[132] The multiple reports of 20p deletions led to the incorrect hypothesis that AGS is a contiguous gene deletion syndrome. In 1997, two groups, Li and colleagues and Oda and colleagues, reported that AGS is due to a single gene defect in *JAG1*.[121,122] *JAG1* encodes a cell-surface protein that functions as a ligand for the Notch transmembrane receptor. The Notch pathway has been well studied in *Drosophila melanogaster* and *Caenorhabditis elegans*.[133] The system is highly evolutionarily conserved. The Notch pathway is present in many cell types during development, where it serves to regulate cell fate decisions. The name was derived from the notched wing appearance seen in *D. melanogaster* mutants. The multiple manifestations of AGS in humans suggest that Jagged and Notch interactions are critical for normal embryogenesis of the heart, kidney, eye, face, skeleton, and other organs affected in AGS.[134,135] Multiple Notch receptors and ligands have been identified in humans. Defects in *Notch3* have been identified as the cause of cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) syndrome.[136] Mutations in another Notch pathway ligand, delta-like 3 (*DLL3*), have been linked to the skeletal disorder spondylocostal dysostosis.[137] The other Notch receptors and ligands have not, as yet, been linked to specific clinical syndromes in humans.

JAG1 encodes a protein with a large extracellular domain, a transmembrane domain, and a smaller intracellular portion. Several regions of the protein are similar in all species studied. Mutations in the gene, however, have not been confined to those regions. A large number of mutations in *JAG1* have now been reported[121,122,128,129,138,139] and are found in approximately 70% of individuals by current techniques.[140] Of these, approximately 4% are gene deletion, 49% are protein truncating frameshift or nonsense mutations, 9% are splice-site mutations, and 9% are missense mutations. In 30%, no mutation was identified.[141] The relatively low percentage of missense mutations is interesting. Although there does not appear to be any phenotypic difference between patients with total gene deletion and those with isolated defects, it is possible that missense mutations might cause milder variants of AGS or perhaps even single-organ abnormalities. Abnormalities in *JAG1* could theoretically cause disease by either of two mechanisms. It appears that having only one normal copy of *JAG1* (haploinsufficiency) is not sufficient for normal embryogenesis in humans. The alternative theory, a dominant negative effect caused by abnormal protein, is not supported by the presence of disease in humans with a total gene deletion. The role of *JAG1* in human embryogenesis is not understood, but studies are under way to assess its role in hepatic and cardiac development.[134,135,142]

Gene testing for AGS is currently available on a research basis. Evaluation by fluorescent in situ hybridization (FISH) for deletions at 20p12 is more widely available but is likely to identify only about 5% of mutations causing AGS. A negative result may be misleading. Molecular testing is available in several centers but is difficult owing to the large size of the gene and the lack of significant mutational hot spots. The majority of mutations identified to date are unique and scattered widely across the gene. In addition, current evaluations cannot identify a mutation in approximately 30% of clearly affected probands.[129] When a defect can be identified in a proband, however, it is then easy and useful to identify relatives who are minimally affected yet carry a significant risk to their offspring. Also, it can help to reassure parents of patients with de novo mutations. Prenatal testing is available but only if the mutation is identified in the proband. Testing has also aided in the diagnosis of AGS for patients with minor or atypical manifestations. Given the relatively large number of patients in whom no mutation has yet been identified, it remains possible that other Notch/ligand gene defects or other unrelated genes may account for some patients diagnosed clinically as having AGS. The similarities between AGS with the 22q contiguous gene deletion syndromes are instructive in that the features of heart disease, posterior embryotoxon, and butterfly vertebrae are common to both disorders.[143] A FISH analysis for 22q deletions should be completed in all patients with potentially overlapping syndromes, particularly if liver disease and duct paucity are not prominent features.

Histopathology of the Liver in AGS

There are numerous publications describing the histopathologic features of the liver in AGS.[106,111,112,117,118,144–149] Although Alagille based his original diagnosis of the syndrome on a bile duct to portal tract ratio less than 0.5,[111]

the presence and extent of bile duct paucity vary, largely as a result of the different ages of the patients at the time of biopsy.[112,117,118,149] There is ample evidence that the number of bile ducts may at first be normal in infants with AGS and that paucity develops with time.[112,144,148,149] Studies of serial liver biopsies have demonstrated that in up to 50% of initial biopsies of patients with AGS less than 6 months or 1 year of age, the bile duct to portal tract ratio is normal, with paucity observed in 90% of those older than 1 year of age.[117,118] The overall frequency of bile duct paucity in AGS also varies with the criteria used in establishing the diagnosis, being a requirement for diagnosis in older studies, whereas in more recent ones, which rely on nonhepatic manifestations and the presence of *JAG1* mutations, paucity is identified in 80 to 85% of patients.[140]

Proliferation of bile ducts has been reported in early biopsies of patients with AGS,[146,150] mimicking extrahepatic biliary obstruction, a diagnostic difficulty compounded by abnormal cholangiograms that fail to visualize the hypoplastic extrahepatic biliary tree.[117,148] Diagnostic confusion with extrahepatic biliary atresia has resulted in Kasai portoenterostomies being performed, with variable results in mortality and progression of disease. Evidence of bile duct damage may be inferred by the findings of a piling up of the epithelium, nuclear pyknosis and pleomorphism, epithelial vacuolization, lymphocytic infiltration of the biliary epithelium, and peribiliary fibrosis. Other morphologic changes observed in early infancy include hepatocellular giant cell transformation with extramedullary hematopoiesis (neonatal giant cell hepatitis) and intralobular cholestasis. Portal spaces are usually not expanded but may contain a mild mononuclear inflammatory infiltrate. A reduction in the number of portal spaces, as observed by Hadchouel and colleagues,[147] may reflect an abnormal vasculature.

Hepatectomy specimens obtained at transplant are typically nodular but variably cholestatic. Late histologic changes include portal expansion and fibrosis, with bridging in approximately 50% of patients and cirrhosis in up to one-quarter.[117] The fibrosis has been observed by Hashida and Yunis to be more severe at the hilum.[149] Paucity of bile ducts is by then usually well established, and there is prominent lobular cholestasis. Bile ductular proliferation at that late stage is uncommon and may indicate a concomitant process, such as cholangitis.[151] Edema of the portal spaces, with dilatation of the lymphatics and veins, has been noted.[149] Other changes noted include hepatocellular degeneration and "pseudoxanthomatous" transformation, pseudorosette formations, increased hepatocellular copper, and sinusoidal fibrosis.[144] Electron microscopic observation of bile pigment accumulation in the Golgi apparatus, in concert with normal-appearing canaliculi, was reported as distinctive ultrastructural features in AGS,[152] although its significance has been disputed by others.[153]

Narrowing and hypoplasia of the extrahepatic biliary tree have been observed by cholangiography in both autopsy and hepatectomy specimens[111,144,146,149,151] and decreased filling of intrahepatic branches by endoscopic retrograde cholangiopancreatography.[154]

Hepatocellular carcinomas have been reported with AGS in children as young as 4 years of age,[155–160] as well as later in life.[161,162] Familial occurrence of hepatocellular carcinomas has also been observed,[162] with three of four siblings affected in one family.[163] Nodular hyperplasia, in some cases involving an entire lobe, has been reported in a few cases.[164,165] The significance of reported associations with thyroid cancer[166] and colonic polyposis[167] awaits further studies.

Renal Histomorphology in AGS

A variety of developmental and acquired renal abnormalities have been reported in AGS,[111,117,118,120,168–176] some leading to renal insufficiency, either early in infancy or later. These include medullary and cortical cysts,[171,174,175] renal hypoplasia,[169] ureteropelvic obstruction and bifid ureters, and renal artery stenoses.[176] The most frequently reported and most characteristic finding is deposition of lipids in the glomerular mesangium and basement membranes, resulting in a membranoproliferative pattern.[170–172] Lipid in the glomeruli can be demonstrated by histochemical stains on frozen sections or by electron microscopy.[171] According to Habib and colleagues, the glomerular lipidosis does not appear to be related either to the age of the patients or the degree of hypercholesterolemia but rather to the severity of cholestasis.[170]

Clinical Manifestations of AGS

AGS usually presents in the first 3 months of life in symptomatic patients. It is one of the more common etiologies of cholestasis and jaundice in the neonatal period and must be distinguished from biliary atresia and nonsyndromic bile duct paucity. In older children, AGS may present as a chronic hepatic disease. It is common for an adult to be diagnosed only after recognition of AGS in a severely affected child. The diagnosis is made when characteristic or compatible liver histology is accompanied by the major extrahepatic findings of the syndrome: chronic cholestasis, characteristic facies, cardiac murmur, vertebral anomalies, and posterior embryotoxon.

The extreme variability of the clinical manifestations and the incomplete penetrance of the syndrome obscure the diagnosis. Some patients demonstrate progressive pruritus, cirrhosis, or liver failure, resulting in liver transplant. Others have few or no symptoms and remain undiagnosed as adults.

Although most patients present with hepatic manifestations, the associated cardiac disease generally accounts for the majority of the early mortality.[111,112,117]

Hepatic Manifestations. The majority of symptomatic patients present in infancy and will have manifestations of hepatic disease ranging from mild cholestasis and pruritus to progressive liver failure. The severity of the disease in the parent is of no prognostic value as to severity in relatives or in subsequent children.[125] The degree of hepatic disease does not correlate with the severity of the other systemic manifestations, such as cardiac disease.

Hepatomegaly, with a firm or normal consistency, is recognized in nearly all patients.[61] Splenomegaly is rare

in infancy but appears in one-third to two-thirds by the second decade.[111,112]

The most common laboratory abnormalities are elevations of serum bile aids, conjugated bilirubin, alkaline phosphatase, and γ-glutamyl transpeptidase, which suggest a defect in biliary excretion in excess of the abnormalities in hepatic metabolism or synthesis. There are elevations of the serum aminotransferases, up to 10-fold, which may persist throughout childhood. However, in general, metabolic regulation of transamination, urea synthesis, glucose homeostasis, and protein synthesis is well maintained.

Jaundice is present in the majority of symptomatic patients and presents as a conjugated hyperbilirubinemia in the neonatal period. In half of these infants, it is persistent, resolving only in later childhood. Jaundice commonly is noted during intercurrent illnesses, but the magnitude of the hyperbilirubinemia is minor compared with the degree of cholestasis. Cholestasis is manifest by pruritus and elevations in serum bile acid concentrations. This pruritus is among the most severe in any chronic liver disease. It is rarely present before 3 to 5 months of age[111,112] but is seen in nearly all symptomatic children by the third year of life, even in those who are anicteric.[111,112]

The presence of severe cholestasis results in the formation of xanthomas, characteristically on the extensor surfaces of the fingers, the palmar creases, nape of the neck, popliteal fossa, and buttocks and around inguinal trauma sites. The lesions persist throughout childhood but may gradually disappear after 10 years of age.[177] The timing for the formation of xanthomas relates to the severity of the cholestasis and correlates with a serum cholesterol greater than 500 mg/dL. Hypercholesterolemia and hypertriglyceridemia may be profound, reaching levels exceeding 1,000 mg/mL and 2,000 mg/mL, respectively, with the expected abnormalities in lipoproteinemia. The incidence of atheromata is unknown, but they have been reported as early as 4 years of age in a child found at autopsy to have extensive aortic and endocardial fat deposition.[112]

Hepatic synthetic function is usually well preserved. Serum albumin and ammonia are typically normal, as is the prothrombin time (with adequate vitamin K supplementation). Nevertheless, progression to cirrhosis and hepatic failure, initially reported to be uncommon, is recognized in approximately 20% of patients with AGS.

Malnutrition and Growth Failure. Diminished bile salt excretion and low intraluminal bile salt concentrations result in ineffective solubilization and absorption of dietary lipid, essential fatty acids, and fat-soluble vitamins. The deficiency of fat-soluble vitamins has profound systemic effects. Coagulopathy (vitamin K deficiency), rickets (vitamin D deficiency), retinopathy (vitamin E and A deficiency), and a peripheral neuropathy and myopathy (vitamin E deficiency) may occur.[178]

Growth failure is a common feature (50–90%) during childhood with delayed pubertal development. This is thought to be the result of caloric deprivation from fat malabsorption, the intrinsic vertebral and skeletal abnormalities, and perhaps a secondary abnormality in endocrine function as demonstrated by elevated growth hormone levels with diminished somatomedin production.[179] Ponderal and linear growth is commonly delayed in the first 3 years of life, and this growth failure is due, at least in part, to significant acute and chronic wasting.[180] Patients with growth failure appear to be insensitive to exogenous growth hormone.[179]

Cardiovascular Manifestations. A wide range of cardiovascular abnormalities have been reported in patients with syndromic paucity.[111,117,181,182] An audible murmur is present at some time in 97% of patients.[117] The most common lesions are pulmonary artery stenoses at various sites in the proximal and distal tree, commonly at bifurcations. The entire pulmonary vascular tree may be hypoplastic, either alone or in association with other cardiovascular lesions. Among these, tetralogy of Fallot (TOF) is the most common (7–12%). Other lesions include truncus arteriosus, secundum atrial septal defect, patent ductus arteriosus, ventriculoseptal defects, and pulmonary atresia. Systemic vascular anomalies, including coarctation of the aorta, renal artery stenosis, and small carotid arteries, occur sporadically.[182] Although the majority of cardiac and vascular lesions are of no hemodynamic consequence, significant lesions do occur and in some series have been the predominant cause of early death.[111,112,117] In a large series of patients, Emerick and others demonstrated that only cardiac disease predicted increased mortality in AGS patients.[117] Many patients with intracardiac structural defects have concomitant pulmonic or peripheral pulmonic stenosis, and there is increased mortality following cardiac surgery for these lesions. Survival of AGS patients with TOF was 66% and with TOF with pulmonary atresia was only 25%.[117] In a recent, large study, McElhinney and colleagues reported the cardiac manifestions of 200 patients with a *JAG1* mutation or clinical AGS (or both), predominantly evaluated by cardiologists and/or by echocardiography.[182] A total of 187 subjects (94%) had evidence of cardiovascular involvement.[182] Of the 150 patients with anomalies characterized by imaging, right-sided anomalies were present in 123 and left-sided anomalies in 22, with both in 12.[182] The most common abnormality was stenosis or hyposplasia of the branch pulmonary arteries in 76%. TOF was seen in 23 (12% of all patients) and was accompanied by pulmonary atresia in one-third, a frequency higher than that seen in non-AGS TOF. The majority of patients with TOF-pulmonary atresia died early on. There was no clear difference in the cardiac disease seen in patients in whom a *JAG1* mutation was or was not identified.[182] Although, in general, genotype-phenotype correlations are rare with *JAG1* mutations, Eldadah and colleagues reported a large kindred with autosomal dominant TOF with reduced penetrance correlated with a missense mutation in *JAG1*.[183] Nine of 11 mutation carriers manifested cardiac disease, including TOF, pulmonic atresia, pulmonic stenosis, and absent pulmonary valve, yet none of the affected individuals met the diagnostic criteria for AGS.

Cardiac disease may also account in part for the increased post–liver transplant mortality seen in some series. Accordingly, it is advisable to seek formal diagnosis for any murmur

in a patient with hepatic disease. Doppler echocardiography is usually sufficient in structural cardiac disease, but cardiac catheterization or digital subtraction arteriography may be necessary for diagnosis in some cases.[184]

Recently, patients with congenital cardiac disease but without apparent AGS have been evaluated by molecular techniques for mutations in *JAG1*. Patients with nonsyndromic TOF patients at one cardiac center were found to have unsuspected defects in *JAG1*.[185] Other lesions, such as multigenerational peripheral pulmonic stenosis without apparent AGS, have also been demonstrated to have defects in *JAG1* by mutational analysis.[185] Loomes and others have shown that the expression of *JAG1* in the developing mammalian heart correlates with the cardiovascular disease seen in AGS.[142] The similarity of the severe cardiac disease, particularly TOF, to other genetic syndromes such as 22q deletion is not yet understood.

Characteristic Facies. Characteristic facies are described in the original reports of syndromic bile duct paucity. These consist of a prominent forehead, moderate hypertelorism with deep-set eyes, a small pointed chin, and a saddle or straight nose that, in profile, may be in the same plane as the forehead (Figure 50.1-9).[111] The facies may be present at birth but, in general, become more obvious with increasing age. The usefulness of the facies as a major criterion for diagnosis of AGS has been challenged because of interobserver differences. It has been suggested that these facies are a common result of early and chronic cholestasis,[186] but the constellation of findings and the finding of typical facies in asymptomatic parents may be striking. In a recent study, clinical dysmorphologists were able to distinguish via photographs infants and children with AGS from others with liver disease with a high degree of sensitivity and specificity.[187] Identification of facies in adults was less accurate. Kamath and colleagues have emphasized that the facies change over time and that the characteristic facies of an adult do not, in fact, directly resemble the facies in childhood but do resemble other adults with the gene defect.[187] With time, the chin becomes more prominent and the forehead becomes less dominant, resulting in a face with a predominant lower portion in contrast to the upper prominence of early childhood (Figure 50.1-10). Identification of these adults, who commonly have minimal signs and symptoms of AGS, would help hepatologists in the evaluation of adults with idiopathic cardiac, hepatic, or renal disease. Further evidence that *JAG1* mutations are directly involved in the development of characteristic facies include the demonstration of *JAG1* expression in the facial structures of developing mouse and human embryos[134,135] and the presence of characteristic facies in subjects with no history or evidence of clincal liver disease or cholestasis.

Vertebral and Musculoskeletal Abnormalities. Vertebral abnormalities are described in the initial reports of this syndrome.[109] The most characteristic finding is the sagittal cleft or butterfly vertebrae (Figure 50.1-11). This relatively uncommon anomaly may occur in normal individuals. The affected vertebral bodies are split sagittally into paired hemivertebrae owing to a failure of the fusion of the anterior arches of the vertebrae. Generally, these are asymptomatic and of no structural significance. The mildly affected vertebrae will have a central lucency. A fully affected vertebrae will have a pair of separate triangular hemivertebrae whose apices face each other like the wings of a butterfly. Although these abnormalities are present from birth, they are often unrecognized at the time of evaluation for neonatal hepatitis, only to be identified on spine films taken later. Other associated skeletal abnormalities include an abnormal narrowing of the adjusted interpeduncular space in the lumbar spine in half of the patients,[109,188] a pointed anterior process of C1, spina bifida occulta,[189] fusion of the adjacent vertebrae, hemivertebrae, and the presence of a bony connection between ribs.[110] The fingers may seem short, with short distal phalanges, broad thumbs, and fifth finger clinodactyly. A characteristic supernumerary digital flexion crease has been reported in 35% of AGS subjects compared with 1% of the general population.[190] Markedly decreased bone density and pathologic fractures are com-

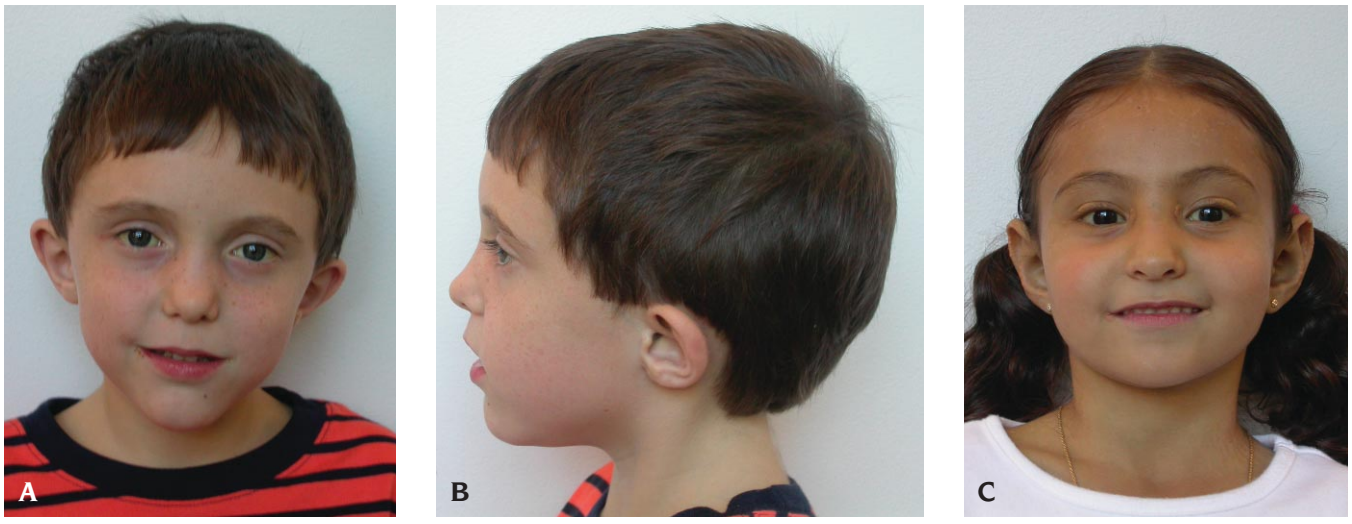


FIGURE 50.1-9 Typical facial characteristics of two unrelated children with Alagille syndrome.

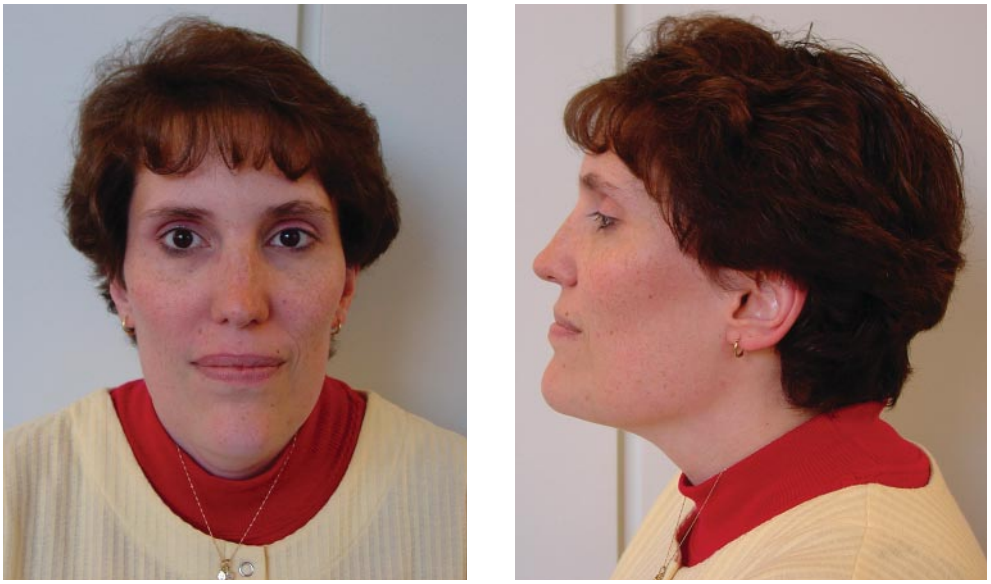


FIGURE 50.1-10 Typical facial characteristics of an adult with Alagille syndrome.

monly seen in patients with AGS and significant cholestasis. This may be due to a combination of fat and calcium malabsorption, vitamin D and K deficiency, magnesium[191] or zinc deficiency, copper excess, or chronic malnutrition.

Ocular Abnormalities. A large and varied number of abnormalities have been described in AGS, including abnormalities of the cornea, iris, retina, and optic disk. A few of the findings are secondary to chronic vitamin deficiencies. Of the primary ocular abnormalities, posterior embryotoxon is the most important diagnostically. Posterior embryotoxon is a prominent, centrally positioned Schwalbe ring (or line), at the point where the corneal endothelium and the uveal trabecular meshwork join (Figure 50.1-12). Posterior embryotoxon occurs in up to 89% of patients with AGS,[111] but it also occurs in 8 to 15% of

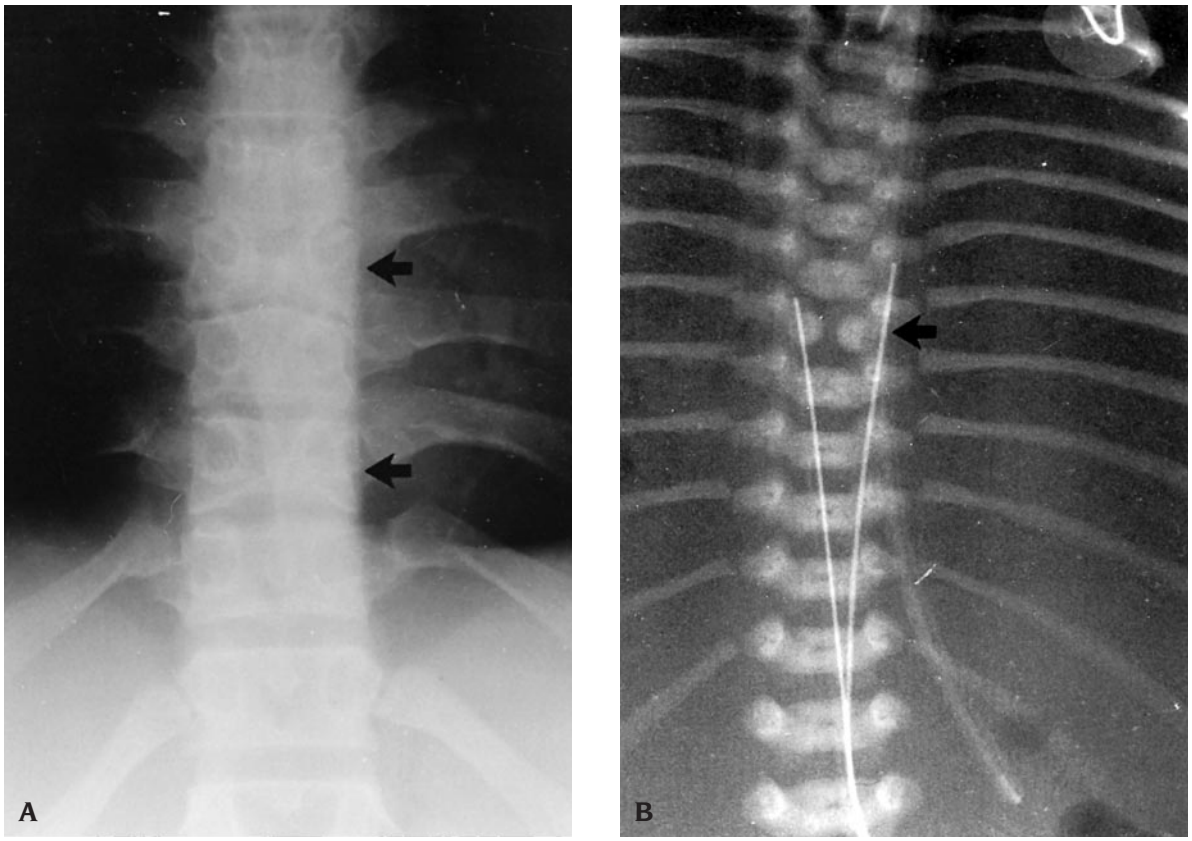


FIGURE 50.1-11 *A*, Multiple butterfly vertebrae (*arrows*) in an adolescent with Alagille syndrome. *B*, Fully affected vertebrae (*arrow*) with separate triangular vertebrae in a neonate with severe congenital heart disease and Alagille syndrome.

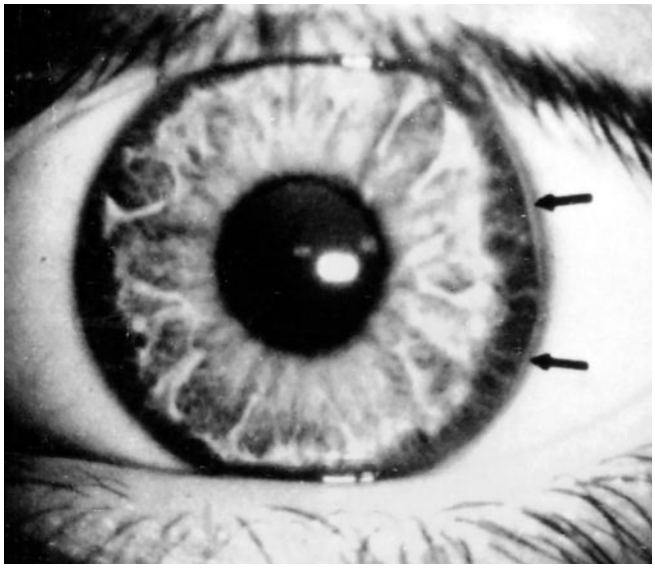


FIGURE 50.1-12 Posterior embryotoxon (*arrow*), prominent Schwalbe line.

normal eyes when evaluated by an ophthalmologist. Posterior embryotoxon can be part of an anterior chamber malformation syndrome. These malformations fall into three groups of peripheral and central abnormalities. Many of these abnormalities have now been reported in AGS. The Axenfeld anomaly is a prominent Schwalbe ring with attached iris strands. In general, about 50% of normal patients with this anomaly develop glaucoma, and glaucoma has been reported likewise in AGS.[192] The Rieger anomaly (primary mesodermal dysgenesis) is a prominent Schwalbe ring with attached iris strands and hypoplastic anterior iris stroma. This autosomal dominant inherited malformation has also been demonstrated in a patient with AGS.[193] Nischal found ultrasonographic evidence of optic disk drusen in at least one eye in 95% and bilateral disk drusen in 80% of patients with AGS but in none of the non-AGS liver patients they studied. This is markedly higher than the incidence in the normal population (0.3–2%), suggesting that this ophthalmologic sign may be an extremely useful diagnostic tool.[194] A peculiar mosaic pattern of iris stromal hypoplasia is present in many patients.[195] In addition, microcornea, keratoconus, congenital macular dystrophy, shallow anterior chambers, exotropia, ectopic pupil, band keratopathy, choroidal folds, and anomalous optic disks have been reported.[196,197] Other ocular findings, including retinal pigmentary changes, are identified in many patients with cholestasis but are not specific for the syndrome and are attributed to fat-soluble vitamin deficiencies.

Central and Peripheral Nervous System Abnormalities. Significant mental retardation (IQ less than 80) is a prominent feature in the initial reports of syndromic paucity.[109,115] More recent estimates are lower, perhaps owing to earlier recognition of the syndrome, the identification of less severely affected individuals, or more aggressive nutritional management. Although only 2% of children had mild mental retardation in one series, 16% had delays in gross motor skills.[117] Current studies emphasize the impact of chronic liver disease on brain development regardless of etiology[198,199] and focus on the role of vitamin E therapy and aggressive nutritional management with intervention programs to optimize outcome. No controlled trials are yet available to fully evaluate these claims. Abnormal visual, auditory, and somatosensory evoked potentials have been noted in AGS patients. These were not explained solely on the basis of fat-soluble vitamin deficiency. Visual evoked potentials returned to normal following resolution of the cholestasis with transplant. Dystonia and tremor associated with elevated whole-blood manganese levels and symmetric hyperintense basal ganglia magnetic resonance signals were seen in one patient with AGS.[200] This resolved following transplant. The possibility exists that neurologic findings in AGS are due to a combination of genetic and vascular abnormalities, chronic nutritional depletion, specific fat-soluble vitamin deficiencies, and toxins accumulated owing to deficient hepatic excretion and chronic cholestasis. A recent report details a family with dominantly inherited deafness, associated with congenital cardiac disease (including TOF) and posterior embryotoxon, without other features of AGS.[201] Affected individuals in this family had a missense mutation of *JAG1*, which segregated with the disease.

Intracranial bleeding has more recently been recognized to be a feature of AGS.[116,117] It occurs in up to 14% of patients and is a significant cause of morbidity and mortality. There is no pattern to the site of bleeding, which varies from epidural to subarachnoid to intraparenchymal. It may be associated with coagulopathy, although most patients had minimal or no abnormalities in clotting. Minor head trauma, to a degree unlikely to cause bleeding, was a factor in a minority of cases. Most were apparently spontaneous bleeds. A prospective study of magnetic resonance angiography was unable to identify any abnormalities, including one small aneurysm that resulted in a fatal subarachnoid hemorrhage (D. A. Piccoli, unpublished data, 1998). The data suggest that a central nervous system vasculopathy may be an intrinsic part of the multisystem abnormalities seen in AGS. Vascular abnormalities have been reported in other organs, including the kidneys and the lungs of patients with AGS. Furthermore, another Notch-related disorder in humans, CADASIL, is a dominantly inherited stroke disorder owing to defects in human *Notch3*. The occurrence and severity of strokes in AGS are not seen in other forms of pediatric liver diseases and thus should be considered as a primary complication of AGS. Careful correction of coagulopathy and observation after head trauma may decrease morbidity and mortality in some cases, but prediction or prevention of intracranial hemorrhage is not currently possible. Moyamoya syndrome, another form of vasculopathy, has been reported in children with AGS.[202] In addition, Lykavieris and colleagues reported a striking frequency of significant bleeding seen in 38 of 174 AGS patients not in liver failure who experienced a total of 49 significant events.[203] Although severe cholestasis was present in most, the majority of patients had normal coagulation and platelet studies. It is unclear if this bleeding tendency is a

pleiotrophic consequence of cholestasis or a primary genetic defect in vascular integrity or hemostasis. In this study, however, eight patients died secondary to the bleeding, half following a surgical episode.

Diagnosis: Clinical Criteria

The specific diagnosis of AGS can be established only by the clinical phenotype. Alagille and colleagues proposed diagnostic criteria for this disorder based on the presence of five major abnormalities. In addition to proper hepatic histopathology, the major criteria are chronic cholestasis, characteristic facies, cardiac murmur, vertebral abnormalities, and posterior embryotoxon.[111] The frequency of these abnormalities compiled from several large series is shown in Table 50.1-5.[111,112,117–119]

Because nearly all patients with significant bile duct paucity will manifest some degree of chronic cholestasis, Alagille and colleagues recommend the use of the other four criteria (facies, murmur, vertebral anomalies, and posterior embryotoxon) to define the syndrome.[111] In 36% of patients, all four features were present. Another 52% had three of the four features, and 12% had only two. Based on these data, Alagille and colleagues recommended that the diagnosis be made with cholestasis and two of the other four abnormalities. The need for more accurate criteria is emphasized by the 8 to 15% frequency of embryotoxon in the general population, the subjective nature of facies assessment, the potential difficulties in assigning a pathologic basis to a mild systolic flow murmur, and the incidence of cardiac disease in biliary atresia (10%), congenital rubella, and deletions of 22q. Furthermore, these data are derived only from significantly affected individuals, who, in the majority of cases, are the affected proband in a family. It is clear from recent genetic studies that family members carrying a mutation in *JAG1* may have few, if any, overt signs of AGS. When tested, most relatives of AGS patients with even isolated manifestations have the mutation.[141] Thus, patients with isolated cardiac disease or apparently nonsyndromic paucity may have mutations in *JAG1*. Although the diagnosis of AGS in a proband should not be established on the basis of fewer than three clinical criteria, it can be suggested. Molecular testing should help to further define these mildly affected patients and their relatives.

In the majority of patients, the hepatic manifestations of the disease dominate the clinical picture. Patients may present with neonatal hepatitis, jaundice, pruritus, cholestasis, or cardiac disease or may be identified as asymptomatic siblings (or parents). The syndrome must be distinguished from other etiologies of neonatal hepatitis and from extrahepatic obstructions such as biliary atresia. The usual evaluation will include an initial laboratory evaluation to identify other etiologies, followed by a sonogram, nuclear scintiscan, liver biopsy, and possibly operative cholangiogram.

An infant with AGS will usually have an elevated conjugated bilirubin and moderately elevated levels of the aminotransferases. The γ-glutamyl transpeptidase, alkaline phosphatase, serum bile acids, and cholesterol may be dramatically elevated, but none of these findings aid in the discrimination of syndromic bile duct paucity from biliary atresia or other causes of extrahepatic obstruction.

Although there is no evidence of mechanical extrahepatic obstruction in AGS, differentiation from biliary atresia can be difficult.[110,111,117,204] Ultrasound examination may

TABLE 50.1-5 CLINICAL MANIFESTATIONS OF ALAGILLE SYNDROME

SYSTEM	FINDINGS
Hepatic	Duct paucity, cholestasis, neonatal hepatitis, fibrosis, cirrhosis, portal hypertension, liver failure, hepatocellular carcinoma, nodular hamartoma
Cardiac	Murmur, pulmonic valvular stenosis, tetralogy of Fallot, pulmonary atresia, truncus arteriosus, ventricular septal defect complex, ventricular septal defect, atrial septal defect, anomalous venous return
Vascular	Peripheral pulmonic stenosis, pulmonic outflow stenosis, coarctation, patent ductus arteriosus, renal artery stenosis, middle aorta syndrome, moyamoya
Skeletal	Butterfly vertebrae, shortened interpedicular distance, shortened phalanges, short stature, spina bifida occulta, fusion of adjacent vertebrae, absent twelfth rib, shortened distal ulna and radius, clubbing, pathologic fractures, osteopenia, rickets
Ocular	Posterior embryotoxon, Axenfeld anomaly, Rieger anomaly, shallow anterior chamber, cataracts, strabismus, exotropia, ectopic pupil, optic disk drusen, iris stromal hypoplasia, band keratopathy, glaucoma, microcornea, keratoconus, congenital macular dystrophy, anomalous optic disks, fundic hyperpigmentation, pigmentary retinopathy, night blindness
Facial/cranial	Characteristic pediatric "particular facies," adult "particular facies," sinus abnormalities, chronic sinusitis, thinned cortical bones, deafness, large ears, high-pitched voice, macrocephaly
Renal	Neonatal renal insufficiency, adult renal failure, solitary, ectopic, or horseshoe kidney, bifid pelvis, duplicated ureter, small kidney, cystic and multicystic kidney, dysplastic kidney, infantile renal tubular acidosis, juvenile nephronophthisis, lipidosis, tubulointerstitial nephropathy, interstitial fibrosis
Central nervous system	Intracranial epidural, subdural, subarachnoid, and intraparenchymal bleeding; stroke; vascular malformation; mental retardation; developmental delay; school dysfunction; abnormal visual, auditory, and somatosensory evoked potentials
Cutaneous	Jaundice, xanthomata, pruritus, thickened, lichenified hands and feet
Growth disorders	Failure to thrive, fat-soluble vitamin deficiency, protein-calorie malnutrition, short stature
Pancreatic	Exocrine insufficiency, diabetes mellitus, pancreatic fibrosis
Other	Tracheal and bronchial stenosis, jejunal atresia, ileal atresia, malrotation, microcolon, otitis media, extrahepatic malignancies

Adapted from references 111, 117, 119, 120, and 149.

not identify the extrahepatic tree owing to diminished gallbladder size, and it is rarely diagnostic. Studies that may demonstrate patency of the extrahepatic biliary tree include technetium 99m disopropyl iminodiacetic acid (DISIDA) and similar scintiscans, radiologic cholangiography via either endoscopic retrograde cholangiopancreatography, percutaneous transhepatic cholangiography, gallbladder cholangiography, or operative cholangiography. A technetium-labeled scintiscan may show excretion into the duodenum in 39% of patients with AGS but in the remainder will not demonstrate communication (as is also seen in biliary atresia).[117]

In addition to the usefulness of DISIDA scintigraphy in the diagnosis of AGS in the neonatal period, there may be a characteristic pattern of excretion of tracer. Distinct retention of tracer in the periphery with central clearing in a young adult has been reported.[205] Studies have suggested that tracer excretion is uncommon in early infancy but may be demonstrable in the same patient later in childhood. In adults, tracer excretion is more common, and the pattern typically involves central clearing. This parallels the clinical progression of severe cholestasis seen in many patients with AGS and suggests that major ducts become the site of functional excretion in AGS.

The liver biopsy is the most useful preoperative study for the discrimination of syndromic bile duct paucity from extrahepatic biliary atresia. However, difficulties in histologic diagnosis may arise early in infancy because bile ductule proliferation may obscure duct paucity or because some ducts may, in fact, be present early in life. In very young infants in whom the percutaneous liver biopsy is not diagnostic, it may be helpful to delay exploration for 1 to 2 weeks and repeat the biopsy (while recognizing that the success of therapy for extrahepatic biliary atresia is correlated with surgery before 60 days of life).[206] If laparotomy is undertaken, an operative wedge biopsy should be obtained. An intraoperative cholangiogram performed by an experienced surgeon must be attempted and carefully interpreted prior to the construction of a portoenterostomy. The extrahepatic bile ducts are anatomically normal and patent in AGS but may be so narrow that operative cholangiography will fail to identify a patent system. Because operative cholangiography alone may result in an incorrect diagnosis of biliary atresia in up to 20% of cases,[117,204] a careful preoperative search should have been performed for the syndromic features. Hepatoportoenterostomy is inappropriate in AGS and may increase morbidity.[111,204] The correct diagnosis is also important for the genetic implications.

In older children, striking abnormalities are seen in fasting bile acid levels, serum lipids, γ-glutamyltransferase, and alkaline phosphatase. Bile acids in severe disease may be elevated 100-fold. The conjugated bilirubin is commonly moderately elevated. The magnitude of the hyperbilirubinemia is usually less than that of the bile acid elevation, and jaundice may disappear during childhood despite persistently elevated bile acids. Most patients have elevated triglyceride and cholesterol, which, in severe cases, may be from 1,000 to 2,000 mg/dL. Moderate elevations of the aminotransferases are common, although to lesser values than the γ-glutamyltransferase. In the majority of patients, the hepatic synthetic and metabolic functions are normal. Prothrombin time following parenteral vitamin K is usually normal. There may be deficiencies in substances requiring bile acids for absorption, such as vitamins A, D, E, and K, and essential fats.

Treatment

Infants with intrahepatic cholestasis may have significant fat malabsorption. Because half of the calories in infant formulas may be from fat, this defect contributes significantly to overall caloric deprivation. Medium-chain triglycerides are hydrolyzed and absorbed in the absence of bile salt micelle formation and thus are a significant caloric additive. Optimal diets include increased amounts of medium-chain triglycerides added to the diet and optimization of the carbohydrate and protein intake. Essential fatty acids may also be malabsorbed, resulting in clinically evident deficiency. This has resulted in acral lesions resembling porphyria, which have responded to parenteral supplementation of essential fatty acids.

Fat-soluble vitamin deficiency is present to a variable degree in most patients with bile duct paucity. Oral or parenteral supplementation is necessary for prevention of vitamin deficiencies. Further exacerbation of these deficiencies may be caused by therapy for cholestasis, such as phenobarbital or cholestyramine. Oral or intramuscular vitamin K will correct the coagulopathy in most patients, and its failure to do so may herald significant synthetic dysfunction. Aggressive therapy should be maintained in patients with clinical bleeding or evidence of significant hypersplenism. Rickets is seen in patients unless supplemented with oral or intramuscular vitamin D. Vitamin D absorption may be enhanced by administration of d-γ-tocopherol polyethylene glycol-1000 succinate (TPGS).[207] Early evidence of elevated serum alkaline phosphatase may be obscured, and serum levels of vitamin D should be checked at frequent intervals.

Deficiency of vitamins E and A may result in significant neurologic abnormalities, including cerebellar ataxia, peripheral neuropathy, abnormalities of extraocular movement, and retinopathy.[120,149,178,208] Vitamin E has been the most difficult to adequately supplement, although TPGS-soluble preparations are widely available. The TPGS is significantly more effective than other oral preparations and has been demonstrated to be effective in reversing neurologic damage in some patients.[209] The serum vitamin E level must be corrected for the serum lipid level in children with marked cholestasis. Vitamin A levels also should be monitored and oral or intramuscular replacement given as indicated. However, measurement of liver concentrations of vitamin A provides a more accurate indication of vitamin A status because serum levels of retinol and plasma retinol binding protein are still normal when hepatic stores of vitamin A are depleted.

Pruritus is the most significant symptom for many patients with chronic cholestasis. Antihistamines may give some relief, and care should be taken to keep the skin

hydrated with emollients. Fingernails must be trimmed. Cholestyramine may improve pruritus in children who can be convinced to take sufficient amounts, but some children will develop a severe acidosis on this therapy.[210] Colesevelam is less well studied but may have some advantages over cholestyramine. Phenobarbital appears to have little effect on either jaundice or on pruritus, although it has proven effective in enhancing bile salt–independent bile formation. Ultraviolet therapy may give temporary relief of pruritus in some cases.[211] Rifampin, which inhibits uptake of bile acids into the hepatocyte, appears to provide significant relief of pruritus in approximately half of patients.[212,213] Ursodeoxycholic acid, a potent choleretic, may have a dramatic effect in reducing symptomatic cholestasis, although, in some patients, it appears to exacerbate pruritus. In other cholestatic diseases, such as sclerosing cholangitis and primary biliary cirrhosis, ursodeoxycholic acid has been demonstrated to improve biochemical parameters and symptoms and may possibly retard disease progression. Naltrexone, an opioid antagonist, has also been useful in some cases.[214] Emerick and Whitington treated nine patients with extreme cholestasis and pruritus with partial external biliary diversion and demonstrated a dramatic decrease in pruritus scores, mean serum bile salt levels, and serum cholesterol.[215] Surgical ileal exclusion has not been well studied in AGS but may provide a more cosmetically appealing alternative to external diversion.

Prognosis

The outcome of syndromic bile duct paucity is highly variable and is most directly related to the severity of the hepatic and the cardiac lesions, with mortality predominantly attributable to these two organs. Complex congenital cardiac disease is a major cause of early mortality, although hepatic complications account for most of the later morbidity and mortality. These data are reflected in a follow-up study that reported a mortality rate of 26% (21 in 80) in 10 years, with only four deaths attributable to hepatic disease (portal hypertension in two and hepatic failure in two).[111] In another series, the predicted probability of survival to 20 years of age for all patients was 75%.[117] The probability of survival to age 20 was 80% for patients who did not require liver transplant and 60% in those who underwent transplant. Hepatic transplant may be required for chronic liver failure, portal hypertension, or severe intractable pruritus. Survival following transplant has varied significantly in different studies, from 45 to 100%.[60,61,116,117,156–158,216–218] Transplant does appear to have a higher risk for patients with AGS, owing in part to the severity of cardiopulmonary disease. Caution should be taken when considering relatives as potential donors for living-related transplant because unsuspected disease in the parent has thwarted donation.[219]

NONSYNDROMIC BILE DUCT PAUCITY

Nonsyndromic bile duct paucity is the term used to designate all instances of paucity except those occurring in AGS. It includes all nonsyndromic cases either with or without an associated primary disease. Thus defined, it covers such a great range of disorders that it is inappropriate to talk of a prognosis for nonsyndromic paucity generally. In some of these disorders, bile duct paucity is a characteristic feature (chronic allograft rejection) or a well-recognized association (α_1-antitrypsin deficiency) or outcome (biliary atresia). In others, bile duct paucity is an unusual and only infrequently reported finding. Most cases of nonsyndromic paucity are acquired, owing to either infections, immune-mediated injury (primary sclerosing cholangitis, primary biliary cirrhosis), or drugs and toxins, or in the setting of graft-versus-host disease (GVHD) or chronic allograft rejection. In those cases associated with a primary disorder, the principal determinant of outcome is usually the primary disease itself. In reviewing reports of supposed nonsyndromic paucity, it should also be kept in mind that there has been an inappropriate tendency to identify progressive intrahepatic cholestasis with paucity in the absence of histologic proof of paucity.

Only a few series of nonsyndromic cases have been published (earlier series of paucity probably include both syndromic and nonsyndromic cases because the syndrome has only relatively recently been recognized). Kahn and colleagues[220] and Alagille[221] have reported series based on histologic criteria. In the series of Kahn and colleagues, of l7 patients with nonsyndromic paucity, 9 were associated with well-defined primary diseases, including Down syndrome, hypopituitarism, cystic fibrosis, α_1-antitrypsin deficiency, cytomegalovirus (CMV) infection, and Ivemark syndrome. (In addition to these, other disorders, including congenital rubella, chromosomal abnormalities, GVHD, rejection of allograft livers, primary sclerosing cholangitis, and possibly Zellweger syndrome, have also been associated with paucity.[222]) In the remaining 8 cases in the series of Kahn and colleagues, the paucity was apparently primary or idiopathic (ie, not associated with any defined disease). The nonsyndromic cases had the clinical and general histopathologic picture of neonatal hepatitis. One of the most striking features in their series was that all of the nonsyndromic patients had paucity before the age of 90 days, whereas syndromic cases did not have paucity before 90 days of age. Their nonsyndromic cases also differed from their syndromic cases in that there was more portal fibrosis and less portal inflammation in the nonsyndromic cases. The clinical course of the patients with nonsyndromic paucity without underlying disease was not outlined in detail, but progressive liver disease was uncommon. Several aspects of this series deserve comment. Most authors have seen histologic paucity in occasional AGS patients before the age of 90 days, so this cannot be taken as an absolute criterion. It should also be noted that this study was conducted using needle biopsy specimens, and there is some lack of agreement as to how many portal tracts must be evaluated to obtain a statistically accurate estimate of bile duct numbers. As previously mentioned, in evaluating liver biopsies for paucity, it must be recognized, as pointed out by Kahn and colleagues, that in premature infants, a ratio of bile duct to portal tracts less than 0.9 may be normal.[107]

Alagille described 24 patients with nonsyndromic paucity who were classified into two groups: group I presented in the first few weeks of life with cholestasis, whereas group II presented later.[221] The groups differ histologically, with group I having portal inflammation, giant cell change, and minimal fibrosis and group II having more portal fibrosis and inflammation in relation to paucity. The outcome of these two groups is highly variable as half developed biliary cirrhosis and 38% died from hepatic failure. About one-third are anicteric, with only biochemical evidence of hepatic disease. Rubella was identified in one patient. It is of note that only 60% of these patients were screened for α_1-antitrypsin deficiency, but 29% of those tested were protease inhibitor type Z. Overall, therefore, it is not clear how many of the cases in this series were truly sporadic or idiopathic and how many were associated with primary diseases.

NONSYNDROMIC PAUCITY WITH PRIMARY DISEASE

Detailed discussions of the various primary diseases are presented elsewhere in this text, so the discussion here will be limited to the pathogenesis of paucity in those few primary conditions in which this pathogenesis is either partially understood or can be plausibly hypothesized.

In terms of paucity associated with well-defined primary diseases, it should be noted that (1) in virtually all of these, paucity is reported in only a small percentage of patients with these diseases, and (2) many of the diseases (eg, trihydroxycoprostanic acid excess, Ivemark syndrome) are themselves quite rare. From these facts, it is evident that a causal association between duct paucity and a number of these disorders is not well established.

Bile Duct Paucity in GVHD

Bile duct injury, sometimes eventuating in duct paucity, is one of the most distinctive hepatic lesions in GVHD. This injury is presumably the basis for the disappearance of ducts and potential paucity that occurs in some patients. It is unusual to find hepatic GVHD lesions in the absence of cutaneous manifestations of GVHD. The duct manifests injury by epithelial atypia, vacuolization, variable staining of nuclei and cytoplasm, and regeneration. Frank necrosis of epithelium can be seen on occasion. Accompanying the epithelial injury, there is often a lymphocytic infiltrate, sometimes with macrophages intermixed. On occasion, there is close proximity of lymphocytes and ducts and even invasion of the ducts by lymphocytes. In any single biopsy, however, it is not uncommon for the injury to be out of proportion to the inflammatory infiltrate, and the presence of endotheliilitis may be useful in indicating that the epithelial lesions reflect GVHD.[223] Centrilobular cholestasis is frequently present and is particularly intense when duct paucity has developed. The duct injury and paucity may be focal. Detailed reconstruction studies have suggested that the injury begins in relatively small ducts (± 30 μm in diameter).[224] When duct paucity is present in a patient with bone marrow transplant or when there is prominent active duct destruction in such a patient, the diagnosis is quite straightforward, particularly in the absence of CMV infection. Reports of duct ultrastructure, which are uncommon, have described a number of rather nonspecific changes involving duct epithelium and basement membrane, as well as close contacts between epithelial cells and lymphocytes.[225,226] Immunohistochemical studies reveal increased numbers of HNK1+ (killer) cells, Leu 3+ cells, and expression of human leukocyte antigen (HLA)-DR (major histocompatibility complex class II) positivity by the epithelial cells.[224,227,228] The latter is not found in normal liver but is found in a variety of conditions affecting the bile ducts, many of which have been speculated to have an immune-related pathogenesis. The precise role and importance of these duct alterations in GVHD and the genesis of the duct lesions remain to be determined, but the effects may be mediated through the action of cytotoxic lymphocytes, as appears to be the situation in mucocutaneous GVHD.[229]

It is interesting to note that despite the rather common occurrence of bile duct injury in GVHD, including a number of cases with paucity of ducts, it is uncommon to find reports of cirrhosis, biliary or otherwise, in GVHD.[230,231] At least superficially, this seems analogous to the similarly infrequent development of progressive liver disease in syndromic bile duct paucity.

Bile Duct Paucity in Liver Allograft Rejection

Bile duct injury is a significant element of the rejection of hepatic allografts,[223,232–235] and evidence of extensive damage (ie, involving greater than 50% of ducts) in a biopsy from a transplanted liver is regarded as strong evidence of acute rejection.[232] This damage is manifest by a variety of histologic features, including vacuolization of epithelial lining cells, variations in nuclei in these cells, and infiltration of the ducts by inflammatory cells. The latter are most commonly lymphocytes, but polys or eosinophils are not uncommon and may occasionally predominate. Active duct injury is accompanied by a lymphocytic or mixed portal infiltrate beyond the ducts. In a full-blown or classic case of cellular (acute) rejection, so-called endotheliilitis (together with duct injury and portal inflammatory infiltrate) forms the third element of a triad diagnostic for rejection.[232] If sufficiently severe, the injury may result in duct loss to the point of paucity, one of the histologic hallmarks of chronic rejection (Figure 50.1-13). The clinical presentation of chronic rejection may be either early or late, with the early-onset form typically occurring within 6 weeks of transplant. These patients often require urgent retransplant owing to the relentless progression of the liver disease. More commonly, patients with chronic rejection present between 6 weeks and 6 months following transplant with progressive jaundice and pruritus, following one or several episodes of acute rejection, or more insidiously, without apparent prior episodes of rejection. Histologically, chronic rejection is characterized by obliterative arteriopathy and bile duct injury, leading to bile duct loss. Degenerative changes in bile duct epithelium may be seen before significant bile duct loss.[236] Focal or transient paucity is not clinically or

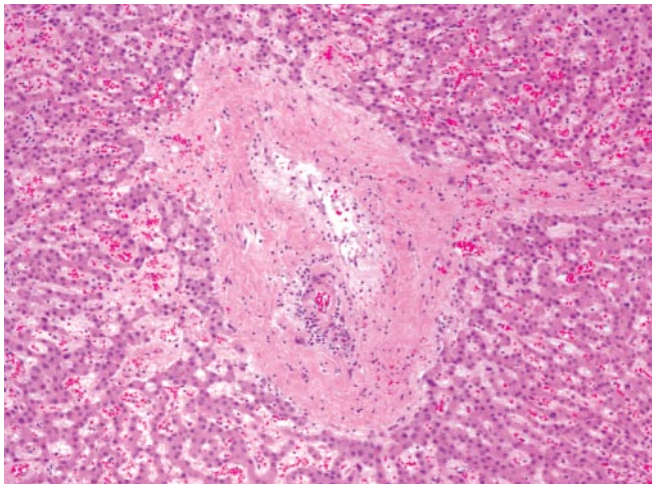


FIGURE 50.1-13 Bile duct paucity resulting from chronic rejection. Chronic rejection of a liver allograft 2 years following transplant. There is loss of bile ducts, with lymphocytic inflammation of the portal tracts and endotheliitis (hematoxylin and eosin; ×100 original magnification).

prognostically significant, but widespread persistent paucity is an ominous prognostic finding.[233] In hepatic allograft rejection, it is characteristic for portal tracts that have lost their ducts to show minimal or no inflammatory infiltrate. This probably speaks to the role of the infiltrating cells in the pathogenesis of the duct injury and loss.

Bile Duct Paucity in Viral Infections

Cholestatic liver disease with hepatocellular giant cell transformation and extramedullary hematopoiesis are frequent in congenital infection with CMV, among which are several well-documented cases of bile duct paucity, postulated to have resulted from an obliterative cholangitis.[237,238] Documentation of CMV-associated paucity in the preterm infant is more problematic because of the reasons stated earlier in the chapter.[239] Obliterative changes with focal loss of bile ducts have also been observed in congenital rubella[240] and in congenital syphilis.[241]

Drug-Associated Bile Duct Paucity

Many different drugs have been reported to cause chronic cholestatic liver disease, defined as the persistence of jaundice for more than 6 months after withdrawal of the causative drug in a patient without previous hepatobiliary disease.[242] More recently, duct injury and loss have been documented, most frequently following the use of antibiotics such as penicillin derivatives, quinolones, tetracyclines, and sulfa drugs, among others.[243–247]

Early biopsies typically reveal an active nonsuppurative inflammatory destruction of bile ducts with evidence of epithelial damage such as vacuolization and nuclear pyknosis. The portal inflammatory infiltrate is usually lymphoid, occasionally with eosinophils, histiocytes, or small granulomas.[247–249] There typically is a lobular hepatic component with canalicular cholestasis. Persistent ductopenia with progression to fibrosis may evolve over the ensuing months even after the cessation of the medication. Progressive loss of intrahepatic bile ducts is noted in untreated cases, as early as 5 to 6 months, and occurs in most patients despite treatment, even when adequate biliary drainage is established.[250–253] Injury by bile stasis, peribiliary ischemia, and continuation of the original insult may all be contributing factors. Heterogeneity and unevenness in the distribution of bile duct loss and occasional nodules of better preserved parenchyma may reflect local differences in biliary obstruction or fibrosis.[250,254]

Bile Duct Paucity in Extrahepatic Atresia

The development of intrahepatic bile duct paucity in relatively long-surviving patients with biliary atresia was recognized prior to development of portoenterostomy and is also seen in patients following portoenterostomy, even when adequate biliary drainage is established.[250,251,253] Injury by bile stasis, peribiliary ischemia, and continuation of the original insult may all be contributing factors. Heterogeneity and unevenness in the distribution of bile duct loss and occasional nodules of better preserved parenchyma may reflect local differences in biliary obstruction or fibrosis.[250,254]

Bile Duct Paucity in Primary Sclerosing Cholangitis

Primary sclerosing cholangitis is a chronic disorder of unknown etiology that is increasingly recognized in children. It is characterized by a generalized beading and stenosis of the biliary tree in the absence of choledocholithiasis, accompanied by histologic abnormalities of the bile ducts (Figure 50.1-14). It may occur in patients who are otherwise well but is often associated with inflammatory bowel disease. Secondary sclerosing cholangitis describes similar bile duct changes when a clearly predisposing factor such as choledocholithiasis or biliary surgery has been identified. There is progressive obliteration of the intra- and extrahepatic bile ducts, which may result in bile duct paucity, biliary cirrhosis, and liver failure.

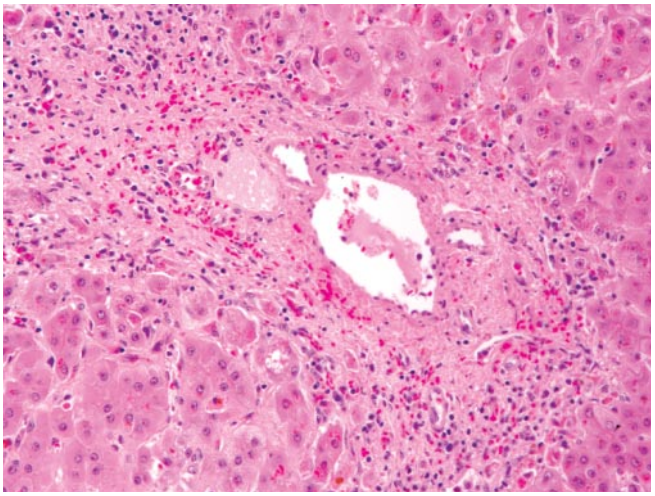


FIGURE 50.1-14 Bile duct paucity resulting from primary sclerosing cholangitis. Bile ducts are absent from the portal tracts. There is cholestasis in the adjacent hepatic lobule (hematoxylin and eosin; ×200 original magnification).

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2. *Biliary Atresia*

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Biliary atresia is a progressive fibroinflammatory cholangiopathy of infancy that results in complete obliteration of the entire or portions of the extrahepatic biliary tree within weeks of birth. This obstruction results in impaired bile flow, reactive proliferation of intrahepatic bile ducts, chronic cholestasis, and ongoing hepatocellular injury. In general, infants appear well but display the classic features of jaundice, acholic stools, and hepatomegaly, features shared with other causes of neonatal cholestasis. Therefore, the initial task is to differentiate biliary atresia from other forms of neonatal cholestasis; timely diagnosis is vital because early surgical relief of biliary obstruction may improve long-term outcome. In the absence of surgical intervention, ongoing injury leads to biliary cirrhosis, portal hypertension, and end-stage liver disease. At this stage, liver transplant is the only therapeutic option for long-term survival.

Biliary atresia is the most common cause of prolonged conjugated hyperbilirubinemia in neonates and is the most frequent indication for liver transplant in the pediatric population, accounting for 40 to 50% of all pediatric liver transplants.[1] The health care costs associated with biliary atresia are significant, reaching $65 million/year in the United States alone.[1] Despite the obvious adverse impact to children's health, advances in understanding of the etiology and pathogenesis of biliary atresia have not kept pace with progress in other cholestatic disorders of childhood.[2] The lack of progress reflects the multifactorial nature of the disease, which has challenged physicians since it was recognized early in the nineteenth century (Table 50.2-1).[3] The development of surgical approaches to re-establish biliary drainage and the acceptance of liver transplant as a treatment for end-stage liver disease owing to biliary atresia have markedly improved clinical outcome. Yet multiple challenges remain, such as early recognition of pathologic jaundice by health care providers, accurate diagnosis of biliary atresia, identification of predictors of outcome and optimal timing of transplant, and design of novel and effective medical therapies. The recent development of research agendas to sponsor multicenter studies in Europe and the United States promises to bring biliary atresia to the forefront of patient- and laboratory-based research priorities. Although the biologic basis of biliary atresia is not yet known, important data exist on epidemiology, clinical course, pathology, treatment, and outcome. A review of these data is the focus of this chapter.

EPIDEMIOLOGY

Biliary atresia respects no geographic boundaries; it occurs worldwide and affects 1 in 8,000 to 1 in 15,000 live births. Within regions, there appears to be a higher incidence of the disease in nonwhite populations (African American, French Polynesian, Chinese). Some studies have suggested time-space clustering of cases and seasonal variation, with the majority of cases occurring in the fall and winter months (December to March).[4,5] Two large population-based studies from France and Sweden, however, failed to identify a significant seasonal variation or time-space clustering.[4–7] After controlling for geographic and racial factors, associations have been observed with advanced maternal age and increased parity, and with a tendency for early fetal losses in mothers of infants with biliary atresia.[4,7] In this context, the disease is rarely seen in stillborns or premature infants, although some patients have a low birth weight for gestational age.[4,8] A slight female predominance (1.25:1) may be present among affected infants, particularly in the "embryonic" form of the disease.[9,10] Although the overwhelming majority of cases of biliary atresia are sporadic, there are reports of apparent recurrences within families.[11–13] This may reflect a shared genetic predisposition, but studies of twins have demonstrated that most sets are discordant for the disease.[14,15]

TABLE 50.2-1 HISTORICAL LANDMARKS FOR BILIARY ATRESIA

YEAR	LANDMARK
1817	Burns's description of an infant with features suggestive of biliary atresia
1892	Review of 50 cases by Thomson
1916	Review of anatomic findings and discussion of surgical approach by Holmes
1928	Ladd reports surgical success in selected infants with biliary atresia
1959	Kasai describes hepatoportoenterostomy in infants with biliary atresia
1982	NIH Consensus Conference: liver transplant as an acceptable treatment modality for end-stage liver disease owing to biliary atresia
1999	European Biliary Atresia Registry: European initiative for collaborative research in biliary atresia
2002	NIH-Biliary Atresia Research Consortium: multicenter consortium to foster clinical, epidemiologic, and therapeutic research in biliary atresia

Adapted from Bates MD et al.[3]
NIH = National Institutes of Health.

Taken together, these epidemiologic data suggest that environmental factors and genetic predisposition may be important determinants of disease. They also highlight the widespread occurrence of biliary atresia and underscore the need for physicians to be knowledgeable of the clinical features of the disease so that a diagnosis can be reached in a timely fashion.

CLINICAL PRESENTATION

Common Features

Despite variability in age at onset of symptoms, extent of hepatobiliary involvement, and presence of nonhepatic abnormalities, infants with biliary atresia share the cardinal features of jaundice (owing to conjugated hyperbilirubinemia), acholic stools, and hepatomegaly. This triad presents in infants who otherwise appear well. Although weight gain is initially adequate, it becomes suboptimal with progression of disease. In addition to these common features, variability in clinical presentation results from complications of liver disease or nonhepatic abnormalities. For example, (1) splenomegaly is common at the time of diagnosis and reflects the degree of hepatic fibrosis and portal hypertension, (2) lethargy is uncommon and when present should trigger evaluation of clotting function and of the central nervous system integrity because coagulopathy owing to vitamin K deficiency may result in intracranial hemorrhage, and (3) poor feeding and recurrent emesis may signal the coexistence of intestinal malrotation or hemodynamic instability secondary to a severe cardiac defect (Table 50.2-2).

Other signs and symptoms may develop with progression of liver disease. Ascites is rarely present at the time of diagnosis but may develop in infants with advanced fibrosis, bacterial peritonitis, or obstruction of portal blood flow (owing to portal vein thrombosis). Sometimes the presence of ascites is suggested by the appearance of inguinal or umbilical hernia, which is treated more appropriately by management of ascites rather than herniorrhaphy. When infants have impaired biliary drainage despite portoenterostomy, progression of liver disease is manifest by variable signs of malnutrition, with decreased subcutaneous mass and muscle weakness. In this setting, infants may also have dilated vascular collaterals ascending from the anterior abdominal wall toward the chest in a distended abdomen (caput medusae) because of intra- and/or extrahepatic portal venous hypertension. Careful evaluation and monitoring of these common clinical features are important for appropriate diagnosis and provide critical clues about the state of evolution of the liver disease. The rate of disease progression may also depend on specific clinical forms that have been identified by the systematic cataloguing of clinical features at the time of diagnosis.

TABLE 50.2-2 MAIN (TYPICAL AND ATYPICAL) CLINICAL FEATURES OF BILIARY ATRESIA

Typical Features for Infants at the Time of Diagnosis
Jaundice (secondary to direct or conjugated hyperbilirubinemia)
Acholic stools
Hepatomegaly—with variable degrees of splenomegaly
Clinical Signs Suggestive of Nonhepatic Complications
Easy bruising, central nervous system hemorrhage—coagulopathy owing to vitamin K deficiency
Poor feeding, vomiting—cardiac insufficiency owing to structural defects
Recurrent (bilious) emesis—intestinal malrotation and midgut volvulus
Features of Advanced Disease
Moderate to severe splenomegaly owing to progressive hepatic fibrosis
Growth failure—poor caloric intake, decreased absorption, or increased metabolic needs
Ascites—secondary to portal hypertension
Caput medusae—secondary to portal hypertension
Umbilical and/or inguinal hernia—complication of ascites
Coagulopathy not responsive to vitamin K—hepatocellular failure

Clinical Forms

There are two well-recognized clinical forms of biliary atresia: embryonic and perinatal.[10,16,17] These forms share the cardinal features of jaundice, acholic stools, and hepatomegaly but differ in the presence of associated anomalies, the timing of onset of jaundice, and perhaps clinical outcome. The embryonic form of biliary atresia (also referred to as "congenital" or "fetal") accounts for 10 to 20% of cases and is defined by the presence of congenital nonhepatic anomalies and earlier onset of disease. Infants present with pathologic jaundice at birth or shortly thereafter, frequently overlapping with physiologic jaundice, such that there is no jaundice-free interval. In addition, affected infants are more likely to have birth weights below the 50th percentile for age, even in the presence of maternal diabetes.[10] Notably, these infants may have complete absence of extrahepatic bile ducts, even a fibrous cord, suggesting a true defect in embryogenesis of the biliary system in a subset of patients.

Although the genetic basis for this form is unknown, hepatic and nonhepatic malformations may result from defects in molecules regulating embryonic development of the hepatobiliary system and the asymmetric organization of single organs, a process named laterality. Analysis of a large cohort identified nonhepatic malformations in 20% of the patients with biliary atresia.[18] Among these patients, defects in laterality were present in 29%, whereas isolated gastrointestinal or cardiovascular anomalies occurred in 59% and intestinal malrotation (with or without preduodenal portal vein; Figure 50.2-1A) was present in 12%. Splenic abnormalities (asplenia, double spleen, and polysplenia) may be present in up to 7.5% of patients with biliary atresia (Figure 50.2-1B), occurring in isolation or in combination with one or more additional defects in a variant known as biliary atresia splenic malformation (BASM) syndrome.[10] This syndrome includes polysplenia/asplenia, preduodenal portal vein, intestinal malrotation, abdominal situs inversus, midline liver, hepatic artery abnormalities, absent inferior vena cava, abnormal lung lobation, annular pancreas, and congenital heart disease. Although infants with the embryonic form of biliary atresia, with or without the BASM syndrome, had no obvious clinical or biochemical differences at the time of diagnosis, 15% were born to mothers with diabetes.[10] There is some evidence that patients with the embryonic form of biliary atresia have

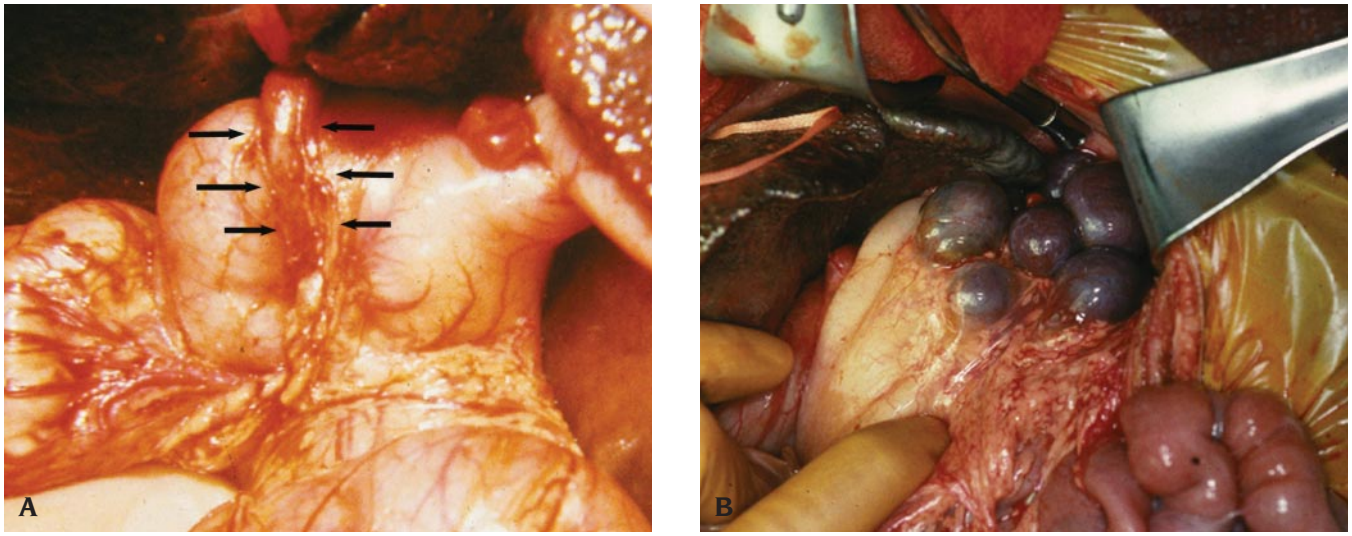

FIGURE 50.2-1 *A*, Operative findings of a preduodenal portal vein (*arrows*) in an infant with the embryonic form of biliary atresia at the time of portoenterostomy. *B*, Polysplenia in an infant with the embryonic form of biliary atresia at the time of portoenterostomy. Courtesy of Dr. Frederick Ryckman, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.

worse outcome following portoenterostomy, with a decrease in actuarial survival (defined as death or transplant) within the first 2 years after surgery.[10] In addition to the challenges that extrahepatic malformations add to the care of infants with biliary atresia, more rapid progression of the hepatobiliary disease in this clinical form may account for a worse outcome.[19]

The perinatal form of biliary atresia (also referred to as "acquired" or "postnatal") accounts for the majority of the cases (80–90%) and occurs in the absence of other congenital anomalies. Most of the infants are born at term with an appropriate weight for gestational age. They have a variable jaundice-free interval after birth but develop jaundice, acholic stools, and dark-colored urine within the first few weeks of life. In contrast to the clinical setting observed in infants with the embryonic form, the combination of jaundice in an otherwise healthy infant and a history of a jaundice-free period is more consistent with a biliary injury that results from a perinatal or early postnatal insult. Efforts to determine the biologic relationship of potential pathogenic mechanisms are particularly important to understand the molecular basis of the clinical forms and to develop new diagnostic/therapeutic modalities for infants with biliary atresia.

PATHOGENIC MECHANISMS OF DISEASE

Any proposed pathogenic mechanism of disease must take into account important clinical features that are exclusive to biliary atresia: (1) onset of disease restricted to the neonatal period, (2) target of injury limited to the biliary system, and (3) lack of recurrence of hepatobiliary lesions typical of biliary atresia following liver transplant. Although inflammation of intra- and extrahepatic biliary tree is universal in biliary atresia, conflicting evidence exists with regard to triggering events, pre- and postnatal timing of onset, and the factors that promote ongoing hepatobiliary inflammation. However, careful observations based largely on epidemiologic and clinical features reported predisposing genetic factors, and the pace of disease progression provided valuable insight into the pathogenesis of disease. Based on these observations, five mechanisms have been proposed: (1) a defect in morphogenesis of the biliary tract, (2) a defect in fetal/prenatal circulation, (3) environmental toxin exposure, (4) viral infection, and (5) immunologic/inflammatory dysregulation (Table 50.2-3).[20]

Evidence for Defective Morphogenesis

Biliary atresia may belong to a spectrum of diseases involving inappropriate persistence or lack of remodeling of the embryonic ductal plate. This concept of ductal plate malformation as a contributing or causative factor for biliary atresia is supported by the persistence of the embryonic shape of interlobular bile ducts in some infants at the time of diagnosis.[17] Also, comparative analyses of the normal embryonic development of the ductal plate and the extrahepatic biliary tree between 11 and 13 weeks of gestation with biliary structures from 205 infants with biliary atresia revealed morphologic similarities in periductular mesenchyme and fibrosis.[21] During this phase of embryogenesis, the ductal plate undergoes remodeling to form large tubular structures surrounded by thick mesenchyme, with shared morphologic similarities to abnormal ductules within the porta hepatis of livers from infants with biliary atresia. Together, these data suggest that abnormal mesenchymal support and improper remodeling of hilar ducts may be important pathogenic factors in early stages of disease development. In agreement with a prenatal onset in some cases, a recent report described segmental cystic dilatation of the biliary system in three fetuses during routine prenatal ultrasonography; postnatally, only duct remnants were detected proximally and distally to the cysts in a fashion similar to the histologic features of biliary atresia.[22]

There is a growing body of evidence pointing to possible roles of specific genes in the control of hepatobiliary

TABLE 50.2-3 POTENTIAL MECHANISMS INVOLVED IN THE PATHOGENESIS OF BILIARY ATRESIA

MECHANISM	SUPPORTING DATA
Defect in morphogenesis	Development of jaundice soon after birth; coexistence of other embryologic abnormalities; polymorphisms in the *Jag1* gene; abnormal remodeling of the "ductal plate"; *inv* mouse: model of biliary obstruction and situs inversus
Defect in prenatal circulation	Intrauterine devascularization results in abnormal extrahepatic bile ducts
Toxin exposure	Time-space clustering of cases
Viral infection	CMV, HPV, reovirus, rotavirus, HHV6 detected in infants with biliary atresia; models of virus-induced injury to biliary tract in suckling mice
Immunologic dysregulation	Increased expression of intercellular adhesion molecules; infiltration of biliary structures by CD4+ and CD8+ lymphocytes; increased frequency of the HLA-B12 allele; expression of proinflammatory cytokines

Adapted from Balistreri WF et al.[20]
CMV = cytomegalovirus; HHV6 = human herpesvirus 6; HLA = human leukocyte antigen; HPV = human papillomavirus.

morphogenesis. From clinical observations, the earlier onset of disease and the presence of nonhepatic malformations in the embryonic form of biliary atresia suggest a prenatal onset and a pathogenesis that differs, at least in part, from that of infants with the perinatal form. The main associated malformations, poly- or asplenia, cardiovascular defects, abdominal situs inversus, intestinal malrotation, and anomalies of the portal vein and hepatic artery, point to potential defects in embryogenesis and asymmetric left-right determination of visceral organs.[18] In support of this concept, abnormalities in organ symmetry and biliary drainage have been identified in the *inv* mouse. In this transgenic mouse line, a recessive insertional mutation of the inversin gene results in complete abdominal situs inversus, severe jaundice, poor weight gain, and death within the first week of life in 100% of homozygous mice.[23] Polysplenia is also occasionally present. In a detailed morphologic analysis of the hepatobiliary system in the *inv* mouse, a defect in patency of the extrahepatic ductular system was identified by trypan blue cholangiography and absent excretion of technetium 99m–labeled tracer.[24] In addition, intrahepatic ductular proliferation was described similar to that seen in biliary atresia. However, the complete absence of inflammation or necrosis within the hepatic parenchyma and the absence of inflammation and fibrosis of the extrahepatic biliary tree are in stark contrast to the histologic features of infants with biliary atresia.[24,25] Furthermore, mutational analyses in children with laterality defects and biliary atresia failed to identify mutations in the inversin gene.[26]

More recently, genetic inactivation of the transcription factors hepatocyte nuclear factor (HNF)-6 and HNF-1β in mice resulted in neonatal cholestasis and growth retardation.[27,28] Morphologic analysis of the HNF-6–deficient mice showed an abnormal development of intrahepatic bile ducts, with a transient formation of cystic structures followed by a new phase of ductular development, with loss of cysts and persistence of a discontinuous ductal plate through the first week of life.[28] In addition, these mice lacked the gallbladder, the extrahepatic bile ducts were replaced by an enlarged structure that connected the liver to the duodenum, and jaundice was present in the first few days of life. Jaundice also developed in HNF-1β–deficient mice but at a later stage of postnatal development. Interestingly, the biliary system of HNF-1β–deficient mice appeared patent, but the gallbladder had a disorganized epithelium, there were variable cystic changes in the extrahepatic bile ducts, and there was a paucity of small intrahepatic ducts.[27] Based on the time-specific onset of biliary abnormalities during fetal development in both mouse lines and on additional gene expression studies in HNF-6–deficient mice, it appears that the development of the biliary system is controlled by a HNF-6 βHNF-1β cascade.[28]

In a different set of experiments, double heterozygozity for the *Jag1* and *Notch2* genes resulted in paucity of intrahepatic ducts in mice, which exhibited some of the cardinal phenotypic features observed in patients with Alagille syndrome.[29–31] Jag1-Notch signaling pathways define a fundamental mechanism controlling cell fate during embryogenesis by modifying the ability of a broad spectrum of precursor cells to progress to a more differentiated state. Although the developmental abnormalities reported for heterozygous *Jag1/Notch2* mice are not similar to those of infants with biliary atresia, the *Jag1* gene may be a modifier of liver disease, as demonstrated by the identification of a high frequency of single-nucleotide polymorphisms of the *Jag1* gene in infants with biliary atresia with poor outcome.[32] Taken together, these data suggest that genetic factors governing morphogenesis of the biliary system may play an important role in development and/or progression of liver disease in biliary atresia.

Evidence for Defective Fetal/Prenatal Circulation

Early interruption to flow in the hepatic artery, which supplies the intra- and extrahepatic biliary system, has been proposed to be an initiating factor in the fibroinflammatory injury of biliary atresia. This is an attractive concept based on the presence of hepatic artery and portal vein abnormalities associated with biliary atresia and the arterial hyperplasia and hypertrophy described in liver specimens of affected infants.[33] Additional data from humans or the development of experimental models to study the impact of blood flow on biliary development are necessary to further validate a potential role of impaired circulation in the pathogenesis of biliary atresia.

Evidence for Environmental Toxin Exposure

To date, the only supportive patient-based data for a role of a toxic insult as a causative factor of biliary atresia are the time-space clustering of cases. In animals, unusual outbreaks of hepatobiliary injury in lambs and calves in New South Wales, Australia, occurred in 1964 and 1988, with pathologic specimens displaying features akin to the pathology seen in humans with biliary atresia. Despite the localized geographic distribution of the outbreaks, an extensive investigation for causative phytotoxins or mycotoxins was unrevealing.[34]

Evidence for Viral Infection

Another environmental factor that may play an important initiating role in biliary atresia is viral infection. The possible role of viral infection as an initiating event in the pathogenesis of biliary atresia was initially suggested by Landing, who viewed biliary atresia as existing along a continuum with choledochal cyst and neonatal intrahepatic cholestasis, which could be linked by a shared infectious insult.[35] Consistent with this concept, different viruses have been detected in the liver of infants with biliary atresia sporadically. For example, hepatitis B virus antigens were detected in the liver of infants with biliary atresia in Japan, but these findings were not reproduced in the United States.[36,37] Likewise, there is little evidence to support the role of hepatitis A or C virus in spite of histologic findings suggesting the presence of non-A, non-B viruses in the liver.[37–39] Subsequent reports using a variety of techniques and substrates for viral detection have implicated cytomegalovirus, retrovirus, human papillomavirus, human herpesvirus 6, reovirus, and rotavirus in specific groups of patients not only with biliary atresia but also with neonatal intrahepatic cholestasis and choledochal cyst.[40–50] The etiologic role for a single agent, however, has not been supported owing to the inability to reproduce the association in other patient populations.[50–54] Nevertheless, among these viruses, reovirus type 3 and rotavirus type C continue to emerge as potential triggering agents for biliary atresia.

Prevalence of antibodies against reovirus type 3 and the detection of the virus in hepatobiliary specimens of patients with biliary atresia have varied according to patient population and laboratory techniques. This is demonstrated by a high prevalence of immunoglobulins G and M to reovirus in infants with biliary atresia,[47–49,55] but at least two studies could not find such an association.[52,56] More recently, the use of virus-specific amplification by reverse transcription–polymerase chain reaction identified reovirus in hepatobiliary samples of 55% of patients with biliary atresia and 78% with choledochal cyst, whereas the virus was present in tissues of only 8 to 21% of appropriately matched controls.[44] The putative association between reovirus and biliary atresia was initially suspected based on studies in young mice, which showed that reovirus infection in the weanling period resulted in the "oily fur syndrome," which is marked by growth failure, jaundice, and oily fur. Histologically, reovirus induced hepatitis and intra- and extrahepatic biliary epithelial necrosis with surrounding edema and inflammation.[57–59] With repeated intraperitoneal injections, weanling mice develop fibrosis of the extrahepatic biliary tree but do not progress to irreversible luminal obstruction.[57]

Administration of rhesus rotavirus type A to newborn mice orally or intraperitoneally produces a notable phenotype resembling biliary atresia, with progressive jaundice, acholic stools, bilirubinuria, and growth failure, eventually culminating in death in many infected animals.[60–62] The histologic appearance of the liver and biliary tree is remarkably similar to that seen in biliary atresia, with inflammation and edema of the intra- and extrahepatic bile ducts 7 days after infection, progressing to sloughing of the biliary epithelium, concentric fibrosis of the extrahepatic bile ducts, and segmental or continuous obstruction of the extrahepatic ductal lumen by cellular debris and inflammatory cells. The precise mechanisms of virus-induced injury have not yet been established, but studies on the potential tropism of the virus to cholangiocytes, the role of inflammatory cells in biliary injury, and the identification of host factors that restrict disease susceptibility of biliary injury by rhesus rotavirus type A to only the immediate postnatal period may provide unique insight into the pathogenic mechanisms of biliary atresia in humans.[60–63]

Evidence for Inflammatory/Immunologic Dysregulation

Several patient-based studies suggest that the inflammation observed in the biliary system is not simply a biologic response to an as yet unidentified insult but rather that it may play a primary role in the targeted destruction of extrahepatic and intrahepatic bile ducts. For example, cholangiocyte pyknosis and necrosis have been associated with infiltration of mononuclear cells into the walls of interlobular bile ducts, as well as lymphocytic infiltration into portal tracts, the duct walls at the porta hepatis, and common hepatic duct remnants of infants with biliary atresia.[64–66] Phenotypic characterization of these inflammatory cells has identified CD8+ T cells infiltrating proliferated bile ducts, although the cells did not express perforin or granzyme B, markers of activated cytotoxic T lymphocytes.[67] In general, however, the lymphocytes infiltrating portal tracts in biliary atresia are CD4+ rather than CD8+ T cells. These cells express markers of T helper (Th) lymphocyte activation and proliferation, such as the interleukin (IL)-2 receptor CD25 and the transferrin receptor CD71.[68–70]

Th lymphocytes are broadly divided into two subtypes: Th1 cells, which regulate cell-mediated immunity, and Th2 cells, which regulate humoral immunity. To initiate a Th-mediated immune response, CD4+ T cells must encounter exogenous antigens complexed with a major histocompatibility complex (MHC) class II molecule on the surface of an antigen-presenting cell. In the context of biliary atresia, cholangiocytes, which normally express MHC class I but not class II antigens, are induced to aberrantly express human leukocyte antigen (HLA)-DR (a major MHC class II molecule) and act as antigen-presenting cells.[68,71,72] Furthermore, intercellular adhesion molecule 1 is expressed on the bile duct cells of patients with biliary atresia, whereas one of its ligands, leukocyte functional antigen 1, is expressed on infiltrating mononuclear cells.[70,73] Interaction between these two molecules is one of the mechanisms necessary for inflammatory cell recruitment and perpetuation of the immune response.

A potential role for Kupffer cells (resident hepatic macrophages) in promoting inflammation and fibrosis has been inferred from their increased number and size in the livers of infants with biliary atresia, as well as from their expression of the MHC class II antigen HLA-DR.[70,74,75] A potential pathway that would explain the interaction of Kupffer cells and lymphocytes in the pathogenesis of biliary atresia involves infiltration of portal tracts by CD14+ Kupf-

fer cells, which are induced to express IL-18, a proinflammatory cytokine that promotes interferon-γ production and Th1-differentiation of lymphocytes.[74,75] Further evidence to support a Th1 proinflammatory immunity in the pathogenesis of biliary atresia has been provided by large-scale gene expression analyses of liver tissue from infants with biliary atresia and neonatal intrahepatic cholestasis. This approach identified a genetic footprint in which genes involved in lymphocyte differentiation (including the regulators of Th1 response osteopontin and interferon-γ) are activated in the early stages of biliary atresia, with simultaneous but transient suppression of markers of humoral immunity.[76] Although circumstantial, these findings point to a potential functional synergism of inflammatory effectors in the development of biliary injury in infants with biliary atresia.

Proposed Model of Disease Pathogenesis

The five proposed mechanisms of disease reviewed above can be grouped into environmental factors (toxins and infections) and processes directly dependent on the host (defective morphogenesis, abnormal fetal/prenatal circulation, and immunologic dysregulation). Collectively, they can be unified in a "working model" in which a toxic or infectious insult to the hepatobiliary system triggers a normal reactive inflammatory response. This response effectively clears the insulting agent and, in the normal infant, stops the injury, repairs the tissue, and restores physiologic homeostasis. In the genetically susceptible infant, however, the injury is perpetuated by a preexisting developmental abnormality or by the predisposition to a proinflammatory differentiation of T lymphocytes, which targets the extrahepatic biliary tract (Figure 50.2-2). Injury to the biliary cells may secondarily result in accumulation of bile acids and activation of apoptosis, which may further exacerbate the tissue injury. As a consequence, the ductular epithelium is destroyed, fibrous material obliterates the lumen, and biliary drainage ceases. The fact that these processes occur in the first few weeks after birth suggests that developmental forces play a key role in disease onset and/or progression.

It remains possible that in a very small number of patients, the biliary injury does not completely destroy the biliary epithelium and allows for the development of different phenotypes: choledochal cyst and chronic hepatitis. This setting is consistent with the model of "infantile obstructive cholangiopathy" proposed by Landing in 1974.[35] Landing's theory was based on several premises. First, biliary atresia is not predominantly a congenital malformation but is probably an acquired obliterative process. Second, biliary atresia displays histopathologic features often found in livers of infants with intrahepatic cholestasis (idiopathic neonatal hepatitis), such as giant cell transformation of hepatocytes and variable degrees of lobular hepatitis. Third, the basic process leading to biliary atresia is inflammatory/immunologic in nature, which is common not only to intrahepatic cholestasis but also to choledochal cyst. Fourth, the inflammation present in choledochal cyst may act in a different manner than in biliary atresia, weakening the duct wall and allowing for aneurysmal dilatation of the affected portion of the duct rather than obliteration of the ductal lumen. Although highly speculative, this theory remains attractive, at least in part, and might be corroborated by recent data from the rotavirus-induced model of biliary injury in neonatal mice. Anatomic analysis of the extrahepatic biliary system in these mice clearly demonstrates a spectrum of hepatobiliary abnormalities, including intrahepatic changes of reactive necrosis and proliferation of small bile ducts, obstruction of the extrahepatic biliary system, and cystic dilatation resembling choledochal cyst.[77]

We have been able to independently reproduce the findings of rotavirus-induced injury in our laboratory. In one set of experiments, intraperitoneal administration of rotavirus type A on the first day of life resulted in biliary injury affecting predominantly the extrahepatic bile ducts, with segmental stenosis, focal dilatation, or complete atresia in 24 of 27 of the mice (Campbell and Bezerra, unpublished data, July 2003). One of the other three mice had a large cystic dilatation of the common bile duct resembling a choledochal cyst and the other two had resolution of acholic stools but continued with jaundice and bilirubinuria. Notably, histopathologic examination of the liver of these two mice with partial improvement of symptoms showed lymphocytic infiltrate in the portal tracts and extensive fibrosis. These data support the concept that one single agent (in this case, rotavirus) induces

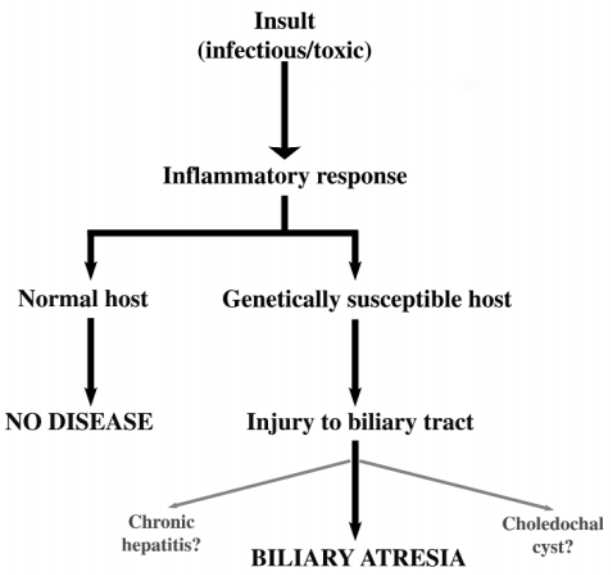

FIGURE 50.2-2 Proposed pathogenic model for biliary atresia. In this model, an infectious or toxic insult to the hepatobiliary system triggers a normal inflammatory response, which results in the clearance of the insulting agent, tissue repair, and restoration of biliary drainage in normal infants. Although the insulting agent may also be cleared in the genetically susceptible infant, a developmental abnormality of the bile ducts and/or a predisposition to a proinflammatory response lead to an ongoing injury that results in the obstruction of the extrahepatic bile ducts by fibrosis (biliary atresia). This model also depicts the potential development of chronic hepatitis or choledochal cyst, rather than biliary atresia, in a very small number of susceptible infants, in whom the injury/inflammation leads to an ongoing injury of intrahepatic bile ducts (chronic hepatitis) or the aneurysmal dilatation of a weak extrahepatic bile duct (choledochal cyst).

a primary phenotype (fibroinflammatory obstruction of the biliary tree) and two secondary phenotypes (choledochal cyst and chronic hepatitis) (see Figure 50.2-2). The use of this animal model and of other in vivo and in vitro models may provide unique insight into the interplay of different factors triggering the biliary injury, modulating the proinflammatory response, and perpetuating disease progression.

DIAGNOSTIC APPROACH

Proper and prompt identification of biliary atresia in the neonate with cholestasis is a high priority because the success of portoenterostomy to restore biliary flow rapidly declines with age.[78–80] Yet the lack of disease-specific clinical signs and laboratory tests makes it difficult to reliably distinguish between biliary atresia, other causes of extrahepatic obstruction, and intrahepatic cholestasis (Table 50.2-4). Therefore, the clinician is challenged to develop a diagnostic algorithm that incorporates ancillary tests with high predictive value for biliary atresia; the approach should be tailored to make full use of center-specific expertise. Our approach to any neonate with cholestasis obeys three diagnostic priorities. First, we establish whether jaundice is due to impaired excretion of conjugated or direct bilirubin. In practice, it is difficult to precisely define the onset of jaundice in the neonate because physiologic and breast milk jaundice may overlap with early phases of pathologic jaundice, defined as serum conjugated or direct bilirubin > 20% of total bilirubin and > 2 mg/dL. Thus, if the neonate is jaundiced beyond 2 weeks, fractionation of serum bilirubin is warranted. Identification of unconjugated hyperbilirubinemia most often points to benign processes, whereas high levels of serum conjugated bilirubin reveal the first clue to an underlying hepatobiliary pathology.

Second, we search for any evidence of systemic or metabolic diseases that may present with neonatal cholestasis and that require immediate treatment. For example, septicemia, galactosemia, and panhypopituitarism are disorders that may present with jaundice and require targeted therapeutics (antibiotics, avoidance of lactose-containing formulas, and thyroid hormone replacement, respectively) to improve survival and optimize long-term outcome. Third, we focus on those diagnostic tools that enable us to differentiate biliary atresia from other causes of neonatal jaundice in a timely fashion. In this context, it must be kept in mind that the most accurate diagnosis derives from careful histopathology combined with intraoperative examination of the extrahepatic ductular system.

HISTORY AND PHYSICAL EXAMINATION

Infants with biliary atresia typically present with jaundice between 3 and 8 weeks of age. They often appear well and develop acholic stools, but in the early stages of disease, the stools may have some bile pigment. Family history of cholestasis is almost always negative in biliary atresia, whereas the history may be positive in 15 to 20% of the cases of intrahepatic cholestasis.[4,81,82] Variable levels of lethargy, emesis, and abdominal distention and a history of bleeding or seizures often reflect coagulopathy secondary to vitamin K deficiency or potentially devastating nonhepatic conditions, such as intestinal volvulus owing to malrotation. On physical examination, hepatosplenomegaly is frequently present; nodular liver surface, prominent splenomegaly, digital clubbing, and arterial desaturation may be seen with advanced disease. Notably, the findings of a liver that is mostly palpable at midline (below the xyphoid process), pathologic murmurs, and dextrocardia in the setting of neonatal cholestasis are highly indicative of biliary atresia, specifically the embryonic form.

Biochemical Analysis

Routine indicators of liver function and injury are helpful but not diagnostic. Although some infants may have prolonged prothrombin time, normalization of coagulopathy following administration of vitamin K and serum levels of albumin above 3 g/dL indicate normal synthetic function. The usual markers of hepatocellular injury, serum alanine and aspartate aminotransferases, are mildly to moderately elevated, whereas the serum levels of alkaline phosphatase and γ-glutamyl transpeptidase (γ-GTP) progressively

TABLE 50.2-4 INTRA- AND EXTRAHEPATIC DISEASES THAT MAY PRESENT IN THE NEONATAL PERIOD AND LEAD TO CHRONIC CHOLESTASIS

INTRAHEPATIC CHOLESTASIS*	EXTRAHEPATIC CHOLESTASIS
α_1-Antitrypsin deficiency	Biliary atresia
Alagille syndrome	Choledochal cyst
Transport defects	Type I: cystic dilatation of the common bile duct
Chronic cholestasis owing to *FIC* mutations	Type II: diverticulum of common duct and/or gallbladder
Chronic cholestasis owing to *BSEP* mutations	Type III: choledochocele
MDR3 deficiency	Type IV: multiple cysts
Cystic fibrosis	Type V: fusiform intrahepatic dilatations (variant of Caroli disease?)
Defects in bile acid synthesis	Spontaneous perforation of the common bile duct
Hypopituitarism/hypothyroidism	Neonatal sclerosing cholangitis
	Biliary sludge and cholelithiasis
	Acalculous gallbladder disease†

BSEP = bile salt export pump gene; *FIC* = familial intrahepatic cholestasis type 1 gene; MDR3 = multidrug resistance protein type 3.
*Detailed differential diagnosis for intrahepatic cholestasis is discussed elsewhere in this book.
†Uncommon in the neonatal period.

increase, indicating more profound biliary injury. γ-GTP levels may have some discriminatory value, with low γ-GTP rarely seen in infants with biliary atresia.[83–85] If infants are evaluated before 4 months of age, conjugated or direct hyperbilirubinemia is present but rarely above 7 mg/dL, despite the existence of an obliterative fibrosis and severe impairment to biliary drainage.

Radiologic Studies and Procedures

A sonographic examination of the upper abdomen is particularly useful in the search for potential causes of anatomic obstruction or cystic abnormalities of the biliary system, such as choledochal cyst, and to survey for congenital malformations, such as midline liver, polysplenia, and vascular malformations. Absence of the gallbladder suggests biliary atresia, but its presence does not rule it out. Examining the hilar structures, the ultrasonographic appearance of a "triangular cord" is suggestive of biliary atresia, with a negative predictive value of over 95% for intrahepatic cholestasis ("neonatal hepatitis").[86,87] This finding corresponds to the fibrous cone of tissue at the bifurcation of the portal vein and is often not present in infants with intrahepatic cholestasis.[86] An evolving technology with a great potential to identify the ductular structures is magnetic resonance cholangiography, but its value is largely unproven to date.[88,89] Despite remarkable improvements in sonographic and magnetic resonance cholangiographic techniques, the main limitation resides in the inability to directly visualize discontinuity of extrahepatic bile ducts with available imaging tools. This can potentially be established using hepatobiliary scintigraphy, a nuclear medicine scan that measures hepatic uptake and excretion of analogs of iminodiacetic acid into the intestine. In infants with intrahepatic cholestasis, uptake is delayed secondary to impaired hepatic function but excretion is not impaired, whereas prompt uptake is not followed by excretion into the duodenum in neonates with biliary atresia. To increase the discriminatory value of the test, phenobarbital may be given at a dose of 5 mg/kg/d for 5 days prior to the study to enhance hepatic uptake and excretion through the biliary system.[90] Despite the potential value of hepatic scintigraphy in establishing patency of the biliary system, the time required for the test may significantly delay the diagnosis.

Aspiration of duodenal fluid is another indirect measure of extrahepatic bile duct patency. If bile-stained fluid is present in a 24-hour collection of duodenal contents, biliary atresia is unlikely.[91,92] A more direct examination can be obtained by endoscopic retrograde cholangiography, which is very useful in adults to determine the patency of extrahepatic ducts but requires an experienced endoscopist for careful examination of the neonate.[93–96]

The combination of specific clinical features and ancillary tests/procedures discussed above may offer a high likelihood of an accurate diagnosis. This was documented in a retrospective review of 288 infants presenting with jaundice before 3 months of age that reported lower birth weight, later onset of jaundice, later onset of acholic stools, and the appearance of pigmented stools within 10 days after admission occurring more frequently in infants with intrahepatic cholestasis rather than in those with biliary atresia.[97] Because of the limited predictive value of scoring systems, however, caution must be exercised and more definitive diagnostic modalities must be pursued because in approximately 10% of infants, intrahepatic disease cannot be reliably distinguished from biliary atresia.[98]

Histopathology

Microscopic examination of a liver biopsy sample is a critical component of the diagnostic approach to the neonate with cholestasis. The initial biopsy is obtained percutaneously and typically shows preservation of basic lobular organization in infants with biliary atresia, with prominent abnormalities in the portal tracts and, to a lesser extent, in the lobule. Portal tracts are expanded by variable levels of edema, proliferation of bile ducts, and fibrosis (Figure 50.2-3). When present, bile plugs within proliferated ducts are highly suggestive of biliary atresia but occur in only about half of the biopsies. Inflammation in the portal space and giant cell transformation of hepatocytes may be seen but are not the dominant features and are more commonly seen in other causes of intrahepatic cholestasis.[99] Canalicular cholestasis, lobular disarray, and extramedullary hematopoiesis do not have discriminatory value between biliary atresia and other causes of neonatal cholestasis. In very young infants, the initial liver biopsy may be inconclusive, in which case, a repeat liver biopsy in 1 to 3 weeks may be necessary before the diagnosis is fully established. Whether these cases represent the sampling error inherent in percutaneous biopsies or the true progression of disease remains to be determined.

Exploratory Laparotomy with Cholangiography

When histopathologic features are suggestive of biliary atresia or the diagnostic workup is inconclusive, exploratory laparotomy must be performed in a timely manner. During laparotomy, direct inspection of the gallbladder and ductular system is the best approach to (1) determine if the ductular system is obstructed, (2) define the site of obstruction, and (3) create a conduit to re-establish biliary drainage. In most cases of biliary atresia, the gallbladder is small and fibrotic, along with diffuse fibrosis of the extrahepatic system extending to or above the level of the porta hepatis. If the gallbladder has a lumen, it may be filled with mucoid clear secretions.

For the cholangiogram, a needle or catheter is inserted into the gallbladder so that diluted contrast material can be injected to document the extent of obstruction and the anatomic variants of extrahepatic disease. These anatomic variants have been proposed by the Japanese Society of Pediatric Surgeons[100] and consist of three main types: type 1, atresia involving primarily the common bile duct; type 2, atresia extending up to the common hepatic duct; and type 3, atresia involving the whole extrahepatic ductular system (Figure 50.2-4). Sometimes a cholangiogram may not be possible owing to extensive fibrosis of the gallbladder or because of an absence of the biliary tree, a condition that has been termed "biliary agenesis." If the contrast delineates the cystic and

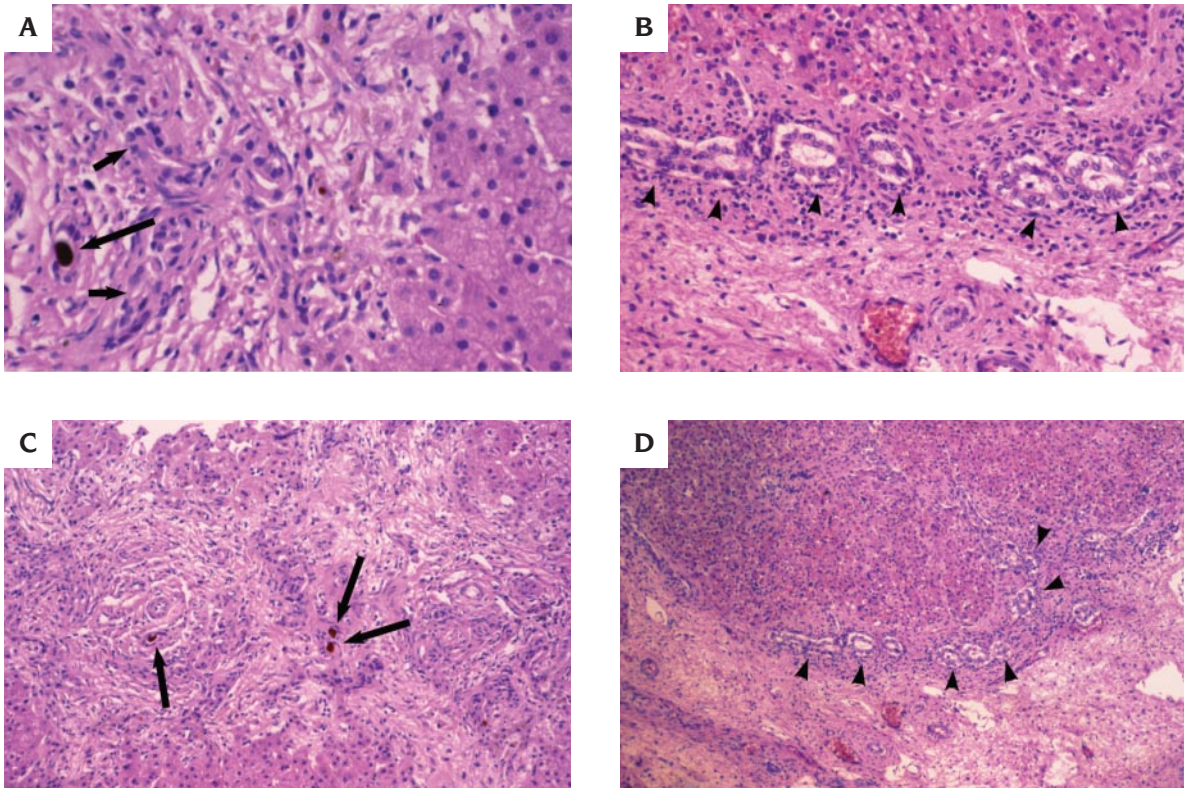

FIGURE 50.2-3 Hematoxylin and eosin staining of liver sections from infants with biliary atresia. *A* and *C* show edema, fibrosis, bile duct proliferation (*short arrows*, *A*) and a bile plug (*long arrow*, *A*) in the liver of an infant with the perinatal form of biliary atresia. In addition to edema and fibrosis, *B* and *D* also show ductal plate malformation (*arrowheads*) in an infant with the embryonic form of biliary atresia. *A* and *B* = ×200 original magnification; *C* and *D* = ×100 original magnification. Courtesy of Dr. Kevin Bove, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.

common bile ducts but fails to show the patency of the hepatic and intrahepatic ducts, gentle and transient clamping of the common duct may be necessary to direct the contract upward, which may enable better filling and visualization of the proximal ductular system by the contrast. Poor filling of intrahepatic bile ducts should be interpreted with caution to avoid the creation of portoenterostomy in patients without biliary atresia. In at least one series, four patients underwent portoenterostomy after inadequate filling of intrahepatic bile ducts at intraoperative cholangiography and were later found to have the phenotypic features of Alagille syndrome.[101] In these patients, portoenterostomy may lead to biliary cirrhosis and a worse prognosis.

Histologically, the extrahepatic biliary remnants show the most typical finding of complete fibrous obliteration of the bile duct at one or more levels (Figure 50.2-5). In some patients, small single-lumen ducts that are present in biliary remnants close to the hilum may show active cholangitis. In other patients, the main duct may be reduced to smaller channels surrounded by moderate chronic inflammatory infiltrate. Although the histologic examination of the biliary structures is essential for a definitive diagnosis, the decision to proceed with portoenterostomy is not delayed until the histologic examination is completed; instead, it is made at the time of laparotomy and is based on data obtained prior to surgery, visual inspection of the biliary tree, and the results of the cholangiogram.

TREATMENT

No medical therapy has been developed to date that effectively halts or reverses cholestasis and hepatic injury in children with biliary atresia. The only therapeutic choice to increase biliary flow and improve jaundice is the portoenterostomy. Prior to the development of this surgical procedure, the mortality from biliary atresia was virtually 100%. Early attempts to relieve obstruction were limited to infants with the "correctable" forms, in which only the common bile duct was obstructed, with patent ducts proximally (type 1 of Figure 50.2-4). In the 1950s, Kasai and Suzuki noted that minute, patent bile duct remnants were present in the fibrous tissue at the porta hepatis. They reasoned that dissection into the liver parenchyma would allow drainage of bile through these ducts, whereas failure to re-establish biliary flow would lead to progressive intrahepatic ductal obliteration. This led to the development of the Kasai portoenterostomy, in which the fibrotic extrahepatic bile ducts are completely excised and an intestinal conduit is anastomosed to the transected surface of the porta hepatis in a Roux-en-Y fashion.[102]

PORTOENTEROSTOMY

Although there have been numerous modifications to the original procedure of portoenterostomy described by Kasai, the concept remains the same: to bypass the fibrotic extra-

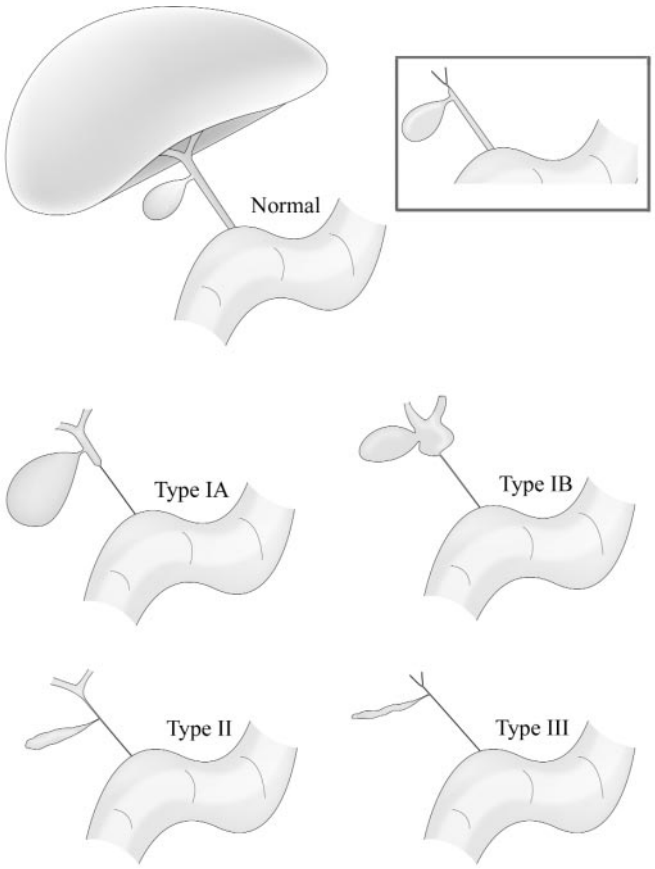

FIGURE 50.2-4 Anatomic variants of the extrahepatic biliary system in infants with biliary atresia. In type 1, the main site of obliteration is the common bile duct, which extends to the hepatic duct in type 2. Type 3, the most common variant, has complete obliteration of the entire biliary system. In the *inset*, a patent common bile duct and gallbladder may be used in lieu of an intestinal conduit to improve biliary drainage by anastomosis of the gallbladder to the hilum ("gallbladder Kasai").

hepatic biliary tree and re-establish bile drainage through intrahepatic ducts that remain patent. On entry into the abdominal cavity, the liver is firm to the touch and appears brown-green, with varying degrees of subcapsular telangiectasia. The liver and intestine are mobilized to expose the ductular and vascular structures, and the gallbladder and extrahepatic ducts are examined. Hilar structures may be edematous in the early phases of disease but are likely fibrotic and more difficult to identify in older infants.

If careful inspection of ductular structures and cholangiography are diagnostic of biliary atresia, the (fibrotic) common bile duct is transected just above the duodenal margin. Next, the gallbladder is mobilized from its hepatic bed, and the bile duct is dissected away from the anterior portal vein wall. Careful dissection toward the hilum is guided by the principle that fibrotic ducts follow the normal biliary position within the portal triad. Therefore, the dissection of the fibrous triangular mass proceeds toward the level of the liver capsule, where the fibrous remnants are transected at the level of the portal vein bifurcation on the right and the umbilical point of the portal vein on the left. Dissection deeper into the hepatic parenchyma has not been shown to significantly increase biliary drainage. At this time, Roux-en-Y anastomosis is achieved with a 35 to 40 cm isoperistaltic retrocolic jejunal limb, which extends from the hilum to the most proximal portion of the jejunum. The Roux-hilar anastomosis is constructed just outside the margin of the hilum where the tissue has been dissected, using absorbable monofilament suture material to avoid the formation of a nidus for future infection. Distally, the intestinal conduit is anastomosed to the proximal jejunum so that biliary drainage optimizes nutrient digestion and absorption. For a small number of infants with anatomic variants of atresia in which there is a patent gallbladder and distal ducts, a "gallbladder Kasai" may be performed (see Figure 50.2-4). In this procedure, the gallbladder is mobilized from its fossa (maintaining intact the cystic artery), the fundus of the gallbladder is transected, and the opening is sutured to the biliary hilum (rather than the use of an intestinal segment). Although logical, this approach may not allow for efficient long-term biliary drainage owing to a potential dysfunction or subsequent obliteration of the common duct.

Medical Treatment following Portoenterostomy

The goals of postoperative management of infants with biliary atresia are threefold: (1) prevention of cholangitis, (2) stimulation of choleresis, and (3) nutritional support. Infants typically receive parenteral broad-spectrum antibiotics for 3 to 5 days postoperatively, followed by oral prophylaxis with trimethoprim-sulfamethoxazole (TMP-SMX; 5 mg TMP/kg/d) or another antibiotic initiated after the patient resumes oral feedings and continued for a variable period of time (3–12 months).[100] Studies do not conclusively demonstrate the efficacy of this approach, but at least one open-label study reported lower recurrence rates of cholangitis when infants received prophylaxis with either TMP-SMX or neomycin.[103]

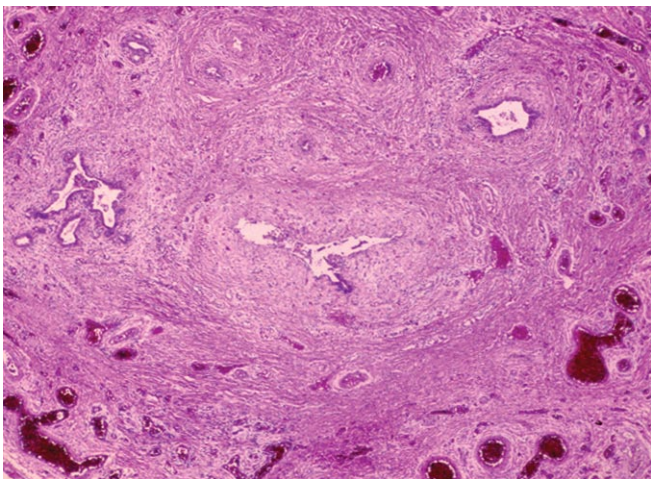

FIGURE 50.2-5 Photomicrograph of a remnant of the extrahepatic bile duct showing marked fibrosis, minute ductules, and vascular channels in a surgical specimen from an infant with biliary atresia. Courtesy of Dr. Kevin Bove, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.

Choleretic and Immunomodulatory Agents. Ursodeoxycholic acid (UDCA; 10–20 mg/kg/d) has been used by many centers to improve choleresis; however, very few studies have documented the efficacy of this approach. In two of these studies, use of UDCA was associated with improved weight gain and decreased levels of serum bilirubin and bile acids in more than half of the patients.[104,105] A similar trend toward improved weight gain, a decrease in the levels of serum aminotransferases and bilirubin, and a reduction in the degree of pruritus has also been reported in infants receiving UDCA, but there were no obvious differences in long-term survival or the need for transplant between treated and nontreated patients.[106]

Corticosteroids are another class of drugs used postoperatively in many centers. At pharmacologic doses, corticosteroids stimulate bile salt–independent bile flow by inducing expression of hepatic Na-K adenosine triphosphatase, a sinusoidal transporter that helps maintain the osmotic and electrical forces necessary for bile formation.[107] In addition, corticosteroids have well-described anti-inflammatory and immunomodulatory properties, including inhibition of leukocyte infiltration and down-regulation of inflammatory mediators, which may lead to decreased hilar edema and scar formation. Dosing regimens and duration of treatment with corticosteroids vary remarkably among published reports. In two early reports, administration of methylprednisolone or prednisolone either the week after portoenterostomy or later in the postoperative course when the infant developed jaundice (3 months to 4 years after surgery) induced a decrease in the levels of serum bilirubin in at least 50% of the patients.[108,109] More recently, two studies in which corticosteroid therapy was initiated soon after portoenterostomy reported improved clinical outcome, as defined by clearance of jaundice and increased survival with native liver, in over 70% of patients.[110,111] Unfortunately, these studies are limited by the use of varying dosing regimens, different indications and timing of treatment, lack of appropriate randomization, and small cohorts of patients. Despite these limitations, the results are promising and provide the basis for future multicenter randomized controlled clinical trials to objectively determine the role of corticosteroids in the treatment of infants following portoenterostomy.

Nutritional Support. Plans for adequate nutritional management should begin at the time of diagnosis and should follow the same general principles for patients with chronic cholestasis: caloric intake to meet energy needs and promote growth and supplementation with fat-soluble vitamins. Serial weight- and height-for-age and weight-for-height measurements are helpful but should be interpreted carefully if the infant develops significant hepatosplenomegaly or ascites. In this setting, high percentiles may derive from organomegaly or fluid retention rather than adequate nutrition. Anthropometrics with triceps and subscapular skin fold thickness may offer more reliable indices. Infants should receive approximately 125% of the Recommended Dietary Allowance based on weight for height at the 50th percentile, with additional calories often needed if biliary drainage is marginal (Table 50.2-5). Breastfeeding should be continued if it fosters adequate weight gain; if weight gain is inadequate or the infant is formula-fed, the use of formulas enriched with medium-chain triglycerides, which are relatively water soluble and directly absorbed into the portal circulation, will increase the pool of available energy and minimize steatorrhea. Intestinal absorption of fat-soluble vitamins A, D, E, and K is strongly dependent on adequate intraluminal concentration of bile acids. Therefore, if cholestasis is present, infants require supplementation and close monitoring to prevent the consequences of vitamin deficiencies. Unfortunately, malnutrition and progressive coagulopathy frequently develop if cholestasis is severe, and liver disease progresses despite adequate nutritional support.

COMPLICATIONS AND SEQUELAE

Most infants with biliary atresia have successful bile drainage after portoenterostomy, but adequate bile flow may be transient. If bile drainage is not achieved, progressive cholestasis and rapid progression to end-stage liver disease are the rule, at which time, orthotopic liver transplant offers the only chance for long-term survival. In the remaining majority of the patients, the clinical course is variable and is often marked by episodes of cholangitis and progressive accumulation of components of the extracellular matrix, which leads to sinusoidal obstruction and portal hypertension.

Ascending Cholangitis

Cholangitis is the rule rather than the exception in infants with successful biliary drainage after portoenterostomy, with an incidence ranging from 40 to 60%.[100,112] Although the pathogenesis is not well defined, clinical observations point to an interplay between the patency of biliary chan-

TABLE 50.2-5 NUTRITIONAL MANAGEMENT OF INFANTS WITH BILIARY ATRESIA

NUTRIENT	TREATMENT OPTIONS	CONSEQUENCES OF DEFICIT
Energy	125% of RDA based on 50th percentile weight for height; protein: 2–3 g/kg/d; lipid: MCT enriched (~ 50% of fat calories)	Malnutrition*
Vitamin A	5,000–25,000 IU/d of water-miscible preparation of vitamin A	Punctate keratopathy[155]
Vitamin D	3–10× RDA for age; 3–5 μg/kg/d as 25-hydroxyvitamin D if rickets is present	Rickets, osteomalacia
Vitamin E	15–25 IU/kg/d TPGS	Neurologic dysfunction
Vitamin K	2.5 mg/twice a week to 5.0 mg/d	Coagulopathy

MCT = medium-chain triglyceride; RDA = Recommended Dietary Allowance; TPGS = *d*-α-tocopheryl polyethylene glycol-1000 succinate.
*Malnutrition may develop in patients with chronic cholestasis and progressive liver disease despite high caloric intake.

nels and enteric pathogens in the development of cholangitis. Pathogens have been proposed to ascend through the intestinal biliary conduit at the porta hepatis, translocate from other intestinal segments, or result from overgrowth favored by bile stasis that may exist in the intestinal conduit regardless of the type of surgical approach.[113–116] One strategy of unproven efficacy, but in widespread use, is the prophylactic administration of TMP-SMX for variable lengths of time during the first year of life to prevent cholangitis. Surgical modifications of the Roux-en-Y, such as partially diverted stoma for decompression of biliary conduit or valve formation in the conduit, have also been employed but have not consistently decreased the incidence of cholangitis.[100] It remains undefined whether the potential immunologic dysfunction that may play a role in disease pathogenesis also makes infants more susceptible to infectious cholangitis.

Typically, infants with cholangitis present with the triad of fever, acholic stools, and increased levels of serum bilirubin. These symptoms can also occur in isolation; therefore, the clinician must keep a high degree of suspicion so that prompt diagnosis is made and antibiotic therapy is initiated. In this context, ascending cholangitis should be suspected following portoenterostomy in any infant with irritability and poor feeding or who suddenly develops fever without an obvious source of infection; acholic stools and jaundice may lag behind by 24 hours. Sudden onset of acholic stools and jaundice in an infant with initial drainage following portoenterostomy should also be considered highly suspicious for cholangitis, even in the absence of fever or additional symptoms. Biochemically, conjugated bilirubin rises and levels of serum aminotransferases, γ-GTP, and alkaline phosphatase may increase above baseline values; leukocytosis with or without immature cells (left shift) may also be present.

Once the presumptive diagnosis of cholangitis is made, blood cultures are obtained, and patients are treated with broad-spectrum intravenous antibiotics. If an organism is identified, dosage and choice of antibiotics should be adjusted based on antimicrobial sensitivity. Intravenous antibiotics are continued for 2 to 3 days after fever resolves. In the event that stools remain acholic and conjugated hyperbilirubinemia persists, a short course of high-dose corticosteroids may be helpful.[108] Surgical revision of the portoenterostomy should be considered when evidence of cholestasis persists despite medical treatment. The surgical approach is made through the anterior wall of the portoenterostomy, followed by débridement of the scarred areas at the porta hepatis. This approach is successful in more than half of patients when they have a history of initial bile flow after primary portoenterostomy, a favorable hepatic histology, and biliary ductal remnants at initial operation or when there is a suspicion of mechanical obstruction in the intestinal conduit.[79,117,118] If revision does not result in improved biliary flow, repeated attempts are discouraged. Repeated revisions and recurrent episodes of bacterial peritonitis may produce perihepatic and intraperitoneal adhesions, which significantly complicate the surgical approach during liver transplant.

Portal Hypertension

In addition to the fibro-obliterative changes in the extrahepatic ducts, variable degrees of intrahepatic fibrosis and portal hypertension are also present at the time of diagnosis of biliary atresia.[119] This fibrosis progresses even in the infants with improved biliary flow[120]; portal pressure gradually increases, and significant portal hypertension develops in 34 to 76% of infants with biliary atresia.[119,121] The clinical consequences of portal hypertension are variable, often resulting in the development of esophageal varices. Interestingly, portal hypertension may resolve spontaneously in older patients. Therefore, the initial approach to portal hypertension in infants with biliary atresia is directed at preventing and treating complications, such as gastrointestinal hemorrhage. A study of a single center's experience in Japan reported esophageal varices in 25% and hypersplenism in 14% of 106 children with biliary atresia without jaundice.[100] Notably, esophageal bleeding occurs in approximately 19% of all long-term survivors[122] and at a higher rate (20–60%) when studies include only those children with esophageal varices.[123]

Primary therapy for children with biliary atresia and hemorrhage from esophageal varices is endoscopic injection sclerotherapy or variceal band ligation.[124–127] Before endoscopy, the patient should be monitored closely, be evaluated for coagulopathy, and receive an infusion of packed red blood cells to restore normal blood pressure. Packed red blood cells should be transfused slowly to avoid sudden expansion of intravascular volume and a new onset of variceal hemorrhage. Continuous infusion of somastomatin analogs to decrease the severity of the bleeding may be particularly valuable to stabilize the patient in preparation for endoscopy. In the setting of active bleeding or in small infants, sclerotherapy is the treatment of choice.

β-Blockers represent another therapeutic option for primary prophylaxis or after an episode of variceal hemorrhage (secondary prophylaxis). Although their use is well accepted in adults, there are few data regarding the efficacy of β-blockers in children. In the one published study to date, 6 of 17 children subjected to primary prophylaxis with propanolol had variceal hemorrhage, whereas 1 of 4 patients receiving secondary prophylaxis bled.[128] In the absence of a control group, it is unclear whether this represents a decrease in the incidence of variceal hemorrhage. Likewise, questions regarding optimum dosage and dosing schedule in children also need to be addressed prospectively. For those patients with recurrent or severe hemorrhage, the utility of the traditional portal shunt (with or without esophageal transection) and transjugular intrahepatic portosystemic shunt should be considered. Decisions regarding these therapies should be made on an individual basis and take into account the available expertise in the medical center. The best therapeutic choice for these patients is timely liver transplant if a suitable organ is available. This is particularly relevant based on recent data suggesting that the outcome of children following the first episode of variceal hemorrhage is related to the coexisting degree of hyperbilirubinemia, with a 12-fold increase in the risk of death or need for transplant if the

serum total bilirubin is > 10 mg/dL versus a 0.6-fold risk when the serum bilirubin was ≤ 4 mg/dL, when compared with children with biliary atresia and no history of variceal hemorrhage.[129]

Other Complications

Hypersplenism is another complication in long-term survivors and is especially problematic in those infants with significant thrombocytopenia and a history of bleeding from esophageal varices. If hypersplenism significantly worsens coagulopathy, splenic embolization has been shown to result in improved blood cell count and hemorrhagic tendency in 60 to 70% of patients, but high fever and abdominal pain are universal complications.[100] Another potential complication in long-term survivors is the development of the hepatopulmonary syndrome, which results from intrapulmonary vascular dilatation with shunting. These patients typically have exercise intolerance, digital clubbing, and cutaneous spider telangiectasia. Currently, no medical therapy alleviates hepatopulmonary syndrome; a definitive cure can be achieved by liver transplant, although children with the syndrome may be more susceptible to complications.[130,131] Lastly, children with biliary atresia may rarely develop cystic dilatation of intrahepatic bile ducts or hepatocellular carcinoma.[132–134]

LONG-TERM OUTCOME

Most patients with biliary atresia have improved biliary drainage following portoenterostomy. Long-term outcome, however, may be better predicted by the serum level of bilirubin 3 months after surgery. In those infants with serum bilirubin levels below 1 mg/dL 3 months after portoenterostomy, 53% had normal growth, no esophageal varix, or hyperplenism at 12 or more years after portoenterostomy.[135] Although the precise mechanisms regulating progression of liver disease are not well defined, ongoing cholestasis, intrahepatic cholangiopathy, persistent inflammatory processes, and progressive fibrosis appear to be important factors.[118,136–139] Specific markers for disease progression and predictors of long-term outcome may emerge from studies of the pathogenesis of biliary atresia. For example, serial levels of serum hyaluronic acid and procollagen propeptides may be unique markers of hepatic fibrogenesis and disease progression.[140]

Actuarial survival with native liver has been estimated at 32 to 61% at 5 years and 27 to 54% at 10 years of life.[100,141,142] Among the several factors that may influence long-term outcome, age and the size of the ductules in biliary remnants at the time of portoenterostomy have been systematically examined. Collectively, studies point to better outcome when portoenterostomy is performed in infants ≤ 60 days of age and when the size of ductules within the biliary remnant measures ≥ 150 μm (Table 50.2-6). The patient's age at operation alone, however, may not be a reliable predictor of outcome, as suggested by a study of infants who underwent portoenterostomy before 30 days of age. In this group, 78% of the patients required liver transplant at a mean age of 6.8 ± 2.3 months.[19] This outcome may reflect a more severe form of disease that leads to early jaundice and early diagnosis of biliary atresia. Other factors that contribute to long-term outcome include episodes of cholangitis after surgery, the decade when surgery was performed, and the experience of the surgical team.[141,143] For example, at Tohoku University Hospital (Japan), actuarial survival of 307 patients treated with the Kasai portoenterostomy improved from ~ 20% before 1971 to ~ 70% between 1971 and 1998.[100] An effect of the "center experience" in achieving high success rates has been emphasized by an improved survival rate to 78% in centers in which a large number of portoenterostomies are performed by the surgical team.[6,144] As long-term survival increases, the quality of life in children with biliary atresia has become a focus of studies designed to improve outcome. In this context, 44 to 47 of 108 patients reported development of pruritus, reduced physical fitness, and the inability to attend school regularly.[143] These results clearly identify areas requiring additional support and therapeutic targets for long-term survivors.

LIVER TRANSPLANT

Liver transplant is a well-accepted treatment option for children with biliary atresia and end-stage liver disease. Access to transplant by a greater number of children has been driven by remarkable improvements in surgical techniques, which have led to the development of reduced-size and living-related donor transplant and to improved immunosuppression.[145–147] As a consequence, biliary atresia has become the most frequent indication for liver transplant in children.[118,148–151] For example, biliary atresia has been the primary indication for liver transplant at the Cincinnati Children's Hospital Medical Center (July 1985 to May 2003), accounting for 44% of 255 transplanted children. Although effective, the timely use of the technique in children with end-stage liver disease is significantly limited by an ever-increasing number of pediatric patients on transplant waiting lists and the scarcity of suitable organs. This is particularly important because the outcome in our center appears to be related to the severity of the patient's illness at the time of transplant.[148,152]

With limited organ availability, the clinician is challenged to identify those patients who may best benefit from liver transplant. In this context, the primary goal should be

TABLE 50.2-6 FACTORS INFLUENCING LONG-TERM OUTCOME OF INFANTS WITH BILIARY ATRESIA

FACTOR	% WITH BILE FLOW	REFERENCE
Age at the time of portoenterostomy		78, 100, 112
< 60 d	67–82	
60–90 d	45–62	
> 90 d	10–44	
Morphology of duct remnants		156
≥ 150 μm lumen size	92	
50–150 μm lumen size	81	
No identified epithelium-lined ducts	18	

to optimize the clinical status and quality of life of all children with their native liver and stay vigilant for the primary indications for transplant evaluation: (1) persistent cholestasis associated with severe malnutrition, growth failure, and hepatocellular dysfunction and (2) decompensated cirrhosis, as evidenced by intractable ascites and hemorrhage. For all children, the overall goals of transplant include restoration of hepatic function, improved nutritional status, adequate growth and development, and improved quality of life with full social reintegration.[149,153]

One additional indication for transplant that generates a significant debate is the use of transplant as the primary treatment modality for infants with biliary atresia who are diagnosed beyond 90 to 120 days of age and have established cirrhosis. Although portoenterostomy in this group has been associated with low incidence of improved biliary flow and poor long-term outcome, data analysis from a large cohort of patients showed a 5-year survival with native liver of ~ 31% when surgery was performed beyond 45 days of age in comparison with ~ 41% when portoenterostomy was performed before 45 days.[144] Therefore, portoenterostomy should remain the first line of therapy in infants with biliary atresia.[154] With appropriate management after portoenterostomy, improved surgical techniques, effective post-transplant immunosuppression, and aggressive management of infectious complications, the overall survival and quality of life of children with biliary atresia will continue to increase.

SUMMARY

Biliary atresia is the most common cause of pathologic jaundice in young infants and results from obliteration of the extrahepatic bile ducts by an inflammatory and fibro-obliterative process. Although the pathogenesis is not fully elucidated, patient- and animal-based studies point to a possible pathogenic model in which a genetically susceptible subject abnormally destroys the extrahepatic biliary system in response to environmental factors. Clinically, the disease is manifest by the triad of jaundice, acholic stools, and hepatosplenomegaly. In approximately one-fifth of the cases, infants also have nonhepatic malformations, such as laterality defects. Because many of the initial features are shared by other causes of neonatal cholestasis, the clinician is challenged to develop a diagnostic algorithm that facilitates the diagnosis so that portoenterostomy is performed in a timely fashion. Postoperatively, infants often require a high-calorie diet, supplementation with fat-soluble vitamins, and medications to induce choleresis and prevent cholangitis. Despite medical and surgical treatments, the disease commonly progresses, with hepatic fibrosis, portal hypertension, and end-stage liver disease occurring in at least half of the patients by 2 years of age. At this stage, liver transplant is an effective treatment modality, but the surgical success presents the infants with new morbidities and carries a high monetary cost to society. Therefore, we are challenged to systematically search for pathogenic mechanisms so that prevention can be attempted and new therapeutic strategies can be developed to stop disease progression and improve long-term outcome.

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3. Other Disorders

Philip Rosenthal, MD

CHOLEDOCHAL CYST

Although uncommon, choledochal cysts are classified as congenital anomalies of the biliary tract with varying degrees of cystic dilatation occurring at varying segments of the biliary tree (extrahepatic or intrahepatic). In general, the term "choledochal cyst" refers to all cystic dilatations of the biliary tree; others, however, restrict the term to only cystic abnormalities of the common bile duct.

Epidemiology

Choledochal cysts occur four times more frequently in girls than in boys regardless of racial differences. It is more frequently diagnosed in Asians, with an estimated incidence of 1 in 1,000 live births in Japan.[1] In the United States, the incidence is estimated at 1 in 13,000 live births.[2] About half of the cases are diagnosed in children before the age of 10 years, and another 25% of cases are diagnosed by 20 years of age. Thus, choledochal cysts are predominantly a disorder of children and young adults.[3,4]

Classification

Choledochal cysts have been categorized into several different types.[3] Anatomic classification is of practical importance in the planning of surgical intervention and treatment (Figure 50.3-1).

Type I, which is the most common type encountered, consists of a cystic dilatation of the common bile duct. Observed may be (1) a large saccular cystic dilatation, (2) small segmental cystic dilatation, or (3) diffuse or cylindrical fusiform dilatation. Type I cysts account for 75 to 85% of biliary cysts.

Type II cysts are diverticuli in the extrahepatic ducts. They may be seen in the common bile duct and/or the gallbladder. They represent only 2 to 3% of reported cases.

Type III cysts are choledochoceles. They are also rare, occurring in only about 3.5% of reported cases. A further anatomic classification of type III cysts has been proposed by Sarris and Tsang.[5] In type A, the ampulla opens into the choledochocele, which communicates with the duodenum via another small opening. Type A choledochoceles can be subclassified into A_1, in which the pancreatic and common bile duct share a common opening into the cyst; A_2, in which the openings are distinct; and A_3, in which the choledochocele is small and entirely intramural. In type B, the ampulla opens directly into the duodenum, with the choledochocele communicating only with the distal common duct.

Type IV cysts are multiple cysts. In type IV A, the multiple cysts are in the intra- and extrahepatic bile ducts and account for about 20% of reported cases. Type IV B cysts are rare and include multiple cysts found only in the extrahepatic system.

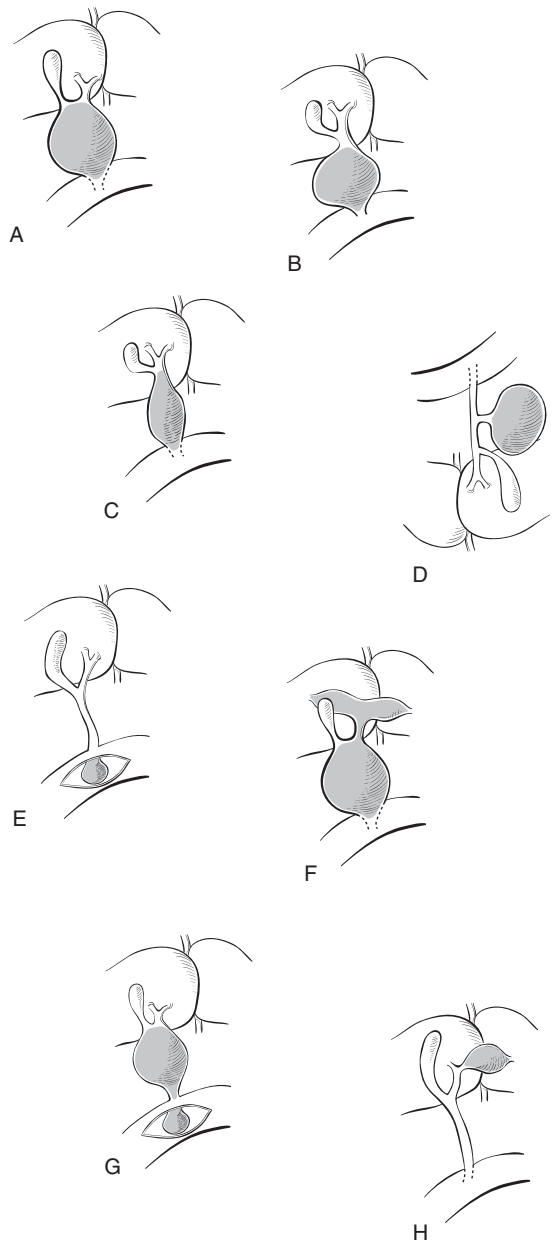

FIGURE 50.3-1 Diagrams demonstrating typing of ductal cysts. Reproduced from Todani T et al[3] with permission from Excerpta Medica.

Type V cysts are either single or multiple intrahepatic bile duct cysts. These are also rare. Type V cysts are identical to the original description of Caroli disease.

Clinical Presentation

Choledochal cysts present clinically with two distinct constellations determined primarily by patient age.[6,7] In infants, jaundice, the result of a conjugated hyperbilirubinemia with or without acholic stools, is observed. The presentation may be indistinguishable from biliary atresia, which is sometimes associated with a choledochal cyst. Abdominal pain may or may not be present. Hepatomegaly may be commonly found, and about half of the children have a palpable abdominal mass if carefully examined. The mass is typically in the right hypochondrium, soft, elastic, round, and mobile and may move with respiration. The classic triad of pain, jaundice, and a palpable abdominal mass occurs in anywhere from 13 to 63% of reported series. Biliary cirrhosis and portal hypertension may also be seen.

In older children and adults, many asymptomatic patients have been reported. Chronic or intermittent abdominal pain is the most common presenting symptom. Also, a history of intermittent jaundice or recurrent cholangitis is obtained. Palpation of an abdominal mass and the presence of cirrhosis or portal hypertension are infrequent in these older individuals compared with infants. However, recurrent pancreatitis is reported only in older children and adults. Frequently, abnormalities of the pancreatic duct system are found. Carcinoma associated with choledochal cysts has been reported only in patients over 10 years of age.

Spontaneous perforation of a choledochal cyst in infancy can occur. In one series, 7% (13/187) of infants with choledochal cysts were encountered with spontaneous perforation of the cyst.[8] Eight patients had biliary peritonitis, and five had sealed perforation. The postulated cause of perforation was biliary epithelial irritation from refluxed pancreatic juice, the result of malformed biliary and pancreatic ducts.

Pathogenesis

The etiology of choledochal cysts is unknown. Theories suggest either a congenital cause or an acquired cause.[9–11] The congenital theory postulates an unequal epithelial cell proliferation during embryogenesis while the embryonic bile ducts are still solid. In support of the congenital theory is the fact that biliary cysts have been reported in utero as early as 15 weeks gestation.[11] Further, after decompression surgery, many cysts fail to shrink, supporting inherently abnormal ductal wall development. The acquired injury theory proposes that choledochal cysts develop owing to an anomalous arrangement of the distal pancreaticobiliary tree. The anomalous merger of the common bile duct and the pancreatic duct proximal to the sphincter of Oddi permits reflux of pancreatic enzymes into the common bile duct, resulting in inflammation, edema, fibrosis, obstruction to bile flow, localized weakness, and dilatation. It is apparent that several factors may be important in the pathogenesis of biliary cysts. No single explanation supports each type of biliary cyst observed.

Diagnosis

Choledochal cysts in the majority of patients are recognized during infancy.[6,7] The diagnosis is suggested if noninvasive imaging studies are sought for vague right upper quadrant symptoms. Ultrasonography is a valued and preferred method to screen for a choledochal cyst (Figure 50.3-2A). Differentiation of a cystic structure that is not the gallbladder is an important consideration (Figure 50.3-2B). Although upper gastrointestinal tract radiographs may outline a mass that displaces the first or second portion of the duodenum, they are unnecessary. If there is doubt following ultrasound examination, the use of nuclear hepatobiliary scans, computed tomography, or magnetic resonance imaging (magnetic resonance cholangiography) may provide important discriminatory information.[12–14]

Percutaneous transhepatic cholangiography (PTC) and endoscopic retrograde cholangiopancreatography (ERCP) can provide excellent detailed information.[15] PTC may be especially helpful in defining complex intrahepatic cysts, whereas ERCP may provide the best visualization of the distal biliary tree. Obviously, PTC and ERCP are more invasive. Ultimate diagnosis can be confirmed by an intraoperative cholangiogram at surgery.

Pathology

The wall of a choledochal cyst is often thickened because of productive fibrosis and inflammation. Histologic examination reveals dense connective tissue, fibrocollagen, and, occasionally, smooth muscle and elastic elements.[16] Often there is no epithelial lining, but islets of preserved cylindrical or columnar epithelium may be found. Externally, duodenal mucosa covers choledochoceles, and, internally, there may be duodenal mucosa, bile duct mucosa, or unclassified glandular epithelium.

Hepatic changes owing to choledochal cyst frequently occur. Biliary cirrhosis, portal fibrosis, or evidence of bile duct obstruction with bile duct proliferation, cholestasis, parenchymal damage, and inflammatory cell infiltration may be seen.[17] Obviously, these changes may simulate biliary atresia.

Treatment

Therapy for choledochal cysts is complete surgical excision.[18,19] It is important that the entire cyst mucosa be removed. Historically, cyst aspiration, external drainage, internal decompression, and drainage into the duodenum or direct anastomosis of the cyst to a jejunal Roux-en-Y loop were used. Each of these techniques retained the cyst abnormal mucosal wall. Poor drainage resulted in stricture formation, biliary lithiasis, and an increased risk of malignant potential in the retained mucosal wall. It is imperative that the extent of any intrahepatic cystic disease be defined at the time of choledochal cyst excision. An intraoperative cholangiogram is well suited for this task. Segmental multifocal cystic disease isolated to a single hepatic lobe can be treated with lobectomy. If the intrahepatic disease is dif-

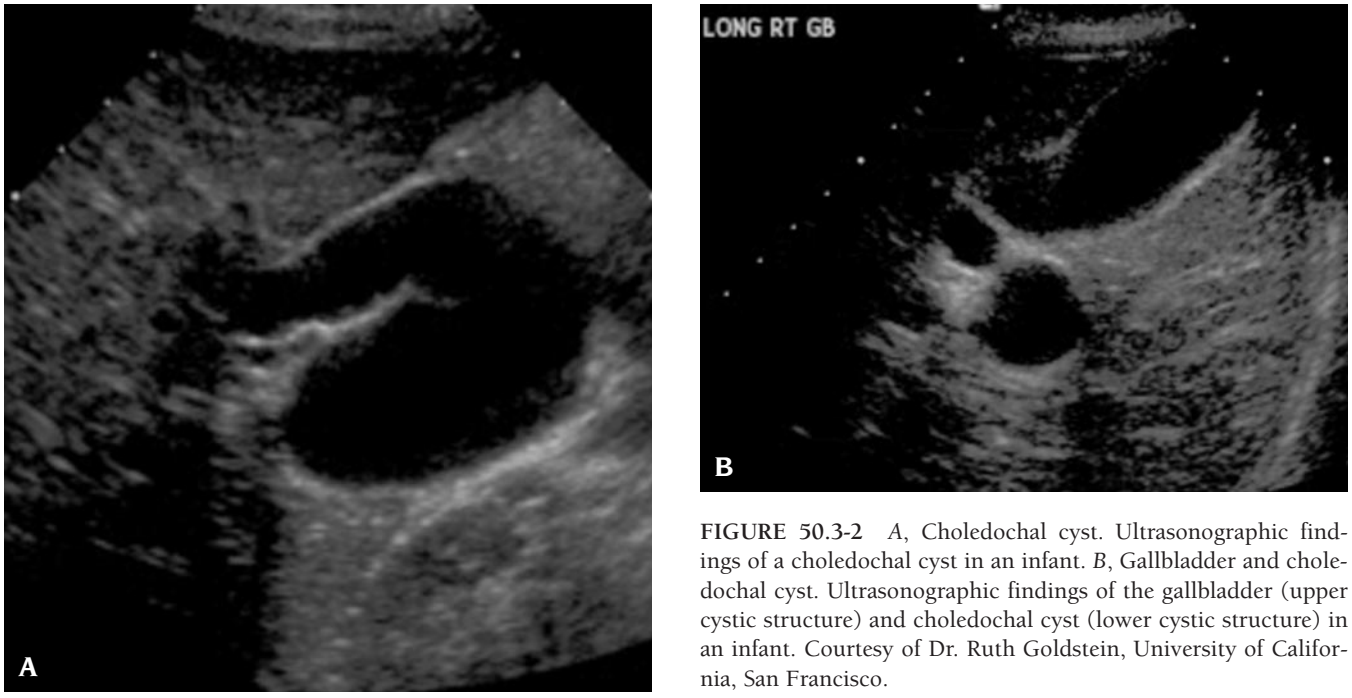

FIGURE 50.3-2 *A*, Choledochal cyst. Ultrasonographic findings of a choledochal cyst in an infant. *B*, Gallbladder and choledochal cyst. Ultrasonographic findings of the gallbladder (upper cystic structure) and choledochal cyst (lower cystic structure) in an infant. Courtesy of Dr. Ruth Goldstein, University of California, San Francisco.

fuse, involving all hepatic lobes, then liver transplant may be required. Following surgery, complications include cholangitis, stricture formation, and pancreatitis. These can be significantly decreased by total cyst excision and careful surgical dissection. Recently, a report of the complete laparoscopic management of choledochal cysts was published.[20] The authors concluded that although feasible, this technique is technically difficult and should currently be restricted to specialized centers dealing with advanced laparoscopic surgery.

Malignancy

The most alarming complication of choledochal cyst is malignancy. Carcinoma has been reported in remaining cystic tissue in up to 26% of patients.[21–24] Although the overall incidence of biliary carcinoma in association with biliary cysts is about 2.5%, the overall incidence of biliary carcinoma in the general population is only about 0.012 to 0.5%. The typical malignancy is adenocarcinoma of the bile duct or gallbladder. Squamous cell carcinoma and cholangiocarcinoma have also been observed. The risk of developing malignancy increases with age. Malignant change can also occur in areas of the biliary tree remote from the cyst. The prognosis if such malignancies develop is grim. Thus, complete cyst removal, even in asymptomatic patients, including those with prior cyst surgery, is warranted.

SPONTANEOUS PERFORATION OF THE COMMON BILE DUCT

Spontaneous perforation of the bile ducts is a rare but well-documented condition in neonates.[25–27] Typically, these infants present with cholestatic jaundice following a postnatal symptom-free interval. There is associated mild jaundice, ascites, acholic stools, poor weight gain, vomiting, and evolving abdominal distention. The combination of a mildly elevated conjugated hyperbilirubinemia with minimally elevated serum transaminases and acholic stools in a neonate should suggest the possibility of the diagnosis. Ultrasonography may reveal ascites or a loculated fluid collection. Nuclear hepatobiliary scintigraphy may demonstrate activity of the isotope outside the biliary tree in the abdomen.[28] Abdominal paracentesis may aid in the diagnosis, revealing clear, bile-stained ascites. Treatment requires surgical intervention to either attempt to repair the perforation or to re-establish bile flow from the liver to the intestine while decompressing the biliary tract. Operative cholangiography may demonstrate the site of the perforation. Often there is stenosis of the distal end of the common bile duct, segmental atresia, or inspissated bile. There is a predilection for the perforation to occur at the confluence of the cystic duct and the common bile duct, suggesting a particular susceptibility to weakness or injury at this site.[29]

NEONATAL SCLEROSING CHOLANGITIS

Sclerosing cholangitis refers to a disorder with irregular narrowing of either the intrahepatic or extrahepatic bile ducts, the result of inflammation and subsequent fibrosis. Obliteration of the bile ducts eventually results in biliary cirrhosis. Cholangiography demonstrates the typical "beading" pattern with alternating areas of strictures and dilatations in the intrahepatic and extrahepatic bile ducts. A neonatal form of sclerosing cholangitis has been reported in infants.[30–35] Jaundice, cholestasis, and acholic stools were observed within the first weeks of life. Cholecystography disclosed abnormal intrahepatic and extrahepatic bile ducts with rarefaction of segmental branches, stenosis, and focal dilation. The early clinical symptoms following birth suggest the possibility of a congenital onset. A history of consanguinity in many of the affected

infants suggests an inherited or genetic basis for this disorder. A case of neonatal sclerosing cholangitis associated with a positive anti–smooth muscle antibody titer (1:320) and elevated immunoglobulin has been reported.[34] Although the cause of neonatal sclerosing cholangitis is unknown, genetic or immunologic factors may be important in its etiology. In all cases of neonatal sclerosing cholangitis reported, jaundice resolved within the first year of life. However, the disease progressed to cirrhosis. Liver transplant has been used to treat some cases of neonatal sclerosing cholangitis.[33] To date, there has been no report of recurrence of disease following transplant. The role of medical therapy, such as ursodeoxycholic acid (UDCA) administration, to alter the progression of neonatal sclerosing cholangitis is unknown.

SCLEROSING CHOLANGITIS

Although more frequently diagnosed in adults, sclerosing cholangitis may also be seen in childhood. Sclerosing cholangitis is defined as a chronic hepatobiliary disorder with inflammation of the intrahepatic and extrahepatic bile ducts. The subsequent periductular fibrosis results in narrowing and dilatation of the bile ducts. Progression of the disorder results in cirrhosis and portal hypertension. Diagnosis can be made by cholangiography and/or histology of the liver parenchyma.

The nomenclature for sclerosing cholangitis is a bit confusing. Primary sclerosing cholangitis (PSC) is the most frequent liver disorder associated with inflammatory bowel disease (IBD). However, PSC may occur in the absence of IBD. Cholangitis related to chronic ascending bacterial infection, stones, bile duct surgery, congenital anomalies of the bile ducts, ischemic injury, neoplasms, or infectious cholangiopathy owing to acquired immune deficiency syndrome (AIDS) is referred to as primary cholangitis. This designation assumes that prior to the onset of PSC, the bile duct anatomy was normal. Secondary sclerosing cholangitis is associated with bile duct injury, the result of choledocholithiasis, postoperative stricture formation, or specific duct involvement from systemic disease. Rather than categorizing sclerosing cholangitis as primary or secondary, Debray and colleagues divide sclerosing cholangitis into three groups: neonatal, postneonatal associated with a disease, and postneonatal not associated with a disease.[36]

The distinction between sclerosing cholangitis and the obstructive cholangiopathies of infancy (ie, biliary atresia, syndromic and nonsyndromic paucity of intrahepatic ducts) is vague. There are histologic and radiographic similarities, suggesting common etiologies. In biliary atresia, there is an inflammatory, progressive, and obliterative destruction of the extrahepatic and intrahepatic bile ducts. Infants with the neonatal form of sclerosing cholangitis may have pathologic and radiographic features that are very similar to biliary atresia early in the course of the disease that do not become characteristic of sclerosing cholangitis until the disease evolves.[31,32] Further, a report of siblings, one with biliary atresia and the other with PSC, has been published.[37]

Sclerosing Cholangitis and Associated Conditions

Reports of PSC associated with both ulcerative colitis and Crohn disease in children are becoming more frequent, potentially as a result of improved cholangiographic techniques being used.[38–50] In adults, PSC is often associated with other disorders with an autoimmune basis, including diabetes mellitus, pancreatitis, and thyroid diseases. These disorders may precede or follow the diagnosis of PSC. In children, besides an association with IBD, PSC may be associated with a wide variety of disorders.[51–58]

Clinical Presentation

Although PSC can occur in infancy, the majority of cases in childhood occur in young adults. As in older adults, there may be a gradual onset of fatigue, malaise, anorexia, and weight loss. Pruritus and intermittent jaundice may then occur. Cholangitis recognized by right upper quadrant pain, fever, and conjugated hyperbilirubinemia is often encountered. In children, growth failure and delayed puberty may also be presenting symptoms. Many children with PSC are clinically asymptomatic, and the disease is detected only as a result of screening serum biochemical abnormalities, which prompts further investigation. There are no pathognomonic laboratory findings in PSC. Commonly in adults, there is an elevated serum alkaline phosphatase. Although this may be variable in children, elevated alkaline phosphatase levels may be difficult to interpret because of growth. An elevated serum γ-glutamyltransferase level appears to be a sensitive indicator for PSC in children. Other variably abnormal biochemical tests include high immunoglobulin G concentrations, positive antinuclear antibodies, anti–smooth muscle antibodies, and antineutrophil cytoplasmic antibodies, suggesting an autoimmune connection. Although the physical examination may be completely normal, often hepatomegaly, splenomegaly, or jaundice is noted.[36,50]

Diagnosis

Cholangiography is considered essential in the diagnosis of PSC (Figure 50.3-3). This may be accomplished by ERCP, PTC, intraoperative cholangiography, or, more recently, magnetic resonance cholangiopancreatography.[38] Characteristic findings are irregular narrowing and stricturing of the hepatic and common bile ducts owing to inflammation and fibrosis. There may be concomitant involvement of the intrahepatic ducts. The strictures may be localized, diffuse, or multifocal. Strictures may be short, with intervening normal duct segments producing the characteristic beaded appearance. The intrahepatic bile ducts may show decreased peripheral arborization, which is referred to as the pruned tree appearance.

Pathology

The characteristic histologic findings in PSC may not be present in many cases in children, limiting the usefulness of this modality for diagnosis.[36,50] Classically, there are focal concentric edema and fibrosis around interlobular bile ducts. This is referred to as an onion skin appearance. Typically, there is portal to portal variability in the biopsy, and a review

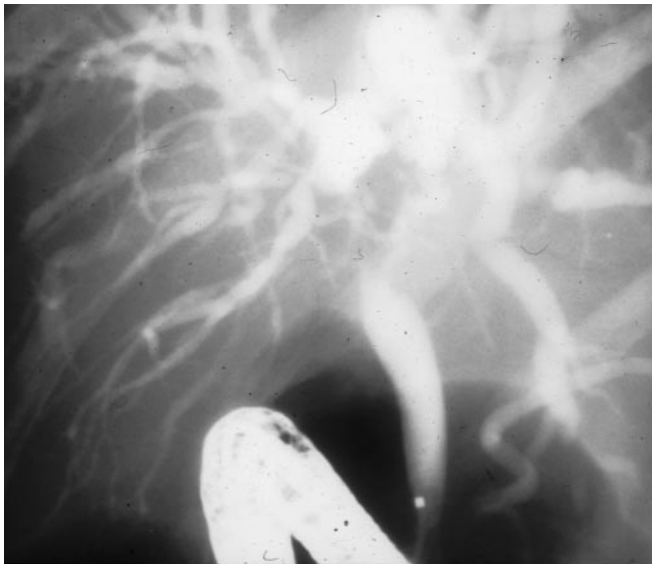

FIGURE 50.3-3 Primary sclerosing cholangitis. Cholangiogram demonstrating irregular narrowing and stricturing of the hepatic and common bile ducts owing to inflammation and fibrosis. Courtesy of Dr. Roy Gordon, University of California, San Francisco.

of serial sections may be required. Serial biopsies demonstrating progressive ductal changes may also be necessary to establish the diagnosis. A fibro-obliterative cholangitis may progress over time to solid cords of connective tissue and loss of interlobular bile ducts, giving the appearance of a paucity of bile ducts per portal zone (Figure 50.3-4). This may continue to progress to frank biliary cirrhosis.

Pathogenesis

The etiology of PSC is unknown. The association of PSC with IBD may suggest potential etiologic mechanisms, but PSC may occur in the absence of IBD.

To incorporate the various proposed theories into a unifying hypothesis, perhaps an immune-mediated destruction of the biliary tree initiated by an infectious agent in a susceptible host with or without intestinal involvement best describes the process. To this end, there is exciting new evidence implicating a retrovirus in the pathogenesis of primary biliary cirrhosis, another obliterative ductal disease.[59] Further, pilot studies using antiviral therapy with lamivudine and zidovudine in adult patients demonstrated significant biochemical and histologic improvement with reversal of ductopenia and normalization of liver function tests.[60] Whether antiviral therapy has a role in PSC remains speculative.

Alterations in the immune system are also proposed to be important in the initiation or perpetuation of PSC (see Chapter 52, "Autoimmune Disease").

Treatment

Unfortunately, there have been no large randomized controlled trials of any potential therapeutic agents for the treatment of PSC in children. UDCA therapy has been used in children with PSC.[40] There were no side effects reported in its use in nine children. There were significant reductions in serum alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, and γ-glutamyltransferase levels with up to 20 months of treatment. The long-term effect of UDCA therapy for children with PSC awaits further trials.

Immunosuppressive therapy has long been used for PSC therapy in children.[61,62] Certainly, there is a rationale for this in children with PSC with IBD. Those children with autoimmune features of disease might also be expected to respond to this therapy. Oral vancomycin was reported to normalize serum transaminase levels in three children with PSC.[45] Although provocative and supporting a role for intraluminal bacteria, further confirmation is awaited before oral vancomycin therapy can be recommended for routine use in children with PSC.

Surgical options have been used predominantly to relieve biliary obstruction, reduce the occurrence of cholangitis, and combat the consequences of cirrhosis and portal hypertension. Procedures performed either surgically, by interventional radiology, or endoscopically range from drainage procedures, stent placement, balloon dilatation of strictures, resection, and transplant.[33,63–67] Certainly, surgical intervention should be avoided if at all possible and reserved for refractory patients after attempts at dilatation and stent placement have been exhausted. Transplant is appropriate for children with PSC who have progressed to cirrhosis and who have developed portal hypertension in the absence of refractory complications. Unfortunately, recurrence of PSC in the transplanted liver has been reported.[68,69] Recently, a report of a 15-year-old boy who underwent liver transplant for PSC was published.[70] What was novel about this case and another five adult cases in this report was the sequential occurrence of autoimmune hepatitis that evolved to PSC over several years.

Prognosis

The true rate of progression of PSC in children is unknown. Presumably, the rate of progression is slow because PSC is

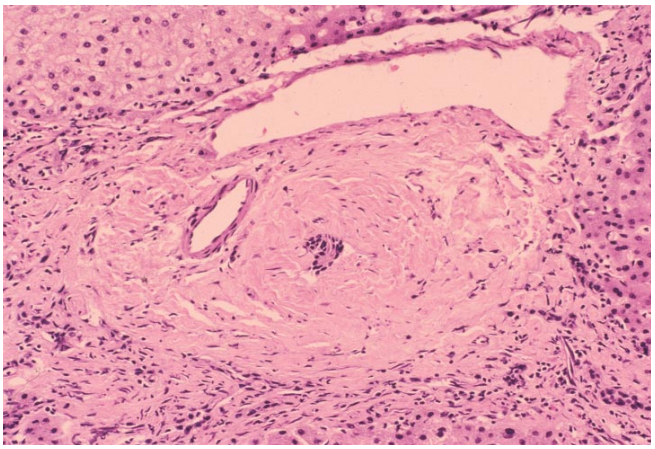

FIGURE 50.3-4 Primary sclerosing cholangitis: portal zone shows prominent periductal fibrosis (onion skin–like fibrosis) around the interlobular bile duct. Ductal remnant remains in this example but can disappear completely. Hematoxylin and eosin stain; ×40 original magnification. Courtesy of Dr. Linda Ferrell, University of California, San Francisco.

not one of the leading indications for pediatric liver transplant. The prognosis may be better for children than for adults, based on the study by Floreani and colleagues.[41] In the larger pediatric series, about a third of children died, the result of cirrhosis and portal hypertension, and another third either were listed for transplant or went on to transplant.[36,50]

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CHAPTER 51

POSTNATAL INFECTIONS

1A. *Viral Hepatitis* B

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Hepatitis viruses can infect individuals of any age. Although most of the complications manifest mainly in adulthood, primary infection may occur in infancy or childhood. Among the hepatotrophic viral infections (A through E), hepatitis A and E run an acute self-limited or fulminant course, whereas hepatitis B, C, and D may follow either an acute or a chronic course. Chronic hepatitis D is very rare in children all over the world, except in a few endemic areas, whereas chronic infection of hepatitis B virus (HBV) is more prevalent and can occur in children of any age, even in the perinatal period. Chronic infection of HBV during childhood may cause chronic hepatitis, cirrhosis, and liver cancer during childhood or later in adulthood. HBV-related carcinogenesis requires time; hepatocellular carcinoma may occur much earlier in those infected in childhood than in those infected in adulthood.

HBV infection is a worldwide health problem. Approximately 2 billion people in the world have been infected by HBV, and 350 million of them are chronic hepatitis B antigen carriers. In hyperendemic areas, where most of the complications of chronic HBV infection develop in adulthood, primary HBV infection occurs mainly during infancy or early childhood.[1] Understanding the long-term natural course of chronic HBV infection in children is very important to evaluate the efficacy and determine the strategy of antiviral therapy for chronic HBV infection in children.

Hepatitis B immunization has effectively reduced the infection and carrier rate of HBV. Immunization is the most important method to achieve the eradication of hepatitis B–related diseases.

HEPATITIS B VIRUS

Hepatitis B virus is a 3.2 kb, circular, partially double-stranded deoxyribonucleic acid (DNA) virus. During active replication in the early phase of infection, viral particles appear in large quantity in the serum in two forms: one is the complete virion of 42 nm diameter, which consists of an envelope, a capsid with capsid protein, a circular DNA molecule, and a DNA polymerase,[2,3] and the other is a 22 nm empty viral envelope, which contains only the hepatitis B surface antigen (HBsAg). In addition, a soluble antigen, hepatitis B e antigen (HBeAg), which is closely related to the nonsecretory capsid antigen (hepatitis B core antigen [HBcAg]), also appears in the serum during the highly replicative phase of HBV infection (Table 51.1A-1).

HBV contains four open reading frames, which encode major structural and nonstructural proteins for HBV. These are the polymerase gene region for polymerase, surface gene region for three surface proteins, precore and core gene regions for HBcAg and HBeAg, and X gene region for

TABLE 51.1A-1 CLINICAL SIGNIFICANCE OF HEPATITIS B VIRUS ANTIGENS, ANTIBODIES, AND DNA

ANTIGEN	CLINICAL SIGNIFICANCE	ANTIBODY	CLINICAL SIGNIFICANCE
HBsAg	Acute or chronic infection	Anti-HBs	Protection by vaccination recovery from infection
HBeAg	Active viral replication	Anti-HBe	Inactive viral replication
HBcAg	Not detectable in the serum	Anti-HBc	Present (IgM, IgG) or detectable in the liver Past infection (IgG)
HBV DNA	Presence of HBV		

Anti-HBc = hepatitis B core antibody; Anti-HBe = hepatitis B e antigen; Anti-HBs = hepatitis B surface antibody; DNA = deoxyribonucleic acid; HBcAg = hepatitis B core antigen; HBeAg = hepatitis B e antigen; HBsAg = hepatitis B surface antigen; HBV = hepatitis B virus; Ig = immunoglobulin.

hepatitis B X protein.[4] HBV has a restricted host range. It infects only humans and chimpanzees.

EPIDEMIOLOGY OF HBV INFECTION IN CHILDREN

HBV infection is prevalent in Asia, Africa, Southern Europe, and Latin America, where the HBsAg-seropositive rate ranges from 2 to 20% in most regions. In hyperendemic areas, HBV infections occur mainly during infancy and early childhood. In Taiwan, the HBsAg carrier rate is approximately 10 to 20%. Before the implementation of a universal HBV immunization program, the HBsAg-seropositive rate in this population was 5% in infants and increased to 10% at 2 years of age, remaining at the same rate thereafter. However, the infection rate, measured by hepatitis B core antibody (anti-HB_c) seropositivity, reached 50% by the age of 15 years. This suggests that most chronic HBsAg carriers are infected before 2 years of age in this population.[1]

Perinatal transmission from HBsAg carrier mothers to their infants is a very important route of transmission, leading to chronicity, in Asia. This mode of transmission accounts for 40 to 50% of HBsAg carriers in Taiwan and many other hyperendemic areas. Approximately 90% of the infants of HBeAg-seropositive carrier mothers become HBsAg carriers,[5] irrespective of a high or low HBsAg carrier rate in the population. The age of infection is an important factor in determining the outcome of infection.[6,7]

In areas of low endemicity, horizontal infection is the main route of transmission. Although Africa is an area of high endemicity, horizontal infection in early childhood is the main route of HBV transmission. The two most important routes of horizontal transmission in children are highly infectious family members, such as siblings, and improperly sterilized syringes.[8] Other sources of horizontally transmitted infections include institutionalized children and multiple or large-volume blood transfusions. In the United States and Europe, HBV is highly prevalent among adopted children from endemic areas of the world.

PATHOGENESIS AND NATURAL COURSE OF HBV INFECTION IN CHILDREN

HBV has an incubation period of 2 to 6 months. Following a primary HBV infection, the host may run an acute, fulminant, or chronic course. The interaction between the host and virus determines the outcome of infection.

Acute and Fulminant Hepatitis B

Acute hepatitis runs a self-limited course. Recovery is marked by hepatitis B surface antibody (anti-HB_s) seroconversion. Fulminant hepatitis is signaled by pathologic mental status changes within 2 to 8 weeks after the initial symptoms in an otherwise healthy child. Trey and Davison defined fulminant hepatic failure as the onset of altered metal status within 8 weeks of initial symptoms in an otherwise healthy individual.[9] Later Bernuau and colleagues defined fulminant hepatitis as hepatic encephalopathy developing within 2 weeks after the onset of jaundice and subfulminant hepatitis as hepatic encephalopathy developing between 2 and 12 weeks after the onset of jaundice.[10]

Symptoms of acute or fulminant hepatitis B may develop as early as 2 months of age in infants of HBsAg carrier mothers. In areas hyperendemic for HBV infection, HBV accounts for around 65% of the etiologic agents for fulminant hepatitis in children. Approximately 65% of pediatric patients with fulminant hepatitis B present in infancy. Maternal transmission is the most important route of transmission in infants with acute or fulminant hepatitis,[11] mainly in infants of hepatitis B e antibody (anti-HB_e)-seropositive mothers.[12]

Fulminant hepatitis carries a very high mortality rate of 55 to 70% without transplant and 30 to 50% with transplant.[11,13] Those who survive usually recover without sequelae. In both acute and fulminant hepatitis B, HBV is cleared rapidly from the host.

Precore mutations have been associated with fulminant hepatitis B in adults, but this has not been confirmed by more recent studies.[14,15] Thirty-three percent of the children with fulminant hepatitis B have the hepatitis B precore stop codon mutant, a prevalence rate similar to that (30%) in children with acute hepatitis B.[16] This suggests that precore stop codon mutations alone do not explain the severe clinical course in fulminant hepatitis B.

Chronic HBV Infection

Children with chronic HBV infection are mostly asymptomatic. They are generally active and grow well, with very rare exceptions. Even with acute exacerbation of liver inflammation, jaundice or growth failure is rare. Although liver damage is usually mild during childhood, serious sequelae, including cirrhosis and hepatocellular carcinoma, may develop insidiously at any age.

The coexistence of high rates of viral replication, normal liver function profiles, and minimal hepatic hisopathology for many years in children with chronic HBV infection suggests that HBV may not be directly cytopathic. An immune-mediated process is the main mechanism for cell damage. During acute exacerbations of chronic HBV infections, CD8-positive cytotoxic T lymphocytes are the predominant cells in the liver in the areas of piecemeal necrosis. As hepatocellular necrosis occurs, there is a gradual decrease of HBV replication and HBeAg serocoversion occurs, along with a decrease in hepatic inflammation (Figure 51.1A-1).

Spontaneous Hepatitis B e Seroconversion. HBeAg is an important marker reflecting active viral replication and infectivity. Its clearance is therefore used as a marker for the success of antiviral therapy. Children with chronic HBV infection are HBeAg seropositive at the initial stage of infection. During this stage, the child is tolerant to HBV, the virus is highly replicative, and serum HBV DNA levels are usually high. The child is thus an important source of horizontal infection in the family and community. Aminotransferase levels fluctuate but are usually normal or mildly elevated, with mean levels higher than those in noncarrier healthy children.[17] Peak alanine aminotrans-

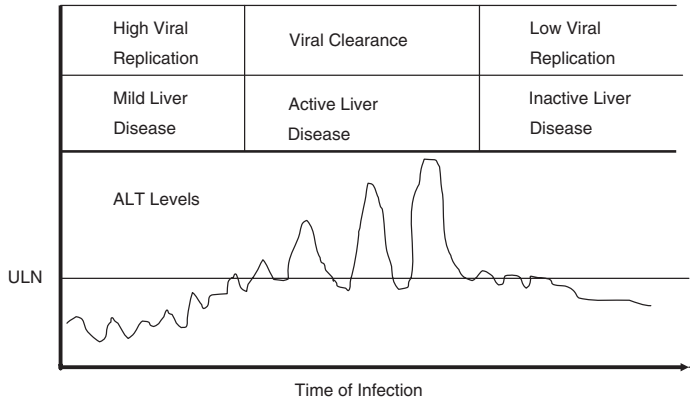

FIGURE 51.1A-1 Natural course of chronic hepatitis B virus infection acquired in childhood. During the early phase of infection, the amount of virus in the liver and blood is usually large, whereas the liver damage is mostly mild. The host immune system gradually recognizes the virus and starts to clear the virus. It results in active inflammation of the liver and elevation of serum aminotransferases. Repeated episodes of elevation of aminotransferases may be followed by hepatitis B e antigen (HBeAg) seroconversion. After HBeAg seroconversion, viral replication declines and the liver inflammation gradually becomes inactive. ULN = upper limit of normal.

ferase (ALT) levels > 100 IU/L are uncommon in this phase. Hepatitis B e antigenemia can persist for years after primary infection.

Spontaneous clearance of serum HBeAg occurs gradually as the child ages. Viral replication is reduced during this process. This process of HBeAg clearance is usually preceded by an elevation of aminotransferases. The peak level of aminotransferase elevation can be mild, transient, and fluctuating. An ALT level > 1,000 IU/mL is unusual. This process of HBeAg seroconversion takes place subclinically in most individuals for a period of 2 to 7 years.[18] After the detection of elevated levels of aminotransferases, around 40% of children will clear HBeAg within 1 year. Children with elevated aminotransferase levels of > 100 IU/mL and HBV DNA levels of < 1,000 pg/mL often seroconvert during the subsequent 1 to 3 years.[17] After HBeAg clearance, aminotransferase levels gradually return to normal limits, and anti-HBe develops spontaneously. In our series, HBV DNA was detectable in only 1% of the anti-HBe–positive sera. However, using polymerase chain reaction (PCR), HBV DNA persists long term in the serum of children with chronic hepatitis B after HBeAg seroconversion. Bortolotti and colleagues. studied 39 children after hepatitis B e seroconversion.[19] They found that 87% of children had detectable HBV DNA by PCR within 5 years of follow-up and in 58% of cases 10 years after seroconversion. ALT levels were persistently normal in 92%, whereas 8% had slightly elevated ALT.

Acute exacerbation of inflammation with reactivation of HBV replication and a rise in aminotransferases is not common in children after hepatitis B e seroconversion.[18,20] Permanent liver damage has occurred, and integration of the genome of HBV has occurred insidiously and gradually, despite clearance of HBeAg. Development of cirrhosis or hepatocellular carcinoma is occasionally observed but is rare during childhood. Most of those who develop these complications of chronic HBV infection are anti-HB_e seropositive.[21]

Factors Affecting Hepatitis B e Seroconversion. Age is an important determinant of the rate of HBeAg seroconversion.[22] Before 3 years of age, the spontaneous HBeAg clearance rate is very low (< 2% per year). The hepatitis B e seroconversion rate gradually increases to around 5% per year after 3 years of age. This might be due to immune tolerance to HBcAg and HBeAg in infected children. Immune tolerance owing to transplacental e antigen has been demonstrated by the absence of a T-cell response to HBcAg in infants and children of HBeAg-positive HBsAg carrier mothers, whereas the T lymphocytes from infants with acute hepatitis of hepatitis B e–negative HBsAg carrier mothers respond very well to HBcAg.[23] The age of hepatitis B e seroconversion varies, ranging from infancy to more than 40 years of age. The most common period is from 15 to 30 years of age. Before 15 years of age, the majority (85% in Taiwan) of HBsAg carrier children are HBeAg seropositive.

Another factor affecting the hepatitis B e seroconversion rate in children is maternal HBsAg.[22] Those with HBsAg carrier mothers have a lower rate of HBeAg clearance than those whose mothers were not HBsAg carriers. Maternal carrier state reflects perinatal transmission or early infection, which will lead to a longer duration of immune tolerance to HBV (Figure 51.1A-2).

HBsAg/Antibody Seroconversion. In our long-term follow-up of HBsAg carrier children, the annual HBsAg clearance rate was very low (only 0.56%),[24] occurring only after clearance of HBeAg. After loss of HBsAg, its antibody (anti-HB_s) remains low or undetectable in the majority (0 to < 100 mIU/mL). The underlying mechanisms of a poor anti-HB_s response in HBsAg carriers who lost HBsAg are multifactorial, including specific failure of antigen presentation or T-cell activation or the lack of a T helper cell–like response to HBsAg.[25] Hepatitis B immunization is not beneficial in these individuals.

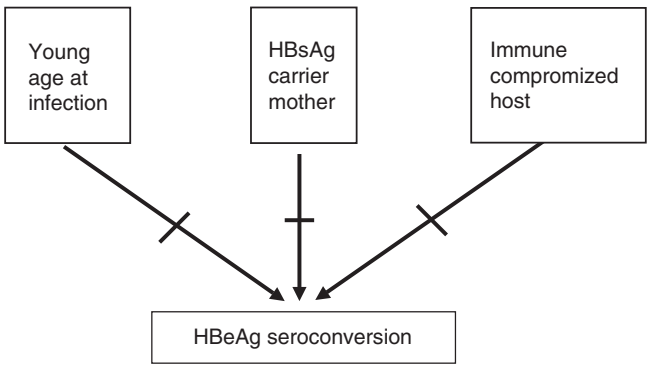

FIGURE 51.1A-2 Factors that may hinder or delay hepatitis B e antigen (HBeAg) seroconversion and viral clearance.

Histopathologic Findings in Children with Chronic HBV Infection. Liver histology in HBeAg-positive HBsAg carrier children generally reveals very mild inflammation and fibrosis.[26] During the process of HBV and HBeAg clearance, the abnormal changes include lobular changes with portal inflammation and fibrosis, with or without piecemeal necrosis. The inflammation is usually milder than that observed in adults. Bridging hepatic necrosis may occur but is uncommon. Within 6 months of hepatitis B e seroconversion, the inflammation is less active and, beyond 6 months, becomes inactive, with mild to minimal inflammation and fibrosis in most children.

The authors have studied the liver histology in 41 asymptomatic HBsAg children at 1 to 9 years of age who were perinatally infected by their HBeAg-positive HBsAg carrier mothers. The histologic findings included chronic active hepatitis (n = 1), chronic persistent hepatitis (n = 8), chronic nonspecific hepatitis (n = 30), and normal histology (n = 2). The histologic abnormalities in the liver begin early in life and may progress to severe liver impairment in later life.

Virologic Factors That May Affect the Clinical Course. During the course of infection, HBV may undergo mutations, which accumulate particularly in some DNA hot spots. Mutations of HBV may affect the outcome of infection or, more commonly, are the result of host immune pressure. HBV precore G to A stop codon mutations at nucleotide 1896 are a common site of mutations detected in patients with fulminant hepatitis, acute hepatitis, chronic hepatitis with acute exacerbation, cirrhosis, or hepatocellular carcinoma in adults.[27,28] We have studied the temporal changes of this mutation in children with chronic HBV infection and found that it emerged before hepatitis B e seroconversion. The proportion of positive precore stop codon mutations increased gradually after HBeAg seroconversion to around 50% of the children.[29]

HBcAg is the target in HBV-infected hepatocytes for cytotoxic T lymphocyte–mediated cell lysis. Mutation of the HBV core gene may change the conformation of core protein and allow the hepatocytes to escape the immune surveillance of the host. Among 31 Taiwanese children with chronic HBV infection, HBV core gene codons 21 (29%), 147 (29%), and 65 (15%) were the frequent sites of mutation.[30]

Genotypes of HBV were reported to correlate with the clinical outcome in patients with chronic hepatitis B. Genotype C was more prevalent in patients with cirrhosis and hepatocellular carcinoma, whereas patients with genotype B experienced earlier hepatitis B e seroconversion and slower progression of liver fibrosis and development of hepatomas.[31,32]

Hepatocellular Carcinoma. Hepatocellular carcinoma is 1 of the 10 most common cancers in the world. In areas prevalent for HBV infection, hepatocellular carcinoma is not rare in children. The rate of seroprevalence of hepatitis B surface antigenemia approaches 100% in children with hepatocellular carcinoma in Taiwan. Maternal transmission or infection in early childhood is an important risk factor.[21,23] Integration of HBV DNA has been detected in the tumor and nonmalignant liver tissue of children with chronic HBV infection and hepatocellular carcinoma.[33]

Core gene mutations were identified much more commonly at some codons (codons 74, 87, and 159) in children with hepatoma than in children with chronic HBV infection but without cancer. Those core gene mutations may induce persistent host immune attacks and lead to severe liver damage, as observed in children with hepatocellular carcinoma.[30]

Hepatitis B Immunoprophylaxis

Prevention of HBV infection can be achieved by passive and active immunization. Passive immunization using hepatitis B immunoglobulin (HBIg) provides temporary immunity. After hepatitis B vaccine became available in 1982, active immunization with three or four doses of HBV vaccine without HBIg has proved to be immunogenic in more than 90% of neonates of noncarrier mothers or HBeAg-negative carrier mothers. In infants of HBeAg-seropositive mothers, it decreased the carrier rate to 24%. Injection of HBIg within 24 hours after birth followed by three doses of HBV vaccine further reduces the carrier rate down to 3% in pilot studies[34] and to 14% in a study of the general population.[35] According to a random sampling study of children in Taiwan 3 years after implementing a universal HBV vaccination program, the protective efficacy was found to be 86% from HBIg plus HBV vaccine and 78% from three doses of HBV vaccine alone.[35]

The first universal hepatitis B vaccination program in the world was launched in Taiwan in July 1984.[36] Pregnant women are screened for both serum HBsAg and HBeAg or titers of HBsAg by a reverse passive hemagglutination test. Infants of mothers with negative serum HBeAg with a reciprocal HBsAg titer lower than 1:2,560 or with negative serum HBsAg receive plasma-derived hepatitis B vaccine at 0, 1, 2, and 12 months for those born before July 1992 or a recombinant hepatitis B vaccine at 0, 1, and 6 months for those born after July 1992. Infants of mothers with positive serum HBeAg or with reciprocal titers of HBsAg by reverse passive hemagglutination ≥ 2,560 receive HBIg within 24 hours after birth in addition to doses of hepatitis B vaccine. The coverage rate of hepatitis B vaccine for neonates is around 94%.

Different immunization strategies are used in different countries, depending on their basic epidemiologic features of HBV infection and available resources. In many hyperendemic countries, HBV vaccination consists of three doses of hepatitis B vaccine and no HBIg for all infants, including infants of an HBsAg carrier mother This has an efficacy of around 75 to 80%. Using this HBV immunization program, the cost of maternal screening and HBIg can be minimized. Other countries, such as the United States, give one dose of HBIg to infants of HBsAg-positive mothers, regardless of their HBeAg status, and three doses of HBV vaccine to all infants.

Impact of Universal Hepatitis B Immunization on Hepatitis B Eradication

The seroprevalence rates of HBsAg and anti-HB_c in Taiwanese children before and 15 years after the initiation of a universal vaccination program were remarkably reduced.[37] The HBsAg carrier rate in Taipei in children < 15 years old decreased significantly from around 10% before to < 1% after the vaccination program (see Table 51.1A-2). The infection rate, measured by the seropositive prevalence of anti-HBc, was decreased in all of the children (anti-HB_c seropositivity declined from 38 to 16% in children below 13 years of age), even in those above 15 years of age who were not vaccinated during infancy. This vaccination program has indeed reduced both the perinatal and the horizontal transmission of HBV.[38] The decrease in horizontal transmission was a result of a decline in the prevalence of sources of infection and the vaccination of the older children.

Although immunoprophylaxis for HBV infection is very successful, around 2.4% of infants of HBeAg-positive mothers still had HBsAg in the serum at birth or shortly after birth.[39] They became HBsAg carriers in spite of complete immunoprophylaxis. Intrauterine HBV infection, although infrequent, is possible. Risk factors for failure of immunoprophylaxis include a high level of maternal HBV DNA, a low level of maternal anti-HB_c, and uterine contraction and placental leakage during the process of delivery.[40,41]

Vaccine Prevention of Hepatocellular Carcinoma

After the implementation of the universal vaccination program for HBV in Taiwan, we have successfully demonstrated a decline in the incidence of hepatocellular carcinoma in children. The average annual incidence of hepatocellular carcinoma in children of 6 to 14 years declined from 0.70 per 100,000 children between 1981 and 1986, to 0.57 between 1986 and 1990, and to 0.36 between 1990 and 1994.[42] In contrast, the incidence of hepatoblastoma in children and the incidence of hepatocellular carcinoma in adults during the same study period were not reduced. Analyzing the incidence of hepatocellular carcinoma according to the birth cohort, the reduction effect is even more impressive. The incidence of hepatocellular carcinoma in children 6 to 9 years of age declined from 0.52 in 100,000 children for those born between 1974 and 1984 to 0.13 in 100,000 children for those born between 1984 and 1986.

Boys may benefit more from HBV vaccination than girls in the prevention of hepatocellular carcinoma.[43] The boy-to-girl incidence of hepatocellular carcinoma decreased steadily from 4.5 in 1981 to 1984 (before the launch of a universal HBV vaccination program) to 1.9 in 1990 to 1996 (6–12 years after the launch of a universal HBV vaccination program) (Table 51.1A-3).

TABLE 51.1A-2 HBSAG-SEROPOSITIVE RATES BEFORE AND AFTER IMPLEMENTATION OF A UNIVERSAL HEPATITIS B VACCINATION PROGRAM IN TAIWAN

	HBSAG-SEROPOSITIVE RATE (%)			
AGE (YR)	1984	1989	1994	1999
< 1	5.1	3.0	0.0	0.0
1–2	10.7	1.5	0.5	1.2
3–4	10.1	2.2	0.3	0.0
5–6	10.6	3.9	0.8	0.0
7–8	9.7	4.7	0.9	2.0
9–10	11.0	9.8	1.5	1.3
11–12	9.1	10.5	6.8	0.0

Universal hepatitis B vaccination was implemented in Taiwan in July 1984.
Adapted from Ni YH et al.[37]

Long-Term Vaccine Immunogenicity

A large-scale prospective community-based study enrolling 1,200 children with complete HBV immunization in infancy was conducted until the children were 14 years of age. Eleven children had new HBV infections with anti-HB_c seropositivity. None of them became positive for HBsAg or HBV DNA by PCR. The proportion of children with protective anti-HB_s gradually decreased from 71% at age 7 years to 37% at age 12 years in 951 children who did not receive booster doses of HBV vaccine during follow-up. Among the 458 children with anti-HB_s levels lower than the protective level at age 7 years, only 1 of the 200 children in the group that received booster doses and 2 of the 258 children in the group that did not developed a new infection with anti-HB_c positivity during follow-up to age 14 years.[44]

Remaining Barriers to Eradication of HBV

Further investigation into the mechanisms of HBV vaccine failures or nonresponders is critical. Interventions to prevent intrauterine infection, the development of HBV vaccines against surface antigen gene mutants, and better vaccines for immunocompromised individuals will further reduce the incidence of new infections.

Those opposed to the HBV vaccines may be reduced in number by a better understanding of vaccine-related side effects. For instance, although there is little supporting evidence, an association between central nervous system demyelinating diseases and hepatitis B vaccine has been suggested.[45] Clarification of this and other poorly documented side effects of the vaccines may help to reduce anxiety about the risks of the vaccine and enhance an appreciation of HBV vaccine benefits. Finally, it is extremely important to find ways to reduce the cost of HBV vaccines and to increase funding for HBV vaccination of children living in hyperendemic areas under poor economic conditions

TREATMENT

The goal of antiviral therapy for HBV infection is to eradicate HBV and to prevent its related liver damage. However, current antiviral regimens are not completely effective in this regard. Inhibition of viral replication with prevention of liver damage and related consequences is also important and is more achievable with available antiviral agents (Table 51.1A-4).

TABLE 51.1A-3 EFFECT OF UNIVERSAL HEPATITIS B VACCINATION ON THE PREVENTION OF HEPATOCELLULAR CARCINOMA IN CHILDREN IN TAIWAN

YEAR OF DIAGNOSIS	BOYS	GIRLS	TOTAL INCIDENCE OF HCC*	MALE/FEMALE
1981–84†	1.08	0.24	0.67	4.5
1984–90	0.87	0.32	0.61	2.7
1990–96	0.49	0.26	0.38	1.9

HCC = hepatocellular carcinoma.
*Per 100,000 population of children aged 6 to 14 years.
†Before the hepatitis B virus vaccination program (universal vaccination was implemented in July 1984).

Strategies for the Treatment of Chronic Viral Hepatitis

Interferon Therapy. Interferons are antiviral and immunomodulatory proteins.[46] They interact with cells by binding to specific receptors on the cell surface. Interferon-α is produced by leukocyte or lymphoblastoid cells when they are stimulated by virus-infected or tumor cells, bacteria, or viral envelopes. In virus-infected cells, interferons can produce antiviral proteins, inhibit synthesis of viral ribonucleic acid, and enhance the expression of human leukocyte antigen class I antigens, thus allowing recognition of the infected hepatocytes by cytotoxic T lymphocytes. Interferon therapy is employed worldwide and is the most frequently used therapeutic antiviral agent for hepatitis B and C. The disadvantages of this drug are the parenteral route of administration and mostly tolerable but frequent adverse reactions (Table 51.1A-5).

Overall, approximately 30 to 40% of adults with chronic hepatitis B achieve a sustained response to interferon-α at a usual dosage of 5 MU/d or 10 MU three times a week for 3 to 4 months. Evidence for the efficacy of interferon therapy in children with chronic HBV is limited by the small number of children enrolled in most reported studies. As shown in Table 51.1A-6, interferon therapy is not effective for hepatitis B e seroconversion in carrier children with normal liver enzymes. In those with elevated aminotransferases, the results of interferon treatment are similar to those in adults, with a rate of hepatitis B e seroconversion and normalization of aminotransferase levels 20 to 40% higher in the treatment group than in the control group.[47–54] A systematic review of the world literature has shown a significant benefit of interferon therapy for chronic HBV infection in children.[55]

Factors that are predictive for a positive response to interferon include high pretreatment levels of aminotransferase, low pretreatment HBV DNA levels, late acquisition of HBV infection, and hepatocellular inflammation.

The recommended dose of interferon for children is 0.1 MU/kg or 3 to 6 MU/m^2 three times a week for 4 to 6 months. Some studies in a small number of children, using higher doses, 6 to 10 MU/m^2, have shown a higher rate of sustained responses but also a higher rate of adverse effects. If severe reactions to interferon occur, the dose should be modified (50% reduction) or discontinued. Extending the duration of therapy for a total of 12 months may be beneficial for those who have a partial response to initial interferon therapy.

Pegylated interferon is a long-acting interferon successfully used in treating chronic hepatitis C[56] and has also been used in treating hepatitis B in adults.[57] The safety and efficacy have not been reported in children.

Nucleoside Analogs. Lamivudine is a 2′,3′-dideoxynucleoside that is phosphorylated by intracellular enzymes to form lamivudine triphosphate. Lamivudine triphosphate can inhibit DNA synthesis by terminating the nascent proviral DNA chain and interfere with the reverse transcriptase activity of HBV. After oral administration, lamivudine is well absorbed and has a mean absolute bioavailability of > 80% in adults and 68% in infants and children.[58] In clinical trials in adults, lamivudine rapidly reduced HBV DNA to undetectable levels in a daily dose of 100 mg or more.[59,60]

TABLE 51.1A-4 THERAPIES FOR CHRONIC VIRAL HEPATITIS B

- Interferon
- Antiviral Agents
  - Nucleoside analog
    - Lamivudine
    - Adefovir dipivoxil
    - Entecavir
    - Emtricitabine
  - Gene therapy
    - Antisense oligonucleotide
    - Ribozyme
    - Interfering proteins or peptides
- Immunomodulatory Therapy
  - Thymosin
  - DNA vaccine
- Combination Therapy

TABLE 51.1A-5 ADVERSE EFFECTS OF INTERFERON, LAMIVUDINE, AND ADEFOVIR DIPIVOXIL

AGENT	ADVERSE EFFECTS
Interferon	Flu-like symptoms, depression, anorexia, weight loss, hair loss, bone marrow suppression, autoantibody induction
Lamivudine	Ear, nose, and throat problems; gastrointestinal symptoms; malaise and fatigue; lower respiratory tract symptoms; pancreatitis; neutropenia; elevation of liver enzyme
Adefovir	Nephrotoxicity

TABLE 51.1A-6 INTERFERON THERAPY FOR HEPATITIS B VIRUS INFECTION IN CHILDREN

PLACE AND STUDY	RATE OF HBEAG SEROCLEARANCE		
	ALT AT THERAPY	CONTROL GROUP	IFN THERAPY GROUP
SPAIN			
Ruiz-Moreno et al,[51] 1995	> 45 IU/L	—	34% (17/50)
ITALY			
Barbera et al,[49] 1994	No limit	14% (5/37)	26% (10/39)
Vajro et al,[53] 1996	> 1.5 × of ULN	11% (1/9)	48% (10/21)
Gregorio et al,[52] 1996	No limit	13% (4/31)	38% (24/64)
UNITED STATES			
Narkewicz et al,[50] 1995	> 2 × of ULN	—	78% (7/9)
HONG KONG			
Lai et al,[47] 1987	Normal	8% (1/12)	8% (1/12)
Lai et al,[48] 1991	No limit	0% (0/30)	8% (5/60)
TAIWAN			
Chang et al,[42] 1997	> 80 IU/L	46% (6/13)	73% (8/11)
EUROPE + AMERICA (MULTICENTERED)			
Sokal et al,[54] 1998	> 1.3 × ULN	11% (8/74)	26% (18/70)
META-ANALYSIS			
Torre and Tambini,[55] 1996	No limit	11% (12/113)	23% (29/126)*

ALT = alanine aminotransferase; IFN = interferon; ULN = upper limit of normal.
*p = .026.

Lamivudine 0.7 to 8 mg/d was well tolerated in 53 European and Canadian children aged 2 to 17 years.[61] Around one-fifth of patients who received 1 month of lamivudine therapy experienced drug-related adverse events. The most common adverse events were ear, nose, and throat problems; gastrointestinal symptoms; general symptoms (malaise and fatigue); and lower respiratory symptoms (see Table 51.1A-5). No serious side effects or need for withdrawal occurred during therapy or follow-up.

Lamivudine was able to eliminate or reduce HBV DNA in pediatric patients after 1 month of therapy in a multicentered study in European and Canadian children.[61] A dramatic fall of HBV DNA of 99.9% and a reduction in HBV DNA of approximately 1,000-fold was noted. However, HBV DNA returned to the pretreatment levels in all patients after cessation of lamivudine. The HBeAg loss rate remained low after 3 months of follow-up. The efficacy of 52 weeks of lamivudine therapy was evaluated in 286 children in a multicentered placebo-controlled study. The complete virologic response (with HBeAg clearance and negative HBV DNA) at the end of treatment was achieved in 23% of the treatment group (191 children) and 13% of the placebo group (95 children). The anti-HB_e seroconversion rate was 22% versus 13% in the treatment group versus the control group. ALT normalization was noted in 55% of the treatment group and 12% of the placebo group. The HBsAg loss rate was minimal (2% in the treatment group and 0% in the placebo group).[62] Higher ALT levels and liver histologic inflammation scores and lower HBV DNA levels before treatment predict a better treatment response.

Lamivudine, at 100 mg/d, is the recommended dose for adults.[60] The recommended dose for children is 3 mg/kg/d up to a maximal dose of 100 mg/day.[61] There is no added benefit to dividing the total daily dose. The optimal duration of treatment requires further investigation. At this time, treatment for 1 year is recommended.

Children aged 2 to 17 years of age who are HBsAg seropositive for more than 6 months with elevated aminotransferase levels and HBV DNA in their serum for more than 3 months may be candidates for lamivudine therapy. This includes HBV carriers who are waiting for a liver transplant, children with repeated acute exacerbations of HBV infections, children with HBV infection < 2 years of age, and children with fibrosing cholestatic HBV hepatitis. Drug compliance should be monitored carefully, particularly in adolescents. Regular physical examinations are mandatory, along with monitoring of serum biochemical markers of inflammation and viral infection, including aminotransferases, coagulation profiles, complete blood cell count, amylase, lipase, urea nitrogen, and creatinine. Liver histology, quantified by the Knodell score, before and 1 year after starting therapy provides valuable information regarding viral clearance, necroinflammatory activity, and fibrosis. Lamivudine should be discontinued if any related adverse reactions develop. HBV DNA, HBeAg, and anti-HB_e should be monitored after discontinuing use of lamivudine because HBV replication frequently resumes.

Mutants of the HBV polymerase gene at the reverse transcriptase region may develop after use of lamivudine for more than 9 months.[63,64] The mutations most frequently found are methionine to valine or methionine to isoleucine mutations at the YMDD (tyrosine-methionine-aspartate-aspartate) motif. The second common site of mutagenesis is leucine to methionine at the polymerase gene nucleotide 528 for gentoype A or nucleotide 526 for genotypes B, C, and F (or reverse transcriptase nucleotide 180).[65] The clinical relevance of these mutations in children infected with HBV is poorly understood at this time. Among the 166 children treated with lamivudine in a muticentered study, 31 developed YMDD mutations at the end of 52 weeks of therapy. Those with YMDD mutants had a

higher (1.5 times) median level of ALT and HBV DNA (19 times) than those without this mutant.[62]

Lamivudine-associated HBV YMDD mutant strains have been reported to be less replicable than the wild-type virus. In fact, the long-term use of lamivudine after the emergence of the YMDD motif mutation may lead to acute exacerbation and subsequent HBV clearance.[66] However, there is a risk of liver failure during these acute exacerbations of infection. Careful monitoring of the quantity of HBV DNA and signs of clinical or biochemical deterioration of liver function is mandatory in children infected with mutant strains.

Adefovir dipivoxil, a nucleoside analog recently approved by the US Food and Drug Administration, is effective in inhibiting the replication of the YMDD motif mutants[67–69] and can be used in patients with acute exacerbation of inflammation during lamivudine therapy. It is an acyclic analog of deoxyadenosine monophosphate that can competitively inhibit deoxyadenosine triphosphate and the first-strand synthesis. It can inhibit > 95% of viral DNA and 30% of pregenomic ribonucleic acid, pre-S (gene of hepatitis B virus middle and large surface protein gene), core proteins, and covalently closed circular hepatitis B virus DNA. Adefovir is nephrotoxic. Other nucleoside analogs, including entecavir and emtricitabine, have good antiviral potential, but their therapeutic benefit in children remains unclear.

Gene Therapy and Immunotherapy

Gene therapy for HBV infection is still under investigation.[70] Antisense oligonucleotides can inhibit hepatitis viral gene expression and may provide a new therapeutic choice for patients with chronic HBV infections.[71] Thymosin α is an immune modifier that has been shown to trigger maturational events in lymphocytes, augment T-cell function, and promote reconstitution of immune defects. It has been reported to be effective in treating chronic HBV infections with clearance of HBeAg and normalization of aminotransferase levels in some[72] but not all studies. DNA vaccines are still in the experimental stage. Injection into mice of a plasmid expressing HBV DNA encoding HBV proteins, under the cytomegalovirus promoter, may induce both cellular and humoral immune responses against this viral antigen.[73,74]

Combination Therapies

Combination therapies are increasingly used for the management of persistent viral infections. The synergistic effect of ribavirin and interferon in treating chronic hepatitis C is one example of successful combination antiviral therapy.

The benefit of different combinations of doses and schedules for interferon plus lamivudine therapy has been controversial in adults.[75] Comparisons between interferon alone versus lamivudine plus interferon have been conducted in children (n = 15 to 47 in each group of treatment) with no statistically significant differences in the outcomes measured. These studies used interferon-α 5 to 10 MU/m^2 three times per week for 6 to 12 months plus lamivudine 4 mg/kg daily (maximun 100 mg daily) for 12 months. A complete virologic response rate of 37 to 47% at 6 months was seen after the end of treatment. It was similar to the 30 to 40% complete virologic response in children treated with interferon alone for 6 to 12 months.[76,77] The use of lamivudine alone for 2 months, then interferon-α 10 MU/m^2 plus lamivudine for 6 months, and then lamivudine alone again for additional 4 months versus interferon plus lamivudine simultaneously for 6 months and then lamivudine alone for another 6 months also showed no differences between the two treatment groups (47% versus 46% complete elimination of HBV).[78] Large-scale studies using other combination schedules or regimens are needed to clarify the efficacy of combination therapy.

FUTURE PROSPECTS

The World Health Organization (WHO) recommended in 1997 that universal hepatitis B immunization should be introduced in all countries. WHO also established the objective to reduce the incidence of new HBV carriers among children by 80% by year 2001 and eventually to eliminate the more than a million deaths that occur annually from HBV-associated cirrhosis and hepatocellular carcinoma.[79,80] Up to now, a total of approximately 140 countries have followed this recommendation. With the integration of the hepatitis B vaccination program into the Expanded Immunization Program in most countries in the world, chronic HBV infection will be further reduced in the twenty-first century. The major issues that remain for hepatitis B prevention include vaccine failure and noncompliance with vaccination programs. Further understanding of the immune defects and pathogenesis of chronic HBV infection will enhance the development of better therapeutic and preventive agents and vaccines focused on the T-cell epitopes of HBV that can be recognized by convalescent patients.

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1B. Hepatitis C Virus

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Although non-A, non-B hepatitis was recognized some 30 years ago,[1,2] its major causative agent was identified only in 1989 and was named hepatitis C virus (HCV).[3,4] Since the discovery of HCV, significant advances have been made in our understanding of the molecular virology and pathobiology of this important viral pathogen. In adults, the epidemiology and natural history of HCV infection are well defined, and approved efficient treatment strategies are available. In contrast, we know much less about the evolution and treatment of HCV infection in children. This part of the chapter summarizes our current understanding of the epidemiology, natural history, and treatment of HCV infection in this young population.

THE VIRUS

HCV is an enveloped, single-stranded, positive-sense ribonucleic acid (RNA) virus that is the major causative agent of non-A, non-B hepatitis. Besides humans, chimpanzees are the only other animals permissive to HCV infection. Based on nucleotide sequence analysis and genetic organization of the viral genome, HCV is classified as an independent genus (*Hepacivirus*) within the Flavivirus family.[5] The HCV genome is approximately 10,000 kb long with one open reading frame that encodes for a polyprotein containing approximately 3,000 amino acids (Figure 51.1B-1).[6,7] The recent detection of a previously unrecognized viral peptide putatively within the core region suggests the presence of a second open reading frame.[8] This new finding, which challenges the prevalent contemporary assertion that the HCV genome consists of only one reading frame, awaits further confirmation. The large viral polyprotein undergoes proteolysis by viral and host-encoded proteases, which results in the formation of the various HCV proteins. The proteolytic processing occurs as the nascent viral polyprotein is newly synthesized (cotranslation) and after it is completely formed (post-translation). The HCV genome is organized so that its 5′ end encodes for the structural proteins. including the core (or capsid) and envelope proteins (E1 and E2), whereas the nonstructural (NS) or functional viral proteins (NS2-NS5) are encoded by the larger subsequent 3′ segment. There are two well-conserved noncoding areas (untranslated regions [UTRs]) flanking the HCV open reading frame that are critically important for the initiation of efficient viral protein synthesis (5′-UTR) and RNA replication (3′-UTR).

The core protein is a highly basic, hydrophobic, and relatively conserved protein that is intimately associated with the viral RNA and forms its "inner shell" or nucleocapsid. In addition to being the integral structural component of HCV, the core protein interacts with several cellular proteins, including apolipoprotein A-2, 60S ribosomal Sub U, hetero-

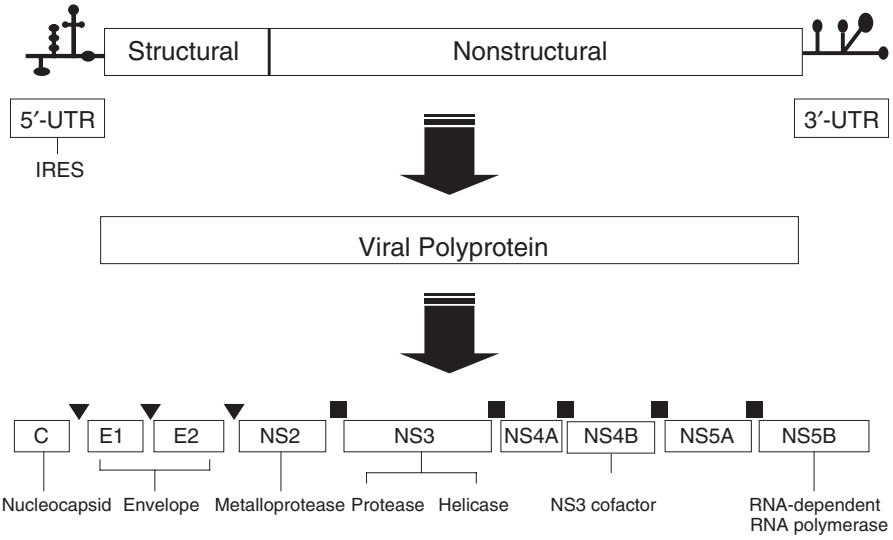

FIGURE 51.1B-1 Processing of hepatitis C virus polypeptide by host (▼) and viral (■) encoded proteases into the various viral proteins. IRES = internal ribosomal entry site; UTR = untranslated region.

geneous nuclear ribonuclear protein, tumor necrosis factor-α receptor, and lymphotoxin-β receptor. Although the precise pathogenetic mechanisms are largely unknown, these virus–host interactions suggest that the HCV core protein may play an important role in directly mediating liver cell damage. Indeed, the HCV core protein has been implicated in the development of hepatic steatosis and liver cancer in a transgenic mouse model.[9] The HCV E1 and E2 proteins are glycoproteins embedded within the viral lipid envelope and are integral constituents of the HCV "outer shell." In contrast to the core protein, the envelope proteins are very heterogeneous and display extensive nucleotide and antigenic variability. This antigenic diversity, greatest within a small area of the 5′ end of the E2 gene (the hypervariable region), is believed to be an important mechanism by which HCV evades host immunologic surveillance, facilitating the establishment of chronic infection. The NS2 segment encodes for a metalloproteinase whose function is limited to specifically cleave the NS2-NS3 site. The 5′ and 3′ ends of the HCV NS3 encode for proteins with serine protease and helicase activities, respectively. The HCV serine protease is the major viral peptidase because it is required for the precise processing of a large segment of the viral polyprotein, including all cleavage sites within the NS3-NS5 segment. The NS3 serine protease interacts with an NS4-derived peptide (NS4 cofactor), which, although not essentially required, augments the proteolytic activity of the viral protease. The HCV helicase encoded by the 3′ end of the NS3 segment is an enzyme that unwinds RNA-RNA duplexes formed during viral replication. Proteolytic hydrolysis of the NS4-encoded protein by viral serine protease yields two peptides, denoted NS4A and NS4B, respectively. In addition to enhancing the activity of the HCV NS3 serine protease activity, as mentioned above, the NS4A product appears to facilitate phosphorylation of the NS5A product. In a similar fashion, NS3-dependent protease cleavage of the NS5 gene results in the elaboration of two viral proteins, NS5A and NS5B. Increased nucleotide heterogeneity within the NS5A product is associated with lower serum HCV RNA levels and more favorable responses to interferon-based therapy,[10,11] suggesting that this protein may be involved in the transcriptional regulation of viral RNA. However, others have not found a link between NS5A mutations and a therapeutic response to antiviral agents[12,13]; the precise function of this gene product remains to be elucidated. The NS5B is an RNA-dependent RNA polymerase, the enzyme responsible for viral replication. The 5′-UTR is an internal ribosomal entry site (IRES) component through which HCV RNA binds to host cellular ribosomes, a necessary step for the initiation of viral protein synthesis. Finally, the 3′-UTR contains a highly conserved region of nucleotide known as the "X region," whose predicted stem-loop structure plays an important role in initiating viral RNA and in enhancing IRES-driven protein synthesis.

Viral Heterogeneity

The NS5B-encoded HCV RNA-dependent RNA polymerase lacks proofreading ability. As a result, like many other RNA viruses, HCV is genetically heterogeneous. Based on phylogenetic analysis of HCV sequences, six major HCV genotypes are currently recognized, and there are multiple subtypes within each viral genotype.[5] Although HCV genotypes 1 and 2 are the most prevalent worldwide, there are unique geographic variations in the global distribution of viral genotypes. Accordingly, HCV genotype 3 is most common in Australia and the Indian subcontinent, and genotypes 4, 5, and 6 are almost exclusively seen in individuals from sub-Saharan Africa, South Africa, and Southeast Asia, respectively. In the United States, HCV genotypes 1, 2, and 3 account for approximately 70%, 15%, and 10% of adult infections, respectively.[14] More recently, some have proposed to divide HCV into six phylogenetically distinct groups called clades. In this new classification scheme, clades 1, 2, 4, and 5 correspond to genotypes 1, 2, 4, and 5; clade 3 comprises genotypes 3 and 10; and clade 6 includes genotypes 6, 7, 8, 9, and 11.[5] The relationship between viral genotypic diversity and clinical disease outcome is a controversial issue because some, but not all, studies correlate HCV genotype 1 infection with more aggressive liver disease and a higher risk for the development of hepatocellular carcinoma.[15] However, HCV genotype 1 is consistently associated with higher serum HCV RNA levels and a significantly poorer response to currently available antiviral regimens than HCV genotype 2 or 3. In fact, based on the results of recent clinical trials in adults, some authorities advocate a shorter duration of therapy for individuals with HCV genotype 2 or 3 than for those infected with HCV genotype 1.[16] Accordingly, determining the HCV genotype has become an essential component of the medical evaluation of infected adults, particularly those who are contemplating initiating antiviral therapy. Much less is known about the geographic distribution of HCV genotypes in children or its impact on the clinical evolution of liver disease in this population. Nevertheless, the viral genotypic distribution in children generally parallels that reported regionally in adults.[17–23] Furthermore, as in adults, HCV genotype 1 correlates with higher serum viral levels and a less favorable response to antiviral treatment.

The degree of genetic heterogeneity is such that even within persons infected with a unique viral genotype, HCV exists as a highly heterogeneous population of different but closely related genomes, which are called quasispecies.[24] HCV quasispecies play an important role in the pathobiology of HCV infection in adults as increased heterogeneity correlates with less favorable response rates to interferon given alone or in combination with ribavirin.[25–27] Assessment of HCV quasispecies by molecular evolutionary analysis has been instrumental in selected situations, such as in verifying the transmission of virus by accidental needle-stick exposures[28,29] or by infected mothers to their offspring (vertical transmission).[30,31] However, little is known about the clinical significance of HCV quasispecies in childhood infection; therefore, its assessment is not currently a component of the routine medical management of patients with HCV infection.

Pathogenesis of Liver Disease

In general, viral infections lead to cellular damage in vivo by either direct cytopathicity or immune-mediated injury, targeted against either viral or autoantigens. HCV may be

directly cytopathic in situations that allow unusually high levels of viral replication and antigen expression, such as in immunosuppressed patients.[32,33] However, a significant amount of accumulating experimental evidence suggests that immune-mediated mechanisms play a more critical role in the controlling and mediating liver cell damage in chronic HCV infection in adults.[34,35] In contrast, our understanding of the pathogenesis of childhood HCV infection is very limited.[36] A Spanish study compared clinical, virologic, and immunohistochemical parameters between 12 children and 24 adults with chronic hepatitis C.[23] Although the HCV genotype distribution and estimated duration of infection were similar between these groups, children had statistically lower serum HCV RNA levels and more benign histologic disease than adults. More importantly, expression of all immunohistochemical markers studied, including CD2+, CD4+ (helper), and activated CD8+ (cytotoxic) lymphocytes; β_2-microglobulin; intercellular adhesion molecule 1; vascular cell adhesion molecule 1; platelet–endothelial cell adhesion molecule 1; and endoglin, were significantly lower in children than in adults. Although limited by the small number of children and adults analyzed, these data suggest that immunologic mechanisms are important in mediating liver cell damage in chronically infected children and that young patients may be more immunologically tolerant to HCV than similarly infected adults.

EPIDEMIOLOGY

The worldwide prevalence of HCV infection is approximately 3%, which represents an estimated 170 million infected persons.[37] Wasley and Alter described several patterns of geographic and temporal variation in the global prevalence of HCV.[37] In the pattern seen in the United States and Australia, individuals younger than 20 years have the lowest prevalence rates, whereas those between the ages of 30 and 49 years have the highest, suggesting that HCV infection is primarily acquired in early adulthood.

Variations in global prevalence rates are observed in children as well and may reflect differences in socioeconomic status. Studies of general pediatric populations have yielded seroprevalence rates as low as 0% (Egypt, Japan, United States) and as high as 14.5% (Cameroon).[38–40] In the United States, the estimated seroprevalence is 0.2% for those children less than 12 years of age and 0.4% among those 12 to 19 years of age.[41] Based on current population statistics, this prevalence rate indicates that there are about 150,000 to 200,000 children who are infected with HCV in the United States.

The prevalence of HCV in the general population of the United States is 1.8% based on serologic analysis from approximately 21,000 subjects in the Third National Health and Nutrition Examination Survey (NHANES III) data, collected between 1988 and 1994.[42] This prevalence rate corresponds to approximately 3.9 million persons nationwide being positive for anti-HCV. It should be noted that children younger than 6 years of age were not represented in this large cohort. The prevalence of viremia as measured by detectable serum HCV RNA among anti-HCV–positive subjects was 73.9%; therefore, an estimated 2.7 million people nationwide are chronically infected with HCV. The factors most strongly associated with HCV infection in persons 17 to 59 years old were illicit drug abuse and practicing high-risk sexual behavior. Age at first sexual encounter was also an important epidemiologic factor because persons who had a first sexual intercourse before the age of 18 years had significantly higher rates of HCV infection than those whose initial sex activity occurred at a later age. Subjects reporting greater use of marijuana had a higher prevalence of HCV infection than those who did not. Because there is no apparent reason to explain the association between increased transmission of HCV and marijuana use, it is presumed that larger amounts of marijuana use serve as a surrogate for other high-risk behaviors such as injection drug abuse or high-risk sexual practices.

Although the seroprevalence among incarcerated adults is as high as 82%,[43] this population was not included in the NHANES III analysis. The prevalence of anti-HCV positivity in incarcerated juveniles in the United States (2%) is significantly higher than that observed in the general adolescent population.[44] High-risk behaviors, which correlated with HCV infection in this confined population, included being sexually active and a history of body piercing. Interestingly, only 6% of the juveniles studied acknowledged injection drug abuse, a well-defined parenteral risk associated with the acquisition of HCV. However, this information was collected by voluntary self-reporting in this study and therefore likely underestimates the prevalence of this illicit practice. Of note, the HCV seroprevalence rate of 2% among incarcerated juveniles in this report is lower than that observed in other studies of homeless or confined young persons, which included a higher proportion of injection drug abusers.[45–47]

Saiman and colleagues retrospectively studied a cohort of 504 internationally adopted children from China, Russia, Southeast Asia, Eastern Europe, and Latin America.[48] Only four children, or 0.8% of the 496 tested, were anti-HCV positive, and none were positive for HCV RNA, compared with 2.8% who tested positive for hepatitis B surface antigen. A study of 169 Romanian orphans found that 36% were positive for hepatitis B surface antigen, whereas only 1 child was anti-HCV positive.[49] Therefore, the seroprevalence of HCV in international adoptees appears to be quite low.

Children who received transfusions of potentially contaminated blood products prior to the institution of routine screening have seroprevalence rates that vary depending on their degree of exposure. Seroprevalence rates of 50 to 95% have been reported in those receiving multiple transfusions or transfusions from pooled plasma, such as factor concentrates.[50–52] Children with fewer but repeated exposures, including those undergoing hemodialysis and cancer survivors, have intermediate seroprevalence rates of 18 to 52%.[53–55]

Since the initiation of routine blood screening in 1992, perinatal transmission has become the predominant source of new HCV infections (see "Natural History"); the seroprevalence of HCV in pregnant women is 1 to 2%.[56,57]

Household nonsexual contacts of infected individuals have a seroprevalence rate of 7%, although this appears to increase with longer duration of exposure to the index patient.[58]

CLINICAL ASPECTS

The mean incubation period of post-transfusion acute HCV infection is 7 to 8 weeks, with a range of 2 to 26 weeks.[59] The majority of acute HCV infections appear within 5 to 12 weeks following transfusion. Acute HCV is usually anicteric or subclinical, and only one-third of patients will develop jaundice or symptoms.[60] Fulminant hepatic failure owing to HCV is exceedingly rare. In adults, 85% of patients exposed to HCV will develop chronic infection, of whom approximately 10 to 20% develop cirrhosis and 1% develops liver cancer. Although the precise mechanisms are unknown, it is now clear that concurrent alcohol ingestion and immunodeficiency accelerate the rate of HCV-related liver disease and thus have a negative impact on its outcome.

In children, the course of HCV infection is generally benign. Most children with acute hepatitis C are asymptomatic and go unnoticed. When symptoms are present, they are often nonspecific (malaise, anorexia) or mild. Jaundice may be evident in only 25% of patients.[41] Children with chronic HCV infection may also remain asymptomatic. Progression to decompensated liver disease (variceal bleeding, ascites) in children can occur, but this is the exception. As in adults, children with immunodeficiency may progress to serious liver disease more rapidly.[61,62]

Biochemical markers such as serum alanine aminotransferase (ALT) typically fluctuate in HCV patients. Normal or only minimally increased ALT levels are usually reported with chronic HCV infection, and serum ALT levels can remain elevated despite anti-HCV seronegativity.[63,64] The histopathologic features of chronic hepatitis C in children are similar to those found in adults.[65–67] Histologic characteristics include portal lymphoid aggregates, bile duct injury, and prominent steatosis. Necroinflammatory activity is commonly mild. Varying degrees of fibrosis and, less commonly, cirrhosis have also been described.

NATURAL HISTORY

The clinical evolution of HCV infection in adults is well defined, but much less is known about the natural history of the disease in children. As in adults, the course of infection in children with parenteral or perinatally acquired HCV may be influenced by host factors such as immunologic status, underlying disease, or the deleterious effects of transfusion-related iron overload on the liver, which further hinders the ability to precisely determine the evolution of this viral infection.

Despite these potential shortcomings, several studies have delineated the clinical impact and evolution of childhood parenteral and perinatal HCV infection (Table 51.1B-1).[68–74] In an early analysis, Chang and colleagues prospectively studied 88 children at risk for HCV infection because of either periodic blood transfusion for hemolytic anemia (33 children), transfusion for cardiac surgery (38 children), or maternal chronic HCV infection (17 infants).[63] Of these, 10 children contracted HCV infection, including 5 with hemolytic anemia, 2 after cardiac surgery, and 3 with HCV-infected mothers. Five children, including the three perinatally infected infants, had detectable HCV RNA and anti-HCV antibodies in serum without symptoms of acute hepatitis. Two of the five patients who developed acute hepatitis exhibited jaundice and malaise. Sixty percent of the 10 children developed chronic infection, and half of these maintained normal ALT during the 3-year follow-up period. As suggested by the authors, these data demonstrate that host factors such as iron overload may play a significant role in the evolution of childhood hepatitis C. This report also shows that HCV seroconversion is a clinically silent process in many exposed children.

In one of the largest natural history reports published to date, Jara and colleagues retrospectively investigated 224 children who were HCV RNA positive.[75] None of the children studied had underlying disease such as malignancy or hemophilia, and the diagnosis of chronic hepatitis was defined by persistently abnormal ALT for more that 6 months or by histologic assessment of liver tissue. Of the 224 children, 45% had an HCV-infected mother and 39% had received transfusion of blood products. At study entry,

TABLE 51.1B-1 SELECTED STUDIES OF THE NATURAL HISTORY OF HCV INFECTION IN CHILDREN

STUDY	NUMBER STUDIED*	TRANSMISSION ROUTE	MEAN FOLLOW-UP PERIOD (RANGE; YR)	CLINICALLY SILENT (%)	PROGRESSIVE HISTOLOGIC DISEASE†	HCV RNA POSITIVE AT END OF FOLLOW-UP (%)
Palomba et al[68]	7	Perinatal	5.4 (2.2–7.5)	100	0/5‡	100
Bortolotti et al[69]	30	Perinatal	1.8 (1–4)	100	0/7	80
Tovo et al[70]	104	Perinatal	4.1 (0.5–12.8)	98	3/20	94
Ni et al[71]	8	Parenteral	NR	100	NR	88
Matsuoka et al[72]	29	Parenteral	7.1 (4–13)	100	1/19	45
Vogt et al[73]	67	Parenteral	17 (12–27)	NR	3/17	55
Sasaki et al[74]	11	Perinatal	3.2 (1.4–5.0)	100	NR	64
	14	Parenteral	4.0 (2.6–6.1)	100	NR	100

HCV = hepatitis C virus; NR = not reported; RNA = ribonucleic acid.
*Number of HCV infected patients included.
†Number with severe hepatitis, fibrosis, or cirrhosis of those who underwent histologic assessment.
‡Complicated by congestive heart disease or hepatitis B virus infection in all cases.

all of the perinatally infected children were asymptomatic, and none of the children exhibited jaundice. Hepatomegaly was a presenting feature in only 15%. The course of infection was studied in 200 of the original 224 children for a period of 1 to 17.5 years (mean 6.2 years). During this time, serum ALT levels normalized and HCV RNA became undetectable in 12 (6%) patients, including 17% of those with perinatal infection. Most children with chronic hepatitis had mild histologic disease, and only one patient, a transfusion recipient, developed cirrhosis and subsequent liver failure in this study. However, older adolescents had greater degrees of hepatic fibrosis than younger children, suggesting that gradual progression of histologic liver disease occurs in young patients.

In general, then, most children exposed to HCV become chronically infected based on persistently detectable serum anti-HCV antibodies and HCV RNA. Acute and chronic hepatitis C in children is usually subclinical, characterized by fluctuating serum ALT and mild histologic abnormalities. However, significant liver disease, including cirrhosis, can occur.

Parenteral Transmission

Liver disease in children with concurrent systemic illness may be related to factors other than HCV infection, and these patients are discussed separately.[76]

Hemophilia. HCV infection is noted in 50 to 98% of children with hemophilia treated with factor concentrates prepared from suboptimally decontaminated pooled plasma.[77–81] The high prevalence rate is particularly important because HCV infection strongly correlates with liver dysfunction in this population.[77,79] In a study from Japan, anti-HCV antibodies were found in 32 of 45 children (80%) with hemophilia who were followed for 1 to 4 years. Twenty-seven of these 32 HCV-seropositive patients were assayed for HCV RNA by polymerase chain reaction. Liver disease was observed in 18 of 22 patients (82%) with detectable HCV RNA but in none of 5 children without this viral marker.[78] In another investigation, HCV infection was demonstrated in nearly 40% of patients with hemophilia with abnormal liver tests, whereas serologic viral markers were found in only 17% of those with normal liver biochemistry.[80] Percutaneous liver biopsies are not usually done in children with hemophilia owing to the potential risk for bleeding. As a result, little is known about the histologic progression of HCV in this group. However, a recent report demonstrated that this procedure can be safely accomplished with appropriate factor replacement therapy.[81] More importantly, mild histologic abnormalities were noted in most of the children with hemophilia analyzed in this report. These data suggest that HCV is associated with liver disease and that clinical and histologic abnormalities are mild in most infected children with hemophilia. In contrast, HCV infection is associated with the development of liver cancer in adults with hemophilia,[82,83] suggesting that earlier viral eradication during childhood may result in reduced HCV-related morbidity later in life.

Thalassemia. Serologic markers of HCV infection are noted in up to 80% of multitransfused children with thalassemia.[84–89] In one prospective 8-year study, 75 of 135 newly diagnosed patients acquired HCV infection, which became chronic in nearly 80% of them, based on persistently elevated transaminase levels. In another prospective study, HCV-infected children were found to have significantly higher levels of transaminases than those without detectable markers of viral infection.[88] The severity of liver disease was not related to HCV genotypes or to HCV RNA levels. Furthermore, hepatic fibrosis was more commonly noted in patients with HCV infection than in uninfected children. To better delineate the rate of liver fibrosis progression, 211 children who had received bone marrow replacement for the treatment of thalassemia underwent serial histologic assessment of the liver by percutaneous biopsy.[90] Of these, 46 (22%) had evidence of fibrosis progression during a median follow-up of 64 months. Using multivariate analysis, hepatic iron overload and HCV infection were noted to be independent risk factors for the progression of liver fibrosis, and their concomitant presence resulted in a striking increase in this risk.

Cancer Survivors. HCV infection has been demonstrated in up to 50% of pediatric cancer survivors and is an important cause of liver disease in this population.[91–94] As a result of an impaired humoral immune response, anti-HCV antibodies are often not detected, despite the presence of HCV RNA in these patients.[91] Thus, the diagnosis of HCV in this population should rely on the detection of HCV RNA by nucleic amplification tests such as polymerase chain reaction or transcription-mediated assays. The clinical impact of HCV infection has been difficult to determine in this group of patients, in whom the pathogenesis of liver disease is likely related to multiple factors. Although elevations of aminotransferase levels are commonly noted during the course of cancer treatment, the biochemical profile and clinical outcomes are similar for children with and without HCV infection.[94,95] These initial retrospective observations, which are limited by the small number of patients included in these reports and relatively brief observation periods, have been verified in large prospective studies. In an Italian study of 114 leukemia survivors, 56 had detectable HCV RNA in serum at the end of chemotherapy, of which 40 had persistent viremia after a mean follow-up of 17 years.[94] At the completion of chemotherapy, a significantly higher proportion of HCV RNA–positive children had elevated serum aminotransferase levels than those without detectable virus. Most patients who underwent liver biopsy at this point had mild histologic abnormalities and the severity of liver disease was similar between HCV RNA–positive and –negative children. During follow-up, all HCV-infected children remained clinically stable, and none developed evidence of decompensated liver disease. Liver tests remained normal in approximately 71% of patients with detectable HCV RNA in serum, whereas 25% and 4% had fluctuating and persistently abnormal levels of serum aminotransferase, respectively. In a preliminary report from a similar ongoing

trial, 77 of 1,175 cancer survivors tested had detectable HCV antibodies.[96] Persistent viral infection, defined by the presence of circulating HCV RNA, was verified in 65 of those with serologic evidence of HCV, most of whom were clinically asymptomatic and had normal liver tests. Although histologic abnormalities were common among the 35 patients who underwent liver biopsy in this report, the degree of disease was mild in most patients at a mean of 6 years after diagnosis of cancer. Of note, three patients had histologic evidence of cirrhosis, and two patients died of complications of HCV-related hepatocellular carcinoma. The two reported cases of hepatocellular carcinoma were leukemia survivors who were transfused prior to routine blood screening.[97] Both patients presented 25 years after their last blood transfusion and were approximately 28 to 30 years old on diagnosis of liver cancer. One of the patients with hepatocellular carcinoma also had chronic HBV infection, another well-known risk factor for this disease. We have also cared for one cancer-surviving adolescent with HCV-related cirrhosis who died of complications of liver cancer, which was discovered while she was being evaluated for liver transplant (R. González-Peralta, personal observation, 1994).

Hemodialysis and Renal Disease. Similar to prevalence rates in adults, anti-HCV antibodies have been detected in 20 to 45% of pediatric patients undergoing hemodialysis but in only 0 to 4% of those on peritoneal dialysis.[98–102] The lower prevalence rate in patients on peritoneal dialysis is likely the result of less exposure to blood products in this group. Most HCV antibody–reactive children develop chronic infection based on persistent detection of viral RNA in serum. More importantly, the majority of children on hemodialysis have persistently normal aminotransferase levels. However, systematic assessment of hepatic histology, a more reliable indicator of liver disease than aminotransferase levels in chronic HCV infection, has not been reported in these children.

Perinatal Transmission

Since the implementation of routine and effective screening strategies, perinatal or vertical transmission has become the primary cause of new HCV infections in children.[103] Perinatal transmission of HCV, suspected even before the virus was discovered,[104,105] has been subsequently confirmed in numerous studies by the detection of HCV RNA in infants born to infected mothers.[106–115] The average rate of vertical transmission is approximately 5 to 6%, which is low compared with those observed for hepatitis B virus and human immunodeficiency virus (HIV). The precise timing and process by which the virus is transmitted from mother to infant are unknown, but high-titer maternal viremia consistently correlates with higher transmission rates and thus appears to facilitate the process. Although the rates of vertical transmission are generally higher in infants born to mothers coinfected with HIV (0–36%; mean 16%),[116–120] other researchers have not documented this correlation.[106,114] Similarly, there are conflicting reports on the influence of mode of delivery on the risk of perinatal transmission of HCV, with increased rates after vaginal delivery noted in some[107,108,115] but not all[112,114] studies. In one report, exposure to contaminated maternal blood and placement of fetal scalp probes during delivery were associated with increased risk for perinatal HCV infection.[121] Interestingly, a recent report noted that human leukocyte antigen DR13–positive infants born to mothers with HCV were less likely to become infected than those who did not express this marker, implying that immunologic mechanisms may play an important role in the transmission of HCV from mother to infant.[122] Of note, there appears to be no increased risk of HCV transmission by breast milk because the rate of viral infection transmission is similar between breast- and formula-fed infants.[110–112,114,115]

Liver Transplant

HCV-related chronic liver disease is a leading indication for liver transplant in adults.[123] However, little is known about the risks, significance, and evolution of HCV infection in children undergoing liver transplant. In a cross-sectional study, the overall prevalence of HCV was found to be 6.5% among 65 children who underwent liver transplant by detection of HCV RNA.[124] All HCV-positive children had undergone liver replacement before the initiation of routine screening for HCV in blood donors. In this cohort, serum transaminase levels were significantly higher in young patients with HCV infection than they were in uninfected children. Recurrent HCV infection is nearly universal after liver transplant, but infection arising de novo is now rare because of improved donor screening.[125] The course of HCV-related liver disease appears to be particularly aggressive in children with de novo infection following liver transplant.[126] In this series, viral infection was verified in 14 of 117 patients who underwent liver replacement before the availability of reliable screening tests for HCV. Nearly all of the children with HCV infection had evidence of histologic progression, varying from nonspecific inflammation to cirrhosis. The overall mortality rate in this group of patients was high (23%), despite intense therapeutic interventions including antiviral treatment and, in a few cases, retransplant.

DIAGNOSIS

The diagnosis of HCV infection is based on detection of antibodies directed against recombinant HCV antigens by enzyme immunoassay (EIA) or recombinant immunoblot assay (RIBA) or by detection of HCV RNA using nucleic acid tests (NATs).

Serologic Tests

Initial, first-generation EIAs were licensed by the US Food and Drug Administration (FDA) in 1990 and detected immunoreactivity against a single viral polypeptide (Figure 51.1B-2).[127] Although the assay provided the first reliable means to easily detect HCV infection, it was limited by occasional false-negative[128] and frequent false-positive results, particularly in patients with elevated globulin levels such as those with autoimmune hepatitis.[129,130] Subsequent

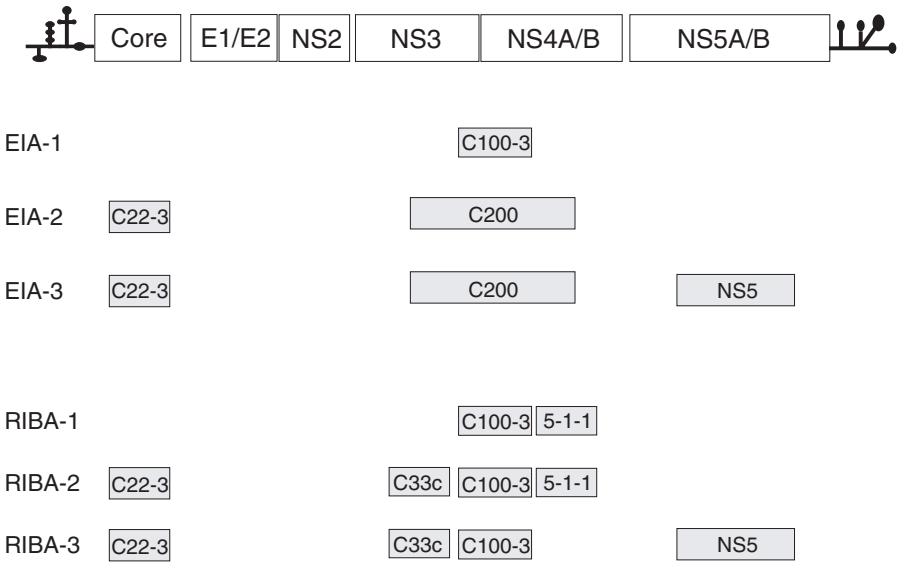

FIGURE 51.1B-2 Hepatitis C virus antigens used in different enzyme immunoassay (EIA) tests. RIBA = recombinant immunoblot assay.

incorporation of additional HCV antigens in second-generation EIAs significantly enhanced the accuracy of the assays (Table 51.1B-2).[131,132] As a result, these more reliable tests were approved by the FDA in 1992 and quickly replaced earlier test versions for the routine detection of anti-HCV antibodies in clinical and laboratory practice. In 1997, the FDA approved a third-generation EIA containing an additional NS5 antigen and reconfigured HCV core and NS3 antigens.[133] However, these modifications did not significantly improve sensitivity and specificity over earlier versions of EIA, and both second- and third-generation assays are currently used. In general, EIAs are easy to perform, yield reproducible results, and are relatively inexpensive, which makes them excellent tools in the initial diagnostic evaluation of and screening for HCV infection. Therefore, the detection of anti-HCV antibodies by these methods in persons with evidence of liver disease and a parenteral risk factor for the virus is probably sufficient to establish the diagnosis of active viral infection. However, the Centers for Disease Control and Prevention advises that anti-HCV EIA–reactive samples be verified by supplemental testing to more precisely identify those harboring HCV.[134]

Like the Western blot test for the detection of HIV antibodies, RIBA detects HCV immunoglobulin (Ig)G antibodies against synthetic HCV recombinant antigens and synthetic peptides immobilized on a solid matrix (nitrocellulose strip) (see Figure 51.1B-2). As in the case of EIAs, more reliable second- and third-generation RIBAs have replaced earlier forms of the test.[135] RIBAs are less sensitive but more specific than EIAs in detecting anti-HCV antibodies. Therefore, RIBAs are not recommended for initial HCV screening and are particularly suited to confirm viral infection.[134]

In addition to IgG anti-HCV antibodies, IgM anti-HCV antibodies are also detected during viral infection. Although the presence of IgM antibodies by EIA correlates well with active viral infection,[136] these are detected in a relatively small proportion of HCV-infected persons. As a result, tests that rely on the detection of IgM anti-HCV antibodies offer little advantage to currently available assays and are not currently used for the diagnosis or clinical management of HCV.

Nucleic Acid Tests

NATs are assays that directly detect circulating virus. There are two NATs currently used for the detection of HCV that rely on different amplification schemes, namely target and signal amplification. In the target amplification process, extracted HCV RNA is transcribed to deoxyribonucleic acid (DNA), which is then directly amplified (polymerase chain reaction) or is converted to RNA during amplifica-

TABLE 51.1B-2 SENSITIVITY AND PREDICTED VALUE OF SEROLOGIC TESTS FOR HCV INFECTION

ASSAY	SENSITIVITY (%)	POSITIVE PREDICTIVE VALUE (%)		TIME TO POSITIVE AFTER INFECTION (WK)
		LOW-PREVALENCE GROUPS	HIGH-PREVALENCE GROUPS	
EIA-1	70–80	30–50	70–85	16
EIA-2	92–95	50–60	88–95	10
EIA-3	97	25	98	7–8

Adapted with permission from Davis GL. Hepatitis C. In: Schiff ER, Sorrell MF, Maddrey WC, editors. Schiff's diseases of the liver.Vol. 1, 9th ed. Philadelphia: Lippincott, Williams and Wilkins; 2003. p. 819.
EIA = enzyme immunoassay; HCV = hepatitis C virus.

tion (transcription-mediated assay). Both quantitative and qualitative (generally more sensitive) versions of target amplification tests are available. By contrast, for signal amplification (branched DNA assay, Bayer Diagnostics, Tarrytown, NJ), extracted HCV RNA is captured by specific oligonucleotides fixed on a solid matrix (Microwell). After incubation with amplifying enzyme–labeled probes and specific substrate, viral levels are determined by comparing the chemiluminescence of test samples to those of controls with known HCV RNA concentrations.

Technical advancements in recent years, in particular the development of automated equipment capable of rapidly and accurately analyzing a large number of samples in a standardized format, have led to widespread use of NATs for the routine laboratory evaluation of HCV in clinical practice. Equally important, the results obtained by different commercial NATs are now normalized against a well-characterized HCV RNA standard developed by the World Health Organization.[137,138] Because these assays were initially developed using different HCV standards, conversion factors are necessary to compare the concentration of HCV RNA copies obtained by the various methods (Table 51.1B-3). One of the major advantages of NATs is their ability to provide direct assessment for the presence of circulating virus, which is only indirectly inferred with serologic assays. Therefore, positive HCV NAT results more precisely reflect active viremia than do antibody-based tests. In addition, exceedingly low levels of circulating virus are detected with currently available NATs, with a detection limit in the range of 30 to 50 IU/mL (see Table 51.1B-3).[139] As a result, NATs markedly reduce the time during which HCV infection can be detected.[140] The ability to recognize HCV infection at the earliest possible phase is particularly important in the clinical practice of transfusion medicine, where the use of NATs has led to reductions in the incidence of post-transfusion hepatitis.[141] Like RIBAs, NATs are also used to confirm HCV infection in anti-HCV EIA–reactive individuals in low-prevalence groups. To minimize the possibility of HCV RNA degradation and thereby avert false-negative results, only serum separated within 4 to 8 hours of venesection and those samples that have not undergone repeated cycles of freeze-thaw should be used for NAT.[134,142]

Nuclear amplification tests are particularly useful in certain clinical scenarios. First, because NATs identify the presence of HCV very early in the course of infection, they can be used to diagnose HCV even before the anti-HCV antibodies have appeared.[139] Therefore, NATs are recommended in HCV EIA–negative patients in whom clinical suspicion for HCV exists. Second, NATs are necessary to detect HCV in infants born to infected mothers, in whom HCV antibodies may be of maternal origin and in immunocompromised patients whose ability to produce HCV antibodies may be impaired. Finally, by identifying persons with active viremia, NATs are critically important to select appropriate candidates for, and monitor the response to, antiviral treatment.

MANAGEMENT

Screening for HCV Infection

HCV testing should be considered for children with risk factors for HCV infection, including recipients of blood product transfusions and organ transplants before the implementation of effective routine donor screening strategies (1992) or of clotting factor concentrates before the widespread use of reliable sterilizing techniques (1987), infants born to HCV-infected mothers, and those with a history of injection drug use. Routine screening of internationally adopted children for HCV is generally not recommended unless the biologic mother has a known high-risk factor, such as injection drug use.[143]

General Care Guidelines

Periodic examination of children with chronic HCV infection is recommended, although there is a lack of consensus about which tests to monitor and how often to check them.[143] In our center, infants, children, and adolescents who are newly diagnosed with HCV undergo thorough medical evaluation to determine the risk factor(s) for infection and detect the presence of liver disease or associated sequelae. This is accomplished by a detailed history and physical examination and comprehensive laboratory evaluation, including complete blood count, liver tests, and coagulation studies. In our practice, any abnormal result promptly triggers further serologic evaluation aimed at excluding other potential concomitant causes of liver

TABLE 51.1B-3 CHARACTERISTICS OF QUANTITATION TESTS FOR HCV

ASSAY	MANUFACTURER	FORMAT	DYNAMIC RANGE (IU/ML)	CONVERSION FACTOR*
Amplicor HCV Monitor V2.0	Roche Molecular Systems (Pleasanton, CA, USA)	Manual RT-PCR	600–500,000	0.9
COBAS Amplicor HCV Monitor V2.0	Roche Molecular Systems	Semiautomated RT-PCR	600–500,000	2.7
LCx HCV RNA	Abbott Diagnostics (Abbott Park, IL, USA)	Semiautomated RT-PCR	25–2,630,000	3.8
SuperQuant	National Genetics Institute (Los Angeles, CA, USA)	Semiautomated RT-PCR	30–1,470,000	3.4
Versant HCV RNA 2.0	Bayer Corporation (Tarrytown, NY, USA)	Manual branched DNA	—	None
Versant HCV RNA 3.0	Bayer Corporation	Semiautomated branched DNA	615–7,700,000	5.2

Adapted from Pawlotsky JM.[139]
DNA = deoxyribonucleic acid; HCV = hepatitis C virus; RNA = ribonucleic acid; RT-PCR = reverse transcriptase polymerase chain reaction.
*HCV RNA IU/mL multiplied by conversion factor = HCV copies/mL.

disease, including other viral hepatitides, autoimmune hepatitis, α_1-antitrypsin deficiency, Wilson disease, and hemochromatosis, as clinically appropriate. Although an arguable practice, histologic assessment of the liver by percutaneous tissue sampling may be valuable in excluding comorbid diseases and determining the severity of liver damage, particularly in those in whom antiviral treatment is being entertained.

Because patients and their families are frequently ill-informed about HCV at the time of diagnosis, an equally critical component of the management of children with HCV is to provide basic concepts about the virus, including ways to prevent its spread and the implications of infection as they specifically relate to potential clinical outcomes and antiviral treatment. Adolescents, in particular, need to clearly understand the negative impact of alcohol on the course of HCV infection (accelerates progression)[144] and should be counseled to abstain from its consumption. The importance of avoiding high-risk behavior such as sharing of intravenous needles needs to be openly discussed and appropriate psychosocial assistance offered to those actively engaged in such practices. The American Academy of Pediatrics also discourages sharing of personal items such as toothbrushes or razors, which may be contaminated. Exclusion of HCV-infected children from child care centers is not justified.[143] Finally, successful completion of hepatitis B virus vaccination should be objectively ascertained and hepatitis A virus immunization should be offered as necessary.

Antiviral Therapy

In an ideal world, the selection of individuals for antiviral therapy would strictly depend on a well-defined propensity to develop severe, chronic liver disease. In such an ideal approach, patients whose liver disease would be more likely to progress would be treated, whereas those expected to have a benign course would be spared treatment and would thereby avert potential treatment-related adverse effects. Unfortunately, the natural history of hepatitis C is incompletely understood, especially in children in whom precise rates of spontaneous viral remission are not reliably known.[103] Accordingly, determining if and when to treat a child or adolescent for chronic hepatitis C is, at the moment, a challenging and controversial task. Nevertheless, compelling reasons exist to consider treating this unique young population with chronic hepatitis C. First, a significant proportion of children exposed to HCV, particularly infants born to infected mothers, develop chronic infection (see Table 51.1B-1). Second, although chronic hepatitis C is usually a mild disease in children with few, if any, symptoms, significant liver damage, including severe hepatitis, cirrhosis, and liver cancer, can occur, as already noted. Finally, factors associated with a favorable response to antiviral therapy in adults, such as short duration of infection, young age, mild histologic inflammation, and absence of cirrhosis, are commonly noted in children with HCV infection. On a theoretical basis, then, a greater global reduction in HCV-related morbidity and mortality may be attainable by treating children (in whom HCV may be easier to eradicate) than adults.

Interferons are a group of naturally occurring agents with antiviral, antineoplastic, and immunomodulatory properties. Multiple large prospective randomized trials confirm that recombinant interferon therapy results in normalization of aminotransferase levels and reductions in serum HCV RNA below detectable levels in approximately 40 to 50% of adults by the end of treatment. Unfortunately, most of these patients relapse, as manifested by elevations of serum aminotransferases and reappearance of serum HCV RNA within 6 months after discontinuation of therapy. Therefore, sustained virologic responses are achieved in only 8 to 35% of adults given interferons alone.[145] Attempts to improve treatment efficacy by using higher interferon doses and prolonging duration of therapy lead to only marginal improvements in virologic responses at the expense of higher rates of side effects. However, significantly higher sustained virologic responses are attained (30–40%) by combining interferons with ribavirin, a guanosine analog. Longer-acting pegylated interferons have been subsequently developed based on the premise that more sustained drug levels would result in greater antiviral activity. Indeed, several randomized clinical trials in adults verify considerably better virologic responses (50–60%) with the use of pegylated interferons, particularly when given in conjunction with ribavirin.[146,147]

Multiple studies have evaluated the efficacy and safety of interferon alone for the treatment of childhood hepatitis C, as comprehensively reviewed.[148] The use of multiple treatment regimens in mostly small and uncontrolled clinical trials in children makes direct comparisons with adult data difficult. However, in general, sustained virologic response rates in children treated with interferon alone (30–60%) appear to be two- to threefold higher than in similarly treated adults.[148] Importantly, biochemical and virologic responses have been accompanied by significant histologic improvement in all treated patients included in these trials, and interferon has been well tolerated in children.

Based on the observed synergy between interferons and ribavirin in adults with chronic hepatitis C, the efficacy and safety of this combination treatment in children have been assessed in several clinical trials. In an initial dose-finding study, sustained virologic response rates were noted in 33%, 35%, and 45% of children treated with interferon and ribavirin 8, 12, or 15 mg/kg/d, respectively.[149] There were no significant differences in the rate of adverse events between the treatment groups. Because a greater sustained virologic response is achieved with the highest dose while maintaining a safety profile similar to that of the lower ribavirin regimens, ribavirin 15 mg/kg/d has become the standard dose for treating children with chronic hepatitis C. Two subsequent pilot studies of childhood cancer survivors with transfusion-acquired HCV given standard interferon and ribavirin doses further verified these initial results because 50% and 64% of treated children had a sustained response, respectively.[150,151] These preliminary results were further confirmed in two subse-

quent larger studies, which together included a total of 110 chronically infected European and North American children and adolescents.[152,153] In a study from Germany, 25 of 40 children (62%) had a sustained response,[152] whereas this was accomplished in 34 of 70 (49%) of young persons in the other trial.[153] As in adults, several important factors are associated with a favorable therapeutic response to interferon therapy given alone or in combination with ribavirin, including infection with an HCV genotype other than type 1, low pretreatment serum HCV RNA levels, younger age, and absence of cirrhosis.[148–153]

Adverse events such as fever, headache, and influenza-like symptoms, ascribed to interferons, are common during the first two to three treatments and generally improve or resolve afterward.[148–153] These frequent side effects may be minimized by administering interferon in the evenings and prescribing routine acetaminophen or nonsteroidal anti-inflammatory agents during the initial weeks of therapy. Hematologic abnormalities are common with antiviral therapy. Ribavirin-induced anemia occurs in nearly all treated patients within 4 to 8 weeks of initiating treatment and then stabilizes, in association with a reactive reticulocytosis. Concomitantly with a reduction in hemoglobin, mild elevations in serum bilirubin and uric acid levels rarely occur. In a similar fashion, neutropenia is consistently seen with antiviral therapy, but this is not associated with clinically significant infections. Mild thrombocytopenia occurs infrequently. Persistent complications include anorexia, weight loss, and depression. It is important to point out that ribavirin is clearly associated with the development of fetal malformations in many animal species tested. Therefore, it is critical that women of childbearing years avoid pregnancy by effective contraception techniques during ribavirin therapy and for up to 6 months after its discontinuation. Finally, there are reported cases of spastic diplegia and seizures occurring in young infants given interferon for treatment of cutaneous vascular anomalies.[154] Although these infants generally received higher doses of interferon than those proposed for chronic hepatitis C, this medication should probably not be used in the very young patient for the treatment of HCV infection.

Because pegylated interferon–based regimens are better than those that rely on conventional interferon in adults, clinical trials are currently under way to assess the efficacy and safety of pegylated interferon in combination with ribavirin in children with chronic hepatitis C. With the development of novel antiviral agents such as HCV protease, helicase, and polymerase inhibitors and efficient immunomodulatory strategies, the future for the eradication of HCV is promising.

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1C. Other Viral Infections

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Numerous viruses have been implicated in causing hepatitis. With the exception of hepatitis B and C, which are covered in the preceding parts of this chapter, these are typically acute, self-limited infections. This part reviews the route of transmission, presentation, and diagnosis for the most important agents in immunocompetent individuals (Table 51.1C-1).

Of the known hepatotropic viruses, hepatitis A virus (HAV) causes the majority of cases of community-acquired viral hepatitis in children throughout the world, whereas hepatitis E virus (HEV) plays a significant role in regions where it is endemic.[1] Because a significant number of cases of both post-transfusion and community-acquired hepatitis are not identified as being caused by hepatitis A–E, investigators have sought to identify additional potentially hepatotropic viral agents. In recent years, candidates have included hepatitis G virus (HGV), TT virus (TTV), and SEN virus (SENV). Whether these agents actually do replicate in the liver and cause hepatitis is now in question; however, the current evidence for and against this is briefly summarized. Finally, a number of viral agents can cause what is typically a milder, nonicteric hepatitis as part of an overall viral syndrome in immunocompetent hosts. The presentation and diagnosis of hepatitis owing to cytomegalovirus (CMV) and Epstein-Barr virus (EBV) are reviewed in this regard. Viral hepatitis as part of a congenital or perinatal infection and in the immunocompromised host is reviewed in Chapter 49, "Approach to Neonatal Cholestasis," and Chapter 51.3, "AIDS and Other Immune Disorders," respectively.

HEPATITIS A

HAV is a nonenveloped ribonucleic acid (RNA) virus in the Picornaviridae family.[2] Within this family, which also includes the rhinoviruses, poliovirus, coxsackievirus, echoviruses, and enteroviruses, HAV is classified in the genus *Hepatovirus*.[3] Oral ingestion of infected material leads to absorption from the stomach or intestine, replication in the liver, secretion into bile, and then either excretion in stool or reabsorption.[2] HAV may also replicate in the intestinal epithelial cells.[4] Viral particles may be detected in the bile and blood, and high titers are present in stool for 2 to 3 weeks before symptomatic liver injury as detected by elevated transaminases has occurred.[5] Fecal excretion then persists for an additional 2 weeks. The hepatitis is due to both a direct cytopathic effect of the virus and the resultant immune-mediated injury, although recent studies have favored the importance of the immune-mediated mechanisms.[6,7]

Hepatitis A is the most common cause of acute hepatitis in the United States; however, in recent years, the incidence in the United States has been declining (Figure 51.1C-1). In 2001, 10,616 cases were reported to the Centers for Disease Control and Prevention. From this, it was estimated that there were 45,000 acute clinical cases and 93,000 new infections in the United States that year.[8] This is as compared with 30,021 reported and 128,000 estimated cases in 1997. The reduction in incidence has been particularly striking in children aged 5 to 14 years. Transmission is primarily via the fecal-oral route, with the most common identifiable source being via personal contact. This includes primarily household contacts and day-care centers. In fact, approximately 10% of cases in the United States each year occur in day-care centers in children who are not toilet trained. Because of the risk of transmission to adults, it is recommended that an index case with hepatitis A be excluded from day-care centers and preschools for 1 week.[9] Foodborne epidemics have been associated with contaminated shellfish and have been transmitted in the United States via produce.[10,11] Waterborne transmission is less important in the United States. Although much less common than fecal-oral transmission, parenteral transmission has also been reported.[12]

Immunoglobulin (Ig)M antibodies directed against HAV are usually present in serum when patients present

TABLE 51.1C-1 AGENTS ASSOCIATED WITH NON-B/C VIRAL HEPATITIS

VIRUS	FAMILY	TYPE	ROUTE OF SPREAD	DISEASE
Hepatitis A	Picornavirus	RNA	Oral/fecal	Acute
Hepatitis E	Hepatitis E–like viruses	RNA	Oral/fecal	Acute
Hepatitis G	Flavivirus	RNA	Parenteral	Unlikely
TT	Circovirus	DNA	Parenteral and nonparenteral	Unlikely
SEN	Circovirus	DNA	Parenteral and nonparenteral	Acute/chronic
Cytomegalovirus	Herpesvirus	DNA	Parenteral and nonparenteral	Acute
Epstein-Barr	Herpesvirus	DNA	Nonparenteral	Acute

DNA = deoxyribonucleic acid; RNA = ribonucleic acid.

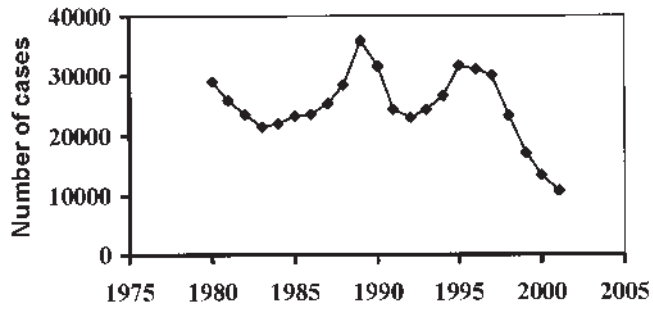

FIGURE 51.1C-1 The annual incidence of reported cases of acute hepatitis A virus (HAV) infection in the United States for the period between 1980 and 2002 is shown. Adapted from the Centers for Disease Control and Prevention.[8]

with clinical symptoms and can first be detected 5 to 10 days after exposure.[13] These are therefore diagnostic of acute infection. An IgG response then occurs that is long-lived and provides resistance against reinfection. The liver injury is likely due primarily to the associated cellular immune response to HAV rather than a direct cytopathic effect of the virus.[6,7] The diagnostic accuracy of the commercially available IgM-specific anti-HAV assay is quite good, with a sensitivity approaching 100%, a specificity of 99%, and a positive predictive value of 88%.[14] Rarely, the test may be negative at the time of presentation but should become positive within 1 to 2 weeks.[15] IgM titers will be detectable for several weeks and then become undetectable. The typical time course of the clinical presentation and viral serology is shown in Figure 51.1C-2.

The clinical presentation of hepatitis A may be classified as sporadic, endemic, or epidemic.[2] Sporadic cases typically occur in older patients and manifest the expected viral prodrome and icteric phase. The endemic form includes a large number of asymptomatic and anicteric cases occurring in younger children.[2] For example, fewer than 10% of children less than 6 years of age will become jaundiced with HAV infection compared with 40% of children between the ages of 6 and 14 years and 70% of children older than 14 years. In endemic areas, 73 to 100% of children have been shown to have been infected in early childhood.[16,17] Epidemics owing to HAV have been reported to have a seasonal pattern, beginning in the fall and peaking in the winter, although the reason for this is not known.[2] The clinical presentation is like that in sporadic cases, with a prodrome featuring anorexia, nausea, vomiting, and malaise leading to the icteric phase. The incubation period averages 30 days, with the prodromal period averaging 7 days. Dark urine is then typically noted before scleral icterus. The duration of jaundice is quite variable but has been reported to range from 7 to 21 days on average.[2] The most prominent finding on physical examination is tender hepatomegaly. The peak bilirubin is typically around 10 mg/dL, and peak alanine transaminase (ALT) is around 3,000 mIU/L (ranging from 20 to 100 times the upper limit of normal). The serum transaminases usually normalize within 2 to 3 weeks, although minor elevations may persist for months, whereas the bilirubin usually normalizes within 4 weeks.

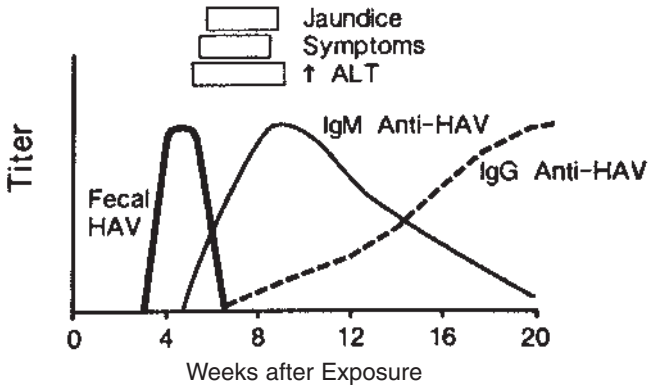

FIGURE 51.1C-2 The sequence and clinical events seen during hepatitis A are shown in this schematic illustration. Fecal hepatitis A virus (HAV) appears during the late phase of the incubation period, peaks near the onset of symptoms, and then declines rapidly; detection of fecal HAV is not used in clinical diagnosis. Diagnosis of acute hepatitis A is usually based on detection of immunoglobulin (Ig)M anti-HAV. Courtesy of the Clinical Teaching Project, American Gastroenterological Association. ALT = alanine transaminase.

Hepatitis A infections may also follow a relapsing, prolonged, or cholestatic course (see Table 51.1C-1). In these individuals, the IgM anti-HAV may persist for 6 to 12 months. Relapses resembling the initial presentation have been documented in 2 to 10% of reported adult series.[18] A prolonged course, with laboratory abnormalities lasting more than 10 weeks, has also been reported in around 9% of cases.[18] Biochemical abnormalities have resolved in these cases by 20 weeks and almost all clinical symptoms by 24 weeks.[2] A cholestatic variant with prominent pruritis, diarrhea, and weight loss has also been described, with eventual complete resolution, although this may take more than 12 weeks.[19] Both the cholestatic and relapsing variants have been associated with an increased frequency of disorders mediated by immune-complex deposition, including cutaneous vasculitis, arthritis, and cryoglobulinemia (see Table 51.1C-1).[20] Finally, there is some evidence that acute HAV infection may trigger an autoim-

TABLE 51.1C-2 ATYPICAL MANIFESTATIONS OF HAV INFECTION

CHOLESTATIC HEPATITIS
Jaundice persists for more than 12 wk
Accompanied by severe pruritis
RELAPSING HEPATITIS
Multiple courses of acute hepatitis
Persistence of IgM anti-HAV in serum
Recurrence of fecal excretion of HAV
IMMUNE COMPLEX DISORDERS
Cutaneous vasculitis
Arthritis
Cryoglobulinemia
AUTOIMMUNE HEPATITIS
Trigger of autoimmune hepatitis in susceptible individuals

HAV = hepatitis A virus; IgM = immunoglobulin M.

mune hepatitis in susceptible individuals.[21] The fatality rate for hepatitis A infection is quite low, reaching a maximum of 1.8% in adults older than age 50 years and less than 0.3% in children under 14 years of age.[8] Older age and underlying liver disease (eg, chronic hepatitis B or C infection) have been associated with more severe disease.

Passive immunoprophylaxis with immune serum globulin is useful for preventing transmission to close contacts of an index case (when given within 2 weeks of exposure) or when traveling to an endemic area. The effectiveness of this strategy has been estimated to be around 90%, although the overall waning of anti-HAV seropositivity in the general population may render this less effective.[22] The US Food and Drug Administration (FDA) has licensed a formalin-inactivated hepatitis A vaccine (Havrix) for administration to children 2 years of age and older. The most recent US recommendations for immunization target children in states with rates of HAV infection twice the national average, or approximately 20 cases per 100,000. Other high-risk populations for which immunization is recommended include travelers to endemic areas (when given within 2–4 weeks of travel), men who have sex with men, users of illegal drugs, persons who are at occupational risk for infection, persons with clotting factor disorders, and persons with chronic liver disease.[23] Individuals with chronic liver diseases should be particularly identified for immunization because coinfection with HAV has been shown to cause more severe liver injury in this setting.[24] However, a recent survey indicated that, in the adult population, HAV immunization in this patient group has not yet been consistently performed.[25] Reported adverse reactions with the vaccine have been minimal, and the seroconversion rate after two doses is 99.8% in healthy individuals.[2] Recent reports have indicated that immunization of the entire population in nonendemic countries would be cost-effective, which may become the policy in the United States in the future.[26,27] Recently, a combination vaccine directed against HAV and hepatitis B virus (HBV) has been approved by the FDA that will be useful in this regard.[28,29]

HEPATITIS E

HEV is a nonenveloped single-stranded RNA virus. It is currently classified within a separate *Hepatitis E–like viruses* genus.[30] Although the general fecal-oral mode of transmission and clinical presentation are quite similar to those in HAV, there are significant differences in its geographic distribution, with endemic areas in subtropical and tropical parts of the world.[31] In contrast to HAV, many outbreaks have been attributable to contaminated water sources, and the highest attack rate is in young adults aged 15 to 40 years.[31] The rate of person to person transmission is relatively lower for HEV relative to HAV, and the prevalence of anti-HEV IgG in endemic areas is much lower, usually not more than 25% for HEV versus 90% for HAV. The case-fatality rate during epidemics is between 0.2% and 4%, with a much higher rate (10–20%) in pregnant women.[31] Endemic areas include parts of India, areas of central and Southeast Asia, northwest China, and parts of Africa (Figure 51.1C-3).

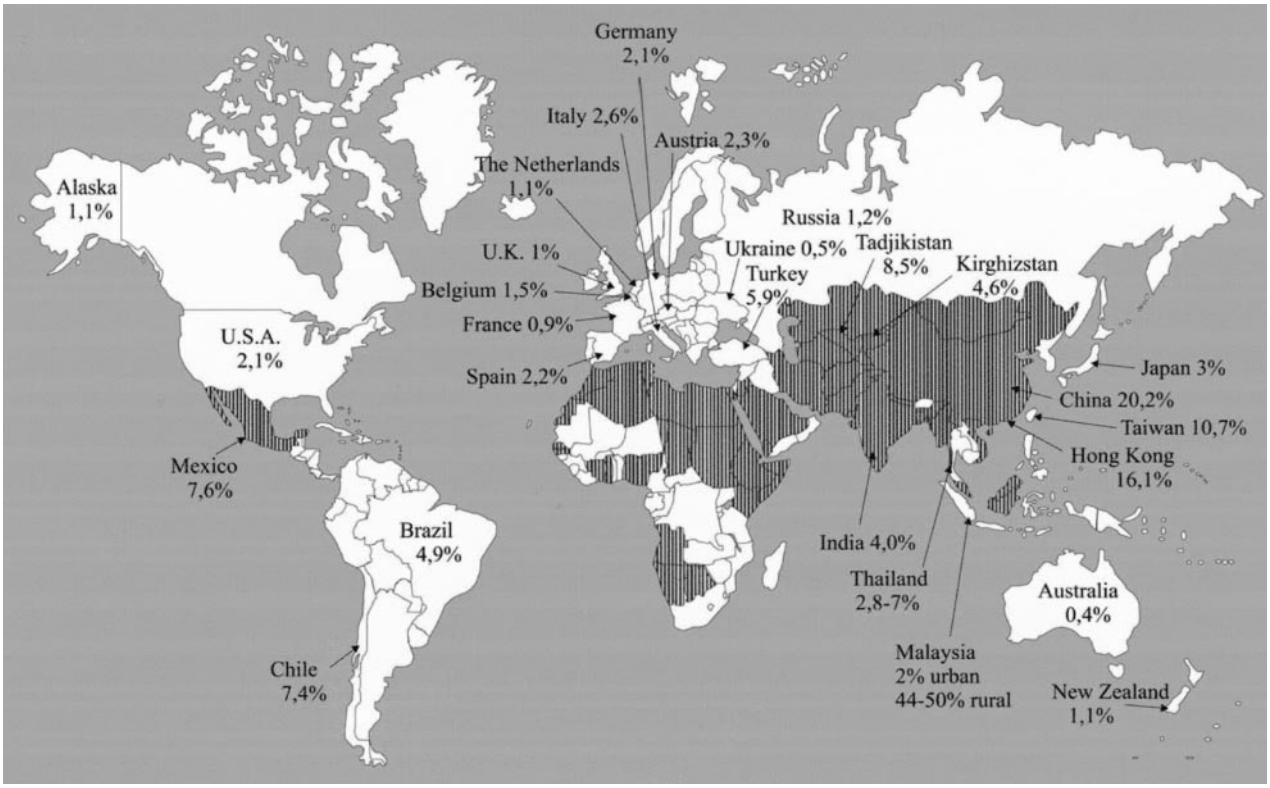

FIGURE 51.1C-3 The prevalence of positive hepatitis E virus serology in various countries is shown. Endemic regions are indicated by the dark vertical bars. Reprinted from Worm HC et al,[30] with permission from Elsevier.

As for HAV, the major mode of transmission for HEV is via the fecal-oral route. Secondary cases in household contacts occur in only 1 to 2% compared with 15% for HAV.[32] Interestingly, cases have not been associated with a foodborne mechanism of transmission. The probability of parenteral transmission is quite low.[33] The prevalence of anti-HEV does not typically exceed 25%, even in endemic areas.[30,31] The reported prevalence in children is much lower (not more than 9%) and increases in adulthood.[34] This is in contrast to other enteric viruses such as HAV, for which the prevalence of anti-HAV may exceed 90% in young children in endemic areas.[35] A low prevalence of anti-HEV ranging from 0.5 to 2% has been consistently detected in the United States and Europe. However, there have been no outbreaks of HEV, and the rare sporadic cases have been primarily limited to travelers to endemic areas. Recent reports have indicated that HEV may also circulate in domestic animals such as swine and in rodents native to industrialized countries; this may represent an increased risk for exposed individuals.[36,37] A recent cross-sectional study identified seropositivity for HEV in 10.9% of North Carolina swine workers compared with 2.4% of nonexposed individuals.[36] This compared with a prevalence of anti-HEV in 34.5% of swine. However, no associated history of past clinical hepatitis or unexplained jaundice was reported in seropositive individuals.

In areas with a low incidence of sporadic HEV, such as the United States and Europe, patients with a recent travel history and acute hepatitis should be evaluated for HEV infection. Otherwise, more common causes should be ruled out first. HEV enters by the oral route and then replicates in the liver.[30] The incubation period ranges from 2 to 9 weeks. The clinical presentation is quite similar to HAV and includes jaundice, malaise, nausea, anorexia, and a variable degree of hepatomegaly. Pruritus may be a prominent feature of the cholestatic form. A flu-like prodrome precedes the acute hepatitic phase. The incubation time is in the range of 15 to 60 days, and the virus is excreted in the stool for an average of 2 weeks after the onset of the illness.[30,32] Abnormalities in transaminases and alkaline phosphatase typically normalize within 1 to 6 weeks.[30] HEV infection may be diagnosed through detection of anti-HEV IgM in serum at the onset of symptoms; this remains positive for 2 to 3 months. Anti-HEV IgG persists long term in about 50% of infected individuals. Although prolonged cases lasting up to 6 months have been reported, there is no evidence that HEV causes chronic hepatitis.[30] As for HAV, HEV superinfection has been reported to worsen liver injury in chronic HBV infection.[38] Although this is typically self-limited, cases of severe liver decompensation have been reported.[39] Thus, patients with chronic liver diseases traveling to or living in endemic areas will be candidates for HEV immunization when a vaccine becomes available.

Strategies to reduce the number of HEV outbreaks and sporadic cases include improved sanitation in endemic areas and the development of an HEV vaccine. This is particularly necessary in endemic areas because of the relatively low prevalence of natural immunity to HEV and the high fatality rate in pregnant women. Because of its high prevalence, HEV is the leading cause of fulminant hepatitis in endemic areas such as India.[40,41] Several efforts are currently under way to develop an effective HEV vaccine, and a candidate vaccine has been evaluated in clinical trials.[42–44]

GB VIRUS C/HEPATITIS G VIRUS

Despite significant advances in the molecular characterization of the primary hepatotropic viruses, A–E, there is still a significant proportion of cases of both post-transfusion and community-acquired hepatitis that are of unknown origin.[45] This includes approximately 10% of cases of transfusion-associated hepatitis and, in some series, up to 20% of cases of community-acquired hepatitis. This and the next two sections briefly summarize the evidence for and against the potential contribution of three recently characterized viruses to these cases: HGV, TTV, and SENV. GB virus C (GBV-C), or HGV, is a recently discovered enveloped RNA virus that belongs to the Flavivirus family.[46] GBV-C and HGV were independently isolated and reported by two different groups; they are 96% homologous, indicating that they are two genotypes of the same virus.[47] Most reported cases have involved parenteral transmission through contaminated blood or blood products; however, cases attributable to intravenous drug use, vertical transmission from mother to child, and sexual transmission have also been reported.[48–50] The risk of vertical transmission is on the order of 50 to 60%, which is much higher than for HCV.[51]

HGV is found throughout the world, with a high prevalence in both healthy populations and in different patient groups.[52–56] This has called into question its actual role in acute or chronic hepatitis.[57–59] Active infection has been detected using a polymerase chain reaction (PCR) assay for HGV RNA in serum, whereas past exposure has been detected using an antibody against the envelope 2 (E2) protein.[50,60] Prevalence rates in healthy populations of 1 to 4% have been reported in Europe and North America and 10 to 33% in South America and Africa.[46] Supporting the parenteral mode of transmission, prevalence rates of 24 to 49% have been reported in intravenous drug use and 18% in polytransfused patients. Rates ranging from 2 to 39% have been reported for acute non-A–E hepatitis and 16 to 43% for fulminant hepatic failure.[46] The majority of adults have a transient infection with clearance of the virus and the appearance of E2 antibodies. However, persistent infections lasting for years in the absence of liver disease have been documented. This has been particularly true in cases of vertical or perinatal transmission.

Although the tissue tropism of HGV is not clearly established, it may replicate in human mononuclear cells rather than in hepatocytes.[61] In this regard, it is interesting to note that coinfection with HGV has been shown to slow the progression of HIV infection, with coinfected patients having higher CD4 counts.[61] Other recent studies have indicated that HGV may also infect and replicate in hepatocytes, albeit without causing any detectable liver injury.[62] Although HGV infection has been implicated in cases of acute, chronic, and

fulminant non-A–E hepatitis, whether it is clearly the cause of these cases has become much less likely with additional negative reports. In most subjects, liver histology has been normal despite evidence of HGV infection in hepatocytes.[62] There is no increase in HGV infection in children with liver disease compared with children without liver disease.[63] Moreover, HGV superinfection has not been shown to worsen liver disease in HCV infection.[64] Overall, most infections are asymptomatic, and whether HGV is truly a cause of non–A-E hepatitis is unlikely.

TT VIRUS

TTV is a nonenveloped single-stranded deoxyribonucleic acid (DNA) virus that was originally identified in an adult with nontypable post-transfusion hepatitis.[65] Although TTV has subsequently been found in patients with non-A–E hepatitis, its overall prevalence has not been different between healthy individuals and patients with liver disease.[66,67] Like HGV, TTV has been detected using PCR from serum samples. A recent study determined the prevalence of TTV in healthy children and children with non-A–E hepatitis and/or a history of transfusion.[68] Depending on the PCR primers used, from 27 to 89% of healthy children had evidence of TTV infection. Children with a history of transfusion had a higher prevalence, ranging from 46 to 100%.[68] However, children with non-A–E hepatitis, whether acute, chronic, or fulminant, did not have a significantly higher prevalence of TTV infection than healthy controls.[68] There was no increase in serum transaminases in children coinfected with TTV and HBV or HCV relative to HBV or HCV alone. Moreover, the treatment response in HCV infection was not affected by coinfection with TTV.[69] Importantly, the data in healthy children also supported a significant nonparenteral route of transmission, beginning in the first year of life and peaking by 4 to 6 years of age. Thus, the data as a whole support both parenteral and nonparenteral routes of transmission for TTV but do not support a significant role for TTV in non-A–E hepatitis in children.[70,71] In fact, more recently, it has been proposed that TTV may represent part of the normal human "viral flora."

SEN VIRUS

The most recent agent to be implicated in non-A–E hepatitis is SENV. SENV is a single-stranded DNA virus from the newly characterized Circovirus family that bears some homology to TTV.[72] Five strains have been characterized, with total SENV detected in 13% of healthy blood donors and up to 70% of transfused patients.[72] The SENV-D and SENV-H strains have been felt to be more promising in terms of potentially causing liver disease, with a prevalence of less than 1% in healthy blood donors and up to 50% in cases of transfusion-associated non-A–E hepatitis.[72] The prevalence of SENV infection was shown to increase with the volume of transfused blood, supporting the parenteral mode of transmission.[72]

In a recent prospective study, 11 of 12 patients who developed nontypable post-transfusion hepatitis were infected with SENV-D and/or SENV-H.[72] However, 55 of 225 patients who did not develop post-transfusion hepatitis were also acutely infected with SENV-D and/or SENV-H. None of the patients were jaundiced; the mean peak ALT level was 396 U/L. In some cases, there was concordance between the level of viremia and ALT. The severity of HCV cases detected in this study was not affected by coincident SENV infection. Most patients cleared SEN-V infection, although 15% were chronic carriers for several years. In two of these patients, chronic viremia was associated with a persistently elevated level of ALT. Overall, this study demonstrated the relatively common occurrence of post-transfusion SENV infection in 30% of patients. Whereas a minority (11/86) of SENV-infected patients did develop acute non-A–E hepatitis temporally related to the appearance of viremia, the majority (75/86) did not. Several recent retrospective studies similarly documented a high prevalence of SENV infection in both healthy individuals and those with acute or chronic liver diseases and also supported a nonparenteral route of transmission.[73–75] Therefore, it is unlikely that SENV infection causes non-A–E hepatitis in most cases. As for TTV, coinfection with SENV has not been shown to affect the severity or response to treatment of HCV; SENV, however, has been shown to be sensitive to interferon therapy.[76] However, further data regarding hepatotrophism and replication will be required to determine whether SENV infection does cause hepatitis in a minority of cases.

CYTOMEGALOVIRUS

It has been estimated that up to 10% of cases of acute viral hepatitis in immunocompetent individuals may be due to non-A–E agents. These may include EBV, herpesviruses, CMV, adenovirus, and parvovirus. The clinical presentation, diagnosis, and management of acute hepatitis associated with CMV and EBV are summarized in the next two sections. A detailed discussion of hepatitis owing to the other viral agents is beyond the scope of this section.

CMV is one of seven members of the herpesvirus family, each of which may infect the liver. Although CMV and other herpes family member infections are a more significant problem in immunocompromised hosts, they may also cause a variety of clinical diseases in immunocompetent patients. This includes what is typically an acute anicteric hepatitis as part of a systemic infection.

By the age of 15 years, approximately 20% of children will have been infected; this reaches a maximum of 50 to 60% by 25 to 30 years of age.[77] The consequences of CMV infection in immunocompromised patients and infants are covered in another chapter. CMV may infect and replicate in both hepatocytes and cholangiocytes.[78] However, whether the resulting liver injury is due to a direct cytopathic effect of the virus or the host immune response is not clear. In fact, this may vary depending on the clinical situation.[78] Whether hepatocytes may harbor CMV in a latent infection is also not well understood and is currently being studied. Finally, it has recently been reported that patients with other forms of chronic viral hepatitis and cir-

rhosis may be more susceptible to developing acute CMV infections that cause additional liver injury.[78]

Although acute primary CMV infection in immunocompetent hosts is usually asymptomatic, it may result in a syndrome similar to EBV mononucleosis.[78] This includes fever, malaise, cervical lymphadenopathy, and splenomegaly. Liver involvement is indicated by a modest elevation in serum transaminases (usually less than threefold) and minimal hepatomegaly.[79] Overt jaundice is rare.[79] The diagnosis of CMV infection may be made by detecting anti-CMV IgM antibodies or CMV antigen in serum or CMV cultured from urine. Typical viral cytomegalic cells or CMV early antigens are rarely detected on liver biopsy, perhaps indicating a vigorous immune response in the normal host leading to destruction of infected hepatocytes. By comparison, cytomegalic cells and both CMV early and late antigens are frequently detected on liver biopsy of transplant patients infected with CMV.[78] Therefore, the liver injury in the normal host may be due primarily to the host immune response, whereas viral cytopathic injury may be more important in the immunocompromised host.[78]

Compared with infection with HAV, patients with hepatitis owing to CMV tend to have fever longer and more prominent cervical lymphadenopathy and splenomegaly.[78] The incubation period ranges from 3 to 12 weeks.[80] Jaundice is much less common, and the maximum elevation in serum transaminases is typically less than 200 U.[80] Transaminases typically peak 2 to 3 weeks after disease onset and normalize by the fifth week.[80] Very rarely, however, acute CMV infection has been reported to cause massive hepatocellular necrosis in a normal host, with attendant fever, jaundice, highly elevated transaminases, and coagulopathy. In severe cases, therapy with ganciclovir or foscarnet may be effective.[81,82]

Ordinarily, CMV infection leads to a lifelong latent phase in the normal host. CMV can then become reactivated in immunocompromised individuals.[78] Patients with chronic liver disease, particularly when it has advanced to cirrhosis, develop a condition of impaired cellular immunity. This may make them more susceptible to CMV reactivation or reinfection. Coincident CMV infection is then associated with an additional impairment in liver function.[78] The specific effect of CMV infection on the progression of chronic liver diseases, including HBV and HCV, is currently the subject of active investigation.[78] A recombinant CMV vaccine has recently completed phase I trials in seronegative toddlers and may prove useful in terms of preventing transmission to woman of childbearing age and other individuals for whom the consequences of primary CMV infection may be severe.[83]

EPSTEIN-BARR VIRUS

As for CMV, EBV may also affect the liver as part of a generalized mononucleosis syndrome in the normal host. This section is limited to a brief review of the hepatitis associated with infectious mononucleosis; the role of EBV in post-transplant lymphoproliferative disease, lymphoma, and acquired immune deficiency syndrome (AIDS) is discussed elsewhere.

Like CMV, EBV is ubiquitous and will have infected over 80% of individuals by the time they are adults.[84] It is a double-stranded DNA virus. Infection of the oropharynx results in ongoing shedding of virus during an active infection. EBV DNA is subsequently incorporated into B cells, resulting in lifelong latent infection. The primary infection in young children is usually asymptomatic.[84] Infection in adolescents leads to the classic infectious mononucleosis syndrome, with fever, cervical lymphadenopathy, sore throat, fatigue, and splenomegaly. Primary infection is confirmed by detection of antiviral capsid antigen IgM in the serum.[84] Liver involvement is similar to CMV, with a mild anicteric hepatitis in most cases. However, from 5 to 10% may develop jaundice.[79,85] Rarely, in about 1 in 3,000 cases of infectious mononucleosis, this may take a more fulminant course, with severe hepatitis, bone marrow failure, and acute respiratory distress syndrome.[84,86] Death may result in these cases from massive hepatocellular necrosis and liver failure. Liver injury owing to EBV is likely secondary to infected cytotoxic T lymphocytes rather than a direct cytopathic effect of the virus on hepatocytes.[87]

FULMINANT HEPATITIS

A more detailed discussion related to evaluation of the child with fulminant hepatic failure is presented in Chapter 58, "Acute Liver Failure." Although each of the viruses previously reviewed may rarely cause fulminant hepatitis in children, a number of cases do not have an identifiable cause. This has led a number of investigators to search for additional causes of severe viral hepatitis.[88] The reported contribution of HAV in developed countries has ranged from < 5% (United States) to 26 to 31% (France and England).[88] Although the reported prevalence of HBV infection in adult fulminant hepatic failure has ranged from 25 to 75%, making it the most common identified viral cause, it remains an uncommon cause in children in nonendemic areas, including the United States.[88] In endemic areas, it plays a significant role, equivalent to the prevalence reported for adults. Also in endemic areas, infection with HEV alone or in combination with HAV has commonly been implicated in pediatric cases of fulminant hepatic failure. This has not been reported in children in the United States or western Europe.[88] As previously reviewed, it is unlikely that HGV or TTV causes fulminant hepatic failure; the potential role of specific strains of SENV remains under investigation. Viruses in the herpes family, including CMV, EBV, herpes simplex virus, and varicella-zoster virus, have each been implicated in causing fulminant hepatic failure, although almost always in an immunocompromised host.

Therefore, a significant proportion of cases of severe hepatitis and, in particular, fulminant hepatic failure in children are presumed to be due to an as yet unidentified virus(es). Survival without liver transplant is lower for this entity than for fulminant hepatitic failure owing to HAV or HBV.[88] The association with aplastic anemia has strengthened the hypothesis that this is a viral infection. In this regard, parvovirus B19 infection has been implicated in

some cases, although its overall contribution is unclear.[89,90] A recent study from Japan identified human herpesvirus 6 in 7 of 11 patients (5 children and 2 adults) with fulminant hepatic failure.[91] With continued improvements in molecular techniques, additional potential viral causes of non-A–E hepatitis will likely be identified in the near future. However, rigorous methods will be required to determine whether these agents are causative or innocent bystanders.

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2. Bacterial, Parasitic, and Other Infections

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The epidemiology of postnatal nonviral infections of the liver varies throughout the world. Whereas in the United States, most nonviral infections of the liver are bacterial, parasites are especially important etiologic agents in developing countries. Although the incidence of hepatic abscesses postappendicitis has decreased substantially owing to the advent of broad-spectrum antibiotics and improvements in surgical care, liver infections in immunocompromised hosts are on the rise. The clinical presentation of hepatic infections may be subtle, and a high index of suspicion is necessary, especially in those patients at high risk. Modern diagnostic techniques and novel therapeutic interventions have afforded significant improvements in mortality rates.[1,2] This chapter discusses the assessment and management of pyogenic liver abscess and some of the other more common nonviral infectious causes of postnatal liver infection.

PYOGENIC ABSCESS

Incidence and Pathogenesis

Pyogenic liver abscess is an uncommon infection in children. The incidence was reported as 0.35% in all pediatric cases coming to autopsy in St. Louis prior to 1967 and as 0.03% of all admissions to Milwaukee Children's Hospital between 1957 and 1977.[3,4] Whereas pyogenic liver abscess in South Africa and India can account for 0.015% and 0.078% of hospital admissions, respectively,[1,5] a hospital rate of 0.025% was noted in a pediatric population from Florida.[6] It is suspected that the incidence of hepatic abscess in children is on the rise, likely owing to improved diagnostic imaging techniques and the changing complexity and survival of pediatric patients at high risk for developing liver abscess, such as those with leukemia or immunodeficiency. Pyogenic liver abscess does occur in neonates, accounting for 0.026% of admissions to a neonatal intensive care unit in one study.[7]

Pyogenic bacteria can reach the liver via the portal vein or hepatic artery from structures adjacent or contiguous to the liver or by direct hepatic trauma. Infections originating within the abdominal cavity (eg, appendicitis, omphalitis, or perforated viscus) can seed to the liver, resulting in abscess formation. Although biliary tract disorders are less common in pediatric patients compared with adults, children who have undergone hepatic portoenterostomy are predisposed to developing ascending cholangitis and liver abscess.[3] In developing countries, malnutrition and helminthic (ascariasis) infestations are the major risk factors associated with liver abscess in children.[1,8] In developed countries, pyogenic liver abscesses occur most frequently in immunocompromised hosts. A review of 92 children with chronic granulomatous disease (CGD) found that 45% of cases were complicated by hepatic or perihepatic abscesses.[9] Children with leukemia on chemotherapy are predisposed to developing bacterial or fungal liver abscesses.[3,10] Patients with congenital neutropenia,[4] hyperimmunoglobulinemia E syndrome,[11] or other congenital or acquired immune deficiency syndromes[12] are also at risk for developing liver abscess. In a review of adult liver transplant recipients, allograft abscess was reported in 1% of cases, with hepatic arterial thrombosis being a significant risk factor.[13] Hepatic "mini-microabscess syndrome" has recently been described in pediatric liver transplant recipients.[14] Liver abscesses have been reported in children with Crohn disease[15] and sickle cell anemia.[16] Ingestion of sharp objects,[17] penetrating injuries to the liver,[18] and the presence of a ventriculoperitoneal shunt[19] can be complicated by the development of liver abscesses. Pyogenic liver abscess also occurs in healthy immunocompetent children, yet no specific etiology is identified in many of these "cryptogenic" cases.[20] The development of liver abscess in neonates is usually associated with umbilical venous catheterization, prematurity, and necrotizing enterocolitis requiring surgical intervention.[7]

Microbiology

A number of pathogenic bacteria can cause pyogenic liver abscess; in some cases, more than one pathogen is recovered (Table 51.2-1). In a review of 96 children with pyogenic liver abscess, *Staphylococcus aureus* was the most common pathogen, seen in 44% of the cases, followed by gram-negative enteric organisms such as *Escherichia coli*, *Pseudomonas*, and *Klebsiella* in 25% of cases and anaerobic organisms in 10% of cases.[21] An increasing role for anaerobes as a source of infection has been appreciated in both adults and children, probably owing to improvement in anaerobic culture techniques.[22] Recently, a severe form of liver abscess owing to *Klebsiella pneumoniae* along with endophthalmitis has been reported in diabetic adults from Taiwan.[23] In neonates with liver abscess, gram-negative enteric organisms are the most common pathogens isolated.[7] Patients with CGD have a high frequency of *S. aureus*, but gram-negative enteric organisms that produce catalase have also been noted.

Knowledge of the source or route of infection can be helpful in predicting a particular pathogen. *S. aureus* is the

TABLE 51.2-1 INFECTIONS OF THE LIVER

BACTERIA
Staphylococcus aureus
Streptococci
Escherichia coli
Pseudomonas aeruginosa
Klebsiella
Proteus
Enterococcus fecalis
Serratia
Salmonella
Brucella
Gonococci
Bartonella
RICKETTSIA
R. rickettsii
Coxiella
PARASITES
Amebiasis
Malaria
Ascariasis
Echinococcosis
Clonorchiasis
Fascioliasis
Schistosomiasis
FUNGI
Candida
Histoplasma
Aspergillus
Cryptococci
Coccidiodes
ANAEROBES
Peptostreptococci
Bacteroides
Clostridium
SPIROCHETES
Leptospira
Borrelia

most likely organism in children with liver abscess following bacteremia or in those who are immunocompromised. Gram-negative enteric organisms and anaerobes are likely culprits when infection spreads to the portal venous system from the gastrointestinal tract. Fungal liver abscesses may occur in neutropenic children with leukemia who have been on broad-spectrum antibiotics.[10] Patients with acquired immune deficiency syndrome (AIDS) are at increased risk for mycobacterial abscesses owing to *Mycobacterium tuberculosis* and *Mycobacterium avium-intracellulare*.[12]

Clinical Features

In children, the clinical manifestations of pyogenic liver abscesses are nonspecific. A high index of suspicion is necessary to establish the diagnosis, particularly in those patients with predisposing risk factors. The presentation may be acute, with rapid onset of severe symptoms, or chronic, with a more insidious onset over weeks to months. The most common presenting complaints are fever and abdominal pain. Many patients have other associated symptoms, including nausea, vomiting, anorexia, malaise, and weakness. Some children present only with fever of unknown origin. Hepatomegaly is present in 50 to 73% of children with hepatic abscess,[6,20] and right upper quadrant tenderness is noted in 40% of cases.[20] Other findings such as abdominal distention or evidence of pleuropulmonary involvement are uncommon on physical examination. The diagnosis of liver abscess in neonates is especially difficult because the presentation is often similar to neonatal sepsis.[24] Children from developing countries tend to have a more acute presentation, with fever, abdominal pain, tender hepatomegaly, and septic shock.[1,25]

Diagnosis

Routine laboratory tests, like the clinical presentation, are nonspecific and are of little value for establishing the diagnosis of liver abscess. Anemia and leukocytosis are typically seen; however, children with underlying malignancy on chemotherapy may be leukopenic.[3] Hepatic transaminases are usually normal or only mildly elevated. Elevation in serum alkaline phosphatase and bilirubin is present in cases secondary to biliary obstruction. Although the yield of aerobic and anaerobic blood cultures is variable and may be quite low, the documentation of bacteremia with the identification of a specific organism is most helpful for directing treatment. Patients with multiple abscesses are more likely to have positive blood cultures than those with a solitary abscess. In a review of neonates with liver abscesses, half of the patients were bacteremic with the same organism that was eventually cultured from the liver abscess.[7]

Radiologic imaging is the most important tool for establishing the diagnosis of liver abscess. Plain chest radiographs may be abnormal, with the presence of an elevated right hemidiaphragm, right pleural effusion, or air in the abscess cavity. Historically, radioactive-isotope liver-spleen scanning or angiography was used to establish the diagnosis. However, the recent advent of noninvasive imaging techniques, including ultrasonography and computed tomography (CT), has allowed for easy, quick, and accurate diagnosis. Ultrasonography is the imaging study of choice in suspected liver abscess because of its high sensitivity and the relative ease with which it can be performed, even in very young infants. On ultrasonography, abscesses appear as hypoechoic lesions with irregular borders (Figure 51.2-1). In children, a solitary (rather than multiple) abscess located in the right (rather than left) lobe of the liver is the most common finding.[25] The differential diagnosis of a solitary hypoechoic liver lesion includes congenital hepatic cyst, liver tumor, or hydatid cyst. On ultrasonography, pyogenic abscesses are more likely to have a "honeycomb" pattern with irregular margins compared with amebic abscesses.[26] CT (Figure 51.2-2) should be requested in the face of a normal sonogram if the clinical suspicion remains high. Magnetic resonance imaging of a hepatic abscess offers no significant advantage over CT or ultrasonography for detecting or characterizing liver abscess.[27]

Treatment

Antibiotic therapy with drainage of the pus collection is the mainstay of therapy for liver abscess. Supportive management in the form of intravenous hydration and analge-

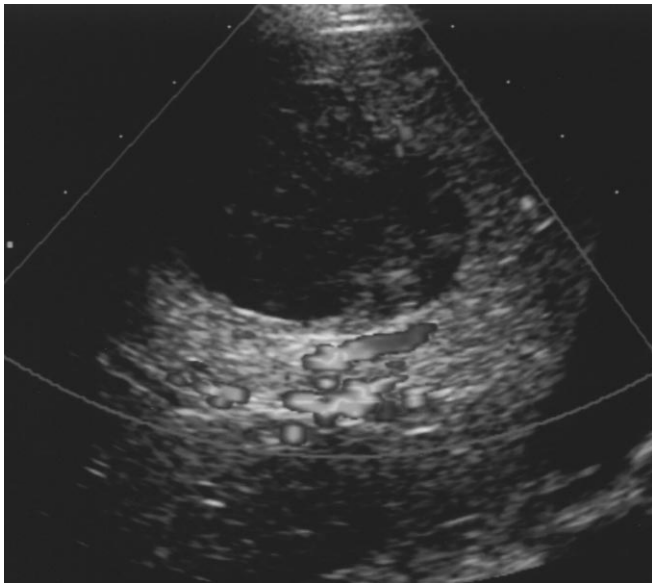

FIGURE 51.2-1 A sonogram of a large solitary abscess in the right lobe of the liver. The abscess appears as a hypoechoic lesion with irregular borders. Courtesy of Dr. D. H. Jamieson, British Columbia's Children's Hospital, Vancouver, BC.

sia may be required. Initial antibiotic therapy should be empiric, broad spectrum, and based on knowledge of the organisms most commonly involved, as discussed earlier. A penicillinase-resistant penicillin should be used, along with an aminoglycoside or third-generation cephalosporin for gram-negative flora, whereas clindamycin, cefoxitin, and metronidazole are appropriate for anaerobes. Empiric parenteral antibiotic therapy should not be delayed, pending the abscess drainage procedure. Once pathogens are identified in blood or abscess cultures, antibiotic therapy can be tailored to susceptibility.

Drainage of the liver abscess can be performed by needle aspiration, percutaneous catheter, or an open surgical approach. In a recent controlled trial, percutaneous continuous catheter drainage was more effective than therapeutic large-needle aspiration.[28] Other reports have documented the safety and efficacy of percutaneous drainage, although left lobe abscesses are more difficult to treat by this route.[6,29] The duration of continuous percutaneous drainage is usually 2 to 3 weeks, whereas parenteral antibiotic therapy should be maintained for at least 2 to 4 weeks, followed by oral antibiotics for a total of 4 to 6 weeks. Longer treatment may be required in immunocompromised children. In a recent report of pyogenic liver abscess in adults, sequential intravenous therapy for 3 weeks followed by oral antibiotics for 3 weeks was safe and equally effective compared with 6-week intravenous antibiotic therapy.[30] Serial ultrasonographic imaging of the abscess cavity and clinical response are helpful in deciding the duration of antibiotic therapy. In a recent series of adults with pyogenic liver abscesses, percutaneous drainage of liver abscesses was associated with a success rate of 90%.[2] Open surgical drainage should be reserved for those cases in which percutaneous drainage is not feasible or fails or cases in which other intra-abdominal pathology is suspected. Drainage procedures may be associated with complications. In a large series of children who were treated with open surgical drainage for pyogenic and amebic liver abscesses, complications such as adhesive intestinal obstruction, incisional hernia, and colonic fistula resulting from erosion of the drain were reported.[25]

Multiple hepatic microabscesses or those abscesses not amenable to continuous drainage have been successfully treated with prolonged antibiotic therapy alone. However, in a large series of South African children with pyogenic or amebic liver abscesses, conservative treatment with antibiotics was successful in only 37% of cases[25]; the remaining cases eventually required surgical drainage owing to worsening of the clinical picture. If a conservative approach is taken, close clinical monitoring with surgical backup is mandatory. The management of children with CGD is difficult. Whereas small abscesses (< 5 cm) may be treated with antibiotics alone, surgical management of larger hepatic abscesses in patients with CGD is crucial for survival.[31] Despite aggressive management, children with CGD may be resistant to the standard therapeutic options of antibiotics and drainage. In this instance, interferon-γ has been employed successfully.[32] Following the successful treatment of a child with liver abscess, appropriate immunologic investigations should be planned because liver abscess may be the presenting manifestation of an immunodeficiency disorder.

Outcome

The complications of pyogenic liver abscess include pleural and pericardial effusions, rupture leading to peritonitis, septicemia, and shock.[25] The mortality associated with pyogenic liver abscess before 1977 was high: up to 27% in children with CGD and 42% in children without CGD.[4] The diagnosis was often missed and was established only at autopsy in many cases. A review of 109 cases of liver abscess in children (excluding neonates) from 1977 to 1988 reported a drop in mortality to 15%.[6] Recently, a mortality figure of 11% was quoted from a series from India of 18 children with pyogenic liver abscesses.[1] Recent

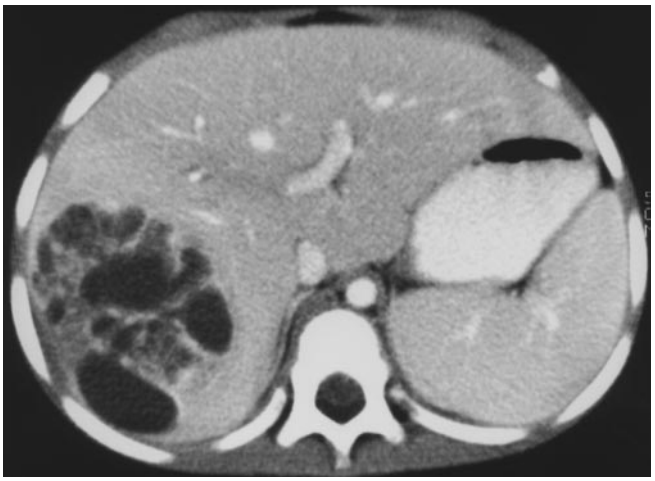

FIGURE 51.2-2 Abdominal computed tomographic scan showing a large multiloculated abscess in the right lobe of the liver.

studies in adults have also reported improvement in mortality rates.[2] In adults, the most important determinant of mortality is the presence of major underlying disease such as malignancy or severe organ dysfunction.[2] Early diagnosis and prompt therapeutic intervention are also important determinants for survival.[2] Thus, the overall outcome for pyogenic liver abscess has been improving in recent years. The principal reasons for this are improvements in imaging techniques (allowing for prompt diagnosis), the introduction of efficacious antibiotic therapy, and novel interventional radiologic techniques.

OTHER BACTERIAL INFECTIONS

Typhoid Fever

Typhoid fever (enteric fever) is an acute systemic illness usually caused by *Salmonella typhi*. It is a major health problem in tropical and developing countries but is uncommon in developed countries. The presenting symptoms, reported in a series of 94 children with typhoid fever from Florida, included fever, diarrhea, vomiting, abdominal pain, and anorexia.[33] Hepatomegaly and splenomegaly were noted in 52% and 23% of children, respectively. A mild elevation of serum hepatic transaminases was noted in the majority of patients in that series, but clinical jaundice was uncommon.[33] In developing countries, clinical and biochemical evidence of hepatic dysfunction is much more obvious, particularly in multidrug-resistant typhoid fever.[34] In one series of adult patients, abnormal liver enzymes were noted in 100% of cases after the first week of typhoid fever, and it was proposed that the liver is always affected to some degree in these patients.[35] Rarely, typhoid fever can mimic an acute viral hepatitis, with fever and moderately abnormal liver enzymes being the sole clinical features.[36] Histologic features on liver biopsy are nonspecific and include focal necrosis with mononuclear infiltrate, inflammation of the portal area, and hyperplasia of Kupffer cells.[35] Other organ complications of typhoid fever in children include encephalopathy, seizures, myocarditis, and circulatory failure. The serodiagnosis of *S. typhi* infection using the Widal agglutination assay is unreliable and not recommended. The diagnosis can be established by positive blood culture or through novel molecular biologic techniques using deoxyribonucleic acid (DNA) probes and polymerase chain reaction (PCR). The abnormal liver function tests seen in typhoid fever completely resolve with appropriate antibiotic treatment.[35] Multidrug-resistant typhoid fever is an increasing problem in tropical countries, and ciprofloxacin and ceftriaxone have been used in such cases.[37] The chronic carrier state for typhoid fever is thought to be due to persistent infection in the liver or gallbladder.

Brucellosis

Brucellosis is primarily a zoonotic infection, caused by *Brucella melitensis*, *Brucella abortus*, or *Brucella suis*.[38] The mode of transmission to humans is direct contact with infected animals, such as cattle and sheep, or consumption of infected unpasteurized milk or milk products. Brucellosis in childhood can present with nonspecific symptoms. In a series of 102 children with brucellosis, the common symptoms were fever, malaise, arthralgia, weight loss, and anorexia.[39] Hepatomegaly was noted in 28% of children in that series, and splenomegaly was present in 35% of cases. Complications of brucellosis include osteomyelitis, pneumonitis, meningitis, and endocarditis. Hepatosplenic abscess has been reported in a 3-year-old child, but this appears to be a rare complication.[40] Common laboratory features include leukocytosis and an elevated erythrocyte sedimentation rate. Elevated hepatic transaminases and alkaline phosphatase were noted in 58% of children.[38] Histopathologic findings in the liver are variable and include inflammation of the portal tract, hepatocellular necrosis, and noncaseating epithelioid granuloma.[41]

The diagnosis of brucellosis should be suspected with a history of exposure, and diagnosis is confirmed by isolation of the organisms in blood or bone marrow cultures. A presumptive diagnosis can be made by high or rising titers of *Brucella* antibodies. Brucellosis responds well to antibiotic therapy, and the prognosis is excellent. In a large multicenter study of children with brucellosis, tetracycline for 3 weeks with initial gentamicin therapy was recommended as the most appropriate therapy for children older than 8 years.[42] For younger children, trimethoprim-sulfamethoxazole can be used in place of tetracycline.

Perihepatitis (Fitz-Hugh–Curtis Syndrome)

Perihepatitis, or Fitz-Hugh–Curtis syndrome, occurs as a complication of pelvic inflammatory disease in young adolescent females. Although the classic cause is *Neisseria gonorrhoeae*, this syndrome has also been described with *Chlamydia trachomatis* infection.[43] The pathophysiology of this syndrome is uncertain, and direct extension of the infection from the genital tract to the hepatic capsule is a possible mechanism.[44] The clinical presentation is one of acute, sharp, right upper quadrant abdominal pain, with or without fever, mimicking the signs of acute cholecystitis.[45,46] Clinical findings may include hepatomegaly, tenderness in the right upper quadrant, and a "friction rub" over the liver. Serum hepatic enzymes and bilirubin are normal.[45]

The history and physical findings of the associated pelvic inflammatory disease are suggestive of the diagnosis, and isolation of the causative organisms from the cervix or urethra confirms the diagnosis. Laparoscopic findings include classic "violin string" adhesions from the liver to the right costal wall.[47] The hepatic parenchyma does not appear to be involved, and liver biopsy findings are normal. Treatment with appropriate antibiotics for the causative organisms is associated with an excellent response.

Cat-Scratch Disease

Cat-scratch disease is an infective illness caused by the gram-negative bacillus *Bartonella* (previously *Rochalimaea*) *hensalae*. Following inoculation of the bacillus, a papule occurs locally, which vesiculates and then encrusts. Within a few days, regional lymphadenopathy (which may suppurate or remain enlarged for a few months) is noted. Although cat-scratch disease occurs in persons of all ages, the highest incidence is among children under 10 years of age.[48] In more than 75% of children, the illness is mild, with generalized

myalgias, malaise, anorexia, fever, and abdominal pain.[49] Unusual clinical manifestations include preauricular lymphadenopathy and conjunctivitis (Parinaud oculoglandular syndrome), pneumonia, erythema nodosum, encephalitis, and granulomatous hepatitis. Cat-scratch disease can also present as a prolonged fever of unknown origin, with or without any obvious adenopathy.[50,51]

Serum hepatic transaminases, alkaline phosphatase, and bilirubin are usually normal, and the erythrocyte sedimentation rate is often elevated. Abdominal imaging (ultrasonography and CT), usually performed as part of the evaluation for fever of unknown origin, may reveal multiple small hypoechogenic lesions in the liver and spleen, with mild enlargement of both organs.[50,52] In a recent retrospective study of 13 children with hypoechogenic liver lesions, 70% were found to have *Bartonella* infection.[51] Liver histology showed epithelioid granuloma with central necrosis and chronic inflammation. Warthin-Starry–stained bacilli may be found in the biopsy specimen. The indirect fluorescence antibody test for *B. henselae* provides a rapid and reliable diagnostic test.[51] The disease is self-limiting in nature, and complete resolution, without any therapy, of radiographically detected hepatic lesions has been reported.[50] However, antibiotics such as ciprofloxacin, azithromycin, and doxycycline have been used in severe cases with good response.[48,53] In a recent report from Texas, 2-week rifampicin therapy resulted in rapid improvement in symptoms and was recommended as an initial antimicrobial treatment of hepatosplenic cat-scratch disease in children.[54]

Spirochetal Infections

Leptospirosis. Leptospirosis is caused by one of several serotypes of *Leptospira interrogans*. Humans acquire infection by exposure to urine or other body fluids of infected animals such as dogs, cattle, hogs, and rats. Leptospirosis is usually a biphasic illness with an initial septicemic or leptospiremic phase lasting 4 to 7 days, characterized by fever, chills, headache, anorexia, abdominal pain, rash, and lymphadenopathy.[55] A second or immune phase, thought to be caused by the host response to the infection, is heralded by a lower-grade fever and complications including hepatitis, jaundice, renal dysfunction, thrombocytopenia, and meningitis. In one series from Brazil, significant jaundice was noted in 70% of infected children, and abnormal liver transaminases were seen in 56%.[56] In another series, 55% of children with leptospirosis had hepatomegaly and acalculus cholecystitis.[57]

Weil syndrome is a rare severe form of leptospirosis associated with hepatic dysfunction, renal failure, hemorrhagic manifestations, and pulmonary involvement, and it portends a high mortality rate.[55] Abnormal laboratory findings in Weil syndrome include direct hyperbilirubinemia, elevated serum transaminases, and prolonged prothrombin time.[55] Histologic changes in the liver include multinucleated cells, proliferation of Kupffer cells, erythrophagocytosis, and cholestasis.[55] Severe hepatic necrosis is unusual, and complete recovery of liver function is seen in those who survive. The diagnosis of leptospirosis requires a high index of suspicion and is confirmed by isolating the organism from blood, cerebrospinal fluid, or urine. A fourfold increase in antibody titers between acute and convalescent sera also establishes the diagnosis.[56] Optimal treatment of leptospirosis is with penicillin or ampicillin, along with supportive management. Antibiotics have been shown to be of significant benefit even in late-presenting or severe cases of leptospirosis.[58] The mortality in children has been reported as 2% in one series, with death owing to respiratory failure secondary to pulmonary hemorrhage.[56]

Lyme Disease. Lyme disease is a multisystem infection caused by *Borrelia burgdorferi*. It is transmitted to humans through the bite of a deer tick. Lyme disease is the most common vectorborne disease among children in the United States.[59] The clinical presentation includes nonspecific symptoms of fever, malaise, and headache associated with a characteristic annular erythematous skin rash (erythema chronicum migrans), which is present in approximately 68% of infected children.[60] The complications of Lyme disease in children include arthritis, facial palsy, aseptic meningitis, and carditis.

Rarely, Lyme disease can present in childhood as acute hepatitis with fever, jaundice, and elevated liver transaminases.[61] In a large prospective series of adults with Lyme disease, 40% of patients had at least one liver test abnormality, whereas 27% had more than one abnormality.[62] These improved significantly or resolved completely after antibiotic therapy. The diagnosis of Lyme disease should be suspected with a history of a tick bite in an endemic area and with finding the classic skin rash on examination. Serologic testing for *B. burgdorferi* is diagnostic. Treatment is with tetracycline except for children under 9 years of age, in whom cefuroxime, amoxicillin, or erythromycin should be used.[63] The prognosis for children adequately treated for Lyme disease is excellent, with no long-term morbidity.[59]

Rickettsial Infections

Rocky Mountain Spotted Fever. Rocky Mountain spotted fever is a clinical syndrome characterized by fever, headache, and a classic maculopapular rash that begins peripherally and spreads to involve the entire body. The disease is caused by *Rickettsia rickettsii*, and ticks serve as vectors for transmission. The clinical presentation is usually with fever, myalgia, rash, headache, abdominal pain, and vomiting.[64] Hepatic involvement may occur in the form of hepatomegaly and jaundice. Variable elevations of serum hepatic transaminases and alkaline phosphatase have been reported.[64] Pathologic changes in one postmortem pediatric study revealed marked inflammation of the portal triad with portal vasculitis and sinusoidal erythrophagocytosis.[65] Rickettsiae have been found in portal blood vessels and in cells of the sinusoidal lining.

A diagnosis of Rocky Mountain spotted fever requires a high index of clinical suspicion. Disease confirmation is established by demonstrating a fourfold rise in antibody titer in the second or third week of illness, using a complement fixation serologic test. About 70 to 80% of

patients will have a positive Weil-Felix reaction, but the test is not specific. Treatment with tetracycline or chloramphenicol is most effective. The treatment should not be delayed pending laboratory diagnosis because untreated infection can lead to severe ilness and fatal outcome.[66]

Q Fever. Q fever is a febrile illness caused by *Coxiella burnetii*. The usual mode of transmission is inhalation, and the animal hosts include cattle, sheep, and goats. The disease presents in acute and chronic forms. Hepatic involvement is common in the acute form, heralded by fever, headache, malaise, vomiting, abdominal pain, and respiratory symptoms. Hepatosplenomegaly may be present on physical examination. In one series, 11 of 13 children with Q fever had elevation of hepatic transaminases.[67] Although quite rare, fulminant hepatic failure and death have been reported in childhood.[68] Liver histology in Q fever shows fatty change with diffuse granulomatous lesions.[69] A classic histopathologic finding is fibrin ring granuloma with a central clear space (Figure 51.2-3). The diagnosis of Q fever is confirmed by serologic testing. The treatment of choice is tetracycline, although most patients recover uneventfully without any specific treatment.

PARASITIC INFECTIONS

AMEBIASIS

Amebiasis is caused by the protozoan *Entamoeba histolytica*. It occurs in all parts of the world and is endemic in southern and western Africa, the Far East, South and Central America, and the Indian subcontinent. In the United States, the prevalence of amebiasis is estimated to be about 4%.[70] The epidemiologic risk factors predisposing to amebiasis are lower socioeconomic status, crowding, poor sanitation, immigration from an area of endemicity, and young age, including infancy.[70] *E. histolytica* is an enteric pathogen that exists in either trophozoite or cyst form. Transmission is by the fecal-oral route, and the infection is acquired by the ingestion of cysts. Cysts dissolve during passage through the small bowel and mature into trophozoites. Trophozoites colonize the colon and may encyst or invade the colonic epithelium, resulting in colitis. Trophozoites reach the liver via the portal venous system and penetrate into the hepatic parenchyma, leading to hepatic abscess. An amebic abscess cavity contains acellular proteinaceous debris surrounded by necrotic hepatic tissue.[71] Fever and right upper quadrant pain are the most common symptoms of hepatic amebic abscess. A history of dysentery may be present; it was reported in up to 16% of childhood cases in one series.[72] On examination, tender hepatomegaly and abdominal distention are usually seen. Clinical jaundice is uncommon. Patients may also present with respiratory symptoms secondary to rupture of the abscess into the pleura or tracheobronchial tree. Rupture of the abscess into the peritoneum may lead to an acute abdomen, and intrapericardial rupture may present as shock.[73]

Routine laboratory tests are of limited value in the diagnosis of hepatic amebic abscess. In one large series, anemia and leukocytosis were seen in more than 90% of children, whereas abnormality of serum transaminases and alkaline phosphatase was observed in only 16% of cases.[72] Examination of stool for cysts or trophozoites is positive in only a minority of cases. Ultrasonography or CT can provide anatomic verification and intrahepatic localization of the abscess cavity. Amebic abscess is usually solitary and is seen in the right hepatic lobe in 75% of cases.[72] Reliable differentiation between amebic and pyogenic abscess is difficult on ultrasonographic appearance alone, but an amebic abscess is likely to have a better defined margin with a peripheral "halo."[26] If a diagnosis of amebic liver abscess cannot be made on the basis of serology and clinical features, needle aspiration of the abscess can exclude the possibility of pyogenic abscess. Aspiration of an amebic abscess yields a reddish brown fluid that has the appearance of anchovy paste.[21] Amebae are usually not recovered from the abscess fluid, and the pus is sterile.

The development of serologic tests for the diagnosis of amebiasis has virtually eliminated the need for needle aspiration. These tests include indirect immunofluorescence, indirect hemagglutination, and enzyme-linked immunosorbent assay.[71] In a recent study, elevated indirect hemagglutination titers (> 1:250) were seen in all children with amebic liver abscess.[72] However, serologic testing may not be as reliable in young infants. In one review, only 62% of infants with amebic abscess had a positive serologic test at presentation.[74] Because each individual test is not always positive, the use of at least two different serologic tests is recommended for diagnostic purposes.

In contrast to pyogenic liver abscess, amebic liver abscess can be successfully treated with antibiotics alone, without aspiration or drainage. Metronidazole (30–50 mg/kg/d in three divided doses for 10 days) is the therapy of choice. A course of intestinal amebicide such as diloxanide furoate (10 mg/kg/d for 10 days) or iodoquinol (30–40 mg/kg/d [maximum 2 g/d] in three doses for 20 days) should be given following metronidazole therapy to eradicate the intraluminal infection.[70] Needle aspiration is recommended when a clinical response is not evident within 48 hours after

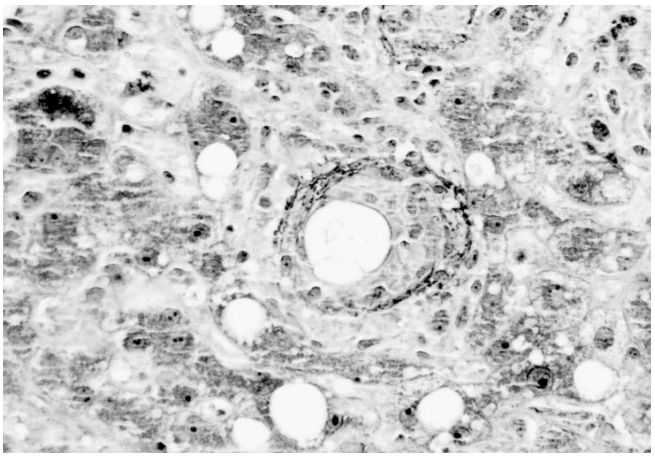

FIGURE 51.2-3 Fibrin ring granuloma with a central clear space in a patient with Q fever (hematoxylin and eosin; ×400 original magnification). Courtesy of Dr. F. A. Mitros, University of Iowa Hospital, Iowa City, IA.

starting medical therapy.[72] Other indications for needle aspiration include an abscess cavity larger than 7 cm and abscesses located in the left lobe (because of the higher risk for pericardial involvement).[72] Surgical intervention is necessary for ruptured amebic abscesses.[73] In one large series, antibiotic therapy along with timely aspiration of the abscess obviated the need for surgical intervention, even in malnourished children who presented late for treatment.[72] The prognosis for uncomplicated amebic abscess is usually good.[71] In infancy, however, the clinical course can be fulminant, with a high mortality rate.[74]

Malaria

Malaria is caused by *Plasmodium vivax*, *Plasmodium falciparum*, *Plasmodium ovale*, and *Plasmodium malariae*. Malaria is an important cause of morbidity and mortality in tropical and subtropical countries, with an estimated incidence of 300 to 500 million cases per year. Malaria is acquired from the bite of infected female anopheline mosquitoes. Sporozoites injected into the bloodstream reach hepatocytes, where further division and maturation take place. Merozoites released from the hepatocytes invade erythrocytes. The symptoms of malaria include fever, abdominal pain, vomiting, and diarrhea.[75] Fever and chills may be periodic (every 48 to 72 hours) or may occur daily. *P. falciparum* infection can result in severe life-threatening disease with complications such as seizures, coma, renal failure, severe anemia, shock, and (rarely) hepatic failure.[76] On examination, tender hepatosplenomegaly is a common finding. Jaundice, usually owing to hemolysis, and mild elevation of liver enzymes may be seen.[75]

Histologic findings in the liver include hyperplasia of Kupffer cells and diffuse periportal infiltration with mononuclear cells.[76] The diagnosis of malaria is established by the detection of parasites in thin and thick peripheral blood smears prepared with Giemsa stains. The drug of choice for all uncomplicated malaria except chloroquine-resistant *P. falciparum* is chloroquine phosphate (10 mg of base per kilogram to a maximum of 600 mg of base [a 1 g tablet] and then half the dose [maximum 500 mg] given once daily beginning 6 hours later, for 2 days). For severe and complicated malaria, quinine is the parenteral drug of choice supplemented with aggressive supportive management.[77] For chloroquine-resistant strains, quinine or mefloquine may be used.

Ascariasis

Ascariasis, caused by *Ascaris lumbricoides* (roundworm), is the most prevalent helminthic infection in the world. Although cases have been reported worldwide, ascariasis mostly occurs in developing tropical and subtropical countries. Ascariasis is most common in childhood and is endemic to areas with poor sanitation. The transmission is via the fecal-oral route. Ingested eggs eventually mature into the adult worm in the small intestine. The clinical manifestations of ascariasis depend on whether the worms reside in the intestinal lumen or whether they invade the pancreatic and biliary ductal system. The usual symptoms are vague abdominal pain and distention.[78] Some heavily infected children may present with an acute abdomen secondary to intestinal obstruction from the mass of worms.[79] Worms invading the ampullary orifice can induce biliary colic or acute pancreatitis.[80] Worm obstruction of the bile duct system can cause acalculous cholecystitis, pyogenic cholangitis, or acute pancreatitis in children.[78] In a report from a children's hospital in Burma, ascariasis was the culprit in acute surgical cases such as biliary obstruction, volvulus, and intestinal obstruction and perforation, accounting for 26.3% of emergency abdominal laparotomies in children.[79] Hepatic abscess has been reported in children and adults owing to worms obstructing the intrahepatic bile ducts.[5,80] In children from developing countries, ascariasis has been considered a predisposing factor for pyogenic liver abscess.[1,5]

The diagnosis of ascariasis is made by the finding of ova in the stools or by a history of passing worms. Elevation of liver enzymes and serum bilirubin may be seen in cases of biliary ascariasis. Ultrasonography of the abdomen is very useful in diagnosing complications, including cholecystitis, biliary tract dilatation, and hepatic abscess.[78] Rarely, an echogenic wormlike structure may be seen within the bile duct on ultrasonography.[81] Worms may also be seen on endoscopic retrograde cholangiopancreatography, and *Ascaris* ova can be found in the bile sample.[80] The treatment of ascariasis is with mebendazole (100 mg twice daily for 3 days) or piperazine salts (50–75 mg/kg/d for 2 days). Surgery may be required for selective obstructive cases, although biliary obstruction and colic symptoms have been treated by the removal of worms with endoscopic retrograde cholangiopancreatography.[80]

Echinococcosis

Echinococcus granulosus infection is found throughout the world and is endemic in sheep- and cattle-raising areas, including the Mediterranean, Australia, New Zealand, and parts of Asia, Europe, and South and North America. Echinococci are small tapeworms that inhabit the intestine of the definitive host, usually dogs, and humans acquire the infection by the ingestion of ova. The parasites may lodge in the liver or lungs of affected patients, leading to the formation of hydatid cysts. The cyst has a thick lamellar layer that supports a thin germinal layer of cells responsible for the budding and production of protoscoleces (Figure 51.2-4). The cyst is surrounded by a fibrous capsule produced by the host. Hydatid cysts may occur in other sites, such as the kidney, spleen, brain, eyes, and pancreas. Although the infection is acquired in childhood, symptoms may not occur for many years owing to the slow growth of hydatid cysts (1 cm in diameter per year).[82]

The clinical presentation of a hydatid cyst of the liver depends on the size of the cyst and on the complications arising owing to the cyst. Common symptoms in children with hepatic hydatid cysts include abdominal pain, abdominal mass, fever, and anorexia.[83] Some children are totally asymptomatic, with the abdominal mass detected incidentally on physical examination. Large hepatic cysts may compress on the venous system or the biliary tract, leading to portal hypertension or obstructive jaundice,

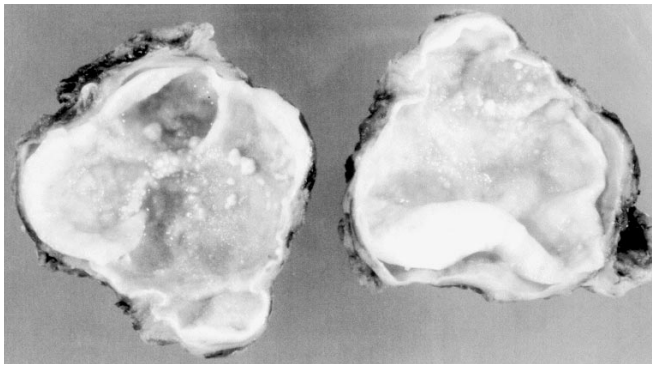

FIGURE 51.2-4 Gross liver resection specimen of a hydatid cyst.

respectively.[84] Cysts may rupture into the biliary tract or the peritoneal or pleural cavities. Anaphylaxis may occur owing to the release of the highly antigenic cystic fluid.[82] Laboratory tests are not specific for the diagnosis of echinococcosis. Eosinophilia, elevated liver enzymes, and hyperbilirubinemia may be present. Plain abdominal radiographs of the abdomen or chest may show a calcified mass. Ultrasonography is useful for diagnosing and localizing the cyst, but imaging does not readily differentiate echinococcal disease from other solitary cysts of the liver unless daughter cysts are present or intracystic septations or calcification of the cyst wall are recognized (see Figure 51.2–4). Definitive diagnosis can be made either by positive serology to *E. granulosus* or by demonstrating the presence of scoleces in the cystic fluid.

Historically, the optimal treatment for hepatic hydatid cyst was surgical, with care taken to avoid spillage of cystic fluid. However, novel reports favor conservative management for uncomplicated cysts. Careful percutaneous drainage combined with albendazole therapy was found to be an effective and safe alternative to surgery in adults.[84] In one long-term follow-up study from Israel, the use of albendazole therapy alone was associated with a cure in 41% of patients with hepatic hydatid cysts and with improvement in another 41% of adult patients.[85] In a recent report from Turkey, 3-month albendazole therapy resulted in successful resolution of hepatic cysts in 27% of children, and medical therapy was recommended as an initial therapy prior to surgery.[86] Medical therapy has also been suggested for children with hydatid cysts under 5 cm in size or for cases in which multiple cysts or multiorgan involvement occurs.[83]

Liver Fluke Infection

Clonorchiasis. Clonorchiasis, caused by *Clonorchis sinensis*, a liver fluke, is endemic throughout the Far East. Infection is acquired by the consumption of raw infected fish, which act as one of the intermediate hosts, along with snails.[87] The parasite migrates from the duodenum into the biliary ductal system and matures in the intrahepatic bile ducts. The injury to bile ducts manifests as adenomatous proliferation and goblet cell metaplasia.[88] Secondary infection occurs frequently, and recurrent pyogenic cholangitis can lead to stricture, periductal fibrosis, and hepatic abscess.[88] Patients with mild disease may be asymptomatic, whereas more severe cases present with recurrent cholangitis, biliary duct obstruction, or portal hypertension.[89] Cholangiocarcinoma has been reported with long-standing disease in adults.[90] Laboratory findings include variable elevation of liver enzymes and bilirubin. Ultrasonography is useful for assessing abnormalities in the biliary ductal system. The diagnosis is made by stool or duodenal aspirate examination for *Clonorchis* eggs. Praziquantel is the drug of choice for treatment.[89]

Fascioliasis. Fascioliasis is caused by *Fasciola hepatica*, the sheep liver fluke, and is found worldwide. Human infection is acquired by ingestion of infected watercress or water. The parasites penetrate the duodenal wall, traverse the peritoneal cavity, and penetrate the hepatic capsule to reach the bile ducts. The disease has two phases: an acute invasive phase, during which the parasite is migrating through the liver, and a chronic phase, during which it resides in the biliary tract.[91] Acute clinical manifestations in childhood include prolonged fever, abdominal pain, and tender hepatomegaly.[92] Significant eosinophilia, raised sedimentation rate, and elevated serum alkaline phosphatase are typically seen. Serum hepatic transaminases are usually normal or only mildly elevated.[91] CT may show abscess-like nodular lesions or multiple hypodense areas in the liver.[93] Liver biopsy findings include eosinophilic abscess (parasitic granuloma) and coagulative necrosis. Chronic fascioliasis can cause biliary colic, jaundice, cholangitis, and pancreatitis owing to obstruction of the bile ducts.[91] The diagnosis is by serologic testing or by finding *F. hepatica* ova in the stool. In a recent report from Egypt, triclabendazole (10 mg/kg single dose) was highly effective and was recommended as the treatment of choice in children.[94]

Schistosomiasis. Schistosomiasis is an important cause of morbidity and mortality in tropical countries. Hepatic schistosomiasis is caused by *Schistosoma mansoni* and *Schistosoma japonicum*. Endemic areas for *S. mansoni* include Africa, the Middle East, and South America, whereas *S. japonicum* infection is endemic in Central and Southeast Asia. Human infection occurs when schistosomes, released from a snail host, penetrate intact human skin. The parasites then migrate to the liver, enter into the portal venous system, and finally reside in the mesenteric veins.[95] Granulomatous hepatic lesions occur as a result of the host's immunologic response to the ova in the portal venous system (Figure 51.2-5). Fibrosis around branches of the portal veins subsequently leads to portal hypertension. Hepatic parenchymal function is usually preserved.

Infection occurs mainly in childhood or adolescence. Acute schistosomiasis (also known as Katayama fever), presumably a consequence of the host's immunologic response to mature worms and eggs, occurs about 4 to 6 weeks after exposure. Clinical features vary and include fever, cough, edema, lymphadenopathy, and eosinophilia.[95] Untreated acute schistosomiasis progresses to chronic disease, resulting in portal hypertension. Children may present with upper gastrointestinal bleeding owing to esophageal varices.

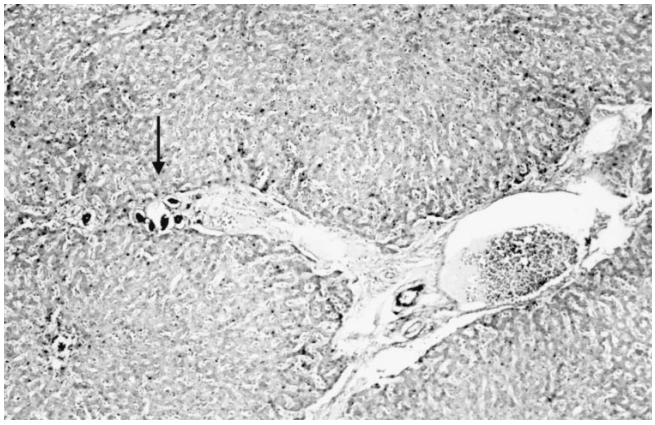

FIGURE 51.2-5 Schistosomiasis with multiple ova (*arrow*) embedded in a fibrotic portal triad (hematoxylin and eosin; ×200 original magnification). Courtesy of Dr. F. A. Mitros, University of Iowa Hospital, Iowa City, IA.

Hepatosplenomegaly can reach massive proportions, and ascites is present in some cases.[95] Other potential complications associated with hepatosplenic schistosomiasis are pulmonary hypertension, myocarditis, and transverse myelitis. Prolonged *Salmonella* infection may be a concurrent feature.

Laboratory features of anemia, leukopenia, and thrombocytopenia may be seen owing to hypersplenism. Eosinophilia and hyperglobulinemia may also occur, but liver function tests are usually normal.[96] Ultrasonography has been used to detect and grade the degree of periportal fibrosis and portal hypertension.[97] Serologic studies using *S. mansoni* egg antigen are helpful for diagnosis. The diagnosis is confirmed by detecting *Schistosoma* eggs in stool or in a rectal biopsy tissue sample. The treatment of choice is a single dose of praziquantel (20–40 mg/kg three times a day for 1 day), resulting in eradication of the parasite in 90% of cases[95] and reversal of periportal fibrosis in some children.[97] The management of portal hypertension and esophageal variceal bleeding may be difficult; however, in most instances, sclerotherapy has been effective. The use of propranolol for the management of portal hypertension in children has not been well studied.[98]

FUNGAL INFECTIONS

Candidiasis

Candida infections are the most frequently encountered systemic fungal infections, with infection of the liver and spleen occurring as a consequence of seeding during fungemia. Candidiasis usually occurs in immunocompromised patients, particularly those on chemotherapy for malignancy. Acute leukemia was the most common malignancy seen in children with hepatosplenic candidiasis.[10] Other risk factors for hepatosplenic candidiasis include neutropenia, recent chemotherapy, and the use of broad-spectrum antibiotics.[10] Clinical symptoms are nonspecific and usually include fever and abdominal pain. On examination, jaundice, hepatomegaly, splenomegaly, and abdominal tenderness may be found. The white blood cell count may be normal, although a history of prolonged neutropenia is usually present. The diagnosis should be suspected in patients with persisting fever and abdominal pain even after resolution of the neutropenia.[99] *Candida* may be isolated from blood, urine, throat, or stool. Serum hepatic transaminases, alkaline phosphatase, and bilirubin are often elevated. Abdominal ultrasonography may show several hypoechoic areas throughout an enlarged liver (Figure 51.2-6). Abdominal CT features include liver enlargement with multiple well-circumscribed low-density areas of 0.5 to 2 cm in diameter, often with accompanying splenic involvement. The appearance of *Candida* abscesses is usually distinct from that of bacterial abscesses, the latter being larger and fewer in number on imaging studies.[100] A percutaneous liver biopsy can confirm the diagnosis in 70% of cases, whereas a laparoscopic or an open liver biopsy provides an even better diagnostic yield.[99] Grossly, the liver surface is studded with yellow to white nodules ranging in size from 1 mm to 2 cm.[99] Histologically, *Candida* abscesses show fungal elements in the necrotic center, surrounded by inflammatory cells and a ring of fibrosis.[101] Granulomatous lesions with occasional giant cells in the liver have also been observed.[10]

The treatment of choice is the use of antifungal agents such as amphotericin B, 5-flurocytosine, or fluconazole. In a recent international consensus conference, fluconazole was favored as first-line therapy for stable patients, whereas amphotericin B was considered the drug of choice for life-threatening infections.[102] The optimal duration of therapy is controversial, and treatment for several months may be required.[103] In the vast majority of cases, drainage of the pus is impractical because of the small size and diffuse nature of the hepatic abscesses. Many of these patients have multisystem disease, and the outcome is poor, with mortality rates in children reported as high as 20%. [10]

Other Fungal Infections

Other fungal organisms such as *Histoplasma capsulatum*, *Aspergillus* species, *Cryptococcus neoformans*, and *Coccidioides immitis* may also infect liver. Similar to candidiasis, these infections usually occur in immunocompromised patients. Children with cancer on chemotherapy are par-

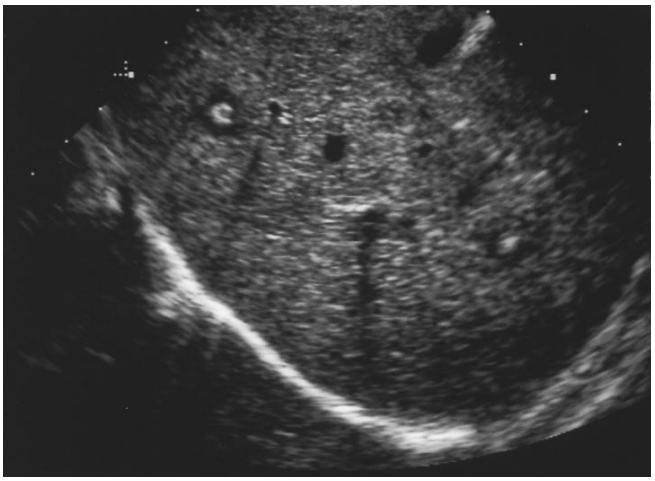

FIGURE 51.2-6 Sonogram of the liver showing multiple foci of *Candida* abscesses in a child with leukemia.

ticularly at risk.[104] Most systemic fungal infections involve organs such as the lungs, skin, central nervous system, and bones, and liver involvement is part of disseminated disease. Hepatic involvement may manifest as hepatomegaly, elevated serum hepatic transaminases, hyperbilirubinemia, and liver abscess formation.[100,105] Histologic findings in the liver include granuloma, inflammatory cells, and fungal organisms.[106] The diagnosis is made by serology or by isolation of the organisms from tissue or body fluids. Optimal treatment is with antifungal agents such as amphotericin B.

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3. AIDS *and* Other Immune Disorders

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Immunodeficiency predisposes the host to the development of recurrent and unusual infections. Liver involvement in these infectious processes is commonly observed. This chapter describes liver infections in three major groups of immunodeficient states: human immunodeficiency virus (HIV) infection, congenital primary immunodeficiencies, and secondary immunodeficiency states associated with bone marrow and solid organ transplant.

As of the end of 2001, the Joint United Nations Programme on HIV-AIDS estimated that more than 40 million adults and children were living with HIV–acquired immune deficiency syndrome (AIDS). In children, there are two important routes of transmission: maternal transfer of virus during pregnancy or in the perinatal period and transmission through blood products. Approximately 90% of pediatric AIDS infections are acquired by the perinatal route.[1] Clinical symptoms in this situation can develop as early as 1 month of age, but the median interval from birth to symptom onset is 8 months. Gastrointestinal problems are typical in patients with HIV, and the liver is commonly involved. Hepatomegaly and abnormal liver function tests are frequently observed in HIV patients.[2–4] Acute hepatitis can be the first manifestation of HIV infection in early infancy.[5] However, significant abnormalities in liver synthetic function, complicated by cirrhosis, hypoalbuminemia, ascites, and portal hypertension, are rare.[2,6]

With newer treatment regimens for HIV, opportunistic infections are decreasing in frequency. On the other hand, mortality from end-stage liver disease among adult patients with HIV is on the rise.[7] Chronic coinfections with hepatitis B and C are the most frequent causes of chronic liver disease in adults with HIV.[3] Because no comparable data are available in the pediatric population, this discussion, for the most part, provides information based on extrapolations from the adult literature. Liver disease in the HIV-infected patient may be caused by a variety of pathogens. The most important ones are listed in Table 51.3-1. Liver damage in HIV-infected patients may also be caused by antiretroviral drugs and medications used for the treatment and prevention of opportunistic infections (Table 51.3-2), as well as tumors. Tumors are exceedingly rare in pediatric patients.

In the earlier stages of HIV infection, liver disease is usually related to drug hepatotoxicity or coinfection with hepatotropic viruses. With disease progression, the systemic opportunistic infections become more problematic. The clinical findings of liver disease in HIV patients are similar, regardless of underlying cause. Fever, malaise, hepatomegaly, right-sided or epigastric abdominal pain, and nausea have all been described. Jaundice is unusual, even in patients with biliary tract disease.

HIV virus has been observed within Kupffer cells and in hepatic endothelial cells,[8] and HIV messenger ribonucleic acid (RNA) has been detected within hepatocytes.[9] Hepatic macrophages and endothelial cells express the CD4 surface molecule and have been shown to support viral replication in vitro.[10] It remains unclear, however, whether HIV can damage the liver directly. There is no correlation between the amount of HIV antigens in the liver and the severity of histologic abnormality, and normal histology can also be seen.[11] There are limited data about hepatic histology in children with HIV disease.[12–14] Steatosis, Kupffer cell hyperplasia, portal inflammation, and focal necrosis are common but nonspecific findings.[15] Certain pathologic features appear somewhat unique to the pediatric age group. The presence of giant cells is common, even in children older than 6 months. The giant cells may be associated with cytomegalovirus (CMV) infection or Kaposi sarcoma but can also be a reaction of the liver to HIV infection.[13,14] Dense lymphoid infiltration of portal spaces with features of chronic active hepatitis has been described in several

TABLE 51.3-1 COMMON PATHOGENS CAUSING LIVER DISEASE IN PATIENTS WITH HIV

HIV VIRUS
COINFECTIONS NONSPECIFIC TO HIV
Hepatitis A
Hepatitis B
Hepatitis C
Adenovirus
Herpes viruses group (EBV, HSV, VZV)
OPPORTUNISTIC INFECTIONS
Viruses
Herpes group (CMV, HHV-6, HHV-8)
Protozoa
Pneumocystis carinii
Cryptosporidium
Microsporida
Fungi
Cryptococcus
Histoplasma
Candida
Bacteria
Mycobacterium-avium
Mycobacterium intracellulare
Mycobacterium tuberculosis

CMV = cytomegalovirus; EBV = Epstein-Barr virus; HHV = human herpesvirus; HIV = human immunodeficiency virus; HSV = herpes simplex virus; VZV = varicella-zoster virus.

cases.[12–14] Cholestatic hepatitis, unique to children less than 1 year of age, has been reported in association with nonspecific or giant cell hepatitis.[5,15–17] A severe course with quick progression is typical for these patients.[15] Peliosis hepatitis has not yet been reported in children.[15]

TABLE 51.3-2 PATTERNS OF BIOCHEMICAL ABNORMALITIES OF DRUGS COMMONLY USED IN PATIENTS WITH HIV

HEPATOCELLULAR
Acetaminophen
Aminosalicylic acid
Ciprofloxacin
Clarithromycin
Clindamycin
Dilantin
Ethionamide
Fluconazole
Foscarnet
Ganciclovir
Itraconazole
Ketoconazole
Mebendazole
Oxacillin
Pentamidine
Pyrazinamide
Ranitidine
Rifabutin
Sulfonamides
Sulfones
Tetracycline
Trimethoprim-sulfamethoxazole
Vitamin A
Zalcitabine
Zidovudine
CHOLESTATIC
Amitriptyline
Carbenicillin
Cimetidine
Clarithromycin
Diazepam
Doxepin
Erythromycin
Naprosyn
Prochlorperazine
Ranitidine
Thiabendazole
Zidovudine
STEATOSIS
Glucocorticoids
Tetracycline
Valproic acid
Zidovudine
MIXED
Amitriptyline
Carbamazepine
Clarithromycin
Diazepam
Doxepin
Naprosyn
Phenobarbitol
Piroxicam
Prochlorperazine
Sulfonamides
Sulfones
Trimethoprim-sulfamethoxazole

Adapted from Weiner FR, Simon D. Liver disease in patients with acquired immunodeficiency syndrome. In: Brandt LJ, editor. Clinical practice of gastroenterology. Vol. 2. Philadelphia: Churchill Livingstone; 1999. p. 975.
HIV = human immunodeficiency virus.

Pitlick and colleagues[18,19] and Guarda and colleagues[20] were the first to report biliary tract involvement in patients with AIDS. The reported infectious agents in these cases include CMV, *Cryptosporidium*,[21] *Microsporida*,[22] and *Isospora*.[23] The spectrum of HIV-related biliary tract disorders includes acalculous cholecystitis, papillary stenosis, and sclerosing cholangitis. Four distinct cholangiographic patterns have been described by Cello.[24] Papillary stenosis occurs in 15 to 20% of patients, sclerosing cholangitis in 20%, and a combination of both in 50% of patients. The pattern of long, 1 to 2 cm extrahepatic bile duct strictures occurs in 15% of cases.

The pathogenesis of the biliary disease in HIV patients is not well understood. It is possible that HIV itself infects and damages the biliary tract. However, to date, HIV has not been isolated from the biliary epithelium.[25] It has been suggested that patients with certain human leukocyte antigen types may develop an immunologic reaction in response to pathogens directed against the biliary epithelium. It is also possible that enteric infection in AIDS patients may lead to portal bacteremia and bile duct injury.[26] Damage to the sphincter of Oddi caused by different pathogens has also been proposed as a possible mechanism.[27,28] The clinical picture of HIV-related cholangitis is similar regardless of the pathogenic organism involved.[24,29] Anicteric cholangitis is a predominant finding. Elevation of serum amylase has also been described, although its significance is unclear.

EVALUATION OF HIV-INFECTED PATIENTS WITH SUSPECTED HEPATOBILIARY DISEASE

Given the wide variety of possible etiologic agents and nonspecific clinical findings of liver disease in patients with HIV infection, an organized approach to the investigation of elevated transaminases, hepatomegaly, and unexplained fever is important. Figure 51.3-1 provides an algorithm for the approach to these patients. The history should focus on prior infections (CMV, hepatitis B or C), recent travel (exposure to tuberculosis, hepatitis A, or parasites), and a history of contact with individuals with highly contagious illnesses (varicella, roseola). Use of potentially toxic medications also has to be evaluated. A complete physical examination can often detect an extrahepatic site of opportunistic infections. Attention should also be paid to the nutritional status of the patient because acute weight loss or precipitous weight gain may cause hepatosteatosis and present as elevated liver function tests.

Although routine liver function tests are of limited value in identifying a specific infection, the magnitude of injury and pattern of injury may be suggested.[4] Two main patterns can be differentiated: hepatocellular dysfunction and cholestasis. With a pattern suggesting predominantly hepatocellular disease, viral hepatitis or drug toxicity should be considered. A cholestatic pattern with right upper quadrant pain, with or without jaundice, should raise suspicion of AIDS cholangiopathy. A markedly elevated serum alkaline phosphatase level in the absence of

bile duct obstruction is suggestive of mycobacterial or fungal infection.[2] Elevation of serum bilirubin and alkaline phosphatase levels without ductal dilatation suggests drug- or virus-induced cholestasis.

The CD4 cell count should be part of the laboratory evaluation because it reflects the degree of immunosuppression. Certain infections, such as *Pneumocystis carinii* and *Mycobacterium avium-intracellulare* complex (MAC), usually occur in severely immunocompromised patients. In contrast, tuberculosis and hepatotropic viruses (hepatitis A, B, or C) can be symptomatic at earlier stages of immunocompromise. Blood cultures, serology, and fungal and viral cultures may help elucidate specific pathogens causing liver dysfunction.

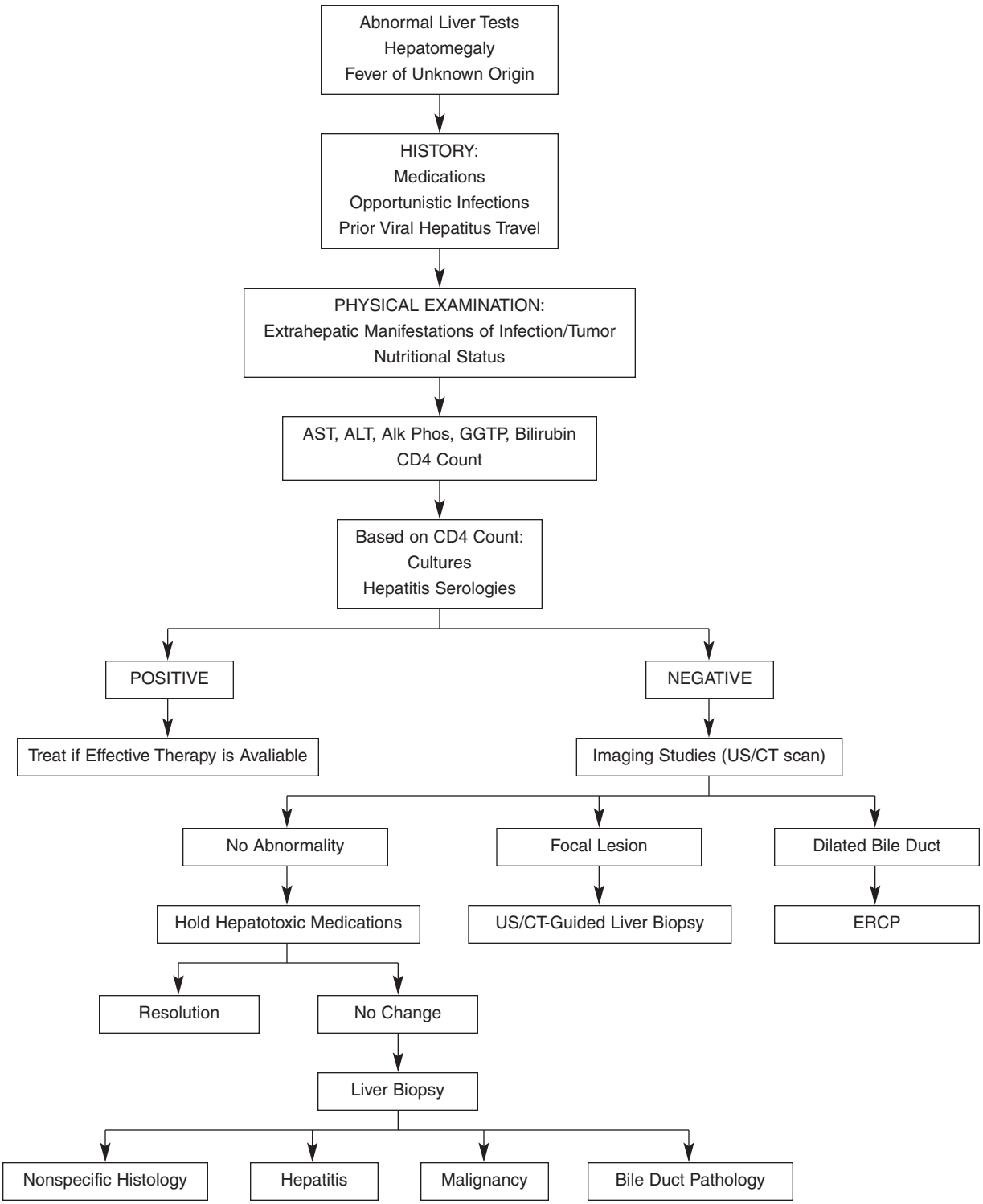

FIGURE 51.3-1 Diagnostic algorithm for the evaluation of human immunodeficiency virus (HIV)–infected patients with hepatobiliary disease. Alk phos = alkaline phosphatase; ALT = alanine transaminase; AST = aspartate transaminase; CD4 count = CD4 count lymphocyte cell count; CT = computed tomography; ERCP = endoscopic retrograde cholangiopancreatography; GGTP = γ-glutamyl transpeptidase; US = ultrasonography.

Ultrasonography is useful in the initial evaluation of suspected biliary tract disease. Although it may miss up to 25% of cases of HIV-related biliary tract disease diagnosed by endoscopic retrograde cholangiopancreatography,[30] it is easily available and inexpensive. Computed tomography (CT) offers a better delineation of focal lesions and intra-abdominal lymph nodes. Both ultrasonography and CT may be useful in performing a liver biopsy. The role of liver biopsy in the evaluation of HIV patients with parenchymal liver disease is controversial. A specific diagnosis may be made by liver biopsy in up to 50% of adult AIDS patients.[31,32] No comparable data are available in the pediatric patient population.

The risk of bleeding after liver biopsy is much increased in AIDS patients, even with normal coagulation. The rate of significant hemorrhage following liver biopsy is described to be as high as 5 to 10%.[15,33,34] Finally, it has not been demonstrated that findings on liver biopsy result in improved quality of life or survival of these patients.[4,35] Despite these issues, a liver biopsy is useful in certain situations. A recent pediatric study has suggested two groups for whom liver biopsy may be very useful when other noninvasive tests are negative: those patients with suspected mycobacterial infections and patients with jaundice.[15] Symptomatic hepatomegaly and persistent fever with a negative noninvasive workup have also been mentioned in the adult literature as indications for biopsy.[4] When a liver biopsy is performed, cultures and special stains should be obtained in addition to routine histology.

In contrast to hepatocellular disease, biliary tract complications of AIDS require an aggressive approach. Patients are frequently symptomatic, and effective palliation is available by endoscopic and surgical means. Endoscopic retrograde cholangiopancreatography remains the gold standard in diagnosing AIDS cholangiopathy.

HIV COINFECTIONS

Hepatitis B

Hepatitis B virus (HBV) and HIV have similar routes of transmission and so frequently coexist. It is unclear whether HIV alters the natural history of chronic HBV infection.[36] Following acute HBV infection, the virus is cleared in 95% of immunocompetent patients. In contrast, 50% of HIV coinfected patients develop chronic infection.[37] Individual case reports suggest that reactivation of HBV infection is associated with progression of the HIV disease.[38–42] Loss of naturally acquired anti–hepatitis B surface antibodies (HBsAB), even in patients who remain hepatitis B surface antigen (HB_sAg) negative, has also been described.[43] Increased prevalence of hepatitis B e antigen (HBeAg) expression and elevated levels of deoxyribonucleic acid (DNA) polymerase in HIV-positive patients has been reported.[37,41,44,45] Therefore, more patients with HIV-HBV coinfection are in a chronic HBV carrier state, with highly infectious serum and body fluids, compared with the HIV-negative population.[45]

Patients with early or well-controlled HIV infection tend to have milder HBV infection, whereas those severely immunosuppressed tend to have severe HBV disease.[46] Spontaneous resolution of chronic HBV infection with an improved immune response in treated HIV patients has also been described.[47] Thus, aggressive treatment of HIV appears to be an important factor in the control of HBV infection. Although HIV-positive patients with HBV may express higher HBV DNA levels and increased HbeAg titers,[36] they often have lower serum transaminases and less histologic damage.[48] Despite these findings, HBV infection in these patients has a greater risk of progression to cirrhosis and development of hepatocellular carcinoma.[49]

The results of interferon treatment in the HIV population are controversial.[50–54] In general, for hepatitis B, response rates to interferon therapy have been less impressive than those seen in the HIV-negative group. Lamivudine significantly lowers HBV DNA and serum transaminases and may result in seroconversion from HBsAg to HB_s antibody.[54,55] Unfortunately, reactivation of hepatitis B may occur following withdrawal of therapy; therefore, long-term treatment appears to be necessary.[38] Mutation of the viral polymerase gene occurs at a rate of approximately 20% per year, similar to that of an immunocompetent host.[56] This mutation leads to antiviral resistance in up to one-third of the patients.[57] The dosage of lamivudine used for HBV treatment in HIV-positive patients is similar to that used for HIV treatment alone (150 mg twice daily) to avoid development of HIV resistance to lamivudine. Minimal published information is available about the role of other nucleoside analogs in the treatment of HBV infection.[47,58–60]

Vaccination against HBV has been recommended for all patients infected with HIV. Unfortunately, HIV infection reduces the efficacy of vaccination in these patients. Several studies demonstrated suboptimal response in both the magnitude and duration of antibody response.[61–64] Loss of anti-HB_s in HIV-infected patients is seen in 43% of persons by 4 years compared with an 8% loss in immunocompetent controls.[65]

Hepatitis C

Hepatitis C virus (HCV) infects an estimated 170 million people worldwide and is five times more widespread than infection with HIV.[66] In the United States alone, approximately 300,000 to 400,000 individuals are HIV-HCV coinfected.[67] For children infected with hepatitis C through vertical transmission, the prevalence of HCV infection is estimated at 8 to 17%.[68,69] With the introduction of highly active antiretroviral therapy (HAART) and improvement in survival, HCV infection has become a major contributor to morbidity and mortality in patients with HIV. End-stage liver disease has become a frequent complication,[67,70,71] and cases of hepatocellular carcinoma (HCC) in HIV-HCV–coinfected adults have been described.[72] HCV also has been implicated as a cause of increased hepatotoxicity of HAART in coinfected patients.[73–75]

In children, HCV is acquired primarily through perinatal transmission. The rate of vertical transmission of HCV alone is 5 to 11%.[76] Maternal coinfection with HIV increases

the rate of perinatal HCV transmission even without simultaneous HIV transmission.[77,78] Likewise, maternal HCV infection has been associated with a higher likelihood of vertical HIV transmission. Perinatal transmission of HIV is approximately two times more frequent in mothers who are infected with both hepatitis C and HIV.[79]

There are no data on the natural history of HCV in HIV-infected children. In adults, HCV infection appears to be more aggressive in HIV-positive patients compared with immunocompetent adults. Hepatic damage from HCV infection is primarily due to direct viral damage. However, an immunologic response is important in the elimination of HCV. Therefore, the rate of HCV replication and levels of HCV viremia are much higher in immunosuppressed individuals.[80] Several studies demonstrated a more rapid progression and more severe course of HCV infection in the HIV population.[81,82]

The US Public Health Service and National Institutes of Health consensus panel has recommended anti-HCV enzyme-linked immunosorbent assay III as a screening test for HCV regardless of the HIV status of the patient.[70] A recent study showed a sensitivity of this test of over 99% in both HIV-infected and HIV-negative patients.[83] The recombinant immunoblot assay produced indeterminate results in 10 to 23% of coinfected subjects.[84] False-negative HCV serology may be associated with CD4 counts less than 200 cells/mm^3. If suspicion of the liver disease is high in those patients, an HCV-RNA test should be performed. The liver biopsy is considered the gold standard for clinical assessment of individuals with chronic HCV infection. Both inflammation and fibrosis tend to be greater in HIV-infected patients, primarily in those with low CD4 counts.[84]

Until recently, treatment of HCV-HIV coinfected patients was with antiretroviral drugs alone. It now has been well documented that HAART does not have any beneficial suppressive effect on HCV.[85] With the improved survival of HIV-positive patients, an aggressive approach to the treatment of HCV infection in this population has been advocated. Monotherapy with interferon-α results in a poor sustained response (0–20%).[79,86] Recent studies with the combination of interferon and ribavirin have demonstrated an end of treatment response rate of 50%, with up to 28% of the patients remaining HCV-RNA negative 3 months after treatment.[86–88] Introduction of pegylated interferon has led to a sustained viral response in 30 to 45%.[89,90] No treatment data are available in the pediatric population.

Hepatitis A, E, and G

The prevalence, morbidity, and mortality of hepatitis A infection are not altered by HIV infection.[91] Vaccination of the HIV-infected patients against hepatitis A is recommended. Vaccination appears to be safe but may be less effective. Prevaccination screening is cost-effective in adults.[91] Hepatitis E virus, a waterborne, fecally-orally transmitted virus, usually causes acute self-limited disease. One study showed an increased incidence of hepatitis E in HIV-infected patients but without clinical significance.[92] Hepatitis G virus (HGV) is present in approximately 20 to 40% of HIV-infected patients.[80] The presence of HGV does not alter the levels of the serum transaminases, CD4 cell count, or plasma HIV-1 levels.[80] Some studies suggest that coinfection of HGV and HIV is a favorable prognostic factor, correlating with a slower progression of HIV disease.[93]

Adenovirus

Adenovirus infections in healthy children usually present as a self-limited upper respiratory tract infection, gastroenteritis, maculopapular rash, and conjunctivitis. In HIV-infected individuals, fulminant multiorgan involvement with bilateral pneumonia, gastroenteritis or colitis, and urinary tract infection is typical.[94–96] Hepatic involvement with rapid progression to liver failure is not uncommon.[95] Ulcerations of the gallbladder wall have also been linked to adenoviral infection.[97] The difference in serotypes affecting healthy and immunocompromised patients may be responsible for the differences in clinical presentation.[98] In adenoviral hepatitis, there is lobular necrosis of the hepatocytes with Cowdry type A basophilic nuclear inclusion bodies. Portal tracts are usually speared. Adenoviral virions can be detected in the hepatocytes by electron microscopy; adenoviral disease is usually more severe. Diagnosis is usually made by viral isolation or histologic examination.

Currently, no effective treatment against adenovirus is available. Use of immunoglobulin (Ig) therapy[99]and intravenous ribavirin[100,101] has been advocated.

Herpes Group Virus Infection

Herpes Simplex Virus (1 and 2). Herpes simplex virus (HSV) dissemination to the liver has been described in patients with HIV.[102] Significant dissemination more commonly occurs with primary infection, although it has also been observed with reactivation. Fulminant liver disease may be the only presenting symptom because mucocutaneous lesions may be absent in up to 50% of the patients.[103] Patients present with significantly elevated transaminases, jaundice, and rapidly progressive liver failure.[80] Liver biopsy demonstrates characteristic intranuclear inclusions (Cowdry type A bodies) in hepatocytes. The intranuclear inclusion bodies produce a typical ground-glass appearance. Although a mild neutrophilic infiltrate is sometimes described, a notable feature of HSV disease is the absence of a significant inflammatory cell response in either the portal areas or parenchyma. Fatty degeneration is frequently observed. Electron microscopy is confirmatory and demonstrates typical intranuclear herpes virions. Viral cultures are usually positive (in more than two-thirds of cases) and should always be obtained.[104] Prompt initiation of acyclovir treatment, as well as discontinuation of all potentially hepatotoxic medications, is recommended.

Human Herpesvirus 3 (Varicella-Zoster Virus). Dissemination of varicella-zoster virus (VZV) infection may occur with primary infection or with reactivation. Certain clinical features distinguish severe VZV and HSV infections. In contrast to HSV, VZV disease is typically accompanied by classic skin and mucous membrane lesions. These lesions are usually obvious on presentation but may be delayed by

3 to 4 days. The main target organ of VZV is the lung. Although the liver is commonly involved in disseminated VZV disease, fulminant hepatitis without significant pulmonary disease is rare.[103] Abdominal pain, nausea, and vomiting are common. Elevated transaminases, with or without bilirubin elevation, and leukocytosis are common with VZV disease, in contrast to the leukopenia associated with HSV dissemination. Coagulopathy, renal failure, and multiorgan system failure are typical of severe VZV.

Immediate administration of varicella-zoster Ig to individuals with no history of chickenpox or without vaccination is recommended within 96 hours of exposure.[103] Prophylactic acyclovir should be prescribed. Intravenous acyclovir is reserved for patients with clinical signs of VZV, and the recommended doses are higher than those for HSV disease.[103]

Among HIV-positive patients, only asymptomatic, nonimmunosuppressed patients should be vaccinated against VZV. Vaccination is contraindicated in all other HIV-positive children.

Human Herpesvirus 4 (Epstein-Barr Virus). Epstein-Barr virus (EBV) infection may present as an acute mononucleosis syndrome, but it has also been implicated in the pathogenesis of non-Hodgkin lymphoma, leiomyomas, and leiomyosarcomas of the liver in children with AIDS.[104,105] If acute mononucleosis syndrome occurs, low-grade fever, abdominal discomfort, nausea, mild hepatomegaly, and lymphadenopathy are common. Hepatitis is frequently present but usually resolves completely. Minimal swelling and vacuolization of hepatocytes associated with lymphocytic and monocytic infiltration of the portal areas are noted in the liver biopsy. Extensive liver necrosis has also been described in HIV patients.

Non-Hodgkin lymphoma is a common feature of AIDS and has been associated with EBV infection. The EBV viral genome has been found within tumor cells by DNA hybridization.[105] It may occur at any stage of AIDS but usually occurs late in the disease. The prevalence of non-Hodgkin lymphoma in HIV is increasing, leading some to suggest that it is the most common AIDS-related neoplasm.[4] Isolated liver involvement without extrahepatic disease has been reported in up to 14% of AIDS patients.[2] Elevation of serum alkaline phosphatase is a very sensitive marker of hepatic involvement; elevation of transaminases and bilirubin is usually observed in the advanced disease. The typical symptoms seen in immunocompetent patients, such as weight loss, night sweats, and fever, are often absent.[105] An abdominal CT scan may help with the diagnosis of non-Hodgkin lymphoma.[4]

Leiomyomas and leiomyosarcomas are extremely rare tumors in children. The incidence of these lesions is increased in pediatric HIV patients. Quantitative polymerase chain reaction (PCR) analysis demonstrated very high levels of EBV in tumor tissue from these patients. No EBV was detected in normal tissues of the same patients or in the tumor tissue of subjects not affected by HIV.[104] The lesions in HIV patients tend to involve the gastrointestinal tract, liver, and lungs.

Human Herpesvirus 6B. Human herpesvirus 6B (HHV-6B) has been implicated in causing roseola (exanthem subitum) in immunocompetent patients. In immunocompromised patients, severe disseminated HHV-6B infection occurs with primary infection and may cause fulminant hepatitis.[106,107] Transaminase elevation, cholestasis, thrombocytopenia, and leukopenia have been reported. There is focal hepatocyte necrosis with mild lymphocytic infiltration of the portal tracts and sinusoids. HHV-6 is detectable in the lymphocytes. The clinical manifestations of HHV-6/-7 reactivation have not been described. Indications for therapy are not clearly defined, but in vitro susceptibility to ganciclovir has been described.[108]

Human Herpesvirus 8. Human herpesvirus 8 viral infection is associated with Kaposi sarcoma, a neoplasm involving skin, mucous membranes, and internal organs. Liver involvement usually occurs as part of a disseminated process, although primary liver disease has also been described.[3,109] Patients are rarely symptomatic from liver disease, although an elevated serum alkaline phosphatase level may be present. Owing to significant sampling error, the diagnosis may be missed if an unguided liver biopsy is performed. Histologically, the lesions appear as dark red to purple nodules in the portal regions, filled with spindle-shaped endothelial cells.

OPPORTUNISTIC INFECTIONS

CYTOMEGALOVIRUS

CMV is one of the most common hepatic infections in HIV-positive adults. CMV is seen in 33 to 44% of all patients and in close to 100% of those who are severely immunosuppressed (CD4 count < 100 cells/mm^3).[80] The incidence of CMV infection associated with symptomatic disease has not been well documented in the pediatric HIV population, with a range of 30 to 60% cited in the literature.[110] The presentation of CMV hepatitis is usually mild, with fever, malaise, hepatomegaly, and transaminitis. CMV typically infects hepatocytes and Kupffer cells, as well as endothelial cells. Inflammation in the portal and periportal areas is usually observed. Intranuclear and intracytoplasmic inclusion bodies are often surrounded by a clear halo ("owl-eye" appearance), and giant cells may be seen in the biopsies.[3,4,13] Less frequently, CMV may affect the biliary epithelium, leading to acalculous cholecystitis, papillary stenosis, and sclerosing cholangitis.[3,4,111]

The diagnosis of CMV may be difficult. The significance of a positive urine culture is unclear because viruria may represent asymptomatic shedding and may persist for months to years.[110] CMV viremia is a much better predictor of active disease.[112] In one study, CMV disease was evident in 57% of children with blood cultures positive for CMV compared with 17% of children with positive urine cultures.[110] There are no controlled trials addressing the use of foscarnet or ganciclovir in the setting of CMV hepatitis. The experience treating nonhepatic infections may suggest their use in hepatitis patients.

Protozoa

Pneumocystis carinii. *Pneumocystis carinii* pneumonia (PCP) is the most common protozoal pathogen among patients with AIDS. The incidence of PCP infection has decreased dramatically, first with the institution of routine PCP prophylaxis and later with the introduction of the HAART regimen. Pulmonary symptoms are the hallmark of *P. carinii* infection. Disseminated infection is not common. Only a few cases of isolated hepatic involvement have been described.[113] It has been suggested that widespread use of aerosolized pentamidine for PCP prophylaxis has contributed to the appearance of *P. carinii* infections of other organs.[114,115] Clinical findings are limited to mild abdominal pain with variable elevation of transaminases. Isolated cases of an obstructive process with increased γ-glutamyl transpeptidase have been reported.[116] Abdominal CT may reveal diffuse punctate calcifications of the liver.[80] Pathology from liver biopsy specimens demonstrates foamy eosinophilic exudate, with the organism demonstrable on silver stain. Acellular nodules in a random or periportal distribution in the lobule, with or without infiltration of the hepatic sinusoids, have also been described.[116,117]

Cryptosporidium. *Cryptosporidium* is the most commonly identified cause of AIDS-related cholangitis. It has been identified in the bile ducts or stools from 20 to 62% of patients with AIDS-related cholangitis.[24] It has been estimated that 10 to 16% of patients with intestinal cryptosporidiosis may develop biliary tract symptoms.[118] Infiltration of portal spaces with eosinophils, plasma cells, and lymphocytes is observed in liver biopsies. Ulceration of the duct epithelium with basophilic Giemsa stain–positive organisms, attached to the luminal surface of the epithelial cells, may be noted.[119]

Microsporida. *Microsporida* is a less common cause of AIDS cholangitis, occurring in less than 10% in most series.[120] The incidence of this infection may be underestimated because techniques for the detection of *Microsporida* are not well developed.[25] The two microsporidia most commonly associated with the disease are *Enterocytozoon bieneusi* and *Enterocytozoon intestinalis*. Clinical symptoms are not different from the cholangitis caused by other organisms and include right upper quadrant pain, nausea, vomiting, and fever. Many patients have associated diarrhea and weight loss. Jaundice is present in only 10% of patients.[121] The diagnosis is based on the detection of *Microsporida* in the stool or bile aspirates. Tissue obtained from the duodenum, ampulla, bile duct, or liver may also be used. Pathogens identified in stools or bile may provide circumstantial evidence that the organism is the etiology of cholangitis. A biopsy specimen from the bile duct revealing the pathogen and inflammation suggests that this pathogen is actually causing cholangitis. Pathogens may be identified in up to 75% of cases.[121]

Fungi

Fungal infections involve the liver only in disseminated disease. These infections share a nonspecific clinical presentation, including fever, hepatomegaly, elevated alkaline phosphatase, and bilirubin.[3] Hepatic involvement may accompany cryptococcal meningitis in the setting of hematogenous dissemination. Opportunistic infections with *Histoplasma*, *Coccidioides*, *Candida*, and *Sporothrix* occur less commonly.[80] Imaging studies may demonstrate diffuse irregularity of the liver parenchyma or, rarely, a formed fungal abscess. Liver biopsy demonstrates nonspecific poorly formed granulomata with minimal inflammatory response.[122] The response to prolonged antifungal therapy is variable, with death resulting from disseminated infection rather than from hepatic involvement itself.

Mycobacteria

MAC includes two closely related species: *M. avium* and *M. intracellulare*. These organisms are small, gram-positive, acid-fast bacilli. Bacterial proliferation typically occurs in the macrophages and is controlled by cell-mediated immunity. With a decrease of the CD4 count, proliferation of the bacteria becomes poorly controlled, and patients are at risk for disseminated MAC infection. CD4 counts of less than 50 cells/mm^3 have been described in patients with disseminated MAC.[123] It is the most common opportunistic pathogen found in AIDS patients. In one series, MAC was found in 38% of cases when liver biopsy was performed.[3] The clinical presentation of disseminated infection is nonspecific and may be attributable to the advanced HIV. Fever, weight loss, hepatomegaly, and anemia have been described. A unique feature of MAC disease in HIV patients is marked elevation of alkaline phosphatase. The enzyme may reach 20 to 40 times the normal level, with little elevation of transaminases, bilirubin, or other parameters of hepatic function.[3] Fractionation shows it to be hepatic in origin. The histologic picture does not show marked abnormalities. This disparity between measured levels and histology suggests interference with enzyme metabolism rather than hepatic destruction.[124]

Diagnosis of MAC infection is based on cultivation of *M. avium* from the liver tissue.

Histology of the liver reveals diffuse, poorly formed noncaseating granulomata, composed mainly of foamy histiocytes. In MAC infection of patients with AIDS, granulomas usually do not contain multinuclear giant cells. Granuloma may be absent in up to 25% of patients. Acid-fast bacilli are found within and around granulomas.

In contrast, *Mycobacterium tuberculosis* is found in patients who are less immunosuppressed (CD4 > 200 cells/mm^3).[3] Extrapulmonary infection with *M. tuberculosis* is seen in over 50% of adult AIDS patients with lung disease.[80] In one study, 7.5% of patients with extrapulmonary disease had hepatic infection.[125] The clinical presentation in these patients is similar to *M. avium* infection; in addition, cough, night sweats, and sputum production may be noted. Tuberculous abscess and biliary obstruction owing to bile duct tuberculoma have rarely been described in adult HIV patients. Liver biopsy reveals well-formed caseating granuloma with a lesser load of acid-fast bacilli compared with MAC infection. Other mycobacteria implicated in liver disease are *Mycobacterium xenopi*, *Mycobacterium genavese*, and *Mycobacterium kansasii*.

Before the availability of HAART, the median survival time in patients with disseminated MAC infection ranged from 5 to 11 months.[123,126] Clinical manifestations of MAC in children receiving HAART have not been described. Treatment regimens have been evaluated in the adult population,[127] with gastrointestinal tuberculosis usually responding to 9 to 12 months of combination antibiotic therapy. Current recommendations for the initial treatment include clarithromycin and azithromycin in combination with ethambutol and rifabutin.[128]

PRIMARY IMMUNODEFICIENCY

Patients with primary immunodeficiency syndromes may have liver disease. Liver infections in these patients can occur due to an increased predisposition to a variety of infectious processes. Causative organisms may vary, based on the type of immunologic defect (Table 51.3-3).

Chronic Granulomatous Disease

Chronic granulomatous disease (CGD) is characterized by the inability of phagocytic cells to reduce molecular oxygen and create the active oxygen metabolites that are necessary for efficient intracellular microbicidal activity. CGD is inherited in an autosomal recessive or X-linked manner. CGD represents a group of four related disorders. The types of infections most frequently encountered include pneumonia, osteomyelitis, skin and soft tissue abscesses, and liver abscess. The increased susceptibility to infection among patients who have CGD is limited to bacteria and fungi that are catalase positive and do not themselves have any production of reduced oxygen metabolites, such as hydrogen peroxide. Catalase-positive organisms are not killed efficiently by the phagocytic cells in patients who have CGD. In contrast, microorganisms that are catalase negative and can produce hydrogen peroxide supply the necessary reactive oxygen metabolites when they are ingested, thereby contributing to their own demise. *Staphylococcus aureus* and *Pseudomonas aeruginosa* are the two most common isolates from a liver abscess in patients with CGD.[129] Needle aspiration of the abscess is needed for the etiologic diagnosis. The use of drainage procedures is limited by poor healing.

Antibiotic therapy should always include penicillinase-resistant penicillin such as nafcillin or oxacillin. These medications may be combined with gentamicin for synergism. Reports of utility of interferon-γ[130] and granulocyte transfusion[131] combined with surgical drainage[132] are present in the literature. Antibiotic prophylaxis with trimethoprim-sulfamethoxazole[133] and use of interferon-γ[130] appear to decrease the incidence of serious infections, including liver abscess, in patients with CGD.

Shwachman Syndrome

Shwachman syndrome primarily affects the exocrine pancreas, bone marrow, and skeleton. The most prominent features seen in this disorder include neutropenia, pancreatic insufficiency, and short stature. Hepatic involvement has frequently been reported with this syndrome and usually manifests as hepatomegaly and elevated transaminases. Biopsy specimens demonstrate steatosis and mild fibrosis. Progression of the liver disease is uncommon.[134,135]

X-Linked Agammaglobulinemia

X-linked agammaglobulinemia presents with recurrent infections in male infants after 9 months of age owing to a failure to synthesize all classes of Igs. Recurrent otitis, sinusitis, pneumonias, and diarrhea are typical. An association with sclerosing cholangitis has occasionally been described. It is not clear whether sclerosing cholangitis is associated with increased autoimmunity in these patients[136] or is caused by opportunistic infections such as cryptosporidiosis.[137]

An unusual syndrome of severe enteroviral infection, most commonly caused by echovirus type 11, has been described in these patients.[138] Patients usually present with meningoencephalitis that has a progressive, usually fatal course. Typical manifestations include fever, dermatomyositis, and significant hepatitis. These symptoms may precede the development of neurologic symptoms. The neurologic disease usually determines survival in these patients.

Common Variable Immunodeficiency

Common variable immunodeficiency is an uncommon disorder with more than 95% of the patients presenting with recurrent sinopulmonary infections. The liver is usually affected by a granulomatous process. This process is thought to be caused by a cytokine disturbance, especially increased production of tumor necrosis factor-α.[139] Approximately 20% of patients have elevated alkaline phosphatase levels that are thought to represent presinusoidal granuloma formation. Most of the patients with liver involvement will develop portal hypertension, esophageal varices, and cirrhosis.

Severe Combined Immunodeficiency

Severe combined immunodeficiency (SCID) is a group of genetic disorders characterized by a block in T-lymphocyte differentiation and may be associated with abnormalities in other lymphocyte lines. At least eight separate diseases comprise this category. Findings indicating combined immunodeficiency include lymphocytopenia; absence of lymph nodes, tonsil tissue, and a thymic shadow on radiographs; low levels of serum Igs; and absent in vitro B- and T-lymphocyte responses to antigens and mitogens. Owing to profound immunosuppression, frequent and severe infections caused by pathogens and opportunistic organisms are commonly observed and may include the liver. In addition, sclerosing cholangitis, possibly caused by biliary tract infection with *Cryptosporidium*, has been reported. Two disorders in this group deserve separate mention with regard to liver disease.

Adenosine deaminase (ADA) deficiency is a systemic metabolic disorder. The enzymatic defect is expressed in all cells, and deoxyadenosine triphosphate, a by-product produced in this disorder, is built up in all cells. Deoxyadenosine triphosphate is extremely toxic to lymphocytes, so 85 to 90% of patients who are ADA deficient present with a picture consistent with severe combined immunodeficiency. These patients are lymphopenic at birth and are pre-

TABLE 51.3-3 COMMON PATHOGENS IN CONGENITAL IMMUNOLOGIC DEFECTS

ABNORMALITY	EXAMPLE	LIVER DISEASE	PATHOGENS			
			BACTERIA	FUNGI	VIRUSES	PROTOZOA
Phagocyte defects						
Quantitative	Neutropenia	Rare	Staphylococci,	*Candida*,		
Qualitative	CGD	Common	streptococci,	*Aspergillus*		
	Shwachman syndrome	Common	*Nocardia*,			
	Leukocyte adhesion defect	Rare	*Escherichia coli*,			
			Klebsiella			
Immunoglobulin deficiencies	X-linked agammaglobulinemia	Rare	*Klebsiella*,		Enteroviruses,	*Giardia*
	Common variable immunodeficiency	Common	*Haemophilus influenzae*,		echoviruses,	
	Hyper IgE (Job) syndrome	Rare	*Streptococcus pneumoniae*		CMV, HSV	
Complement deficiencies		Rare	*S. pneumoniae*,			
			H. influenzae,			
			Neisseria			
Cellular and combined immunodeficiencies	SCID	Common	Mycobacteria,	*Candida*,	CMV,	*Pneumocystis carinii*,
	DiGeorge syndrome	Rare	*Salmonella* spp,	*Histoplasma*,	VZV,	*Toxoplasma gondii*,
	Wiskott-Aldrich syndrome	Rare	*Listeria* spp,	*Cryptococcus*	HSV,	*Cryptosporidium*,
	X-linked lymphoprolipherative syndrome	Common	*Legionella* spp		EBV	*Microsporida*,
						Isospora spp

CGD = chronic granulomatous disease; CMV = cytomegalovirus; EBV = Epstein-Barr virus; HSV = herpes simplex virus; IgE = immunoglobulin E; SCID = severe combined immunodeficiency; VZV = varicella-zoster virus.

disposed to recurrent illnesses caused by both pathogens and opportunistic organisms. Symptoms often begin within a few weeks of birth. Pneumonia, intractable diarrhea, and extensive candidiasis are common. Profound failure to thrive is also frequently present. A growth abnormality of costochondral junctions causing cupping and flaring of rib ends on radiograph has been associated specifically with ADA deficiency. Elevated serum levels of hepatic transaminases are not uncommon in ADA-deficient patients, but the cause usually is not determined. Severe bridging fibrosis has been described in liver specimens during autopsy.[140] Bollinger and colleagues described a patient with ADA deficiency and persistent neonatal hepatitis, apparently unrelated to infection or graft-versus-host disease (GVHD), that responded to ADA replacement therapy.[141] It is thus possible that the toxic effects of ADA substrates may be responsible for the liver disease in these patients.

Omenn syndrome is another variant of SCID. T-lymphocyte infiltration of the skin and gut is the hallmark of this condition. Patients present with erythroderma, alopecia, protein-losing enteropathy, and failure to thrive. Life-threatening infections are common. Marked hepatosplenomegaly and lymphadenopathy may be present within the first weeks of life. Laboratory evaluation usually demonstrates elevated white blood cell and eosinophil counts, increased IgE, hypogammaglobulinemia, and histiocytosis. Severe liver disease, likely owing to infiltration with T cells and histiocytes, is associated with this condition.[142]

X-Linked Lymphoproliferative Syndrome

In 1975, Purtilo and colleagues described a kindred with 18 boys, of whom 6 died of a lymphoproliferative disorder.[143] This syndrome has since been linked to infection with EBV. The affected boys had a T-cell regulatory defect that made them extremely vulnerable to EBV infection, which often is fatal.[144] The immune defect allows an explosive proliferation of B cells after exposure to EBV. Liver involvement is a prominent feature, with progression to multiorgan system failure, and death is typical. Of the patients who survive the initial infection with EBV, 24% will acquire a malignant lymphoproliferative disorder at a median age of 4.9 years. All neoplasms have a B-cell phenotype. The majority of the non-Hodgkin lymphomas occur in the intestinal region, especially the ileocecal area. The overall risk of having a lymphoma has been estimated to be 200 times higher than that for the general population.[144]

INFECTIONS IN TRANSPLANT RECIPIENTS

Infectious complications following solid organ transplant may be divided into three time periods.[145] The early post-transplant period (first 4 weeks) is characterized by nosocomial bacterial and fungal infections. The only significant viral infection observed during the early period after transplant is caused by recurrent HSV.

The risk of infection is directly related to the surgical procedure itself, invasive monitoring with intravascular catheters, or preexisting infection in the donor or recipient. Typically, the specific sites of transplant and allograft are the most likely sites of infection. For example, liver transplant recipients are at risk for biliary sepsis and liver abscesses.

From 1 to 6 months post-transplant, infections are often related to the use of immunosuppressive agents. CMV is the dominant pathogen causing disease during this period. Other major pathogens are *Cryptococcus*, *Candida*, tuberculosis, HBV and HCV, and EBV. Infectious complications that occur later than 6 months after transplant are usually due to one of two factors: the effect of chronic viral infection acquired earlier or chronic graft dysfunction, which requires repeated courses of immunosuppression and puts patients at risk for infection with opportunistic organisms.

The course of the patients who undergo bone marrow transplant may also be divided into three phases.[146] The early phase corresponds to the first 2 to 4 weeks post-transplant. The predominant risk factor in this period is profound neutropenia and damage to mucosal surfaces. Invasive fungal and bacterial infections predominate during this period.

Between the period of engraftment and weeks 15 to 20 post-transplant, infections with opportunistic pathogens (CMV, *P. carinii*) predominate. The major predisposing factors for these infections are the immunosuppressive effects of acute GVHD and its treatment. Serious infections occurring 4 to 6 months after transplant are seen predominantly in patients with chronic GVHD.[146] Liver infections are rare in this period, with the most common being EBV infection.

The majority of patients undergoing solid organ or bone marrow transplant show evidence of CMV infection (a rise in titers or viral isolation from the urine or blood). However, not all patients develop clinical disease. The major determinant of the progression to disease is whether the infection with CMV is primary or secondary. If the organ from a CMV-positive donor is transplanted into a CMV-negative recipient, this will result in primary infection. Primary infection is more likely to be associated with the disease than is CMV reactivation.[147] The severity of the immunosuppression is another determining factor. In general, bone marrow transplant patients have more severe manifestations of CMV disease compared with solid organ transplant patients. Clinical manifestations of CMV infection in transplant recipients are usually a mononucleosis-type syndrome with fever, leukopenia, and elevated transaminases. Interstitial pneumonitis may be life threatening. Liver disease is rarely severe.[146] CMV infection in liver transplant patients may be associated with a higher risk of acute rejection[148] and possibly with an increased incidence of hepatic artery thrombosis owing to endothelial cell infection.[149] HSV and VZV infections are also common during this time, with a clinical course that is similar to that in HIV-infected patients.

EBV is responsible for a number of disorders, but the major concern in solid organ transplant recipients is post-transplant lymphoproliferative disorder (PTLD). Acute infection with EBV leads to polyclonal activation of B cells with expansion of lymphoid tissues. Because the cellular immune responses provided by natural killer and cytotoxic T cells are critical to clearing the virus, the T cell–targeted immunosuppression used in organ transplant puts allo-

graft recipients at risk for PTLD.[150] It has now been well established that as the level of immunosuppression increases, so does the incidence of PTLD.[151,152]

Primary EBV infection, young age, and receiving an EBV-positive donor organ are interrelated predisposing factors to PTLD and are particularly important in pediatric transplant recipients.[153] Many young children, usually EBV negative, receive grafts from older donors who are much more likely to have had previous EBV infection.

Sites of involvement include the small bowel, the intra-abdominal lymph nodes, the tonsillar bed, and the liver. Patients may present immediately post-transplant or months to years after successful transplant. Fever, weight loss, lymphadenopathy, hepatosplenomegaly, and abdominal pain are typical presenting findings. Hepatitis may resemble rejection episodes in liver transplant patients.[150]

How best to prevent EBV infection in the EBV-naive recipient is debatable.[154] Some protocols use intravenous ganciclovir for variable lengths of time; others rely primarily on CMV hyperimmunoglobulin. Along with a preventive strategy, there should be a method of detecting when EBV infection or reactivation first occurs so that immunosuppression levels can be decreased promptly. This determination is best accomplished by serially monitoring the peripheral blood for EBV by quantitative PCR techniques.[155–157]

Hepatitis B infection tends to be more aggressive in immunosuppressed patients. An accelerated course of the disease was described after kidney transplant.[158]

Reactivation of previously quiescent disease has been described in children undergoing chemotherapy[159] and in patients after bone marrow transplant.[160] Hepatitis B may result in mortality rates as high as 12% in bone marrow transplant patients.[161] Increased mortality from liver disease in recipients of kidney transplants has been found in some studies, but if it occurs, it is seen 10 years or more following renal transplant.[162] Fibrosing cholestatic hepatitis is a rare, early, severe complication of renal transplant in HBV-infected recipients of kidney transplants.[163] It is characterized by cholestasis, with only a mild to moderate increase in aminotransferase levels, and by a rapid deterioration in liver function that can lead to short-term liver failure. The liver histology demonstrates periportal and perisinusoidal cholestasis, scarce mixed infiltrates, hepatocellular ballooning, and histologic cholestasis.

In addition, the acquisition of HBV soon after renal transplant carries a risk of early death from liver failure.[162] Reactivation of HBV after renal transplant in patients with preexisting HBV surface antibodies has been reported.[164] Use of immunosuppressive medications has been demonstrated to increase viral replication in vitro.[165] Viral mutations may also be responsible for the more aggressive course of the disease.[166] Hepatitis B–induced chronic liver disease is one of the main indications for liver transplant in adults. Thus, viral reinfection of the allograft is a major challenge in this population but is a less common issue in pediatrics.

The use of hepatitis B immunoglobulin during and after transplant has been proven to decrease the incidence of the recurrence rate in up to 80% of the patients.[167] The limitations of this treatment are indefinite length of the therapy, need for parenteral administration, and drug cost.[168] Other medications used to prevent HBV include agents used in the treatment of HBV in the nontransplant setting. Interferon-α is poorly tolerated and has limited efficacy in this population. Lamivudine and adefovir have been successfully used to prevent HBV recurrence.[58,169] The combination of lamivudine with hepatitis B immunoglobulin has completely eliminated recurrent HBV disease in some series.[170] Previously vaccinated patients who are HBsAg negative should be tested annually for anti-HBV antibodies and should receive booster vaccinations[171] when the titer decreases to < 10 mIU/mL. Allografts from donors who are positive for HBsAg should not be used. Controversy exists with respect to whether allografts from donors who are HBV core antibody positive should be used.

Hepatitis C infection may be caused either by exposure to contaminated blood products or tissues or via recurrence of preexisting HCV infection following liver transplant. In the pediatric population, the former is the more common route. HCV can also be transmitted with other allografts in addition to the liver. In one study, 42% of patients receiving kidneys from donors seropositive for HCV developed clinical hepatitis.[172] Organs from donors with HCV antibody who are HCV RNA positive are most likely to transmit HCV infection, but organs from donors who are HCV antibody positive and HCV RNA negative cannot be regarded as completely safe.[173]

Virtually all liver transplant recipients who are viremic with HCV at the time of transplant will become reinfected.[174–176] Factors associated with a more rapid progression of the disease after transplant may include the degree of immunosuppression,[177] coinfection with CMV,[178] and HCV genotype 1b.[179] In bone marrow transplant patients, HCV infection is a major cause of post-transplant cirrhosis. Clear association with veno-occlusive disease has also been observed.[180] The evolution of the liver disease is typically slow, with the disease following a similar course in liver, heart,[181] kidney,[182] and bone marrow transplant patients. Short-term survival is not affected in kidney transplant patients, but long-term (10 and 20 years) outcome was significantly worse in HCV antibody–positive patients.[183,184] Fibrosing cholestatic hepatitis is the only early but serious complication of HCV infection observed in transplant patients.[180] The clinical and pathologic features are similar to the one described in hepatitis B. Diagnosis is made by detection of HCV RNA by reverse transcriptase PCR and other assays because serology is a poor marker of HCV infection in transplant recipients.[185,186] Liver biopsy is important to confirm the diagnosis histologically and to delineate the severity of the disease.

Treatment is similar to that in the normal host. Interferon alone or in combination with ribavirin is typically used. The concern with the use of interferon is its association with graft rejection in renal transplant; however, these issues do not appear to be valid in liver transplant recipients. The response rates to treatment are lower than those seen in immunocompetent patients.[187] Preemptive therapy prior to liver transplant tends to reduce the viral load and is associated with milder disease and slower progression.

Invasive candidiasis is a major cause of mortality and morbidity in patients with hematologic malignancies. Hepatosplenic involvement is typical, as was initially described by Bodey and colleagues.[188] Risk factors for invasive disease include the presence of acute leukemia, prolonged neutropenia, intravascular catheters, disruption of mucosal barriers, and the administration of broad-spectrum antibiotics.[189] Invasive candidal infection is extremely rare in nonleukemic patients.

Hepatosplenic candidiasis initially presents as neutropenia and fever without focal signs or symptoms that fails to respond to broad-spectrum antibiotics. Liver function tests, as well as ultrasonography and CT scan of the abdomen, are generally normal at this point. Often the patient's leukemia goes into remission, and the neutrophil count recovers. However, high fever, anorexia, and weight loss persist. Right-sided abdominal pain or pleuritic chest pain may be present. Substantial elevation of alkaline phosphatase is noted and may persist for months. Elevation of other liver function tests is usually present but is less impressive.[189]

CT scan identifies lesions in about 90% of the patient, and ultrasonography visualizes pathology in 70 to 75% of patients.[190] CT scan demonstrates small, round, low-attenuation lesions scattered throughout the liver and spleen. The appearance on CT scan is not pathognomonic and may mimic metastatic disease or bacterial abscesses. Histologically, three patterns have been observed: necrosis with minimal inflammatory reaction, microabscesses with severe inflammation, and granulomas.[191] Granulomatous inflammation is usually seen in the liver. Central areas of necrosis or fibrosis, surrounded by granulation tissue, are seen; macrophages, fibroblasts, and giant cells are typically observed.[189]

The diagnosis of invasive candidiasis requires a high index of suspicion. No single noninvasive study is sufficient to establish a diagnosis, and liver biopsy is frequently performed. Because many infections may cause granulomatous inflammation, and given the small size of the lesions, percutaneous liver biopsy may fail to establish the diagnosis. Open liver biopsy is considered by many to be the most reliable way of diagnosing hepatosplenic candidiasis.[192] The optimal management of this condition is not yet established. The response rate to the liposomal formulation of amphotericin B is much better than the response rate to the regular formulation: 85 to 90% versus 50%.[193–195] Fluconazole has a response rate of about 80% and may be effective even in patients who failed to respond to amphotericin B treatment.[193,194,196] It is believed that fluconazole should be the drug of choice for hepatosplenic candidiasis because it is at least as effective as amphotericin B, is less toxic, may be given orally, and is less expensive than liposomal amphothericin B.[197] Usually, it takes 2 to 3 weeks to see a measurable response.

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CHAPTER 52

AUTOIMMUNE DISEASE

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Autoimmune liver disorders are inflammatory liver diseases characterized histologically by a dense mononuclear cell infiltrate in the portal tract and serologically by the presence of nonorgan and liver-specific autoantibodies and increased levels of immunoglobulin G (IgG), all in the absence of a known etiology. These disorders usually respond to immunosuppressive treatment, which should be instituted as soon as a diagnosis is made. The onset of these conditions is often ill-defined, and they frequently mimic acute hepatitis; the previously accepted requirement of 6 months duration of symptoms before a diagnosis of autoimmune disease can be made has been abandoned.[1,2]

There are three liver disorders in which liver damage is likely to arise from an autoimmune attack: autoimmune hepatitis (AIH), autoimmune sclerosing cholangitis (ASC), and de novo autoimmune hepatitis after liver transplant.

According to data collected at our tertiary center, there appears to be an increase in the yearly incidence of AIH and ASC in childhood. Thus, in the 1990s, these conditions represented 2.3% of children older than 4 months referred to our unit during 1 year, whereas in the past 3 years, their incidence has increased to 12%.

AUTOIMMUNE HEPATITIS

Clinical Features

Two types of AIH are recognized according to the presence of smooth muscle antibody (SMA) and/or antinuclear antibody (ANA) or liver/kidney microsomal type 1 antibody (LKM-1). A major target of SMA is the actin of smooth muscle,[3] whereas the molecular target of LKM-1 is cytochrome P-4502D6 (CYP2D6).[4] Pediatric series, including our own, report a similarly severe disease in ANA/SMA-positive and LKM-1–positive patients.[5,6] We reviewed the clinical, biochemical, and histologic features and outcomes of ANA/SMA-positive or LKM-1–positive AIH in 52 children referred between 1973 and 1993 (Table 52-1).[6] Thirty-two patients were ANA and/or SMA positive, and 20 were LKM-1 positive. All other known causes of liver disease were excluded. Only one child with the LKM-1 antibody presented with acute liver failure and had received plasma transfusions abroad before referral, as well as had evidence of exposure to hepatitis C virus (HCV) infection, being positive for anti-HCV antibody by second-generation assay. There was a predominance of girls (75%) in both groups. Although LKM-1–positive patients presented at a younger age (median 7.4 versus 10.5 years), the duration of symptoms before diagnosis and the frequency of hepatosplenomegaly were similar in the two groups. There was also no significant difference in the frequency of associated autoimmune disorders and a family history of autoimmune disease between the two groups. Associated autoimmune disorders included nephrotic syndrome, thyroiditis, Behçet's disease, ulcerative colitis, insulin-dependent diabetes, and urticaria pigmentosa in ANA/SMA patients and thyroiditis, vitiligo, hypoparathyroidism, and Addison disease in LKM-1 patients.

We observed three clinical patterns of disease: (1) In 50% of ANA/SMA-positive and 65% of LKM-1–positive patients, the presentation was indistinguishable from that of acute viral hepatitis (nonspecific symptoms of malaise, nausea/vomiting, anorexia, and abdominal pain, followed by jaundice, dark urine, and pale stools); six children (five LKM-1 positive) developed acute hepatic failure with grade II to IV hepatic encephalopathy from 2 weeks to 2 months (median 1 month) after onset of symptoms. In the remaining children, the duration of disease before diagnosis ranged from 10 days to 5 months (median 1.8 months). (2) Twenty-five percent of LKM-1–positive and 38% of ANA/SMA-positive patients had an insidious onset, with an illness characterized by progressive fatigue, relapsing jaundice, headache, anorexia, and weight loss, lasting from 6 months to 2 years (median 9 months) before diagnosis. (3) In six patients (two LKM-1 positive), there was no history of jaundice, and the diagnosis followed presentation with complications of portal hypertension, such as hematemesis from esophageal varices, bleeding diathesis, chronic diarrhea, weight loss, and vomiting.

The mode of presentation of AIH in childhood is therefore variable, and the disease should be suspected and excluded in all children presenting with symptoms and signs of prolonged or severe liver disease.

Laboratory features at presentation are summarized in Table 52-2. Overall, LKM-1–positive patients had higher median levels of bilirubin and aspartate aminotransferase than those who were ANA/SMA positive, but if the six patients presenting with acute hepatic failure are excluded, the differences for these two parameters are not significant. A severely impaired hepatic synthetic function, as assessed by the presence of both prolonged prothrombin time and

TABLE 52-1 CLINICAL FEATURES AT PRESENTATION IN 52 PATIENTS WITH AUTOIMMUNE HEPATITIS DIVIDED ACCORDING TO ASSOCIATED AUTOANTIBODIES

FEATURE	ANA/SMA POSITIVE AIH (*n* = 32)	LKM-1 POSITIVE AIH (*n* = 20)	*p* VALUE
Age at diagnosis (yr), median (range)	10.5 (2.3–14.9)	7.4 (0.8–14.2)	.011
Female *n* (%)	24 (75)	15 (75)	1.00
Duration of illness before diagnosis (mo; median [range])	4 (0.2–24.6)	1.7 (0.03–15.4)	.06
Fulminant liver failure *n* (%)	1 (3)	5 (25)	.05
Associated autoimmune disorders before or after diagnosis of AIH, *n* (%)*	7 (22)	4 (20)	.85
Nephrotic syndrome	1	0	
Autoimmune thyroiditis	1	2	
Behçet disease	1	0	
Ulcerative colitis	3	0	
Insulin-dependent diabetes	2	0	
Urticaria pigmentosa	1	0	
Vitiligo	0	1	
Hypoparathyroidism and Addison disease (APS-1)	0	1	
Autoimmune disorders in first-degree relatives, *n* (%)*	13 (43)	8 (40)	.81
Thyroid disease	3	4	
Insulin-dependent diabetes	3	4	
Autoimmune hepatitis	3	0	
Crohn disease	2	0	
Behçet disease	1	0	
Rheumatoid arthritis	1	1	
Psoriasis	0	1	
Systemic lupus erythematosus	0	1	

AIH = autoimmune hepatitis; ANA/SMA = nuclear and/or smooth muscle antibody; APS-1 = autoimmune polyendocrine syndrome type 1; GGT = γ-glutamyl transpeptidase; LKM-1 = liver kidney microsomal type 1 antibody.
*Some subjects have more than one disorder.

hypoalbuminemia, tended to be more common in ANA/SMA-positive patients (53%) than in LKM-1–positive patients (30%). The majority (80%) of the patients had increased levels of IgG, but 10 (5 LKM-1 positive) had a normal serum IgG level for age, including 3 patients who presented with acute hepatic failure, indicating that normal IgG values do not exclude the diagnosis of AIH. As previously reported, we found that partial IgA deficiency is significantly more common in LKM-1–positive patients than in ANA/SMA-positive patients (45% versus 9%).[7]

When compared with those of controls, the frequencies of the human leukocyte antigen (HLA)-DR3 were significantly higher in patients with ANA/SMA-positive AIH but not in those with LKM-1–positive AIH. Recent data suggest that possession of DR7 predisposes the patient to type 2 AIH.[8] Although these results should be confirmed in a

TABLE 52-2 LABORATORY FEATURES AT PRESENTATION IN 52 PATIENTS WITH AUTOIMMUNE HEPATITIS DIVIDED ACCORDING TO ASSOCIATED AUTOANTIBODIES

	ANA/SMA POSITIVE AIH (*n* = 32)	LKM-1 POSITIVE AIH (*n* = 20)	*p* VALUE
Total bilirubin, μmol/L (nv: 20)	62 (6–462)	188 (13–773)	.007
Aspartate aminotransferase, IU/L (nv: 50)	632 (81–2,500)	1,146 (93–2440)	.047
GGT, IU/L (nv: 50)	126 (11–871), *n* = 26	91 (36–299), *n* = 17	.055
Alkaline phosphatase, IU/L (nv: 350)	376 (131–1,578)	377 (102–1,677)	.87
Total protein, g/L (nv: 60–80)	75 (58–117)	72 (45–92)	.20
Albumin, g/L (nv: 35–50)	32 (20–43)	38 (25–54)	.02
International normalized ratio (nv: 0.9–1.2)	1.6 (1–2.5)	1.6 (1–8.6)	.39
Immunoglobulin G, g/L (nv: 5–18)	28 (13.4–73.3)	21 (10.2–40)	.06
Immunoglobulin A, g/L (nv: 0.8–4.8)	2.3 (0.9–4.2)	1.4 (0.07–3.6)	.059
Immunoglobulin M, g/L (nv: 0.5–2)	1.7 (0.4–9.3)	2.0 (1.05–8.0)	.53
C3, g/L (nv: 0.6–1.2)	0.7 (0.03–1.6), *n* = 19	0.7 (0.2–1.2), *n* = 12	.79
C4, g/L (nv: 0.2–0.6)	0.1 (0.03–0.34), *n* = 19	0.1 (0.03–0.24), *n* = 12	.52
ANA titer	120 (10–5,120), *n* = 20	NA	
SMA titer	160 (10–2,560), *n* = 26	NA	
LKM-1 titer	NA	640 (40–10,400), *n* = 19	
Anti-LSP titer	1,000 (0–3,300), *n* = 21	1,250 (0–2,400), *n* = 7	.61
Anti-ASGPR titer	0 (0–1,500), *n* = 21	200 (0–750), *n* = 7	.27

AIH = autoimmune hepatitis; ANA/SMA = nuclear and/or smooth muscle antibody; ASGPR = asialoglycoprotein receptor; LKM-1: liver kidney microsomal type 1 antibody; LSP = liver-specific lipoprotein; NA = not applicable; nv = normal value.

larger number of patients and in different centers, they suggest that the immunopathogenic mechanisms involved in the development of the two forms of AIH may be different. Patients with AIH, whether LKM-1 or ANA/SMA positive, have isolated partial deficiency of the HLA class III complement component C4, which is genetically determined, being associated with the possession of the silent gene *C4AQ0* at the C4A locus.[9,10] C4AQ0, either in linkage with DR3 or on its own, has been reported in association with other autoimmune disorders.[11]

The severity of portal tract inflammation, lobular activity, and periportal necrosis, features characteristic of interface hepatitis, at diagnosis was similar in both groups. Cirrhosis on initial biopsy was more frequent in ANA/SMA-positive patients (69%) than in LKM-1–positive patients (38%). Of note is that 57% of patients already cirrhotic at diagnosis presented with a clinical picture reminiscent of that of prolonged acute virus-like hepatitis. Multiacinar or panacinar collapse, which suggests an acute liver injury, was present in eight patients (five LKM-1 positive), six of whom had acute liver failure. In these patients, it was not possible to ascertain the degree of fibrosis or the presence or absence of cirrhosis. The question as to whether the acute presentation in these patients represented a sudden deterioration of an underlying unrecognized chronic process or a genuinely acute liver damage remains open. Progression to cirrhosis was noted in four of seven ANA/SMA-positive patients and in two of five LKM-1–positive patients on follow-up biopsies done between 17 and 56 months from the initial biopsy. Overall, 74% of ANA/SMA-positive and 44% of LKM-1–positive patients showed evidence of cirrhosis on initial or follow-up histologic assessment, indicating that, apart from the higher tendency to present as acute liver failure, the severity of LKM-1–positive disease is not worse than that of ANA/SMA-positive disease. Recently, we have demonstrated that a more severe disease and a higher tendency to relapse are associated with the possession of antibodies to soluble liver antigen, which are present in about half of the patients with AIH type 1 or 2 at diagnosis.[12]

Pathophysiology

The typical histologic picture of AIH, which is characterized by a dense mononuclear cell infiltrate eroding the limiting plate and invading the parenchyma (interface hepatitis), first suggested that autoaggressive cellular immunity might be involved in its causation.[13,14] Immunocytochemical studies have identified the phenotype of the infiltrating cells. T lymphocytes mounting the alpha/beta T-cell receptor predominate. Among the T cells, a majority are positive for the CD4 helper/inducer phenotype, and a sizable minority are positive for the CD8 cytotoxic phenotype. Lymphocytes of non–T-cell lineage are fewer and include (in decreasing order of frequency) natural killer cells (CD16/CD56 positive), macrophages, and B lymphocytes.[15] The recently described natural killer T cells, which express simultaneously markers of both natural killer (CD56) and T cells (CD3), are involved in liver damage in an animal model of autoimmune hepatitis.[16]

A powerful stimulus must be promoting the formation of the massive inflammatory cell infiltrate present at diagnosis. Whatever the initial trigger, it is most probable that such a high number of activated inflammatory cells cause liver damage. There are different possible pathways that an immune attack can follow to inflict damage on the hepatocyte (Figure 52-1). Liver damage is believed to be orchestrated by CD4-positive T lymphocytes recognizing a self-antigenic peptide. To trigger an autoimmune response, the peptide must be embraced by an HLA class II molecule and presented to uncommitted T helper (Th)0 cells by professional antigen-presenting cells (APCs), with the costimulation of ligand–ligand (CD28 on Th0, CD80 on APC) interaction between the two cells. The Th0 cells become activated, differentiate into functional phenotypes according to the cytokines prevailing in the microenvironment and the nature of the antigen, and initiate a cascade of immune reactions determined by the cytokines they produce. Arising in the presence of the macrophage-produced interleukin-12, Th1 cells secrete mainly interleukin-2 and interferon-γ, which activate macrophages, enhance expression of HLA class I (increasing the vulnerability of liver cells to cytotoxic attack), and induce expression of HLA class II molecules on hepatocytes, which then become able to present the autoantigenic peptide to Th cells, thus perpetuating the immune recognition cycle. Th2 cells, which differentiate from Th0 if the microenvironment is rich in interleukin-4, produce mainly interleukin-4, -5, and -10, which induce autoantibody production by B lymphocytes. Physiologically, Th1 and Th2 cells antagonize each other. The process of autoantigen recognition is strictly controlled by regulatory mechanisms. If these regulatory mechanisms fail, the autoimmune attack is perpetuated. Over the past two decades, different aspects of the above pathogenic scenario have been investigated.

An impairment of immunomodulatory mechanisms, which would enable the autoimmune response to develop, has been described in several reports. Children and young adults with AIH have low levels of T lymphocytes expressing the CD8 marker.[17] The notion of defective immunoregulation is supported by the finding that suppressor cell function is also impaired in AIH.[18] Nouri-Aria and colleagues[19,20] have shown that (1) the impairment of immunoregulation segregates with the possession of the HLA haplotype B8/DR3,[19] the haplotype that predisposes to AIH,[21] and (2) this immunoregulatory defect is correctable by therapeutic doses of corticosteroids.[20] In addition, it has been shown that patients with AIH have a specific defect in a subpopulation of T cells controlling the immune response to liver-specific membrane antigens.[22]

Lobo-Yeo and colleagues have shown that hepatocytes from patients with AIH, in contrast to normal hepatocytes, express HLA class II molecules.[23] These hepatocytes, although lacking the antigen-processing machinery typical of APCs, may present peptides through a bystander mechanism.[24] Given the impaired regulatory function and the inappropriate expression of HLA class II antigens on the hepatocytes, the question arises as to whether an autoantigenic peptide is presented to the helper/inducer

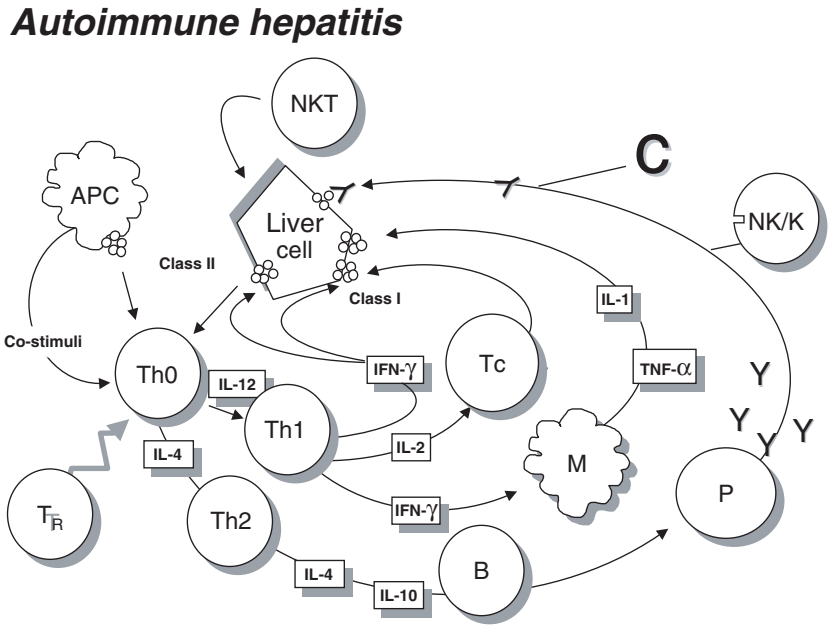

FIGURE 52-1 Autoimmune attack on the liver. A specific autoantigenic peptide is presented to an uncommitted T helper (Th0) lymphocyte within the human leukocyte antigen (HLA) class II molecule of an antigen-presenting cell (APC). Th0 cells become activated and, according to the presence in the microenvironment of interleukin (IL)-12 or IL-4 and nature of antigen, differentiate into Th1 or Th2 and initiate a series of immune reactions determined by the cytokines they produce: Th2 secrete mainly IL-4 and IL-10 and direct autoantibody production by B lymphocytes; Th1 secrete IL-2 and interferon-γ (IFN-γ), which stimulate T cytotoxic (Tc) lymphocytes, enhance expression of class I and induce expression of class II HLA molecules on hepatocytes and activate macrophages; activated macrophages release IL-1 and tumour necrosis factor-α (TNF-α). If T "regulatory" (T_R) lymphocytes do not oppose, a variety of effector mechanisms are triggered: liver cell destruction could derive from the action of Tc lymphocytes; cytokines released by Th1 and recruited macrophages; complement (C) activation or engagement of killer (NK/K) lymphocytes by the autoantibody (Y) bound to the hepatocyte surface. Natural killer T cells (NKT), cells with markers of both natural killer and T cells, are involved in liver damage in an animal model of autoimmune hepatitis. Adapted from Vergani D, Chodhuri K, Bogdanos DP, Mieli-Vergani G. Pathogenesis of autoimmune hepatitis. Clin Liver Dis 2002;6:439–49.

cells, leading to their activation. Although there is no direct evidence as yet that an autoantigenic peptide is presented and recognized, activation of helper cells has been documented in AIH.[15,25] These activated cells possess the CD4 phenotype, and their numbers are highest when the disease is most active.

What triggers the immune system to react to an autoantigen is unknown. A lesson may be learned by the study of humoral autoimmune responses during viral infections. Thus, recent studies aimed at determining the specificity of the LKM-1 antibody, present in both the juvenile form of AIH and in some patients with chronic HCV infection, have shown a high amino acid sequence homology between the HCV polyprotein and CYP2D6, the molecular target of LKM-1, thus implicating a mechanism of molecular mimicry as a trigger for the production of LKM-1 in HCV infection.[4,26,27] It is therefore conceivable that an as yet unknown virus infection may be at the origin of the autoimmune attack in AIH.

Titers of antibodies to liver-specific lipoprotein, a macromolecular complex present on the hepatocyte membrane, and to its well-characterized component asialoglycoprotein receptor, correlate with the biochemical and histologic severity of AIH.[28,29] Antibodies to alcohol dehydrogenase, a second well-defined component of liver-specific lipoprotein, have been described in patients with AIH.[30] Immunofluorescence studies on monodispersed suspensions of liver cells obtained from patients with AIH showed that these cells are coated with antibodies in vivo.[31] A pathogenic role for these autoantibodies has been indicated by cytotoxicity assays showing that autoantibody-coated hepatocytes from patients with AIH are killed when incubated with autologous or allogeneic[32] lymphocytes. The effector cell was identified as an Fc receptor–positive mononuclear cell.[32] More recently, T-cell clones obtained from liver biopsies of children with AIH and expressing the gamma/delta T-cell receptor have been shown to be cytotoxic to a variety of targets but to preferentially kill liver-derived cells as opposed to cell lines derived from other organs.[33–35]

Data from our laboratory show an elevation of the circulating levels of cytokines produced by both Th1 and Th2 cells at diagnosis of AIH, whereas Th1 cytokine levels significantly decrease and Th2 cytokines remain elevated during remission (D. Vergani and colleagues, unpublished data, 2001). This observation, implicating Th1 cytokines in the causation of liver damage, finds support in immunohistochemical studies showing a significantly higher number of cells that produce interferon-γ and interleukin-2 in

the liver tissue of patients with active disease, whereas the number of cells producing interleukin-4 and interleukin-10 is similar in active and inactive biopsies (D. Vergani and colleagues, unpublished data, 2001).

The establishment of cell lines and clones has enabled Wen and colleagues[33,34] and Löhr and colleagues[36,37] to show that the majority of T-cell clones obtained from the peripheral blood and a proportion of those from the liver of patients with AIH are CD4 positive and use the conventional alpha/beta T-cell receptor. Some of these CD4-positive clones were further characterized and were found to react with partially purified antigens, such as crude preparations of liver cell membrane or liver-specific lipoprotein,[34] and with purified asialoglycoprotein receptor[34,37] or recombinant CYP2D6[36] and to be restricted by HLA class II molecules in their response. Because CD4 is the phenotype of Th cells, both Wen and colleagues[34] and Löhr and colleagues[37] investigated whether these clones were able to help autologous B lymphocytes in the production of immunoglobulin in vitro and found that their coculture with B lymphocytes resulted in a dramatic increase in autoantibody production. All of the above experimental evidence suggests that cellular immune responses are involved in the liver damage of AIH even though the evidence that the trigger is an autoantigen is still incomplete.

Treatment

Unless it presents with acute liver failure (which usually requires urgent transplant), AIH responds satisfactorily to immunosuppression. Treatment should be started with prednisolone 2 mg/g/d (maximum 60 mg/d), which is gradually decreased over a period of 4 to 8 weeks if there is progressive normalization of the transaminases, and then the patient is maintained on the minimal dose able to sustain normal transaminase levels, usually 5 mg/d. During the first 6 to 8 weeks of treatment, liver function tests are checked weekly to allow a constant and frequent fine-tuning of the treatment, avoiding severe steroid side effects. If progressive normalization of the liver function tests is not obtained over this period of time or if too high a dose of prednisolone is required to maintain normal transaminases, azathioprine is added at a starting dose of 0.5 mg/g/d, which, in the absence of signs of toxicity, is increased up to a maximum of 2 mg/g/d until biochemical control is achieved. Azathioprine is not recommended as first-line treatment because of its hepatotoxicity, particularly in severely jaundiced patients. A preliminary report in a cohort of 30 children with AIH suggests that the measurements of the azathioprine metabolites 6-thioguanine and 6-methylmercaptopurine are useful in identifying drug toxicity and nonadherence and in achieving a level of 6-thioguanine considered therapeutic for inflammatory bowel disease.[38]

In our experience, although an 80% decrease of initial transaminase levels is obtained within 6 weeks from starting treatment in most patients, complete normalization of liver function may take several months. In our own series, normalization of transaminase levels occurred at medians of 0.5 years (range 0.2–7 years) in ANA/SMA-positive children and 0.8 years (range 0.02–3.2 years) in LKM-1–positive children.[6] Relapse while on treatment is common, affecting about 40% of the patients and requiring a temporary increase of the steroid dose. The risk of relapse is higher if steroids are administered on an alternate-day schedule, often instituted in the unsubstantiated belief that it has a less negative effect on the child's growth. Small daily doses should be used because they are more effective in maintaining disease control and minimize the need for high-dose steroid pulses during relapses (with attendant more severe side effects). If a liver biopsy shows minimal or no inflammatory changes after 1 year of normal liver function tests, cessation of treatment should be considered but not during or immediately before puberty, when relapses are more common. In 13 children (4 LKM-1 positive), the only ones fulfilling these criteria in our series, discontinuation of treatment was attempted. This was successful in six children, all ANA/SMA positive, after a median duration of 3.2 (range 1–11) years of treatment. All six have remained in remission for a period of 9 to 13 years. The remaining children (three ANA/SMA positive and four LKM-1 positive) relapsed between 1 and 15 months (median 2 months) after immunosuppression was discontinued. They all responded to the reintroduction of treatment. These data indicate that most children with AIH, particularly those who are LKM-1 positive, are likely to require lifelong immunosuppressive treatment. An important role in monitoring the response to treatment is the measurement of autoantibody titers and IgG levels, the fluctuation of which is correlated with disease activity.[39] Despite the efficacy of current treatment, severe hepatic decompensation may develop even after many years of apparently good biochemical control. Thus, four of our patients who responded satisfactorily to immunosuppression ultimately required transplant 8 to 14 years after diagnosis. Overall, in our series, 46 of the 47 patients treated with immunosuppression were alive between 0.3 and 19 years (median 5 years) after diagnosis, including 5 patients after liver transplant.

Sustained remission of AIH has been reported in adult patients maintained on azathioprine alone.[40] Following this observation, we have attempted to stop prednisolone, maintaining azathioprine, in five children, two who were ANA/SMA positive and three who were LKM-1 positive. Although the attempt was successful in the ANA/SMA-positive cases, all LKM-1–positive children relapsed and required reinstitution of steroid treatment.

Remission was achieved in 25 of 32 children with AIH treated with cyclosporin A alone for 6 months followed by combined low-dose prednisone and azathioprine for 1 month, after which cyclosporin A was stopped and the other two drugs were continued.[41] The side effects of cyclosporin A were mild, and high-dose steroid side effects were avoided. A disadvantage of this schedule was that all patients were eventually treated with the prednisone-azathioprine combination, whereas by using the conventional treatment schedule, about a third of the children can maintain remission with very-low-dose steroids alone. In addition, longer follow-up of the patients is necessary to establish possible long-term toxicity of cyclosporin A.

Mycophenolate mofetil has been successfully used in adult patients with type 1 AIH who have been either intolerant of or not responsive to azathioprine.[42] Mycophenolate mofetil is an inhibitor of purine nucleotide synthesis and has a mechanism of action similar to that of azathioprine. It is not hepatotoxic or nephrotoxic, and its main side effects are diarrhea, vomiting, and bone marrow suppression. In our experience, the drug was able to resolve laboratory abnormalities in 5 of 12 children who did not tolerate or respond to azathioprine. In four others, it reduced serum aminotransferase levels to a degree that allowed a decrease in the dose of prednisolone. Only three patients did not respond to mycophenolate mofetil, and the side effects were minor, apart from severe nausea and dizziness in one of these three children.

Children who present with acute hepatic failure pose a particularly difficult therapeutic problem. Although it has been reported that they may benefit from conventional immunosuppressive therapy,[43,44] only one of the six children with acute liver failure in our own series responded to immunosuppression and survived without transplant. Of the four LKM-1–positive patients, one died before a donor organ could be found and two died soon after transplant. Encouraging results have been reported using cyclosporin A in LKM-1–positive patients presenting with fulminant hepatitis.[44,45] These results should be evaluated on a larger number of patients because our own experience has not confirmed the value of this therapeutic approach.

AUTOIMMUNE SCLEROSING CHOLANGITIS

Clinical Features

Sclerosing cholangitis is an uncommon disorder, characterized by chronic inflammation and fibrosis of the intrahepatic and/or extrahepatic bile ducts. In childhood, sclerosing cholangitis may occur as an individual disease or may develop in association with a wide variety of disorders, including Langerhans cell histiocytosis, immunodeficiency, psoriasis, cystic fibrosis, and chronic inflammatory bowel disease. An overlapping syndrome between AIH and sclerosing cholangitis has been reported both in adults[46–48] and children.[49–52] In a retrospective study, we have shown that 40% of patients with sclerosing cholangitis have clinical, biochemical, immunologic, and histologic features that are indistinguishable from those of AIH.[49] In both AIH and sclerosing cholangitis, the serum IgG level is similarly increased, and non–organ-specific ANA and/or SMA are frequently present. Both diseases also commonly have portal tract inflammation and interface hepatitis. Most of the reported cases of overlap were originally diagnosed as AIH.[46–48,51] Typically, the overlap with sclerosing cholangitis was not recognized until years later, when biliary features on follow-up liver biopsy examination justified the performance of cholangiography. The sequence of diagnoses was then interpreted as an evolution from AIH to sclerosing cholangitis, when, in fact, the concurrence of these diseases had not been excluded by cholangiographic studies performed at presentation.

In a prospective study over a period of 16 years, we found that 27 of 55 children who presented with clinical and/or laboratory features characteristic of AIH had evidence of sclerosing cholangitis when assessed by cholangiography at presentation.[53] Bile duct abnormalities on cholangiography were both intra- and extrahepatic in two-thirds of patients and intrahepatic in one-third. Because of the cholangiographic changes, a diagnosis of ASC was made in these patients (Figure 52-2).

Of the 27 patients with ASC, 26 were seropositive for ANA and/or SMA and 1 for LKM-1.[53] Fifty-five percent were girls, and the mode of presentation was similar to that of 28 patients with typical AIH. Symptoms were those of acute hepatitis or chronic liver dysfunction. In some instances, symptoms were absent, and the diagnosis was revealed after the incidental discovery of abnormal liver tests. Inflammatory bowel disease was present in 44% of children with cholangiopathy compared with 18% of those with typical AIH, and more than 75% of children with ASC had greatly increased serum IgG levels. Perinuclear antineutrophil cytoplasmic antibodies were present in 74% of patients with ASC compared with 36% of patients with typical AIH.

There was only a partial concordance between the histologic and radiologic findings, and six patients with an abnormal cholangiogram had histologic features more compatible with AIH than sclerosing cholangitis.[53] Interestingly, all patients fulfilled the criteria for the diagnosis of "definite" or "probable" AIH established by the International Autoimmune Hepatitis Group.[1] Indeed, the diagnosis of sclerosing cholangitis was possible only because of the cholangiographic studies. The similarities and differences between type 1 AIH, type 2 AIH, and ASC are summarized in Table 52-3.

Treatment

Children with ASC respond to the same immunosuppressive treatment described above for typical AIH.[53] The liver test abnormalities resolved in almost 90% of our patients within a median of 2 months after starting treatment. This good response is in contrast to the outcome in adults with primary sclerosing cholangitis (PSC) who have no beneficial effects from corticosteroid treatment.[54,55] The PSC of adults, however, is usually diagnosed at an advanced stage and may be the result of various etiologies. Disappointing results with immunosuppressive agents have been reported in a small number of children with sclerosing cholangitis associated with autoimmune features, but these children may have had more advanced disease than those recruited into our prospective study.[51]

Ursodeoxycholic acid (UDCA) was added to our treatment schedule in 1992 following preliminary reports of its value in the treatment of adult PSC.[56,57] The small number of patients and the relatively short follow-up period do not allow us to determine whether treatment with UDCA from onset is successful in arresting the progression of ASC. In adults with well-established PSC, UDCA treatment has been disappointing, possibly because of the advanced stage of the disease at the time of diagnosis.[58]

Measurement of autoantibody titers and IgG levels is useful in monitoring disease activity and response to treatment, not only in AIH but also in ASC.[39]

Follow-up liver biopsies in our series have shown no progression to cirrhosis, although one patient did develop vanishing bile duct syndrome. Follow-up endoscopic retrograde cholangiograms have shown static bile duct disease in half of our patients with ASC and progression of the bile duct abnormalities in the other half. Interestingly, one of the children with AIH who was followed prospectively developed sclerosing cholangitis 8 years after presentation despite treatment with corticosteroids and no biliary changes on several follow-up liver biopsies. This observation suggests that AIH and ASC are part of the same pathogenic process and that prednisolone and azathioprine may be more effective in controlling the liver parenchyma inflammatory changes than the bile duct disease.

The medium-term prognosis of ASC is good.[53] All patients in our series were alive after a median follow-up of 7 years. Four patients with ASC, however, required liver transplant after 2 to 11 years of observation (median interval of follow-up 7 years). In contrast, liver transplant has not been required by any of the 28 children with typical AIH who have been followed for this same time.

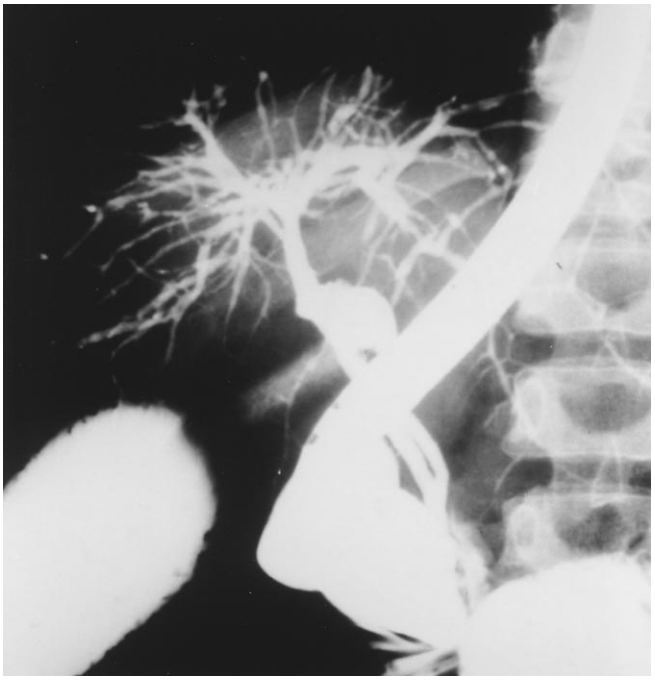

FIGURE 52-2 Endoscopic cholangiography demonstrating widespread intrahepatic changes predominantly affecting the second- and third-order bile ducts.

Pathophysiology

It is unclear if the juvenile autoimmune form of sclerosing cholangitis and AIH are two distinct entities or different aspects of the same condition. Akin to AIH, liver-specific autoantibodies, including antibodies to liver-specific lipoprotein, asialoglycoprotein receptor, alcohol dehydrogenase, and soluble liver antigen, are found in ASC.[12,30,59] HLA-DR3, -DR13, and -DR15 occur as commonly in patients with ASC as in healthy control subjects, but HLA-DR4 occurs less commonly. This HLA profile has also been associated with susceptibility to sclerosing cholangitis in adults.[60]

DE NOVO "AUTOIMMUNE" HEPATITIS AFTER LIVER TRANSPLANT

Clinical Features

Late graft dysfunction not attributable to recognized causes such as rejection, infection, or vascular and/or biliary complications may occur after liver transplant. We have observed and reported a particular type of graft dysfunction associated with autoimmune features in 7 (4%) of 180 children transplanted at our center between 1991 and 1996.[61] They developed an unexplained but characteristic form of graft dysfunction at a median postsurgery period of 24 months (range 6–45 months). Of the seven children, five were boys, and the median age at presentation was 10.3 years (range 2–19.4 years). None of the children had been transplanted for autoimmune liver disease. Indications for transplant were extrahepatic biliary atresia in four children, Alagille syndrome in one child, drug-induced acute liver failure in one child, and α_1-antitrypsin deficiency in one child. At the time of graft dysfunction, four were on triple immunosuppression with cyclosporin A, azathioprine, and prednisolone, whereas three were on tacrolimus. Common causes of graft dysfunction, such as infectious and surgical complications, were excluded. Liver biopsy showed the histologic changes of chronic hepatitis, including portal and periportal hepatitis with lymphocytes and plasma cells, bridging collapse, and perivenular cell necrosis, without changes typical of acute or chronic rejection. All patients had increased levels of IgG and positive autoantibodies, ANAs, SMAs, or atypical liver/kidney microsomal antibodies (LKMs, which, on immunofluorescence, stain the proximal renal tubules similarly to LKM-1, in the absence of liver staining).

Following this report,[61] autoimmune phenomena and liver disease mimicking AIH after liver transplant have been described in adults and children.[62–66] The graft dysfunction has been responsive to therapy with corticosteroids and azathioprine but not to increased doses of cyclosporin A or tacrolimus. [62–66]

Treatment

In our study, all patients but one responded to the conventional treatment regimen for AIH based on prednisolone (2 mg/kg/d) and azathioprine (1.5 mg/kg/d).[61] Antirejection therapy with cyclosporin A or tacrolimus was not changed. Serum aminotransferase abnormalities resolved within a median treatment period of 32 days (range 7–316 days). The one child who did not respond had a history of poor compliance with therapy. One responder relapsed owing to poor compliance, but remission was again obtained after retreatment. All six responders remained in remission on a reduced dose of prednisolone (5–10 mg/d) and azathioprine (1.5 mg/kg/d) during a median follow-up interval of 283 days (range 108–730 d). Similar satisfactory results

TABLE 52-3 COMPARISON BETWEEN AUTOIMMUNE HEPATITIS AND AUTOIMMUNE SCLEROSING CHOLANGITIS

CLINICAL FEATURES AT PRESENTATION	TYPE 1 AIH	TYPE 2 AIH	ASC
Median age (yr)	11	7	12
Females (%)	75	75	55
Mode of presentation (%)			
Acute hepatitis	47	40	37
Acute liver failure	3	25	0
Insidious onset	38	25	37
Complication of chronic liver disease	12	10	26
Associated immune diseases (%)	22	20	48
Inflammatory bowel disease (%)	20	12	44
Family history autoimmune disease (%)	43	40	37
Abnormal cholangiogram (%)	0	0	100
ANA/SMA (%)	100	25	96
LKM-1	0	100	4
pANCA	45	11	74
Increased IgG level (%)	84	75	89
Partial IgA deficiency (%)	9	45	5
Low C4 level (%)	89	83	70
Increased frequency of HLA-DR3	Yes	No	No
Increased frequency of HLA-DR7	No	Yes	—
Interface hepatitis (%)	66	72	35
Biliary features (%)	28	6	31
Cirrhosis (%)	69	38	15
Remission after immunosuppressive treatment (%)	97	87	89

Adapted from Mieli-Vergani G, Vergani D. Autoimmune hepatitis in children. Clin Liver Dis 2002;6:335–45.
AIH = autoimmune hepatitis; ANA = antinuclear antibodies; ANCA = antineutrophil cytoplasmic antibody; ASC = autoimmune sclerosing cholangitis; C4 = C4 component of complement; HLA = human leukocyte antigen; IgA = immunoglobulin A; IgG = immunoglobulin G; LKM-1 = anti–liver/kidney microsomal type 1 antibody; SMA = anti–smooth muscle antibody.

have been reported by other authors using prednisolone and azathioprine,[62–66] whereas the condition has worsened in the absence of such treatment.[66] These findings underscore the importance of prompt recognition of the condition and institution of appropriate treatment.

Pathophysiology

Whether the liver damage observed in these patients is a form of rejection or the consequence of an "autoimmune" injury, possibly triggered by drugs or viral infection, remains to be established. Further characterization of the target specificity of intrahepatic lymphocytes may provide information regarding its pathogenesis. The administration of cyclosporin A or tacrolimus to rodents after bone marrow transplant can result in a "paradoxical" autoimmune syndrome in which the immunosuppressive drugs interfere with maturation of T lymphocytes and favor the emergence of autoaggressive T-cell clones.[67–69] This experience in animals may explain, in part, the development of this disorder in immunosuppressed children after liver transplant.

The manifestations of the autoimmune condition in rodents vary in different strains and depend on genetic factors possibly encoded by the major histocompatibility complex.[67] Analysis of the HLA phenotypes of the recipients and donors in our study did not show an association between the development of autoimmune features, the possession of either HLA-DR3 or -DR4, or the degree of donor-recipient HLA mismatch.[61] Five of the seven patients, however, had received livers from donors with HLA markers known to be associated with susceptibility to AIH, including two with DR4, one with DR3, and two with DR3 and DR4.[21]

RECURRENCE OF AUTOIMMUNE LIVER DISEASE AFTER LIVER TRANSPLANT

Recurrence of AIH after transplant has been repeatedly reported in some 30% of patients.[70–75] The recurrence rate may be as high as 68% 5 years after transplant.[76] The diagnosis is based on the reappearance of clinical symptoms and signs, histologic features of periportal hepatitis, raised transaminases, circulating autoantibodies, and elevated IgG, associated with a response to prednisolone and azathioprine. Possession of the HLA-DR3 allele appears to confer predisposition to disease recurrence, as it does to the original AIH,[71] but this has not been universally confirmed.[75] Recurrence has been noted both in adult and pediatric series, and although the rate of this complication increases with the post-transplant interval, it may appear as early as 35 days postsurgery.[77] The reported recurrence rates are likely to be influenced by differences in study design, immunosuppressive regimens, and lengths of follow-up. Most transplant recipients with recurrent AIH respond to an increase in the dose of corticosteroids and azathioprine, but AIH recurrence can lead to graft failure and to the need for retransplant. Caution should be taken in weaning immunosuppression in patients who undergo transplant for AIH because discontinuation of corticosteroid therapy may increase the risk for recurrent disease.

Recurrence of sclerosing cholangitis after transplant has also been reported in 6 to 20% of patients transplanted for this condition, but it is particularly difficult to prove.[78–81] The diagnosis of sclerosing cholangitis is based on the radiographic documentation of biliary tree lesions, which can also arise as a consequence of the transplant surgery or post-transplant complications. Most patients transplanted for sclerosing cholangitis have a Roux-en-Y loop rather than a duct-to-duct anastomosis, with an increased risk of biliary obstruction and radiographic appearances of sclerosing cholangitis in the graft, making it difficult to distinguish secondary from recurrent disease. Moreover, radiologic and histologic features indistinguishable from sclerosing cholangitis may result from ischemic biliary complications.[82] Whether patients with the autoimmune form of sclerosing cholangitis are more likely to experience recurrence than those without is unclear at the present time. Similarly to AIH, immunosuppression may modify or delay the disease expression within the graft. Patient and graft survival, however, do not appear to be affected by recurrence of sclerosing cholangitis in the intermediate term.

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CHAPTER 53

DRUG-INDUCED HEPATOTOXICITY

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The liver plays a central role in drug action. It chemically transforms many drugs to their active form and acts on most drugs to expedite their excretion from the body. These functions put the liver at risk for toxicity from these chemicals and their metabolites. Because of its anatomic and physiologic complexity, drug-induced liver disease represents a broad spectrum of biochemical, histologic, and clinical abnormalities. This can make it difficult to diagnose drug-induced liver disease or determine its pathogenesis. The problem of drug-induced liver disease in children is further complicated by a widely held notion that drug hepatotoxicity does not happen very often in children. Children may indeed be protected in some way from drug hepatotoxicity. Whether or not this is true, because the child's liver is in the process of metabolic maturation, the manifestations of drug-induced liver disease may differ in children from those in adults. Because drug hepatotoxicity often imitates other more common diseases, arriving at a diagnosis of drug hepatotoxicity in a child can be especially difficult.

The purpose of this chapter is to address special features of drug hepatotoxicity in children. Mechanistic information gained from studying such processes in children is important for understanding the pathogenesis of drug hepatotoxicity. However, much more is known about the diversity of hepatic drug reactions in adults than that in children. For encyclopedic reviews of which hepatotoxicities a given drug has caused in patients of any age, the reader should consult broader references[1–3] or computerized adverse drug reaction indices (such as <www.fda.gov/medwatch> and <www.pharmacovigilance.org>).

ROLE OF THE LIVER IN DRUG METABOLISM

Hepatic drug metabolism, or biotransformation, contributes to drug hepatotoxicity and to some hepatic neoplasia. Biotransformation in the liver is divided into two broad aspects: activation (phase I) and detoxification (phase II) (Figure 53-1). For hepatotoxicity, the balance between these two processes is critical. Factors that influence this balance include age or stage of development, fasting or undernutrition, coadministered drugs, and immunomodulators resulting from viral infection. Inducing chemicals may affect phase I and phase II processes differently. The pharmacokinetics of the toxic drug also affects hepatic biotransformation. Whether the drug is taken as a single dose or many doses chronically may change its hepatic metabolism. Finally, polymorphisms of cytochromes P-450 and various phase II enzymes also influence this balance.

The cytochromes P-450 are hemoproteins that are found in numerous body tissues but are particularly important in the liver. They carry out most phase I reactions. These reactions are diverse and include various types of hydroxylation, dealkylation, and dehalogenation. The common feature in all reactions is that one atom of molecular oxygen is inserted into the substrate, whereas the other combines with protons to form water. Hence these enzyme activities are monooxygenases. Cytochromes P-450 themselves are diverse and have overlapping substrate specificity. An important characteristic of many cytochromes P-450 is inducibility. Another is functional polymorphism.

The cytochromes P-450 were initially classified on the basis of the predominant inducing chemical: basically either phenobarbital or the polycyclic aromatic hydrocarbon 3-methylcholanthrene.[4] Thirty-six subfamilies of cytochromes P-450 have been distinguished on the basis of similarities in primary amino acid sequence. The cytochrome P-450 1A (CYP1A) subfamily includes those cytochromes induced by polycyclic aromatic hydrocarbons. Two major forms within the CYP1A subfamily have been identified in the rat, mouse, and rabbit and in humans. Apart from various carcinogens, other chemicals, such as caffeine and theophylline, are metabolized by these cytochromes to a varying extent. Induction of the cytochromes in the CYP1A subfamily is regulated through a cytoplasmic receptor protein, the Ah receptor, which has been characterized in humans. The cytochrome P-450 2B (CYP2B) subfamily includes cytochromes induced by phenobarbital. Cytochrome P-450 2E1 (CYP2E1) represents ethanol-inducible cytochrome P-450. The cytochrome P-450 3A (CYP3A) subfamily includes cytochromes induced by pregnenolone and by glucocorticoids. CYP3A4 is the most abundant cytochrome P-450 in human liver. Induction of cytochromes P-450 by phenobarbital involves

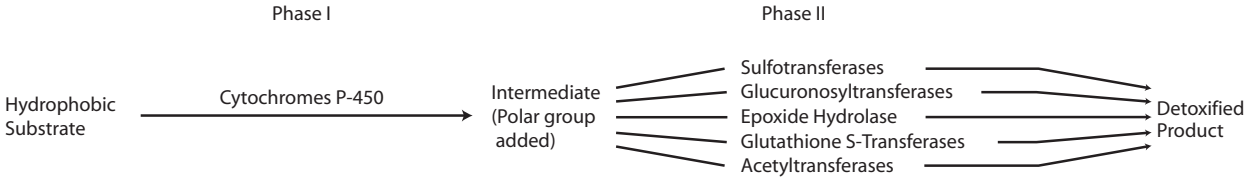

FIGURE 53-1 Phase I and phase II metabolism. Although the main objective is to convert a hydrophobic substance to a detoxified, water-soluble product so that it can be excreted from the body, phase I metabolism is also capable of converting some drugs to their active form or transforming other chemicals to toxic intermediates.

the constitutive active receptor (CAR) for CYP2B cytochromes and the pregnane X receptor (PXR) for CYP3A cytochromes through response elements, but not all details of these regulatory mechanisms are yet known.[5] Drugs that cause proliferation of peroxisomes appear to induce yet another cytochrome P-450 subfamily, P-450 4A (CYP4A). As the hepatic metabolism of common drugs is studied more extensively, these subfamilies, as well as CYP2C cytochromes and CYP2D6, are found to be involved. This has important implications for hepatotoxicity.

Polymorphisms for certain cytochromes P-450 have also been identified in human populations and in laboratory animals. In general, these polymorphisms relate to differences in the rate of enzyme action. An important polymorphism is that for debrisoquine 4-hydroxylation, for which individuals may be "extensive metabolizers" or "poor metabolizers." Other drugs whose metabolism shows the same pattern include sparteine, metoprolol, and dextromethorphan. The poor metabolizer phenotype is associated with absence of cytochrome CYP2D6 protein; several different mutations in the structural gene for CYP2D6 have been described, which account for this phenotype.[6] Poor metabolizers appear to be at greater risk for adverse drug reaction from drugs that are metabolized by this route, although clear relationships to any specific hepatotoxicity have been difficult to prove.[7]

For many drugs, the effect of phase I biotransformation reactions is to create a more polar chemical with a substituent poised for conjugation via a phase II reaction. Phase II detoxifying reactions are performed by a variety of different types of enzymes, including glutathione *S*-transferases, glucuronosyl transferases, epoxide hydrolase, sulfotransferases, and *N*-acetyltransferases. In general, these reactions complete the transformation of a hydrophobic chemical to a hydrophilic one that can be excreted easily in urine or bile. Certain phase II enzymes, such as some glucuronosyl transferases, are subject to induction. Some are polymorphic, notably *N*-acetyltransferase (either rapid or slow acetylators). In some metabolic diseases, the activity of phase II enzymes may be abnormal; for example, in 5-oxoprolinuria, conjugation to glutathione is reduced because of deficiency of glutathione owing to decreased levels of glutathione synthetase.[8] In hereditary tyrosinemia, glutathione *S*-transferase activity is depressed because intermediates in the abnormal tyrosine pathway consume glutathione.

Hepatic drug metabolism shows developmental changes. Caffeine, which is metabolized in part by cytochromes P-450 in the 1A subfamily, exemplifies these changes. The elimination half-life is very long in the newborn period[9] and drops to approximately 3 to 4 hours around 6 months of age.[10] For the balance of childhood, that is, until puberty, caffeine metabolism remains somewhat more rapid than in adults.[11] Clearance of many drugs is more rapid in children than in adults. Prominent examples include theophylline, phenobarbital, and phenytoin. Among phase II processes, a well-known example of late maturation of a detoxifying enzyme is the glucuronosyl transferase for bilirubin conjugation, which is frequently deficient for a short time after birth. Hepatic bile acid metabolism also shows maturational changes in the first months of life. These variations may influence the occurrence and character of hepatotoxicity in children.

The product of a phase I reaction may be an unstable or reactive metabolite. Phase II reactions may inactivate such chemicals before they do much harm. However, it is possible, as in the case of benzo[*a*]pyrene, for the product of phase I to recycle through the same cytochrome a second time and then be metabolized to a proximate carcinogen. Apart from the adequacy of the detoxification systems, whether reactive metabolites actually damage the cell will also depend on how much reactive metabolite actually binds to cellular components, whether these components are critical to cellular function, and whether they can be repaired. If the reactive metabolite binds to intracellular proteins or membranes that are vital to cellular integrity, the hepatocyte may die. If it binds to a genetic apparatus, mutagenesis, carcinogenesis, or teratogenesis may ensue (Figure 53-2).

Toxic metabolites are electrochemically unstable, and thus highly reactive, species derived from drugs, xenobiotics, or endogenous chemicals. Electrophilic intermediates (or electrophiles) are formed when electrons are lost from the original chemical; they carry a net positive charge. Examples include hydroxylamines, quinoneimines, and arene oxides. Tissue nucleophiles, such as glutathione, preferentially combine with these species. Not all nucleophiles are necessarily protective. For example, reactions that involve activation of oxygen produce negatively charged species, which are nucleophiles. They tend to bind to intracellular lipids, leading to lipid peroxidation. Examples include halocarbon and nitroso radicals. Besides lipid peroxidation, membranes can be altered by alkylation (addition of an aliphatic radical such as methyl or ethyl groups), arylation (addition of an aromatic group such as a phenyl group), or acylation (adding a radical derived from a carboxy acid). Glutathione, which is found in most mammalian cells in high concentrations, can react with electrophiles via conjugation reactions catalyzed by glutathione *S*-trans-

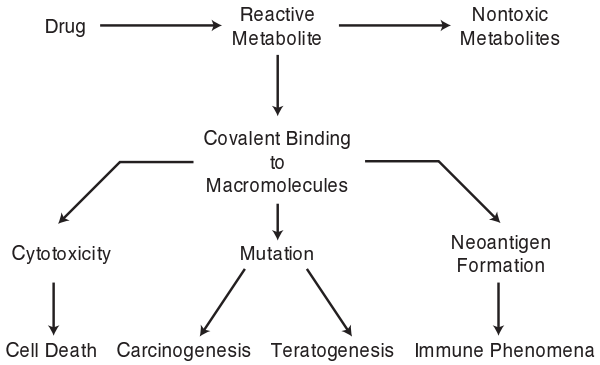

FIGURE 53-2 The potential fates of a toxic intermediate.

ferases. It can also interact with hydrogen peroxide and activated oxygen species via a different enzyme, glutathione peroxidase. In general, when toxic metabolites are the important cause of cell damage, high tissue concentrations of the parent drug are not found. Metabolite(s) covalently bound to cellular constituents may be detected.

The cellular specialization of hepatocytes accounts in part for the diversity of patterns of hepatotoxicity. Binding to certain subcellular elements may interfere with specific metabolic functions such as protein or lipid synthesis or energy production. The parent compound or its reactive metabolites may interfere with biliary excretion or damage proteins within the biliary excretion apparatus, thus leading to cholestasis.[12] In particular, the bile salt excretory pump (BSEP; abnormal in progressive familial intrahepatic cholestasis type 2) appears to be an important target in forms of drug hepatotoxicity with prominent cholestatic features. Additionally, polymorphisms in the genes for these bile canalicular transporters may influence their susceptibility to damage by toxic intermediates. Binding to nuclear deoxyribonucleic acid (DNA) may lead to carcinogenesis. Binding to mitochondrial DNA or otherwise interfering with production of normal mitochondrial DNA may have important consequences for hepatocellular metabolism because, unlike nuclear DNA, mitochondrial DNA has limited resources for DNA repair. Drug-induced injury may occur to other cells in the liver besides hepatocytes. Cytotoxic damage to biliary epithelial cells, hepatic stellate cells, or endothelial cells accounts for some of the clinical diversity of drug-induced liver disease. For example, damage to sinusoidal endothelial cells by reactive metabolites leads to veno-occlusive disease.

CLASSIFICATION OF DRUG HEPATOTOXICITY

The clinical spectrum of drug hepatotoxicity in adults is wide. The clinical patterns of drug hepatotoxicity presented in Table 53-1 form the basis for considering hepatotoxicity in children. It encompasses a combination of clinical presentations, histologic findings, and other factors. Nonspecific elevation of serum aminotransferases is omitted. This form of hepatotoxicity is probably the most common of all, but its causes are extremely heterogeneous and the least understood.

Most drug-induced liver disease is cytotoxic. Clinically, serum aminotransferases are elevated, and hepatic insufficiency may develop. Hepatocellular damage is classically defined in adults as having an alanine aminotransferase (ALT) level greater than two times the upper limit of normal or the ALT-to-alkaline phosphatase (ALP) ratio is ≥ 5.[13] The exact mechanism of cell death is not known and is probably different for different drugs and toxins. Hepatocyte damage may be zonal, reflecting metabolic specialization in various parts of the hepatic lobule. Specifically, hepatocytes in zone 3 of the Rappaport acinus have the highest concentration of drug-metabolizing enzymes and thus the greatest potential for producing toxic intermediates. Zonal hepatocellular necrosis suggests that metabolic activation of toxic metabolites has an important role in the pathogenesis of the toxicity, but spotty necrosis scattered throughout the lobule does not necessarily exclude a mechanism involving toxic metabolites. The same drugs that can cause this spotty hepatocyte damage can, on occasion, cause damage affecting most hepatocytes, leading to massive hepatocellular necrosis. Whenever hepatocellular damage is sufficiently severe, some degree of cholestasis will develop.

Some drug-induced liver disease, however, is predominantly cholestatic. For adults, this is classically defined as serum ALP greater than two times the upper limit of normal or the ALT-to-ALP ratio is ≤ 2.[13] Clinically, this type of reaction is characterized by jaundice, pruritus, prominent elevation of ALP, and mild elevations of serum aminotransferases. Traditionally, these cholestatic injuries have been classified on the basis of histologic inflammation. In hepatocanalicular jaundice, with agents such as chlorpromazine or erythromycin, liver cell injury and inflammation

TABLE 53-1 SPECTRUM OF DRUG-INDUCED LIVER DISEASE

TYPE	EXAMPLES
Acute hepatitis	Methyldopa, isoniazid, halothane, phenytoin
Hepatitis-cholestasis	Erythromycin, chlorpromazine, azathioprine, nitrofurantoin, cimetidine
Zonal liver cell necrosis	Acetaminophen
Bland cholestasis	Estrogens, cyclosporines
Steatonecrosis (like alcoholic hepatitis)	Perhexiline, amiodarone
Phospholipidosis	Amiodarone
Microvesicular steatosis	Valproic acid, tetracycline
Granulomatosis	Sulfonamides, phenylbutazone, carbamazepine
Biliary cirrhosis	Practolol, chlorpropamide
Sclerosing cholangitis	Floxuridine via hepatic artery
Hepatic vascular changes	
Peliosis	Estrogens, androgens
Hepatic vein thrombosis	Estrogens (oral contraceptives)
Veno-occlusive disease	Thioguanine, busulfan, pyrrolizidine (*Senecio*) alkaloids
Noncirrhotic portal hypertension	Vinyl chloride, arsenic
Liver cell adenoma	Estrogens (oral contraceptives), anabolic steroids
Malignant tumors	Estrogens, anabolic steroids, vinyl chloride
Porphyria	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin, chloroquine

are relatively prominent. This mixed picture is characterized biochemically in adults because the ALT-to-ALP ratio is 2–5, and the ALT and ALP are each at least twice the upper limit of normal. In bland cholestasis, with agents such as contraceptive steroids, inflammation is minimal.

It is sometimes useful to think about drug hepatotoxicity in terms of the duration of the hepatotoxic process. Acute hepatotoxic injuries develop over a relatively short time and cause a lesion without any features of chronicity. Subacute hepatotoxicity refers to lesions that have developed over weeks to months as indicated by areas of fibrosis and possibly regeneration. Chronic hepatotoxic lesions include those with fibrosis or cirrhosis, vascular changes, and neoplasia. Some drugs can cause clinical liver disease indistinguishable from autoimmune hepatitis (the so-called "chronic active hepatitis" picture): these include oxyphenisatin, methyldopa, isoniazid, nitrofurantoin, and minocycline.

Our knowledge of the mechanisms of hepatotoxicity is evolving. For many years, hepatotoxicity has been categorized on the basis of predictability. Intrinsic hepatotoxins are differentiated from idiosyncratic heptatotoxins. The intrinsic hepatotoxin causes predictable hepatic damage in almost any individual. The toxicity is dose related: higher doses cause worse damage. Animal models can be developed that exhibit the same type of hepatotoxicity. However, most instances of hepatotoxicity, mainly those associated with medications, are unpredictable, infrequent, and apparently sporadic. If such a reaction is accompanied by systemic features such as fever, rash, eosinophilia, atypical lymphocytosis, and possibly other major organ involvement, then, classically, it has been regarded as an idiosyncratic hypersensitivity reaction, where hypersensitivity, with its connotation of allergy, is left undefined.

An alternate explanation is that idiosyncratic hepatotoxicity has a biochemical basis and is due to metabolic idiosyncrasy. It occurs in individuals who have specific abnormalities in drug metabolism. If this abnormal metabolism is expressed in liver cells, then these rare individuals will develop hepatotoxicity if exposed to the appropriate drug. In most instances, a metabolite, not the drug itself, is responsible for hepatotoxicity (see Figure 53-2). Frequently, the problem seems to be a defect in detoxification of the reactive metabolite because the detoxification system is itself focally defective and cannot meet the normal demands of metabolite production. Sometimes these individuals show systemic features interpreted as hypersensitivity: it is likely that interaction of the reactive metabolite with cellular components, such as the cell membrane, elicits an immune response. In such cases, hypersensitivity is itself the consequence of metabolic idiosyncrasy, not a separate mechanism of drug hepatotoxicity. There may be strictly allergic drug hepatotoxicity, but investigations of the mechanism of drug-induced hepatotoxicity suggest that metabolic idiosyncrasy is much more common than formerly supposed. It seems likely to account for hepatotoxicity with drugs that show two main patterns of toxicity: mild reversible toxicity in a comparatively large segment of patients and severe hepatotoxicity in a few individuals. Toxic metabolites are probably involved in both patterns of toxicity. Severe reactions occur in rare persons with abnormal generation of toxic metabolites or detoxification, irrespective of the appearance of drug allergy. Transient reaction may reflect spontaneous adjustments in hepatic biotransformation for the particular drug.

The major implication of the metabolic idiosyncrasy thesis is that most drug hepatotoxicity is predictable if one understands the pathways of hepatic biotransformation and detoxification for each drug. Given the plethora of drugs and hepatic biotransformation pathways, it is no wonder that most clinically important drug hepatotoxicity appears sporadic and fortuitous. However, enough experimental data are available now to warrant rethinking the standard classification of drug hepatotoxicity that regards all drug-associated hepatotoxicity as either intrinsic, owing to the drug being a poison, or else idiosyncratic-allergic. The definable metabolic defects in hepatic drug metabolism are particularly common in the types of drug hepatotoxicity that occur in children.

Recent research has focused on the role of the immune system in drug hepatotoxicity.[14] It seems likely that for many, if not most, individuals, drug hepatotoxicity depends on immune reactivity as well as personal peculiarities of drug biotransformation. With some drugs, the connection between immune-mediated mechanisms and hepatic damage may be very direct: autoantibodies are elaborated against specific components of the hepatic biotransformation machinery. The target cytochrome P-450 varies with different drugs: CYP2C9 for tienilic acid, CYP1A2 for dihydralazine, and CYP2E1 for halothane. In addition, the herb germander is associated with antibodies directed against the phase II enzyme epoxide hydrolase.[15] Reactive metabolites may alter other components of hepatocytes to form neoantigens. Hepatocyte damage mediated through immune mechanisms involves apoptosis or necrosis. Bile acid–associated hepatocyte injury involves Fas (CD95) activation leading to apoptosis. When toxic metabolites or reactive oxygen species or cytokines stimulate Kupffer cells, specific mechanisms of cell damage are set into motion involving tumor necrosis factor-α or nitric oxide produced by Kupffer cells. Nitric oxide elaborated by Kupffer cells and hepatocytes appears to play a role in acetaminophen hepatotoxicity. Other cytokines, including interleukin-8[16] and other CXC chemokines regulating leukocyte action, may modulate these effects. Moreover, Kupffer cells also elaborate a number of factors that are cytoprotective to hepatocytes.[17] The vigor of the immune response in general, an individual polygenic trait, may also determine the importance of immune mechanisms in drug hepatotoxicity. Thus, the individual's "immunogenetic" makeup and "pharmacogenetic" makeup are important.

In summary, as our knowledge of the complex and numerous individual mechanisms of drug hepatotoxicity continues to evolve, it remains useful to categorize drugs as follows: intrinsic hepatotoxins, contingent hepatotoxins, and drugs eliciting an immunoallergic response. The intrinsic toxin is a true poison and causes predictable damage in all persons in a dose-dependent fashion. The contingent hepatotoxin causes drug hepatotoxicity in individuals who

are susceptible under circumstances that favor imbalance of production of a toxic intermediate and its detoxification. These circumstances may be inborn (such as genetic defects in a phase II detoxification enzyme or genetic polymorphism in a cytochrome P-450) or acquired (such as induction of CYP2E1 by chronic ethanol abuse or depletion of hepatic glutathione by fasting and malnutrition). Genetic variations in expression of cytokines that effect hepatic cytoprotection (such as interleukin-10 promoter region polymorphisms, which regulate its expression) also contribute to the mechanism of contingency. Drugs eliciting an immune response can produce a variety of clinical phenomena: the drug hypersensitivity syndrome (fever, rash, atypical lymphocytosis, eosinophilia, lymphadenopathy, multisystem involvement including hepatitis, renal dysfunction, myocarditis, thyroiditis), hepatic granulomatosis, autoantibodies, and "chronic active hepatitis" fully resembling idiopathic autoimmune hepatitis. Individual immunogenetic makeup probably determines the pattern and extent of this response. These categories are not mutually exclusive. Acetaminophen is an intrinsic toxin at very high doses and a contingent toxin at therapeutic doses. Phenytoin is a contingent hepatotoxin and the archetypal drug for eliciting an immunoallergic response.

INCIDENCE OF DRUG-INDUCED LIVER DISEASE IN CHILDREN

Drug-induced liver disease is generally regarded as rare in children. In a survey of 10,297 pediatric hospital admissions to teaching and community hospitals in Boston, Mitchell and colleagues found that only 2% of hospital admissions were due to any sort of adverse drug reaction.[18] In a subset of 725 patients with cancer, however, 22% of admissions were related to adverse drug reactions. Adverse drug reactions in the whole population were somewhat more common in the 0- to 5-year-old age group than in older children. The most commonly implicated drugs included phenobarbital, aspirin, phenytoin, ampicillin or amoxicillin, and sulfa. Only phenytoin-associated hepatitis was specifically mentioned in this large survey as drug hepatotoxicity. An outpatient study of 1,590 children in Britain also failed to detect drug hepatotoxicity as a problem in children.[19] A recent review of the Yellow Card Scheme for reporting adverse drug reactions in the United Kingdom revealed that between 1964 and December 2000, there were 331 deaths in children possibly owing to drug toxicity, and 50 of these involved hepatic failure.[20] The median age was 5 years old, and the types of drugs most often associated with fatal adverse events were anticonvulsants and antineoplastic drugs. Despite the limitations of this tracking system, these data help to objectify this problem in the pediatric age bracket.

Why childhood drug hepatotoxicity seems uncommon is not certain. Underdiagnosis and underreporting remain a possibility. Another simple reason is that most children take relatively few medications. They do not use ethanol chronically or smoke cigarettes; most are not obese. Thus, they are free of many predisposing factors to drug hepatotoxicity. Interestingly, where drug therapy plays a key role—epilepsy and childhood neoplasia—drug hepatotoxicity is somewhat frequent. Advanced age is a risk factor for severe hepatotoxic reactions. The aging liver metabolizes some drugs more slowly. In view of the increased risk attached to some drug hepatotoxicities in women, one can speculate that changes in drug metabolism possibly associated with puberty may influence the differing incidence of drug hepatotoxicity in childhood and adulthood.

SPECIFIC DRUG HEPATOTOXICITIES IN CHILDREN

Drug hepatotoxicity does occur in children. Hepatotoxicity attributable to most of the following drugs has been diagnosed in children at the Hospital for Sick Children in Toronto in the past 15 to 20 years. Other drugs are included because they are commonly used in pediatric practice and are known to be hepatotoxic in children.

ACETAMINOPHEN

Acetaminophen is commonly used in children as an effective antipyretic and analgesic because its metabolism is rapid enough in most children that it does not accumulate and is not influenced by dehydration. A single large dose is extremely hepatotoxic. The mechanism for this toxicity involves the formation of a toxic metabolite.[21–24] The important role of drug metabolism in this hepatotoxicity is reflected in the predominance of hepatocellular injury in zone 3 (Figure 53-3). Acetaminophen is usually metabolized via sulfation and glucuronidation (Figure 53-4). If a sufficiently large amount is taken, these pathways are saturated, and an otherwise relatively minor pathway through

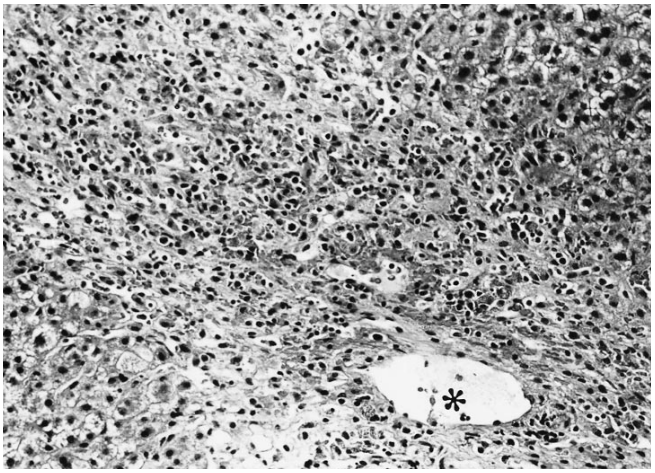

FIGURE 53-3 Liver biopsy in acetaminophen hepatotoxicity. There is a wide zone of necrosis occupying zone 3 of the liver, to which there is only a modest inflammatory cell response. The transition between the necrotic cells and the surrounding hepatocytes, which are swollen, vacuolated, and contain fat, is abrupt. In zone I (not shown), the liver parenchyma is normal. This zonal distribution of necrosis surrounded by swollen fatty hepatocytes is characteristic of acetaminophen hepatotoxicity. Terminal hepatic venule (*asterisk*) (hematoxylin and eosin, ×250 original magnification). Courtesy of Dr. M. J. Phillips.

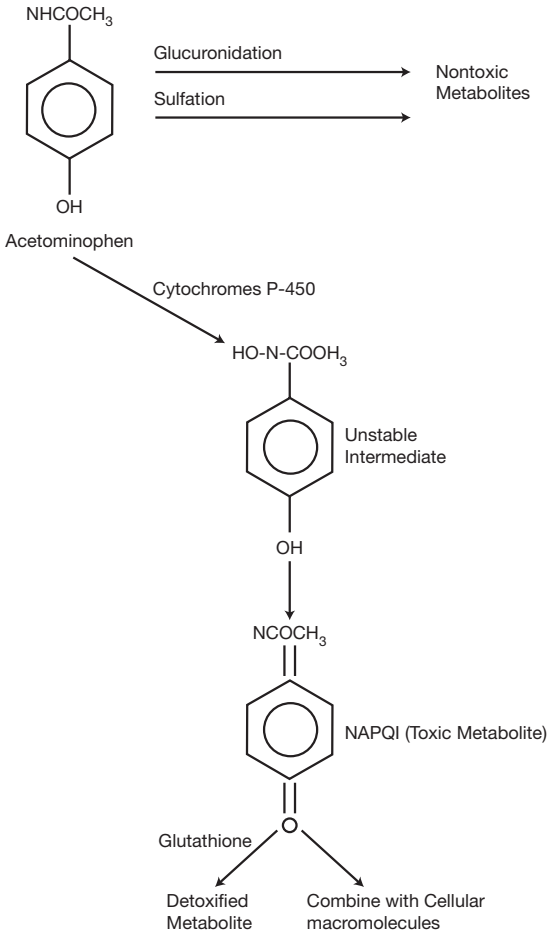

FIGURE 53-4 Metabolism of acetaminophen.

cytochromes P-450 (CYP3A4, CYP2E1, and CYP1A2) becomes quantitatively important. The product of this pathway is a highly reactive species *N*-acetyl-*p*-benzoquinoneimine (NAPQI),[25] a potent electrophile. It is conjugated by glutathione as long as sufficient glutathione is available; otherwise, NAPQI reacts with cellular proteins, causing cell damage and cell death. *N*-Acetylcysteine acts by providing substrate for making more glutathione[26] and thus can minimize hepatotoxicity if given early enough. It does not reverse the toxic effects of the toxic intermediate once they have occurred. Other factors may influence the metabolism of acetaminophen. Cimetidine, which inhibits cytochromes P-450, interferes with acetaminophen toxicity in laboratory animals if given early, but the comparable dose for humans is probably toxic in itself. Concomitant drug treatment may induce cytochromes P-450 involved in the production of NAPQI. Fasting decreases the amount of glutathione in cells and thus increases acetaminophen toxicity. Additionally, recent reports indicate that nitric oxide may play a role in the mechanism of acetaminophen hepatotoxicity[27,28] and that interleukin-10 acts as an immunologic cytoprotective mediator by interfering with nitric oxide production and possibly with the action of other proinflammatory cytokines.[29]

The clinical course of single high-dose acetaminophen hepatotoxicity is distinctive. Immediately after taking a large single dose of the drug, there is nausea and vomiting. These symptoms clear, and then there is an interval before hepatic toxicity becomes clinically apparent. At that point, jaundice, abnormal serum aminotransferases, and coagulopathy develop. Serum aminotransferases may be extremely high in this condition, and the degree of abnormality is not necessarily predictive of outcome. Finally, hepatic failure may supervene with progressive coma. In adults, clinical findings predicting poor outcome (ie, extremely severe hepatic failure) are the concurrent findings of serum creatinine > 300 µmol/L *and* prothrombin time > 100 seconds (international normalized ratio > 7) *and* grade 3 or 4 hepatic encephalopathy in patients with a normal pH or the single finding of arterial pH < 7.3 in a patient who is not hypovolemic.[30]

Whether to use *N*-acetylcysteine can be decided on the basis of plotting on a semilogarithmic graph the patient's plasma acetaminophen concentration against time[31]; if it falls in the zone for probable hepatic toxicity, *N*-acetylcysteine should be given. *N*-Acetylcysteine is most effective if given within 10 hours of acetaminophen ingestion and may be of less benefit if given more than 24 hours after ingestion of the acetaminophen. However, even if there is doubt as to its usefulness, it should be given anyway. Indeed, there is a strong case for treating all patients with *N*-acetylcysteine because some patients who appear to be at low risk by the nomogram develop severe hepatotoxicity.[32] In adults, late administration of *N*-acetylcysteine has been associated with greater survival after acute acetaminophen intoxication; no adverse side effects of the *N*-acetylcysteine were observed.[33,34] A 72-hour regimen of oral *N*-acetylcysteine appears to be as effective as the 20-hour intravenous regimen; the oral regimen may be more effective if treatment is delayed.[35] This regimen consists of a loading dose of 140 mg of oral *N*-acetylcysteine per kilogram of body weight, followed 4 hours later by 70 mg per kilogram given every 4 hours for an additional 17 doses, and it can be given by a nasogastric tube. A recent retrospective study of children 1 to 17 years old who presented with acetaminophen poisoning showed that the best predictor for low risk of acetaminophen hepatotoxicity in this setting was normal prothrombin time *and* aspartate aminotransferase *and* ALT at 48 hours after ingestion, and these authors concluded that treatment with *N*-acetylcysteine should be used for at least 48 hours in children—longer if the clinical situation warranted it.[36] Activated charcoal may be effective but only if administered early (less than 1 hour after the ingestion); interaction with *N*-acetylcysteine reduces the effectiveness of charcoal.[37] Inducing vomiting may not provide any benefit. Hemodialysis, if it is to be effective at all, must be used early when acetaminophen plasma concentrations are high.

Extensive reviews of acetaminophen poisoning in children suggest that younger children tend to be resistant to this hepatotoxicity.[38,39] The incidence of hepatotoxicity was 5.5% in a study of 417 children 5 years old or less compared with 29% in adolescents and adults at comparable toxic blood levels.[38] Various studies of acetaminophen pharmacokinetics, metabolism, and toxicity in children

suggest a biochemical basis for this observation. The elimination half-life is essentially the same in children and adults, although, with interindividual variation, it ranges as much as 1 to 3.5 hours.[40] The elimination half-life is somewhat longer (2.2–5.0 hours) in neonates. The profile of metabolites differs greatly in early childhood from adolescence and adulthood: sulfation predominates over glucuronidation.[41] The switch to the adult pattern seems to occur around 12 years of age. However, even in newborns, urinary metabolites reflecting cytochrome P-450–generated intermediates can be found; thus, the capacity for producing toxic metabolites seems to be present from an early age.[42,43] In vitro studies with fetal human hepatocytes have shown that the cytochrome P-450–generated intermediates can be formed and conjugated to glutathione as early as at 18 weeks of gestation, but the rate of formation is approximately 10% of that in adult human hepatocytes; sulfation, but not glucuronidation, of acetaminophen also can be detected in human fetal liver cells.[44] Studies in young rats showed less susceptibility to hepatotoxicity in the 11-day-old rat compared with the adult rat.[45] In other studies, hepatocytes from young rats were shown to have a higher capacity for synthesizing glutathione than those from older rats and to be able to also increase synthesis when glutathione is depleted.[46] Perhaps human infants also have a greater capacity for synthesis of glutathione than adults and thus can detoxify acetaminophen toxic metabolites more effectively.

Despite their relative resistance to acetaminophen hepatotoxicity, young children definitely can develop severe hepatotoxicity from acetaminophen. The threshold dose for severe toxicity in children has not been determined but is probably in the range of a single dose of 120 to 150 mg/kg.[47] In contrast to single high-dose ingestion, therapeutic misadventure is an important pattern of hepatotoxicity in young children. This term denotes hepatotoxicity that develops after taking repeated doses of relatively small amounts of acetaminophen (three to four times the recommended dose) for a short time, usually a few days.[48–52] Typically, the improper use of acetaminophen occurs because the dosage schedule was not understood, a different preparation of acetaminophen was substituted for the age-appropriate version (eg, tablet substituted for elixir), or the wrong measuring device was used. Incorrect use of sustained-release preparations or inadvertent overdosing because it was not appreciated that many over-the-counter cold remedies contain acetaminophen may also lead to hepatotoxicity. The threshold dose for hepatotoxicity under these circumstances has been estimated at 90 mg/kg/d.[53] The liver disease presents as acute liver failure, which is often attributed to another etiology, usually viral hepatitis. The typical clinical course of the single large-dose ingestion is either not present or not noticed. Serum concentrations of acetaminophen are frequently not in a toxic range. Diagnosis requires an extremely meticulous drug history to find out exactly what preparation of acetaminophen was used and how often. The nomogram for treatment with *N*-acetylcysteine does not apply; however, finding a detectable concentration of acetaminophen at 24 hours or more after the last dose suggests this etiology. An estimated elimination half-life > 4 hours also suggests acetaminophen hepatotoxicity. Detection of serum acetaminophen-cysteine conjugates by high-performance liquid chromatography is a sensitive diagnostic test.[54] These children should be treated with *N*-acetylcysteine as soon as possible. The intercurrent illness for which the acetaminophen was used may have caused enough anorexia to deplete normal glutathione stores. Hepatotoxicity with extreme prolongation of the elimination half-life of acetaminophen has been reported in infants born after maternal self-poisoning with acetaminophen.[43,44]

Rectal administration of acetaminophen may also cause hepatotoxicity. Absorption from the suppository is often highly variable. In general, it takes longer to attain peak blood levels, but bioavailability can vary within the length of a single suppository and between different types of suppository depending on carrier composition. Overall, this appears to be a highly unsatisfactory way to give the drug because it is difficult to predict efficacy, determine dosing interval, and calculate cumulative dose. Hepatic failure associated with rectal acetaminophen has been reported, probably owing to high-dose acetaminophen, although the child also received diclofenac and underwent appendectomy.[55]

Initial studies on the mechanism of acetaminophen toxicity showed that toxicity was worse when animals were pretreated with the polycyclic aromatic hydrocarbon 3-methylcholanthrene, a potent inducer of CYP1A1. It has become evident that chronic alcoholics are more sensitive to acetaminophen than nonalcoholics in that they can develop subacute acetaminophen hepatotoxicity after taking ordinary therapeutic doses over time because of induction of CYP2E1.[56] Some adolescents may be at risk for this type of acetaminophen hepatotoxicity. Whether exposure to environmental toxins such as polychlorinated biphenyls or aromatic hydrocarbons, cigarette smoking, or chronic use of proton pump inhibitors that induce CYP1A2 increases susceptibility to acetaminophen hepatotoxicity remains unproved. Whether obesity (often associated with CYP2E1 induction) enhances the risk of acetaminophen hepatotoxicity has not been established. Available data relevant to children do show that concomitant treatment with medications that induce cytochromes P-450 lowers the threshold for acetaminophen hepatotoxicity. These drugs include phenobarbital, carbamazepine, phenytoin, rifampin, and isoniazid (INH). Mercury poisoning through exposure to elemental mercury apparently enhanced acetaminophen hepatotoxicity in one child.[57]

Phenytoin

Although the commonly used anticonvulsant diphenylhydantoin has been associated with a broad range of adverse effects, phenytoin-induced hepatitis is important because severe hepatic necrosis and liver failure often develop. The perception that it is rare in children is misleading. Phenytoin-associated hepatitis was the only hepatitis mentioned specifically among adverse drug reactions in a large prospective study of adverse drug reactions in children.[18] There are at least 30 cases in the literature[58,59] and an addi-

tional 18 cases of hepatic dysfunction in patients (9 of whom were children) whose adverse reaction to phenytoin was dominated by other organ system involvement.[60]

Phenytoin hepatotoxicity typically presents as part of a systemic disease with fever, rash (including morbilliform rash, Stevens-Johnson syndrome, and toxic epidermal necrolysis), lymphadenopathy, leukocytosis, eosinophilia, and atypical lymphocytosis. Serum aminotransferases are elevated, and the patient may be moderately jaundiced. In severe cases, clinical features of hepatic failure (coagulopathy, ascites, altered level of consciousness) are also present. Histopathologic examination of the liver shows spotty necrosis of hepatocytes, along with features reminiscent of mononucleosis in some cases or of viral hepatitis in others; cholestasis may complicate more severe hepatocellular injury; granulomas are sometimes found.[61] Although treatment of severe phenytoin hepatitis with high-dose intravenous corticosteroids has not been tested in a controlled trial and anecdotal reports do not uniformly show clear benefit, intravenous methylprednisolone 2 mg/kg/d has been effective in some patients. In patients with severe Stevens-Johnson syndrome, use of intravenous gammaglobulin has also been advocated.[62]

The typical clinical presentation of phenytoin hepatotoxicity is termed a "drug hypersensitivity reaction." There is reason to believe that this clinical syndrome develops as a result of abnormal handling of a toxic metabolite of phenytoin. Phenytoin is metabolized via an arene oxide intermediate that is ordinarily metabolized and thus detoxified by epoxide hydrolase.[63] When lymphocytes, which are readily isolated cells complete with most phase II biotransformation pathways, are incubated in vitro with phenytoin and a murine microsomal system that can generate the intermediate metabolites of phenytoin, lymphocytes from persons who have developed the drug hypersensitivity syndrome to phenytoin are killed in excess of control lymphocytes.[63] If lymphocytes from normal individuals are pretreated with chemicals that inhibit cellular epoxide hydrolase, these lymphocytes behave like those from affected individuals.[64] Studies of parents indicate an intermediate sensitivity to the toxic metabolite(s), consistent with an inherited defect in drug detoxification. Instead of causing cell death, binding of the toxic metabolite may create haptens for initiating an immune response. This may account for the appearance of hypersensitivity clinically and for positive immune challenges noted by others.[65] More recent studies have failed to confirm the thesis that deficient epoxide hydrolase activity is responsible for phenytoin hepatotoxicity.[66] Others have shown elaboration of reactive intermediates, including quinone derivatives.[67,68]

Three of four children reported with fatal diphenylhydantoin hepatotoxicity were taking phenobarbital at the same time. Because in vitro studies indicate that some patients who cannot detoxify toxic intermediates of phenytoin are similarly sensitive to phenobarbital, this combined treatment may have made the liver damage worse. Another patient was switched from phenytoin to phenobarbital and then relapsed; he improved when high-dose corticosteroids were given along with phenobarbital.[58]

Carbamazepine

Carbamazepine is a dibenzazepine derivative, similar structurally to imipramine in that it has fundamentally a tricyclic chemical structure. Hepatotoxicity is relatively uncommon. In adults, the predominant hepatotoxicity has been granulomatous hepatitis presenting with fever and right upper quadrant pain, suggestive of cholangitis.[69,70] In children, the clinical picture has been more of a hepatitis, sometimes dominating a drug hypersensitivity syndrome like that of phenytoin. One child died of progressive liver failure when carbamazepine was not stopped in time.[71] Four children with fatal acute liver failure were taking carbamazepine, phenytoin, and primidone.[72] More recently, severe hepatitis was reported in three children taking only carbamazepine: one recovered with corticosteroid treatment, but the others died or required liver transplant.[73] Another child developed acute severe hepatitis 5 months after beginning treatment with carbamazepine; she survived with prednisone treatment.[74] Two other children presented with a mononucleosis-like illness with rash, lymphadenopathy, hepatosplenomegaly, hepatosplenomegaly, and, eventually, neutropenia.[75,76] This is similar to a child treated at the Hospital for Sick Children in Toronto (Figure 53-5), who presented with fever, rash, incipient liver failure, lymphopenia, and eosinophilia. In vitro rechallenge of her lymphocytes with metabolites of carbamazepine provided evidence of defective detoxification mechanisms. An infant boy also presented here with only hepatotoxicity and three other children with drug hypersensitivity to carbamazepine, with hepatitis not the dominant feature, have been described.[60] Carbamazepine may also be metabolized via arene oxides, although not all data support this thesis.[77]

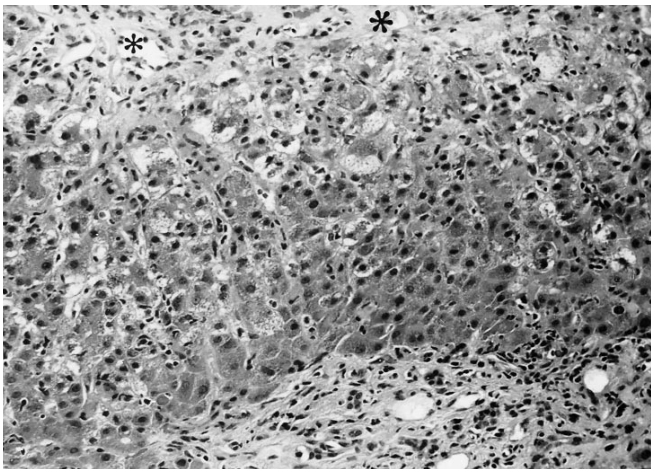

FIGURE 53-5 Liver biopsy in carbamazepine hepatotoxicity. The portal area shows widening with fibrosis, ductular proliferation, and mild chronic inflammatory changes. The lobular parenchyma shows variability in the size of the hepatocytes, with many swollen ballooned hepatocytes in zones 2 and 3 and occasional inflammatory cells. In zone 3, there is central bridging necrosis (*asterisks*). The pathologic diagnosis is drug-induced acute hepatitis with bridging necrosis; these findings are fully representative of the hepatic lesion with carbamazepine (hematoxylin and eosin, ×250 original magnification). Courtesy of Dr. M. J. Phillips.

Recent work indicates that carbamazepine is metabolized to a reactive iminoquinone species that can react with glutathione.[78] Persons with the metabolic idiosyncrasy that renders them susceptible to carbamazepine hepatotoxicity may also be susceptible to phenytoin and phenobarbital hepatotoxicity.

Phenobarbital

Hepatitis is a rare complication of phenobarbital use. When it occurs, it, too, is usually part of a multisystemic drug hypersensitivity reaction, but it may dominate the clinical picture. Seven of 13 patients reported in the world literature were children.[79] Two additional children, a girl aged 3 years and a boy aged 18 months, have been treated at the Hospital for Sick Children in Toronto; both had severe hepatic dysfunction with coagulopathy or ascites but ultimately survived. Two further cases in children have been treated at the Hospital for Sick Children in Toronto, but hepatitis was not the dominant clinical feature.[60] In most cases of major hepatotoxicity, jaundice began 1 to 8 weeks after starting phenobarbital, along with generalized rash and fever. Usually, the liver disease was moderately severe but self-limited; however, a few patients developed severe hepatitis with coagulopathy and ascites, and one died fulminantly. One child developed chronic liver disease. Severe phenobarbital hepatitis may be treated with intravenous methylprednisolone.[79]

The mechanism of this hepatotoxicity remains unclear. Results from in vitro rechallenge indicate an inherited defect in detoxification of active metabolite. Phenobarbital may also be metabolized via arene oxide intermediates, which are typically detoxified via epoxide hydrolase. In in vitro rechallenge, if lymphocyte epoxide hydrolase is inhibited, the extent of cytotoxicity of metabolites generated from phenobarbital, as from phenytoin, increases.[64]

Persons who develop hepatotoxicity from phenobarbital also cannot detoxify other barbiturates and may get worse if so treated. Sedation for a diagnostic procedure in a child is an important opportunity for such a drug exposure. It is also important to bear in mind that persons who cannot detoxify the toxic metabolite(s) of phenobarbital often cannot detoxify those of carbamazepine or phenytoin either.[60] Thus, substituting either may worsen the hepatitis.

Lamotrigine

Lamotrigine may cause hepatotoxicity with a typical anticonvulsant hypersensitivity syndrome clinically.[80–82] Hepatic involvement varies from elevated serum aminotransferases to severe acute hepatitis. Lamotrigine can generate an arene oxide intermediate, which is a candidate for mediating this hepatotoxicity.[83] Whether cross-reactivity between lamotrigine and the phenytoin-carbamazepine-phenobarbital anticonvulsant group exists has not been conclusively proven.

Valproic Acid

Valproic acid (VPA) is chemically very different from the other anticonvulsants above: it is an eight-carbon, branched fatty acid. Hepatotoxicity is mild or severe. In a certain proportion of patients, estimated at 11% overall,[84] serum aminotransferase levels become abnormal, typically within a short time of starting treatment. This biochemical abnormality returns to normal when the dose of VPA is decreased. Much more rarely, patients develop progressive liver failure that, in some cases, looks similar clinically to Reye syndrome. This severe hepatotoxicity does not always regress when the drug is withdrawn. Its occurrence cannot be predicted by regular monitoring of serum aminotransferases and other liver function tests.[85] The time from initiating treatment with VPA and onset of liver disease is usually less than 4 months, but hepatotoxicity may develop later in treatment. A distinctive feature of severe VPA hepatotoxicity is that it is more common in children than in adults.[86] Special identifiable risk factors include age less than 2 years, multiple anticonvulsant treatment along with VPA, and coexistent medical problems such as mental retardation, developmental delay, or congenital abnormalities.[87] In children with these predisposing factors, the risk of fatal hepatotoxicity is 1 in 600.[88] Hyperammonemia, not associated with liver failure, is another metabolic adverse effect of VPA.[89,90]

Severe hepatotoxicity was described with rising use of VPA,[91,92] and the total experience has been reviewed in detail.[84,93,94] The severe hepatotoxicity typically presents with a hepatitis-like prodrome, mainly malaise, anorexia, nausea, and vomiting. Seizure control is often found to deteriorate over the same time period. An intercurrent illness with fever may precede the onset of liver failure. Coagulopathy is often present early; jaundice and other signs of progressive hepatic insufficiency, such as ascites and hypoglycemia, develop later. Death owing to liver failure, complicated by renal failure or infection, is the frequently reported outcome; liver transplant may be performed. Liver histology reviewed in one large series shows evidence of hepatocellular necrosis, which may be zonal, with extensive loss of hepatocytes and severe damage to those remaining.[93] Acidophilic bodies, ballooned hepatocytes, and cholangiolar proliferation may be present. Microvesicular steatosis is the most common finding overall and is often present in addition to the features of cell necrosis. Hepatocellular mitochondria may be prominent on light microscopy so that the hepatocytes have a granular, very eosinophilic appearance (Figure 53-6). In cases presenting clinically like Reye syndrome, fever, coagulopathy, progressive loss of consciousness, severe acidosis, and variably abnormal serum aminotransferases are present, but the patient is not jaundiced.[92] Hepatocellular necrosis, as well as microvesicular fat, is found on histologic examination of the liver, unlike the histologic findings of Reye syndrome. Electron microscopically, the mitochondrial changes associated with VPA toxicity differ from those of Reye syndrome.

The mechanism of this severe hepatotoxicity is thought to involve generation of toxic metabolite(s) plus some type of metabolic idiosyncrasy. Metabolic idiosyncrasy is probable not only because severe hepatotoxicity is rare but because toxic ingestions do not necessarily lead to liver necrosis.[95] VPA is related structurally to two known hepatotoxins: hypoglycin, which causes Jamaican vomiting sickness, characterized by microvesicular steatosis, and 4-pentenoic acid,

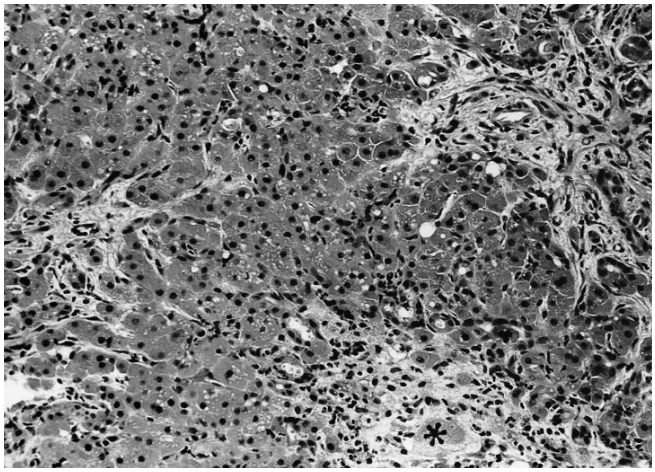

FIGURE 53-6 Liver biopsy in valproic acid hepatotoxicity. The liver lobule shows great reduction in the number of hepatocytes. There is portal tract widening with increased numbers of bile ducts. Hepatocytes are swollen, and most contain multiple microvesicular fat droplets. In zone 3, there is an area of necrosis (*asterisk*) with tubular transformation of hepatocytes surrounding the necrotic zone (hematoxylin and eosin, ×250 original magnification). Courtesy of Dr. M. J. Phillips.

which causes microvesicular steatosis in rat liver and inhibits β-oxidation. The partly unsaturated metabolite 4-ene-valproic acid (4-ene-VPA), produced by Ω-oxidation, which is a minor pathway of VPA metabolism, is chemically very similar to these toxins. Formation of 4-ene-VPA has been demonstrated in a primate model[96] and in patients with liver failure developing on VPA treatment.[97] Administration of 4-ene-VPA to rats caused accumulation of microvesicular fat in hepatocytes along with changes in hepatocyte organelles, including mitochondrial abnormalities and elaboration of myeloid bodies,[98] as well as inhibition of β-oxidation.[99] Both VPA and 4-ene-VPA inhibit β-oxidative metabolism of decanoic acid, a fatty acid of medium length; in contrast, 4-pentenoic acid is only a weak inhibitor in this system.[100] Thus, VPA and its metabolite(s) are capable of causing adverse changes in liver cell metabolism, which may lead to the observed features of this hepatotoxicity. The similarities and differences in these metabolic toxicities compared with those of hypoglycin and 4-pentenoic acid merely reflect the complexity of this metabolic system. The β-oxidation metabolite of 4-ene-VPA, (*E*)-2,4-diene-VPA, may also mediate VPA hepatotoxicity as a toxic intermediate. Urinary levels of thiol conjugates of 4-ene-VPA and (*E*)-2,4-diene-VPA were found to be elevated in children less than 7.5 years old receiving VPA monotherapy and were also elevated in older children if they were receiving treatment with an antiepileptic drug capable of inducing cytochromes P-450 along with the VPA.[101] Concentrations of the thiol conjugates of (*E*)-2,4-diene-VPA were higher than those of 4-ene-VPA.

VPA is capable of inhibiting β-oxidation in humans. Investigations of VPA metabolism in patients with severe hepatotoxicity indicate that β-oxidation is inhibited in these patients,[102] although the step in β-oxidation at which the apparent block occurred varied individually.[103] Increased amounts of 4-ene-VPA were detected in some cases.[97,104,105] Biochemical abnormalities indicating inhibition of β-oxidation have also been found in children on VPA treatment at low risk for hepatotoxicity.[106] These observations suggest a complex pattern of inhibited β-oxidation and increased utilization of alternative pathways, including those associated with cytochromes P-450 and δ-dehydrogenation to produce, among other metabolites, 4-ene-VPA.

In VPA hepatotoxicity, the target organelle appears to be the mitochondrion. An individual who develops severe VPA hepatotoxicity may not be able to detoxify these metabolites or subsequent toxic intermediates before significant mitochondrial damage occurs. The defective detoxification pathway is not yet known. Studies of VPA toxicity in vitro in human liver slices indicate important interindividual variation in susceptibility to toxicity.[107] The metabolic idiosyncrasy might be a functional defect in the mitochondrion itself. An intercurrent environmental problem, such as a viral illness, might additionally inhibit β-oxidation. Experimental data in the ornithine transcarbamylase–deficient mouse support the hypothesis of an intrinsic metabolic defect in the mitochondrion. The ornithine transcarbamylase–deficient mouse develops hepatocellular necrosis and microvesicular steatosis at doses of VPA that do not affect the normal control adversely.[108] Individuals who develop severe VPA hepatotoxicity may have mitochondria biochemically predisposed to this injury. Ornithine transcarbamylase deficiency may be one such definable abnormality and has been suspected in one instance.[109,110] Other mitochondrial abnormalities may have predisposed some patients to VPA hepatotoxicity.[111]

Serum carnitine has been found to be abnormally low in persons with Reye syndrome. Decreased serum carnitine has also been found in VPA hepatotoxicity.[102,112] Serum carnitine is also low in patients treated chronically, without evidence clinically of hepatotoxicity.[112–114] Conjugation to carnitine is a unique metabolic pathway for VPA.[115] Whether this pathway is important for the development of hepatotoxicity is not known. Equally, the value of carnitine repletion as treatment for severe hepatotoxicity remains unproven, and most evidence to date suggests that it is ineffective.[106,116] However, a recent retrospective study indicated efficacy if carnitine was started very early in the course of severe hepatotoxicity.[117]

Sulfonamides

Hepatotoxicity may occur with any sulfonamide antibiotic. In children, this problem arises most commonly in connection with treatment for otitis media and upper respiratory infections or for inflammatory bowel disease. Sulfanilamide, trimethoprim-sulfamethoxazole, and pyrimethamine-sulfadoxine have all been associated with major hepatic injury.[118,119] Sulfasalazine has been associated with severe liver disease in adolescents and young adults.[120–123] Although the liver abnormality may be manifested only by elevated serum aminotransferases or may be a granulomatous hepatitis, the hepatic dysfunction may be severe enough to cause acute hepatic failure, which is fatal in some

cases. In general, hepatotoxicity is part of a clinical drug hypersensitivity reaction. Fever, significant rash, periorbital edema, atypical lymphocytosis, lymphadenopathy, and renal dysfunction with proteinuria have all been described.

Sulfonamide hepatotoxicity is due to elaboration of an electrophilic toxic metabolite in the liver. The intermediate appears to be the hydroxylamine derived from the particular sulfonamide or, more likely, the nitroso species derived from the hydroxylamine.[124–126] Patients who develop severe adverse reactions, including significant hepatotoxicity, have been shown to be slow acetylators (in the rapid/slow polymorphism for *N*-acetyltransferase) and are also unable to detoxify this reactive metabolite. On in vitro rechallenge of their lymphocytes with sulfonamide and a metabolite-generating system, the patient's lymphocytes show significantly more cytotoxicity than controls.[119] Glutathione *S*-transferases may be important for detoxifying the toxic intermediate.[127,128] How this reactive intermediate causes hepatocellular damage is not yet known. The multisystemic hypersensitivity features of this adverse drug reaction appear to be subsequent to metabolic events in that the reactive metabolite probably acts as a hapten to initiate the immune response. Thus, sulfa hepatotoxicity fundamentally represents metabolic idiosyncrasy, not simply allergy.

Erythromycin

Erythromycin estolate and other salts are used frequently in children. Although the estolate was originally associated with a cholestatic hepatic lesion, it is now clear that the ethylsuccinate and other salts are also potentially hepatotoxic.[129–132] Indeed, all forms of erythromycin are potentially hepatotoxic. The clinical presentation includes anorexia, nausea, predominantly right upper quadrant abdominal pain, and jaundice. Pruritus owing to cholestasis has been reported in some adults. Hepatomegaly, sometimes accompanied by splenomegaly, appears to be frequent in children. Erythromycin ethylsuccinate hepatotoxicity in a child was a relatively mild, self-limited disease.[130]

Histologic findings include prominent cholestasis, which is particularly severe in zone 3, focal necrosis of hepatocytes also tending to predominate in zone 3, and eosinophils in the portal infiltrates and in the sinusoids.[129] These histologic findings are different from those of extrahepatic biliary tract obstruction, although the clinical presentation may suggest biliary tract obstruction.

The mechanism of this hepatotoxicity remains obscure. Erythromycin itself, not a specific erythromycin salt, may be the cause of the hepatotoxicity. However, in the perfused rat liver model, erythromycin estolate led to decreased bile secretion, altered canalicular permeability, and decreased activities of Na/K–adenosine triphosphatase and Mg^{++}–adenosine triphosphatase, unlike erythromycin base.[133] Earlier studies in Chang liver cells suggested that erythromycin derivatives cause intrinsic hepatocellular damage. In various types of primary rat hepatocyte culture systems, erythromycin estolate leads to cytotoxicity.[134,135] Erythromycin and other macrolide antibiotics are metabolized in the liver by the CYP3A subfamily. Hepatocellular damage may be due to a toxic metabolite, but this is by no means proven. Cholestasis may also reflect damage to the cellular biliary apparatus. The association of eosinophilia with erythromycin hepatotoxicity in some patients probably represents a forme fruste of a drug hypersensitivity syndrome.

Propylthiouracil

Hepatitis is a rare complication of propylthiouracil treatment for hyperthyroidism. It tends to occur in girls, but this may reflect the greater frequency of thyroid disease in girls.[136–140] The clinical picture typically was a nonspecific hepatitic presentation with anorexia, nausea, vomiting, and jaundice. Serum aminotransferases were moderately elevated. Symptoms began typically within 2 to 3 months of starting treatment, but in one child, liver disease began at least 9 months after starting treatment and in another after 15 months. A more cholestatic picture has been reported in some adults. Liver histology shows mild to severe hepatocellular necrosis, which was characterized as submassive in three cases.

A single case of propylthiouracil hepatotoxicity associated with chronic active hepatitis has been reported in a child.[141] Hepatomegaly with elevated serum aminotransferases developed after more than 1 year of treatment. Both anti–smooth muscle antibodies and anti–liver/kidney microsomal (anti-LKM1) antibodies were negative. Liver biopsy showed portal inflammation with moderate piecemeal necrosis.

Aspirin

Hepatotoxicity has been associated with high-dose aspirin treatment. Approximately 60% of the 300 reported cases have been in patients with juvenile rheumatoid arthritis (JRA) (not necessarily all children), and a further 10% have occurred in children with acute rheumatic fever.[142] Hepatotoxicity may be more frequent in girls. The hepatotoxicity appears to be dose dependent, and patients without rheumatoid disease can develop hepatotoxicity. The preponderance of cases in patients with rheumatologic diseases, however, raises the possibility that these patients have a predisposition to this toxicity. One theory is that chronic inflammation favors generation of oxygen radicals.[143]

In most cases, salicylate hepatotoxicity presents with anorexia, nausea, vomiting, and abdominal pain, along with elevated serum aminotransferases.[144–148] Hepatomegaly is usually present, and the liver may be tender. Progressive signs of liver damage, such as jaundice and coagulopathy, are rare, occurring in approximately 4% of all reported cases.[142] However, even in uncomplicated cases, serum aminotransferase levels may be quite high, greater than 1,000 IU.[146] In some cases, encephalopathy (not related to Reye syndrome) was present.[149,150] Clinical and laboratory abnormalities resolve when aspirin is stopped. Rechallenge with aspirin may lead to recurrent hepatotoxicity. Liver histology frequently shows a rather nonspecific picture with acute, focal hepatocellular necrosis.[144]

A different clinical syndrome with hepatotoxicity has been reported in seven children with JRA, of whom all but one received aspirin.[151] Clinical features included high

fever, drowsiness, vomiting, hepatosplenomegaly, and bleeding owing to disseminated intravascular coagulation and suboptimal clotting factor synthesis. Liver histology showed steatosis (predominantly large droplet) and prominence of reticuloendothelial cells in the liver. Rechallenge with aspirin did not reproduce this syndrome, and it may have been related to other drug treatment or to intercurrent infection. Two children died of coma, but there was neither cerebral edema nor severe hyperammonemia noted, and other features were not typical of Reye syndrome. However, Reye syndrome can occur in children with rheumatologic diseases who receive aspirin chronically,[152] and its clinical presentation may be atypical.

Methotrexate

Methotrexate hepatotoxicity in children appears to be similar to that in adults. Chronic low-dose treatment used for treatment of psoriasis or connective tissue disease can cause hepatic fibrosis with steatosis.[153,154] Histologically, it may resemble alcoholic hepatitis with fibrosis. Cirrhosis can develop, and liver transplant has been performed in some adults treated for psoriasis.[155] In adults, obesity, diabetes, chronic alcohol abuse, older age, and large cumulative dose appear to be factors associated with increased risk of methotrexate hepatotoxicity. Serum aminotransferase levels may not indicate reliably the extent of ongoing liver damage, and liver biopsy prior to treatment and at regular intervals during prolonged treatment has been advised. Recent guidelines for monitoring methotrexate hepatotoxicity in adults with rheumatoid arthritis advocate a more formal approach to monitoring aspartate aminotransferase, ALT, ALP, albumin, and bilirubin (γ-glutamyltransferase to corroborate hepatic origin of ALP should be added to this list) every 4 to 8 weeks; liver biopsy is performed only when sustained abnormalities are found.[156] Liver biopsy prior to starting treatment is reserved for patients with known liver disease or specific risk (chronic hepatitis B or C infection). Meta-analysis indicates that there is a tangible risk in adults for significant hepatotoxicity owing to long-term, low-dose methotrexate.[157]

It is difficult to transpose these data from adults with various diseases directly to children. The risk factors identified in adults have limited applicability to children except for obesity. Regular monitoring of liver function tests in children receiving chronic methotrexate treatment is advisable. Children with JRA on methotrexate should have serum aminotransferases checked frequently (monthly or bimonthly). Those with elevated aspartate aminotransferase or ALT on ≥ 40% of tests in 1 year should be considered for liver biopsy. Generally, the surveillance regimen should be individualized for each child, depending on many variables, such as existence of previous liver disease (including chronic viral infection), concomitant drug therapy, cumulative methotrexate dose, chronic hypoalbuminemia, obesity, and diabetes mellitus. Liver biopsy is indicated to determine the extent of liver damage. Performing a liver biopsy after a large cumulative dose of methotrexate has been taken may be appropriate in some cases, especially if continued treatment is anticipated. A pretreatment liver biopsy is also sometimes appropriate since several studies indicate that hepatic abnormalities may be present prior to treatment, which would otherwise be wrongly attributed to methotrexate hepatotoxicity.

Several recent studies have examined the occurrence of hepatotoxicity in children with JRA treated with methotrexate over the long term. The risk of methotrexate hepatotoxicity in JRA appears to be comparatively low. No child in a cross-sectional study of 14 children with JRA who had received a methotrexate cumulative dose > 3,000 mg or > 4,000 mg/1.73 m^2 body surface area had significant fibrosis; one had moderate to severe fatty or inflammatory changes or hepatocellular necrosis on liver biopsy.[158] In a study of 37 liver biopsies from 25 patients with JRA, most were normal or near normal; 4 had moderate to severe fatty or inflammatory changes, and 2 had mild portal fibrosis. Weak yet statistically significant correlations were found between abnormal histology and percent frequency of serum aminotransferase elevations and body mass index.[159] In similar previous studies, normal or near-normal liver histology was found.[160,161] Two patients with JRA treated with methotrexate have been reported as developing some degree of liver fibrosis.[162]

High-dose methotrexate treatment used in some oncology regimens may cause acute hepatitis.[163,164] After more protracted treatment, hepatic damage may be relatively slight, apart from ultrastructural changes including steatosis, fibrosis, and damage to some hepatocellular organelles.[165] Others have also found steatosis, portal inflammation, or portal fibrosis on light microscopic examination of liver biopsies from children with acute lymphoblastic leukemia, treated with various drugs, including methotrexate.[166] Serum aminotransferase abnormalities did not predict histologic findings, which were, in general, mild after 2 years of treatment.[166,167]

The mechanism of this hepatotoxicity is not known. The dosage schedule may be important. Chronic intermittent administration of methotrexate may lead to recurrent hepatocellular damage superimposed on partial repair and regeneration, not unlike experimental models of carbon tetrachloride–induced hepatic fibrosis.

Antineoplastic Drugs

Besides methotrexate, many drugs used to treat neoplasia in childhood can cause hepatotoxicity.[168,169] A common and perplexing problem is elevation in serum aminotransferases without other evidence of severe liver toxicity. Antineoplastic drugs that commonly produce this reaction include nitrosoureas, 6-mercaptopurine, cytosine arabinoside, cisplatinum, and dacarbazine. Doxorubicin, cyclophosphamide, dactinomycin (actinomycin D), and vinca alkaloids are infrequently associated with hepatotoxicity, although drug interactions may increase their hepatotoxicity. Indeed, the difficulty in assessing the hepatotoxic potential of all of these drugs is that they are rarely used separately, and patients receiving them are usually at risk for multiple types of liver injury.

In children and adults, L-asparaginase has been associated with more severe damage characterized by severe steatosis, hepatocellular necrosis, and fibrosis. This is usu-

ally reversible after the L-asparaginase is stopped.[170] The most likely mechanism for this hepatotoxicity is a profound interference with hepatocellular protein metabolism. Dactinomycin (actinomycin D) is exceptionally associated with severe liver damage. Several patients treated with dactinomycin at the Hospital for Sick Children in Toronto developed acute severe hepatitis, with extremely elevated serum aminotransferases and coagulopathy, all of which resolved spontaneously off the drug. The mechanism is not known. Mithramycin has been associated with acute hepatic necrosis. Thrombocytopenia and acute liver failure have been reported in an 18-year-old patient receiving carboplatin.[171]

Thioguanine and other antineoplastic agents, including cyclophosphamide, cytosine arabinoside, busulfan, dacarbazine, and carmustine, have been associated with veno-occlusive disease (VOD) at conventional or high doses.[172] It most frequently develops after allogeneic bone marrow transplant. It presents acutely with an enlarged tender liver, ascites or unexplained weight gain, and jaundice; serum aminotransferases may be elevated; it may progress to cirrhosis. Although irradiation can, in itself, lead to this type of hepatic vascular damage,[173] the combination of irradiation and chemotherapy in conditioning regimens may lead to earlier development of VOD than after single-agent (irradiation or chemical) injury.[174] Clinical predictors of likelihood for development of VOD in children have not yet been identified, but patients with chronic hepatitis, for example, owing to chronic hepatitis C or possibly nonalcoholic steatohepatitis, preceding bone marrow transplant are at increased risk.

The pathogenesis of VOD involves direct toxicity to the sinusoidal endothelial cells in the liver. When it is due to dacarbazine, the damage to endothelial cells occurs through production of toxic metabolite(s) in the endothelial cells; glutathione appears to protect against toxicity.[175] With cyclophosphamide, the toxic intermediate may be produced in hepatocytes.[176] Treatment with *N*-acetylcysteine may reverse the process.[177] Unchecked, the damage progresses from congestion and hemorrhage into the space of Disse in zone 3 of the Rappaport acinus to damage to terminal hepatic venules, which has been described as sinusoidal obstruction syndrome, and, subsequently, to fibrosis.[178] The term sinusoidal obstruction syndrome emphasizes that damage to sinusoidal endothelial cells is the initiating event in this kind of hepatotoxicity and that terminal hepatic venules may be patent as the lesions evolve; however, in many patients, only the resulting lesion with obliterative damage to terminal hepatic venules (namely, VOD) is identified.

Cyclosporine

Cyclosporine is a calcineurin inhibitor with potent immunosuppressive effects. It is extremely lipophilic and has a novel cyclic structure composed of 11 amino acids. It is metabolized in humans by CYP3A4. Although, at high dosage, jaundice with abnormal serum aminotransferases may develop, the more common hepatic abnormality is mainly cholestasis: direct hyperbilirubinemia without other evidence of hepatocellular damage.[179] Cholestasis without biochemical or histologic evidence of hepatotoxicity after cyclosporine administration has been demonstrated in a rat model.[180] Cyclosporine inhibits the bile salt excretory pump (BSEP) directly,[181] down-regulates expression of the multidrug resistance–associated protein (Mrp2), which is located on the apical membrane, and interferes with enzymes responsible for synthesizing glutathione.[182] It may also affect membrane fluidity and thus impair canalicular transporter function indirectly.[183] Increased incidence of gallstones has been reported in children receiving cyclosporine after organ transplant.[184]

Pemoline

Pemoline, a second-line drug for treatment of attention-deficit disorders, has been associated with significant hepatotoxicity: asymptomatic elevation of serum aminotransferases, hepatitis with jaundice, and acute liver failure.[185–190] The largest series documented hepatitis of variable severity, including one patient who died with fulminant hepatic failure[191]; male patients predominated. Two other deaths associated with hepatic dysfunction while on pemoline have been reported. Both patients were boys: one may have had previous chronic liver disease, and the other may have taken an overdose of pemoline.[192] Other cases of severe hepatotoxicity leading to liver transplant have been reported. Pemoline has been withdrawn in some countries. If it is used, serum aminotransferases and other liver function tests must be monitored frequently throughout the entire term of treatment. When pemoline is associated with elevated serum aminotransferases, it must be discontinued. The mechanism of this hepatotoxicity is not known but is probably related to the hepatic metabolism of the drug, not to immune response. Pemoline should not be combined with other hepatotoxic drugs and should not be used in patients with a history of liver disease.

Risperidone

Risperidone is a new drug for various psychiatric problems, including autistic disorders. A few cases of possible hepatotoxicity in children have been reported in adults,[193,194] although they are difficult to interpret because of prior liver disease or concomitant drug treatment. A report of risperidone hepatotoxicity in two children may actually have been concurrence of nonalcoholic steatohepatitis and possible drug toxicity,[195] and a subsequent case series review did not corroborate these findings.[196] Nevertheless, serum aminotransferase elevations after starting risperidone have been observed in children. Risperidone typically leads to weight gain. Body mass index should be assessed before and during treatment. Children taking risperidone should have serum aminotransferases and bilirubin monitored regularly until the risk of hepatotoxicity is determined.

Isoniazid

In adults, INH is capable of causing a wide spectrum of toxic liver disease.[197,198] Clinically, the common finding is

an asymptomatic patient with elevated serum aminotransferases. The development of a hepatitis-like illness with fatigue, anorexia, nausea, and vomiting is ominous. On histologic examination, INH hepatotoxicity frequently looks like acute viral hepatitis. Submassive hepatic necrosis can occur, and, occasionally, the hepatocellular damage is zonal.

There have been scattered reports of INH hepatotoxicity, including fatal hepatic necrosis, occurring both in children being treated for tuberculosis and in those receiving prophylaxis.[199–205] The overall incidence of symptomatic INH hepatitis in children is 0.1 to 7.1%.[206] Large studies of INH hepatotoxicity as evidenced by abnormal serum aminotransferases in children receiving INH alone as prophylaxis showed a 7% incidence in a series of 369 children[207] and a 17.1% incidence in 239 patients aged 9 to 14 years.[208] The discrepancy in these two studies is partly methodologic. However, these findings are nearly the same as in adults, where the incidence of transiently elevated serum aminotransferases is estimated at 10 to 20%.[198] Several studies of children being treated with INH and rifampicin for tuberculosis also show a high incidence of hepatic dysfunction. Thirty-six of 44 patients receiving INH and rifampicin had some elevation of serum aminotransferases and 15 patients (42%) had elevated AST and were jaundiced.[209] These children received comparatively high doses of INH and rifampicin, and many had severe infection. In another study, 37% had hepatotoxicity, including four of seven under 17 months old.[210] These children received conventional, lower doses of INH and rifampicin and brief sequential courses of streptomycin and ethambutal. Inducers of cytochromes P-450 may contribute to INH hepatotoxicity. Severe INH hepatotoxicity occurred in a 10-year-old boy concurrently treated with carbamazepine.[211] As in adults, hepatotoxicity typically developed in children in the first 8 to 10 weeks of treatment. In most children, it resolved with either no change in dose or else a modest dose reduction. Children with more severe tuberculosis, such as tuberculous meningitis, seemed to be at greater risk for hepatotoxicity.

INH hepatotoxicity appears to be due to a toxic metabolite, although the mechanism remains obscure. Acetylisoniazid or its derivatives have been thought to be the toxic intermediate. Susceptibility to hepatotoxicity has been linked to the polymorphism for *N*-acetylation—rapid acetylators being at greater risk.[198] Clinical studies in children have not shown a universal trend implicating rapid acetylators as more susceptible.[210,212] Metabolism by cytochromes P-450 may also be implicated because pretreatment with phenobarbital appears to increase toxicity in laboratory animal models. Rifampicin may enhance INH toxicity by inducing certain cytochromes P-450.[204]

It is probably inaccurate to regard INH hepatotoxicity as uncommon in children. Some of the hepatotoxicity appears to be dose related, and recent downward revisions of dosage recommendations may eliminate some instances of hepatotoxicity. Children who have more severe tuberculosis or who receive simultaneous treatment with rifampicin, phenytoin, or phenobarbital may be at increased risk. The genetic predisposing factors remain unclear. Monitoring with frequent measurement of serum aminotransferases and direct inquiry for symptoms of hepatitis is important in the first 10 to 12 weeks of treatment. INH should be discontinued if anorexia, nausea, or vomiting develops.

Halothane

Halothane hepatotoxicity shows two major clinical patterns. One is hepatitis indicated by abnormal serum aminotransferases in the first or second week after the anesthetic exposure. The other pattern is severe hepatitis with extensive hepatocyte necrosis and liver failure.[213] It is remarkably infrequent in children. Large retrospective studies in children estimate that the incidence is approximately 1 in 80,000 to 1 in 200,000,[214,215] in contrast to an incidence of 1 in 7,000 to 1 in 30,000 in adults.[216] The infrequency of this hepatotoxicity is not due to lack of exposure to the drug because halothane is a mainstay of pediatric anesthetic practice. However, despite its rarity, it is evident that halothane hepatitis can occur in children. Eight cases have been documented in detail in children aged 11 months to 15 years, all of whom had multiple exposures to halothane; one died of fulminant liver failure, but all others recovered.[217,218] In addition, three cases of halothane hepatitis were found retrospectively,[214,215] as well as three further children who succumbed to fulminant hepatic failure after halothane.[219–221] Perhaps an additional eight cases may be found among other reports of hepatitis or hepatic failure in children after halothane anesthesia where inadequate data or the presence of complicated, and thus confounding, systemic disease make evaluation difficult. Clearly, this problem cannot be discounted in children. There has been some speculation that children with α_1-antitrypsin deficiency may tolerate halothane poorly.

Halothane is metabolized by various cytochromes P-450, and toxic metabolites are generated.[222–224] Depending on the prevailing tissue oxygen tension, oxidative or reductive metabolic pathways predominate (Figure 53-7). The reductive pathway generates a toxic intermediate identified as a chlorotrifluoroethyl radical, which leads to lipid peroxidation,[222] and the oxidative pathway generates a trifluoroacetyl intermediate, which can acetylate cellular membranes. The contribution of these metabolic systems to halothane hepatotoxicity in humans is complex. The oxidative pathway is probably predominant in humans. CYP2A6 and CYP3A4 are associated with the reductive metabolism,[225] and CYP2A6 and CYP2E1 (mainly the latter) are associated with the oxidative pathway.[226]

Recent studies of the mechanism of halothane hepatotoxicity are beginning to show the connection between cytotoxic damage from reactive intermediates and immunologic phenomena often associated with this hepatotoxicity. Patients surviving halothane hepatotoxicity have been found to have an antibody to altered hepatocyte membrane constituents.[227] In rabbits, only oxidative metabolism of halothane has been associated with production of this altered hepatocyte membrane antigen, and the effect is greater after pretreatment with the polycyclic aromatic hydrocarbon β-naphthaflavone.[228] Other investigators have shown that tri-

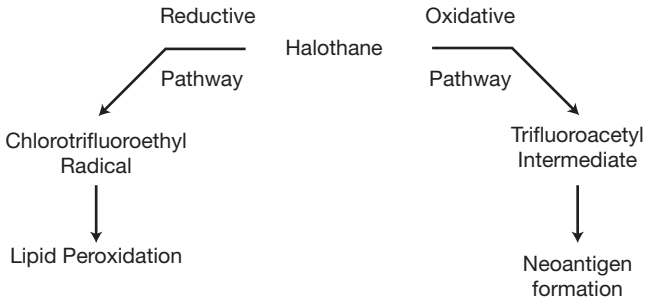

FIGURE 53–7 Metabolic fates of halothane. Whether the reductive or oxidative pathway predominates depends on the prevailing tissue oxygen tension.

fluoroacetyl adducts can be identified with fluorescent-tagged antibodies, mainly in zone 3 hepatocytes in the rat after phenobarbital pretreatment and also on the hepatocyte plasma membrane.[229] Antibodies to these neoantigens have been identified in sera from patients with halothane hepatitis.[230] Further studies have shown that neoantigens, analogous to these neoantigens derived from halothane-treated animals, are expressed in human liver in individuals exposed to halothane.[231] One of these neoantigens has been purified and identified as a microsomal carboxylesterase.[232] Kupffer cells may participate in the process by which the trifluoroacetyl adducts initiate an immune response.[233] Other studies in rats suggest that factors such as gender and previous exposure to specific inducers of cytochromes P-450 may influence the expression of halothane-associated neoantigens.[234] Thus, the oxidative pathway appears to be associated with hepatocellular membrane damage and immune phenomena typical of the clinical hepatotoxic syndrome.

In summary, severe halothane hepatotoxicity involves several factors whose interdependence can be partly defined. Formation of toxic metabolites depends on tissue oxygenation and possibly on which cytochromes P-450 are involved. Idiosyncratic susceptibility with inadequate detoxification of an electrophilic intermediate may play a role.[216] The extent of immune phenomena may further depend on the immunogenicity of adducts formed and the innate immune responsiveness of the host.[229] Halothane hepatotoxicity provides the best example currently available for demonstrating a link between drug metabolism and an immune reaction in hepatotoxicity.

Penicillins

Semisynthetic derivatives of penicillin may cause liver damage. Amoxicillin-clavulinic acid has also been associated with cholestasis[235] or a mixed hepatic-cholestatic reaction.[236,237] The cholestatic effect of flucloxacillin may be mediated by an intermediate produced by hepatocellular cytochromes P-450 that may be toxic to bile duct epithelial cells.[238] With prolonged cholestasis owing to semisynthetic penicillins, the development of small portal ("interlobular") bile duct paucity, known as ductopenia, has been observed in adults.[239] Although small bile duct paucity has been associated with amoxicillin alone,[240] the combination drug containing clavulinic acid appears to be more toxic.[241] Hepatotoxicity has been reported in children with intravenous oxacillin.[242] Ductopenia has been reported with amoxicillin-clavulinic acid in one child, who eventually required liver transplant.[243]

Minocycline

The tetracycline derivative minocycline is often used to treat acne in adolescents. It appears to have a greater potential for causing liver damage than is generally appreciated. Several cases of hepatotoxicity in teenagers have been reported: symptoms of hepatitis, jaundice, elevated serum aminotransferases, and positive antinuclear antibodies were common.[244–248] One typical presentation was polyarthritis with biochemical hepatitis; jaundice was present when hepatitis was severe. Some cases had features of autoimmune hepatitis. In many patients, liver damage resolved when the drug was discontinued. Two cases of acute liver failure in adolescents have been reported[249,250]: one died before transplant and the other underwent liver transplant. Careful monitoring of liver function is indicated when minocycline is used chronically.

Herbals

Complementary medicines are frequently used, usually without a physician's knowledge or review. Problems relating to the purity and strength of the specific preparation and to potential drug interactions complicate the issue of inherent risk for hepatotoxicity.[251] Children may be given herbal medications as tonics, and adolescents may choose to take them on their own. Herbal drugs known to be hepatotoxic include bush teas containing pyrrolizidine alkaloids, comfrey,[252] germander,[253,254] chaparral leaf,[255] kava-kava,[256,257] jin bu huan,[258–260] and ma huang (Ephedra). Comfrey also contains pyrrolizidine alkaloids, which can cause sinusoidal obstruction syndrome.[261] Germander undergoes biotransformation by CYP3A4, leading to diterpenoid toxic metabolites.[262,263] Some of these herbal medications are taken to promote weight loss, and the role of obesity in predisposing patients to hepatotoxicity deserves consideration.

"Ecstasy" (3,4-Methylenedioxymethamphetamine)

This drug is frequently used by adolescents as a recreational drug. It can have severe multisystemic adverse effects, including cardiac arrhythmias, hyperthermia, and rhabdomyolysis. The severity of hepatotoxicity can be extremely variable, including mild to moderate elevations of serum aminotransferases with acute or chronic hepatitis, fibrosis, and, occasionally, a picture resembling autoimmune hepatitis.[264] Acute liver failure occurs, sometimes at the first use, and is either fatal or requires liver transplant.[265,266] This has become an important problem for young adults and, to a lesser extent, adolescents. The mechanism of hepatotoxicity remains unclear. Hepatic biotransformation pathways are complex and involve CYP2D6 as the high-affinity component for the major pathway and also CYP1A2, CYP3A4, and CYP2B6.[267] Whether slow metabolizers in the CYP2D6 polymorphism are at greater risk for Ecstasy-associated hepatotoxicity is disputed.[268,269] Production of Ecstasy is clandestine; thus,

drug potency and purity are always uncertain, factors that may also contribute to hepatotoxicity.

PRINCIPLES OF TREATMENT

Most drug-induced liver disease resolves spontaneously when the hepatotoxic drug is withdrawn. Severe chronic changes should not be expected to regress. However, the histologic finding of bridging necrosis on liver biopsy does not tend to presage aggressive chronic liver damage in drug-induced liver disease. Certain hepatotoxins require timely treatment with specific antidotes, such as *N*-acetylcysteine for acetaminophen hepatotoxicity. Intravenous steroid treatment has been beneficial when severe acute hepatitis dominates a multisystemic hypersensitivity reaction, as with phenytoin, carbamazepine, or phenobarbital. In general, the use of steroids in drug-induced liver disease remains controversial. The treatment of fulminant hepatic failure owing to drug hepatotoxicity is otherwise essentially the same as in viral hepatitis. Liver transplant may be lifesaving in these circumstances.

When the major intervention is to stop a drug treatment, arriving at the diagnosis of drug-induced liver disease becomes all important. A high index of clinical suspicion is critical to making the diagnosis. A meticulous history of the illness with detailed attention to all drugs taken, including over-the-counter preparations, and to the potential for exposure to environmental or industrial toxins is of utmost importance. In children, it is important to ensure that the appropriate dosage was actually given. Liver biopsy, with electron microscopic examination if possible, is often very informative and sometimes definitive. Algorithms for determining the likelihood of an adverse drug reaction,[270] especially those specifically developed for drug hepatotoxicity,[271–273] may be helpful diagnostically. A recent study[274] showed that the Council for International Organizations of Medical Sciences scoring system[271] is superior to that of Maria and Victorino[273]; however, neither is specifically geared for children. In vitro rechallenge of the patient's lymphocytes with generated toxic metabolites usually provides important corroborative evidence,[275] but this remains a research investigation. Rechallenge assays using immunologic end points are often difficult to interpret.

SUMMARY

Drug-induced hepatotoxicity is more common in children than is generally thought. As in adults, the spectrum of disease is wide. Although cytotoxic processes, presenting as hepatitis, predominate, virtually every major type of hepatic pathology can occur. Hepatic drug metabolism has an important role in most of the drugs that most frequently cause hepatotoxicity in children: an imbalance between generation of toxic metabolite and detoxification processes can be identified. Focal defects in detoxification, often responsible for this imbalance, may be inherited. Developmental changes in drug disposition and metabolism further complicate the clinical spectrum of drug hepatotoxicity in children. Developmental and genetically determined aspects of immune function may also play a role. The possibility of drug hepatotoxicity should be considered in every instance of childhood liver disease.

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CHAPTER 54

LIVER TUMORS

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Understanding and treating liver tumors in children continues to be a formidable task. The very rarity of such tumors contributes to the difficulty because few individuals or centers compile sufficient experience to provide definitive direction. Additionally, the remarkable diversity of conditions that fall under the term "tumor" makes it difficult to design a unifying formula to approach the subject or individual patient. Another problem is the extraordinary functional capacity of the liver to compensate for the intruding mass, so clues to the presence of a tumor are often few and so late as to make simple removal impossible. Finally, the liver's anatomy encourages internal dissemination of neoplasms and taxes the skills of the most experienced surgeon. Nevertheless, recent advances in knowledge of the molecular biology of gene expression and cellular differentiation, experimental carcinogenesis, monoclonal antibodies for diagnosis and perhaps even treatment, imaging techniques and anesthesia, and transplant immunology make this a time for optimism. But of all of the scientific advances, the single most important advance has already been achieved and is being implemented: vaccination against hepatitis B virus (HBV). In regions where hepatitis B is endemic, such as Taiwan, hepatocellular carcinoma (HCC) has accounted for 13% of all cancers in patients less than 15 years of age. By interrupting the cycle of mother to newborn transmission, vaccination has already begun to eliminate the most important single cause of hepatic malignancy.[1] Prevention of hepatitis C virus (HCV) transmission in blood products will further reduce the incidence of cirrhosis and HCC in adults. Thus far, no children with HCV and liver cancer have been reported. However, two 20-year childhood leukemia survivors developing HCC exhibited antibodies to hepatitis C at the time of diagnosis.[2]

Estimates of the incidence of primary hepatic tumors suggest that they account for about 0.04 to 0.16% of 1,000 US hospital admissions and 0.5 to 2.0% of all pediatric cancers. About three-quarters of the collected tumors in large series worldwide (Table 54-1) are malignant, and 85% of those are of hepatocellular origin.[3] Hepatoblastomas comprise about 43% of all primary hepatic tumors. They occur in about one child per million under the age of 15 years (about 100 cases per year) in the United States.[4]

There is a strong possibility that all series and reports are biased toward malignancy, unusual cases, and unusual circumstances. The relative contribution of referral centers to surgical surveys and national statistics is uncertain, and the use of death certificates without autopsy verification is unreliable. The incidence of hepatoblastoma appears to be increasing,[4] possibly because of the survival of extremely premature infants (< 1,000 g).[5] It was found that 58% of all hepatoblastomas in the Japanese Children's Cancer Registry occurred in children who weighed less than 1,500 g at birth.[6] The relative risk for hepatoblastoma in premature versus term infants was 15.6 for those weighing less than 1,000 g, 2.5 for birth weights of 1,000 to 1,499 g, and 1.2 for birth weights in the 2,000 to 2,499 g range. The Children's Cancer Group (CCG) of the United States found that 13.9% of 72 patients who had gestational histories recorded in their series were premature.[7] Ten of the 18 patients weighed less than 1,000 g at birth—a 16- to 23-fold excess versus term infants. Four percent of the hepatoblastoma patients in the German Registry (3 of 77) were premature infants who required parenteral nutrition,[8] suggesting a potential source of mutagens. Three premature infants treated with long-term parenteral nutrition have developed hepatocarcinoma,[9] but the German Registry reports the first hepatoblastoma–parenteral nutrition association.

ETIOLOGY

Worldwide, HBV has been responsible for more malignancy than any other environmental agent. Among adults, there is a definite relationship to chronic hepatitis and macronodular cirrhosis, and at least 20 years of infection

TABLE 54-1 PRIMARY LIVER TUMORS IN CHILDREN (18 SERIES WORLDWIDE)

TUMOR	NUMBER (%)
Hepatoblastoma	539 (43)
Hepatocarcinoma	287 (23)
Adenoma	23 (2)
Hemangioma, hemangioendothelioma	171 (13)
Mesenchymal hamartoma	76 (6)
Sarcoma	80 (6)
Focal nodular hyperplasia	22 (2)
Other	58 (5)
Total	1,256 (100)

Adapted from Weinberg AG and Finegold MJ.[3]

seem to be required for neoplastic transformation. The occurrence of HCC in children as young as 3 years of age following perinatal exposure to carrier mothers is surely an important clue to the carcinogenic process. Only 2 of 173 black South Africans with HCC under age 30 lacked serologic evidence of HBV infection. One hundred percent of Taiwanese children with HCC are HBV carriers.[10] The younger the child, the less often there is evidence of active hepatitis and cirrhosis. HBV functions like a retrovirus, with reverse transcriptase activity providing the means toward deoxyribonucleic acid (DNA) replication. As with other carcinogenic retroviruses, the DNA may become integrated into the host genome, and there may be associated deletions of portions of the cellular genome, but the integration site is variable, and mutations in the virus may be important.[11] Increasing reports of cell-cycle gene disruptions[12,13] may lead us to anticipate additional specific mutations as molecular methods are applied to HBV-associated HCC. The reason why children exposed to HBV as infants develop HCC so quickly may be that the integration of viral DNA is facilitated by the rapid rate of cell division in the developing liver. Perhaps the effects of early viral DNA integration on hepatocyte differentiation would explain why three of the five HCCs in children with HBV infection described by Ohaki and colleagues[14] contained primitive hepatoblastic foci. We have examined seven tumors containing both HCC and hepatoblastoma in children having a mean age of 8.5 years. All were HBV positive.

At least three-quarters of the mothers of children with HBV infection and HCC in Africa, China, and Japan display hepatitis B surface (HB_s) antigenemia. Curiously, only a minority of the fathers have antigenemia.[10] Even more perplexing is the observation that 27 of 28 fathers of children with HCC in Senegal lacked antibody to the HB_s antigen compared with 48% of age-controlled males in the same population.[15] Eighteen percent of those fathers had HB_s antigenemia, in contrast to 71% of the mothers. The role of gender in the development of liver tumors is indeed noteworthy. Among adults, the male-to-female HCC ratio is said to be 8:1 to 10:1. Most of the difference is attributed to chronic HBV hepatitis with cirrhosis, industrial or occupational exposure, and alcoholism. Only 1 of 15 HB_s antigen–positive mothers of Taiwanese children with HCC had HCC herself, and no malignancy developed in any of their 9 HB_s antigen–positive sisters. However, 5 of 13 HBV-carrying brothers had HCC.[10] Even with underlying genetic diseases and metabolic errors having an autosomal recessive (sex neutral) basis, such as familial adenomatous polyposis,[16] familial cholestatic cirrhosis,[17] and type I glycogen storage disease, the incidence of hepatocellular malignancy in boys is at least double that of girls. Coire and colleagues described liver adenomas in 24 males and 12 females with von Gierke disease.[18] All four patients who later developed carcinomas were males (Figure 54-1).

It is stimulating to consider these clinical observations in light of studies on steroid hormone receptors in normal and neoplastic liver tissue. Iqbal and colleagues found androgen receptors in fetal liver and HCC but not in normal adult liver, whereas estrogen receptors were detected in both tumor and adjacent liver.[19] Nagasue and colleagues were able to detect androgen receptors in normal male liver, and 18 of 23 carcinomas had a significantly higher concentration.[20] The cirrhotic liver of one woman also had androgen receptors. But one of two HCCs in that same liver lacked androgen receptors. Both Nagasue and colleagues[20] and Ohnishi and colleagues[21] found a loss of estrogen receptor activity in HCC versus the surrounding liver tissue. When aplastic or Fanconi anemia patients of either sex are treated with C17–alkylated anabolic steroids, tumors develop with significant frequency, and tumors have also been observed when testosterone was given to correct sexual immaturity in boys.[3] Boys accounted for 25 of the 34 androgen-associated liver tumors in patients less than 20 years of age reviewed by Chandra and colleagues.[22] Although half were discovered at autopsy, just two were judged to be carcinomas. Some of the benign tumors regressed on withdrawal of steroids.

Estrogens in oral contraceptives are definitely associated with hepatic adenoma development. After 8 years of oral contraceptive use, the incidence of hepatic carcinoma in women is 4 to 20 times that of age-matched controls when alcoholism, hepatitis, and cirrhosis are excluded.[23] Focal nodular hyperplasia is a non-neoplastic process that has to be distinguished from adenomas and carcinomas.[24] For a short time, it appeared that this lesion was more common in women. It now seems that the lesion is more often symptomatic in women because oral contraceptives make it more vascular and more likely to bleed. Case reports associate angiosarcoma and cholangiocarcinoma with oral contraceptives. Additionally a 19-year-old girl was found to have a hepatoblastoma after 15 months of "pill" use.[25] Prenatal exposure to synthetic estrogens or gonadotropins has been reported in two infants with hepatoblastoma[3] and one with angiosarcoma.[26] The Children's Cancer Study Group (CCSG) looked for risk factors in

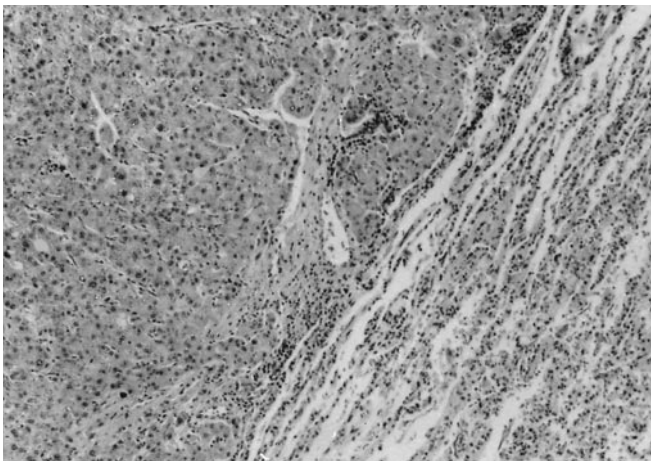

FIGURE 54-1 Hepatocarcinoma metastatic to the lung. A well-differentiated malignancy from a 17-year-old boy who had glucose-6-phosphatase deficiency, managed successfully by frequent and nocturnal feedings. Adenomas were recognized in this liver 3 years earlier. If the illustrated tumor were in the liver, it would be impossible to predict its behavior from histology alone (hematoxylin and eosin; ×63 original magnification).

75 cases of hepatoblastoma versus age-matched controls and found a significantly higher frequency of maternal exposure to metals and petroleum products. Paternal exposure to metals was also excessive.[27]

The importance of oncogene expression in the genesis of hepatic malignancy has been augmented by recent studies of tumor suppressor genes, cell-cycle genes, and growth factors. Baffett and colleagues found expression of N-ras messenger ribonucleic acid (RNA) in 11 of 11 hepatocarcinomas.[28] In 7 of 11 tumors, c-Ki-ras and c-Ha-ras were detected, whereas c-myc and fos were expressed in 2 of 11 tumors. The finding that *c-met* is overexpressed in a significant (8 of 18) proportion of HCCs is intriguing because the protein is a receptor for hepatocyte growth factor, a potent stimulant of hepatocyte mitosis.[29] Overexpression of c-met conferred a worse prognosis (33% 5-year survival versus 80% in low expressors).[30] Likewise, the role of transforming growth factor-α (TGF-α) in hepatocellular malignancy appears to be important. It is produced by fetal hepatoblastoma cells with low proliferative activity[31] and by 65% of hepatocarcinomas.[32] It is a strong stimulus of hepatocyte replication,[33] and, interestingly, only male mice overexpressing TGF-α develop hepatocarcinomas.[34] Telomerase activity in nontumoral regenerative nodules of patients with cirrhosis and hepatocarcinoma was equal to that in the neoplasm and predictive of early recurrence or second primaries.[35] In hepatoblastomas, the most significant genetic abnormalities involve the chromosome 11p15 region, in which the Beckwith-Wiedemann locus is found (see below). Genes for insulin and insulin-like growth factor 2 (IGF-2), both stimulants of fetal cell growth, are located in the same region. Loss of imprinting and loss of heterozygosity for IGF-2 and IGF-2 receptor have both been observed in hepatoblastoma.[36,37] Overexpression of IGF-2 has also been demonstrated in hepatoblastomas by microarray expression technology.[38] That report also revealed surprising breast cancer (BRCA2) expression in hepatoblastomas and binding of antibody to BRCA2 protein by six of six hepatoblastomas but not normal fetal liver.[38]

The exact role of *p53* in the pathogenesis of hepatic neoplasia remains uncertain. No liver tumors were found when the *p53* gene was inactivated by homologous recombination in mice (in whom several other cell types developed neoplasms).[39] When small early HCCs in humans were examined, *p53* expression was normal, whereas 8 of 22 cancers at an advanced stage had mutations.[40] Interestingly, six of those eight cases that were informative also had deletions of the retinoblastoma gene product. The activation of HIC-1, hypermethylated in cancer, a candidate tumor suppressor, by *p53* may be meaningful because messenger RNA for HIC-1 is markedly reduced in precancerous nodules of cirrhosis and even more so in poorly differentiated carcinomas.[41] In Japan, serum antibodies to *p53* were associated with decreased survival.[42]

Many metabolic defects and congenital malformations are associated with and possibly contribute to hepatocellular malignancy.[3] They are listed in Table 54-2. The highest frequency of HCC in a metabolic disease of childhood was found with tyrosinemia type 1, owing to inactivity of fumarylacetoacetate hydrolase.[43] The 18 to 37% incidence of HCC reported in survivors of the infantile period has been reduced by the recent discovery that an inhibitor of an upstream enzyme (4-hydroxyphenylpyruvate dioxygenase) reversed the clinical and chemical abnormalities in four of five children with fumarylacetoacetate hydrolase deficiency.[44] By 2000, over 200 children with tyrosinemia were treated with the inhibitor 2-(2-nitro-4-trifluoromethyl benzoyl)-1,3 cyclohexane dione prior to the age of 2 years. Just two have developed hepatocarcinoma, one of whom (whose treatment began at 5 months of age) was 15 months old when the malignancy was discovered.[45]

Kingston and colleagues found that hepatoblastoma occurred in five families with intestinal polyposis.[46] With an incidence of familial adenomatous polyposis of 1 per 8,300 and an incidence of hepatoblastoma of one per million children under the age of 15 years, this cannot be coincidental. One of 20 hepatoblastomas occurs in a polyposis kindred. Hughes and Michels have pointed out that despite the 500 to 1,000 excess of hepatoblastoma in families with familial adenomatous polyposis, less than 1% of the families are at risk.[47] Garber and colleagues expanded the number of affected families to 25; there were 18 boys and 7 girls.[48] Eleven of the 25 patients have survived, including all but one of the girls but only five of the boys. Six of the seven survivors examined developed colonic polyps as early as 7 years of age. Bodmer and colleagues localized the adenomatous polyposis coli gene (*APC*) for familial polyposis to chromosome 5q21,[49] and it is now possible to screen young children in families with polyposis (Gardner syndrome) for the presence of the gene by a variety of molecular tools. Mutations of *APC* were found in 8 of 13 sporadic hepatoblastomas,[50] but no cytogenetic

TABLE 54-2 PRECURSORS OF HEPATIC NEOPLASIA

PERINATAL EXPOSURE
Oral contraceptives
Phenytoin
Ethyl alcohol
NEONATAL FACTORS
Extreme prematurity
METABOLIC DISEASE
Tyrosinemia
von Gierke disease, glycogenosis type I
Glycogenosis III and IV (case reports)
MALFORMATIONS
Hemihypertrophy, Beckwith-Wiedemann syndrome
Von Recklinghausen neurofibromatosis
Soto syndrome
Multiple hemangiomatosis
Ataxia-telangiectasia
Fanconi syndrome
Budd-Chiari syndrome
BILIARY TRACT DISEASE
Extrahepatic atresia
Familial cholestatic cirrhosis
Alagille syndrome
Parenteral alimentation
DRUGS
Oral contraceptives
Anabolic steroids

abnormalities at chromosome 5q21 have been described (Table 54-3). The mechanism of carcinogenesis by germline *APC* mutations appears to be mediated via a cell adhesion protein that accumulates in cells with the mutation.[51] Activating mutations in β-catenin have been observed in 48% of sporadic hepatoblastomas[52] and 20 to 26% of hepatocarcinomas.[53,54]

Several other cytogenetic abnormalities have been seen in children with hepatic neoplasms (see Table 54-3). Trisomy 2 was complete or partial in 26 of 40 hepatoblastomas, and trisomy 20 was found in 29 of 40 cases.[55,56] A unique t(10;22) was reported in one small cell hepatoblastoma.[57] The relatively small number of reported cytogenetic aberrations in hepatoblastoma may relate to the lack of suitable dividing cell samples. When conventional cytogenetic analyses are limited by the lack of suitable metaphase cells, fluorescent in situ hybridization techniques applied to frozen or paraffin-embedded tissues may reveal abnormalities in chromosome number and structure.[58] Among HCCs, the frequent allelic deletions at 17p (38 to 54%) correlate with deficient p53 function.[59] Among the many other deletions observed in HCC, those at chromosomes 16q and 4q have not been observed in other cancers, so they may be the most meaningful. Molecular genetic analysis of hepatocellular neoplasms has begun to provide important insights into dysregulation of growth. Those occurring with significant frequency are shown in Table 54-4.

Also noteworthy are reports of HCC in relation to chronic biliary tract disease, including extrahepatic biliary atresia, Byler disease, and bile salt export protein deficiency.[60,61] A 6-month-old boy received parenteral alimentation all his life and was found at autopsy to have a microscopic focus of carcinoma.[62] Two more cases have been described.[8] Arteriohepatic dysplasia (Alagille syndrome) was previously not regarded as a preneoplastic condition because 85 to 90% of affected individuals survive without serious hepatic complications of their childhood-onset cholestasis and bile duct paucity. But several patients with the syndrome have been described with HCC, even in the absence of biliary cirrhosis; the youngest was a $3^1/_2$-year-old girl,[63] and there were three affected siblings in one family.[64]

CLINICAL MANIFESTATIONS

Regardless of cell type, the great majority of hepatic tumors are first detected as a mass or abdominal swelling. Upper abdominal pain is the next most frequent presenting complaint, followed by anorexia and weight loss, vomiting, and diarrhea. Infants with vascular hamartomas (hemangiomas) may display signs of congestive heart failure, as have rare patients with mesenchymal hamartomas. Pruritus and frank jaundice are observed when tumors obstruct bile flow. In children, obstruction suggests rhabdomyosarcoma at any level of the biliary tract. Minor blunt trauma or apparently spontaneous hemorrhage of a liver tumor can be the earliest sign of its presence, especially among adolescent girls and young women taking estrogens for oral contraception, when tumors are especially vascular.

Several metabolic effects or paraneoplastic syndromes occur with a variety of hepatic tumors. Hypercalcemia with marked osteopenia can be very severe in children with hepatoblastoma, carcinoma, or sarcoma. As in other malignancy-related hypercalcemias, the mechanism is not fully understood, but ectopic parathormone production is not the reason.[65] Hyperlipidemia has been associated with epithelial malignancies; when caused by hepatoblastoma in infants, it has been associated with early fatality.[66] Both hyperlipidemia and hypoglycemia are thought to be secondary to injury to the remaining liver or dysfunction of the neoplastic epithelial cells rather than a sign of underlying enzymatic error, such as glucose-6-phosphatase deficiency. The rare carcinomas in von Gierke disease do not appear until the midteens or early adulthood.[18] A few case reports of HCC in children and adults with debrancher and

TABLE 54-3 CYTOGENETIC ABNORMALITIES IN LIVER NEOPLASMS

NEOPLASM	ABNORMALITY	INCIDENCE (%)
Hepatoblastoma	Trisomy 20	72 (40 cases)*
	Trisomy 2	65 (40 cases)*
	Trisomy 8	32.5 (40 cases)
	Chromosome 1 translocation	8 cases
	Trisomy 18	3 cases
Hepatocarcinoma	1q excess by CGH	58–72
	4q losses by CGH or LOH	43–77
	8q excess by CGH or LOH	48-77
	16q LOH	52–70
	17p loss or LOH	49–51
Mesenchymal hamartoma	Translocations of 19q13.4	3 cases
	Aneuploidy	2 of 8 cases
Undifferentiated (embryonal)	Monosomy 20	2 cases
	Sarcoma aneuploidy dicentric telomeric association (4;22)(p16;q13)	1 case
Inflammatory myofibroblastoma (pseudotumor)	Hyperdiploidy	4 of 9 cases
	2p23 rearrangements (*ALK* abnormalities)	Unknown for liver

CGH = comparative genomic hybridization; LOH = loss of heterozygosity.
*Two cases each in which trisomy 20 or 2 was the only karyotypic abnormality.[55,56]

TABLE 54-4 MOLECULAR GENETICS OF HEPATOCELLULAR NEOPLASMS

GENE OR LOCUS	ABNORMALITY	TUMOR TYPE	TUMORS AFFECTED (%)
HIC1 (17p13.3)	Hypermethylation, LOH, ↓ expression	HCC	90
CDK4 (12q13–15)	↑ Expression	HB	88
Telomerase (3q26.3)	↑ Expression	HCC	74
Cyclin D_1 (11q13)	↑ Expression	HB	70
p53 (17p13)	Codon 249 mutation	HCC	67 in aflatoxin regions, < 15 elsewhere
FAS (CD95L)	↓ Expression	HCC	64
IGF2 (11p15.5)	LOH or LOI	HB	64
E-cadherin (16q22.1)	Hypermethylation ↓ Expression	HCC	58
TGF-β (2p13)	↑ Expression	HCC	54
β-catenin (3p22–p21.3)	↑ Expression	HB	48
RB (13q14)	LOH	HCC	47

HB = hepatoblastoma; HCC = hepatocellular carcinoma; IGF = insulin-like growth factor; LOH = loss of heterozygosity; LOI = loss of imprinting; TGF = transforming growth factor.

branching enzyme deficiencies (glycogen storage disease III and IV) have appeared recently. Thrombocytosis and polycythemia have been observed in some HCC patients, and some 60% of children with hepatoblastoma have thrombocytosis at presentation. Precocious puberty in males has been observed with hepatoblastomas and carcinomas. In most cases, ectopic gonadotropin production is responsible, but a few instances of tumor testosterone synthesis have been reported.[67]

IMAGING TECHNIQUES

Real-time ultrasonography is the hepatologist's "stethoscope." In Japan, where the incidence of hepatic malignancy is high and screening resources are readily available, Okuda compared the various imaging modalities in adults.[68] They found none to be sufficiently sensitive to detect HCCs smaller than 2 cm or to discriminate between regenerating nodules or adenomas and HCCs. Repeat ultrasonography has shown the doubling time for carcinomas to be variable, with growth from 1 to 2 cm taking an average of 3 months. The most proliferative tumors grew from 1 to 3 cm in 4.6 months. Ultrasonography, combined with serum α-fetoprotein measurements, can detect early lesions susceptible to resection in the presence of cirrhosis. Intraoperative ultrasonography has proven a useful aid to partial hepatectomy in children.[69]

Arterial injections of enhancing compounds during computed tomography (CT) or magnetic resonance imaging (MRI) provide more expensive and complex means of detecting small intrahepatic lesions with questionably greater sensitivity than ultrasonography. The application of MRI is especially useful for distinguishing small and common hemangiomas from solid tumors.[70] MRI also shows spread of tumor into large abdominal veins very clearly. By including iodized oil (Lipiodol) in the arterial infusate and taking delayed CT images, cancers as small as 3 mm have been observed because the oily material is retained only by the tumor. CT is useful for scanning the chest and abdomen for other sites of involvement when a liver mass is present. Enhanced CT and MRI have superseded angiography in delineating the extent of disease prior to partial hepatectomy (Figure 54-2).[70] Scintigraphy with radiolabeled sulfur colloids has been used to distinguish lesions containing Kupffer cells (focal nodular hyperplasia) from those without Kupffer cells (adenomas and carcinomas).[71] However, some hepatoblastomas have produced scintigraphic images indistinguishable from those of benign masses. Imaging with radiolabeled monoclonal antibody to α-fetoprotein proved helpful in managing a child whose hepatoblastoma could not be fully resected.[72]

LABORATORY TESTS

Serum α-fetoprotein measurement is the most useful marker of malignant liver tumors. Eighty to 90% of hepatoblastoma patients and 60 to 90% of HCC patients have elevated levels at diagnosis. Small cell undifferentiated hepatoblastomas and rhabdoid tumors in infancy do not produce α-fetoprotein.[73,74] Except for a very few infant mesenchymal hamartomas and germ cell and yolk sac tumors, there are no false indications of hepatocellular malignancy when serum α-fetoprotein levels exceed

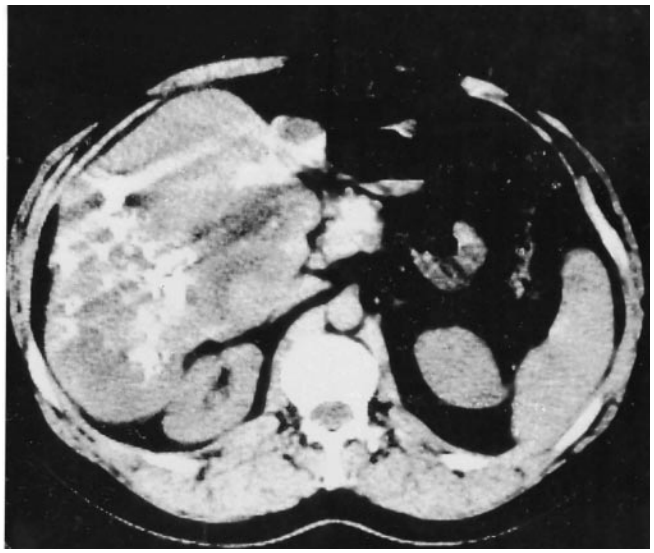

FIGURE 54-2 Computed tomography of the hepatoblastoma shown in Figure 58-4. The larger inner zones of calcification are visualized as stellate radiopaque zones, whereas the epithelial portions of the mass are difficult to distinguish from host liver.

500 ng/mL after 3 months of age. Serum α-fetoprotein is elevated, although not to such high levels, in the absence of demonstrable carcinomas in both hereditary tyrosinemia and ataxia-telangiectasia.[3] Both conditions are associated with a high frequency of hepatic malignancy, and the secretion of the fetal protein is indicative of defective regulation of gene expression in the hepatocyte. Regrettably, α-fetoprotein levels may not be elevated in time for effective therapy. For example, elevations may not occur until ordinary HCCs exceed 4 to 5 cm in diameter, which can take several years.[68] Intrahepatic portal vein dissemination has been observed in both hepatoblastomas and primary carcinomas less than 3 cm in diameter. Measuring serum α-fetoprotein to follow recurrences of resected liver tumors can be helpful. However, a return to normal levels has been observed in some patients even though their tumors continued to grow.

Pseudouridine, a catabolic product of transfer RNA, was detectable in the urine of 9 of 13 patients with HCC whose serum α-fetoprotein concentration was normal.[75] Plasma transcobalamin I (vitamin B_{12}–binding protein) and neurotensin have been elevated in patients with fibrolamellar carcinoma (FLC), which is potentially very useful because only 10% of those tumors have increased α-fetoprotein.[71,76,77] An abnormal form of prothrombin, desgamma-carboxyprothrombin, was present in the serum of 74% of 70 adult HCC patients and in blood and tumor cells of three children with hepatoblastoma.[78] Coagulation tests were unaffected. There was no correlation with serum α-fetoprotein levels, and the test was insensitive for carcinomas less than 3 cm in diameter.

Because of the low incidence of childhood hepatocellular malignancy in the United States, screening of children in the United States or elsewhere would be unrewarding. But application of some of these sensitive tools to the small groups of patients with precursor or associated conditions, such as extreme prematurity, tyrosinemia, hepatic glycogenoses, and chronic cholestatic conditions, could be lifesaving.

PATHOLOGY

HEPATOBLASTOMA

This is an embryonal tumor in the classic sense of incomplete differentiation. Ninety percent of the cases are manifest by the fourth birthday, and several have been present at birth. The usual composition reflects the complex origin of the organ, with endodermal derivatives from the original midgut outgrowth and mesodermally derived offspring of the septum transversum. Thus, parenchymal elements include hepatocytes of varying maturity, resembling the early embryonal or later fetal liver, in association with hematopoietic cells (Figure 54-3). Primitive ducts are characteristic of the embryonal pattern, but well-differentiated ductal elements are unusual except in relation to diffusely infiltrating mesenchymal or blastemal cells. In that situation, they represent residual and sometimes proliferating cholangioles of the host liver because they are not found in metastases. However, when ductal epithelium and tumor cells in the middle of a mass are in continuity on ultrastructural examination, it is difficult to regard them as normal remnants.[79] It is not unusual to find portions of the epithelial hepatoblastoma to be indistinguishable from HCC, even in the youngest patients.[3] Undifferentiated mesodermal and/or mature stromal derivatives are present in 20 to 30% of cases. The differentiated stroma usually includes osteoid-like material but less often skeletal muscle or cartilage (see Figure 54-3). The osteoid is clearly related to epithelial rather than mesenchymal cells. Not infrequently, keratinizing squamous nests are found among the embryonal cells. Rarely, ducts resembling primitive intestine, neural rosettes, and melanocytes suggest the possibility of a true teratoma.[80]

Depending on the proportions and degree of maturation of the various elements, the gross appearance ranges from yellow-brown (well-differentiated epithelium) to pink-gray (undifferentiated mesenchyme). Focal necrosis and hemorrhage are found in rapidly growing tumors, and firm, even gritty, areas are present when osteoid is abundant. Generally large multinodular expansile masses, hepatoblastomas appear to be well demarcated from the normal host liver but are not encapsulated (Figure 54-4). They may invade hepatic veins and disseminate to the lungs by the time of discovery or penetrate the capsule to reach contiguous tissues and the peritoneum. Hilar lymph nodes are early targets. Staging of hepatoblastomas is done at diagnosis. Stage I indicates complete resection; II, microscopic residual tumor (IIA, inside the liver; IIB, outside the liver); III, gross residual tumor (IIIA, spillage during surgery or gross nodal involvement; IIIB, incomplete resection with or without spillage or node involvement); and IV, metastatic disease (IVA, primary completely resected; IVB, primary not completely resected). Our review of 316 hepatoblastomas from US Pediatric Oncology Group (POG) contributors from 1986 to 2002 had 79 (25%) stage I, 24 (7.5%) stage II, 153 (48%) stage III, and 60 (19%) stage IV cases. The hepatoblastoma histology classifications by Ishak and

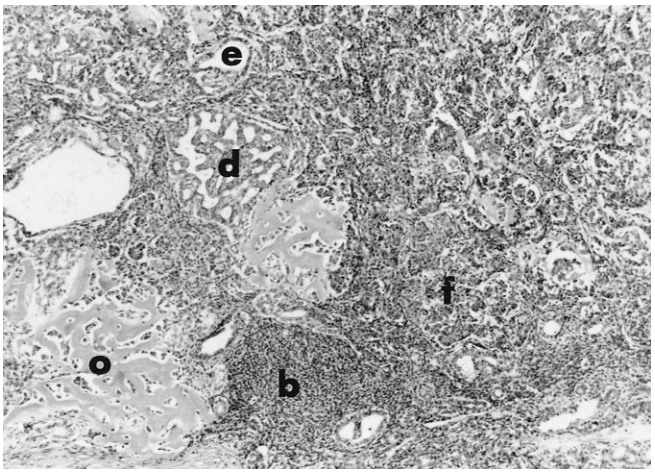

FIGURE 54-3 Hepatoblastoma. The diversity of cell types and varying degrees of maturation are demonstrated in this classic embryonal neoplasm. b = undifferentiated blastemal cells; d = ductular epithelium; e = embryonal epithelium; f = fetal epithelium; o = osteoid (hematoxylin and eosin; × 63 original magnification).

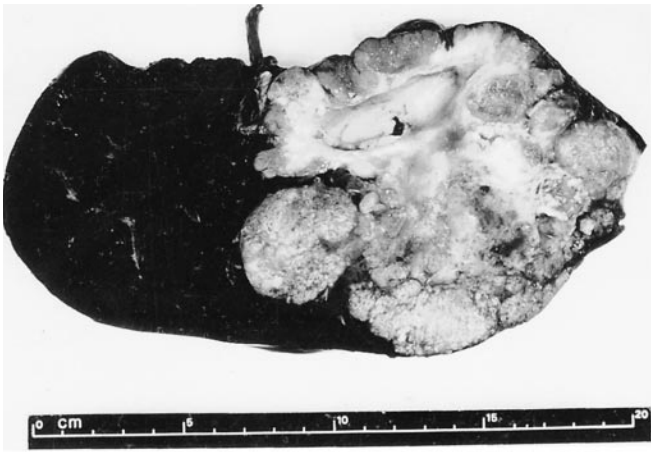

FIGURE 54-4 Hepatoblastoma. A multilobular expansile yellowish tan mass of mixed epithelial and mesenchymal tissues has foci of cystic degeneration and bone. It appears clearly demarcated from the host liver, which is normal, but there is no encapsulation.

TABLE 54-5 CLASSIFICATION OF HEPATOBLASTOMA

MAJOR CATEGORIES
Epithelial
Fetal, well differentiated (mitotically inactive, diploid)
Crowded fetal (mitotically active)
Embryonal
Macrotrabecular
Small cell undifferentiated
Mixed
Undifferentiated mesenchymal-blastemal
MINOR COMPONENTS
Ductal (cholangioblastic)
Osteoid
Keratinizing squamous epithelium
Intestinal glandular epithelium
Neuroid-melanocytic (teratoid)
Rhabdomyoblastic
Chondroid
EXCEPTION
Rhabdoid

Glunz[81] and Kasai and Watanabe[82] provide the foundation for current microscopic assessment of hepatoblastoma. Incorporating minor modifications from Gonzalez-Crussi and colleagues[83] and Manivel and colleagues,[80] we evaluated tumors in the POG-CCG intergroup study according to the scheme shown in Table 54-5. In the current US intergroup study, the stage I (well-differentiated fetal hepatoblastoma with less than 2 mitoses per 10 high-power fields) has been singled out for surgical treatment without adjuvant therapy. Only 9 of the 316 cases (2.8%) in the POG series qualified, but the decision to spare children with such completely resected tumors the morbidity of adjuvant therapy seems soundly based. Early observations of 100% survival rate in a small group of such tumors treated with surgery alone[3] were augmented by similar cures when surgery was combined with minimal doses of doxorubicin[84,85] or standard chemotherapy.[86]

Quantification of DNA by image analysis and flow cytometry demonstrated good correlation with prognosis (71% 3-year survival for diploid tumors versus 31% for aneuploid lesions).[87] Six of seven pure fetal tumors were diploid (ie, "favorable"), whereas each of two embryonal epithelial tumors was aneuploid. Ruck and colleagues described another important correlation: p53 expression by immunostaining was lacking in fetal areas of eight tumors and mesenchymal areas of four tumors.[88] However, p53 was expressed in both small cell tumors and in embryonal regions of two of eight tumors examined.

Unresolved questions about the relation of histology to outcome relate to the following:

1. The prognostic import of the degree of differentiation of the fetal epithelial component, which often displays gradual but sometimes abrupt transition from a uniformly well-differentiated pattern with few mitoses to a more crowded but still cord- or plate-like architecture in which nuclei are more pleomorphic and mitoses are more numerous (Figure 54-5). Do such tumors require standard chemotherapy if totally resected?
2. The significance of the proportion of prognostically important histology. Rowland recently provided insightful quantitative analysis pertinent to the limitations of prior published associations between histology and outcome.[89] The relation of completely excised well-differentiated pure fetal histology with low mitotic activity to outcome is unquestionable. However, the lack of uniformity in definition of other histotypes combined with limited sampling of unresectable tumors prior to chemotherapy presents challenges to the desired histology outcome paradigm.

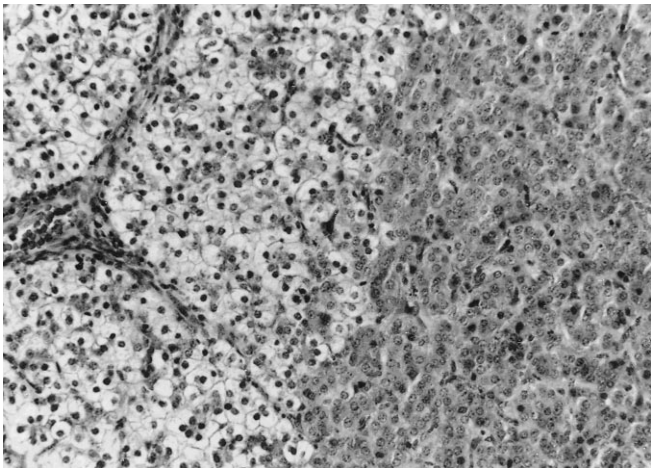

FIGURE 54-5 Hepatoblastoma. The well-differentiated fetal pattern is on the left. Regular cords or plates, one to three cells thick, contain hepatocytes having glycogen-rich clear cytoplasm and regular uniform nuclei and rare mitoses (none in this picture). Immediately adjacent, on the right, the regular cordlike pattern is maintained, but the cells are more numerous and crowded without being macrotrabecular (five to six cells in thickness) or primitive, as in embryonal tumors. The growth rate is increased, as reflected by the presence of two mitoses. A well-differentiated fetal pattern has been associated with an excellent rate of resection and cure. The "crowded" pattern is of uncertain prognostic significance (hematoxylin and eosin; ×160 original magnification).

3. The influence of the differentiated stromal derivatives on prognosis. Muraji and colleagues[66] and Haas and colleagues[84] found that mixed tumors, particularly in stage II–III cases, responded better to chemotherapy than did pure epithelial lesions. However, the impossibility of determining the totality or proportion of all histologic constituents from biopsies of unresectable tumors must be acknowledged.
4. The impact of small undifferentiated cells on prognosis. As the name indicates, these cells fail to demonstrate histologic or ultrastructural features of any known hepatocellular or ductal element, but they contain both cytokeratin filaments typical of epithelial cells and vimentin, a mesenchymal component.[74] Their mitotic activity is variable. Of the 5% of hepatoblastomas that are pure small cell, all but two cases occurred in infants less than 6 months of age, and all such patients have died of disease despite chemotherapy that is highly effective for other histologic subtypes.[85] The role of small cell undifferentiated histology in completely resected hepatoblastoma is becoming more clear.[90] Ten patients with such tumors suffered a relapse in periods ranging from 2 to 21 months. Five whose tumor recurred died. The proportion of small cell histology in four of the fatal cases exceeded 75% of the tissue available for examination, whereas the other fetal case and all of the five survivors had less than 5% small cells. It may be important to note that just three of eight such patients treated with contemporary adjuvant therapy suffered relapse, but none died.[90]
5. The histogenesis of the "rhabdoid" cell type. These monomorphic malignancies of diffusely infiltrating, noncohesive cells with large quantities of intermediate filaments have arisen in several tissues. They tend to occur in young infants, to disseminate widely, and to resist chemotherapy.[73] Immunohistochemical and ultrastructural studies indicate the cells to have both epithelial and mesenchymal characteristics.[91] In the liver, this cell may represent a neoplastic derivative of a stage in the maturation of the undifferentiated mesoderm to the hepatocyte. Except for the presence of epithelial membrane antigen, the immunohistochemical reactions resemble the small undifferentiated or "blastemal" cells of the hepatoblastoma exactly.[92] We have observed transition to "rhabdoid" morphology in otherwise typical hepatoblastomas (Figure 54-6).[3]
6. The significance of postchemotherapy microscopic residual disease. In addition to the greater abundance of osteoid, it is common to find complete necrosis of tumor within hepatic and portal vein branches that are obliterated by scar.[93,94] Nevertheless, nodules of viable tumor are usually found despite marked clinical regression. Most often these have bland fetal histology, but all patterns may be observed, as they are in metastases. The expression of the cell-cycle regulatory protein kinase inhibitory protein (Kip 1 or P27) was markedly reduced in nuclei of residual fetal hepatoblasts after chemotherapy compared with the same cell type before treatment: from a mean of 75% positive tumor cells to 12%.[95] This difference was not associated with increased DNA synthesis, as determined by Mib1 immunostaining. It is not clear which factors govern the susceptibility or resistance of hepatoblasts to current therapeutic regimens, but von Schweinitz and colleagues have emphasized the importance of post-chemotherapy complete resection before development of drug resistance.[96]

HEPATOCARCINOMA

The frequency of cirrhosis in pediatric patients with HCC is much less than in adults (20–25% versus 60–70%), but its presence compounds the therapeutic problem. The appearance of the tumor is similar in children and adults. HCC has a higher frequency of multiple nodules than does hepatoblastoma and exhibits intrahepatic portal vein and lymphatic dissemination more often at the time of diagnosis. A wide range of histologic appearance seems to have little or no influence on resectability or responsiveness to therapy, with one possible exception: the FLC may express different behavior in adults than it does in children.

FLC is rarely associated with cirrhosis, rarely produces α-fetoprotein, and tends to affect young persons. In contrast to the more typical HCCs, just one FLC has been associated with Fanconi anemia.[97] Thirty-nine percent of FLC patients are less than 20 years old, and 90% are less than 25 years of age. Forty-five of 80 tumors occurred in girls, and Malt has called attention to the high incidence of reproductive dysfunction among the group.[71]

The POG-CCG intergroup study has demonstrated that children with FLC do not have a favorable prognosis and do not respond any differently to current therapeutic regi-

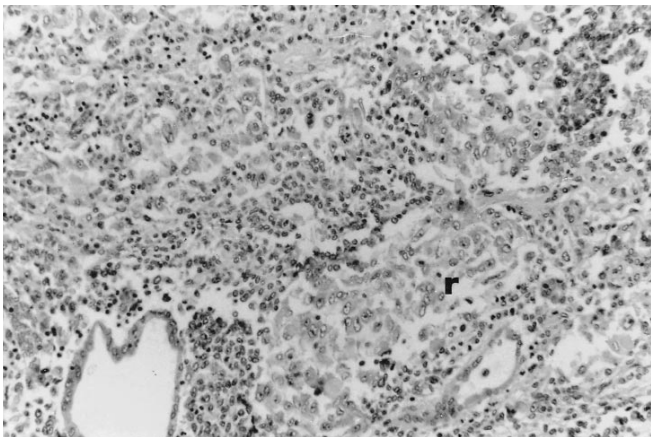

FIGURE 54-6 Hepatoblastoma with focal "rhabdoid" transformation. On the left, fetal-type epithelial components of the neoplasm admixed with hematopoietic cells abut what is probably a residual (non-neoplastic) bile duct. A transition to noncohesive cells of uncertain differentiation is seen in the center right (r). Those cells have larger, vesicular nuclei with prominent nucleoli and perinuclear cytoplasm that is rich in intermediate filaments, producing eosinophilic inclusions in some cells. When tumors are homogeneous for such cells, they are called "rhabdoid." In the liver, they tend to occur in infants younger than 1 year of age, and they behave badly (hematoxylin and eosin; ×160 original magnification).

mens when compared with typical HCC.[98] Although children with initially resectable typical HCC or FLC have a good prognosis (75% 5-year event free survival) irrespective of histologic subtype, outcome is uniformly poor for children with advanced-stage disease.

FLC tends to be a single large bulky light tan to yellow-orange mass with distinct borders. Histologically, large polygonal cells cluster in small groups separated by bands of well-organized collagen (Figure 54-7). Carcinoembryonic antigen and fibrinogen are abundant in the tumor, and the serum concentration of carcinoembryonic antigen has been observed to fall after resection.[99] The tumor cells also tend to be rich in copper and to express the biliary cytokeratin 7 that is not found in ordinary HCC.[100] Intense immunohistochemical staining of TGF-β demonstrable in 9 of 11 FLCs was present in just 3 of 14 typical hepatocarcinomas.[101] The morphologic differences between FLC and ordinary HCC are further reflected in the secretion of neurotensin and by histochemical and ultrastructural evidence of neurosecretory granules.[102] None of these tumors has been associated with clinically evident hormonal effects, and they do not morphologically resemble any of the several cases of primary hepatic carcinoid tumors examined.[3] The exact histogenesis of this epithelial neoplasm is unresolved.

Adenoma and Focal Nodular Hyperplasia

Each condition contributes 2% of the collected series of childhood liver tumors (see Table 54-1). Both adenomas and focal nodular hyperplasia have been observed in patients with glycogen storage disease[18] and in women using oral contraceptives, but only adenoma seems to be causally related to "the pill."[23,24] Both are usually solitary and expansile, but adenoma is more often multiple and is generally encapsulated. Both consist primarily of well-differentiated hepatocytes arranged in cords or plates but without the normal lobular pattern. However, the cells of adenomas may be slightly larger than normal hepatocytes, and their nuclei can be slightly pleomorphic. Adenomas lack bile ducts or portal tracts, and focal nodular hyperplasia has septa radiating from a central region of scarring in which ducts can be numerous. In a needle biopsy, these distinguishing features may be unavailable.

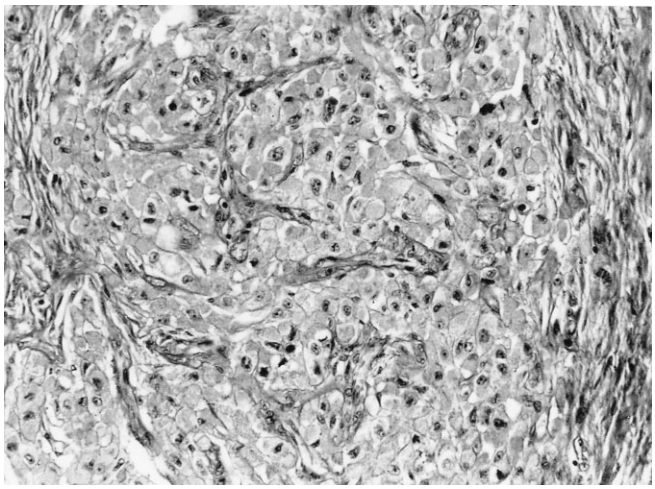

FIGURE 54-7 Fibrolamellar carcinoma. Nests of large polygonal cells with abundant eosinophilic cytoplasm and slightly pleomorphic nuclei are separated by distinct bundles of collagen. Ordinary hepatocarcinomas have little fibrous tissue unless there is a ductal (cholangiolar) component. This was from a 19-year-old girl with normal serum α-fetoprotein. Resection was curative (trichrome; ×160 original magnification).

Vascular Tumors

Hemangioendotheliomas of infancy are the most common benign tumors of the liver and are generally regarded as hamartomas rather than neoplasms. Nevertheless, they may be symptomatic as mass lesions or may produce high-output congestive heart failure owing to arteriovenous shunting. They may rupture and cause intraperitoneal hemorrhage. Occasionally, thrombocytopenia and intravascular coagulation have been observed.[103] Classification depends on the degree of endothelial cell proliferation and the size of the channels. When actively dividing vasoformative cells are plentiful and not quite organized into channels, the lesions are called type II hemangioendotheliomas.[104] The type II lesions have reportedly disseminated occasionally.[104] Type I lesions consist of definite vessels, with few mitoses and no nuclear atypia. Calcification is frequent. Most cases of both types regress spontaneously or respond well to corticosteroid therapy. However, perfectly bland type I lesions have been followed, on at least four occasions, by hepatic angiosarcomas. At that point, atypical rapidly dividing neoplastic cells were widely dispersed through the sinusoids as well as filling vascular lumens and replacing parenchymal tissues.[3] Two additional angiosarcomas in children appeared histologically to have arisen in preexisting hemangioendotheliomas.[26] Sometimes the spleen and other organs have been involved. One infant had documented exposure to arsenicals. The other had been exposed to metals, glue, and oral contraceptives during the first trimester.[26] Two young adults with neurofibromatosis have had hepatic angiosarcomas.[105] A lymphangioendothelioma confined to the liver almost completely replaced the hepatic parenchyma of a newborn.[106] Hepatic angiomyolipomas have been reported in 24% of children with tuberous sclerosis.[107]

Mesenchymal Tumors

The mesenchymal hamartoma of infancy can be present at birth, grow to an enormous size, and cause heart failure because of arteriovenous shunting. Over 90% of cases are manifest in infancy, but individual cases have been observed in adults, the oldest patient being 28 years old.[108] The mass may bulge from the liver and even become pedunculated, but it has no capsule (Figures 54-8 and 54-9). Typically, there are multiple large cystic spaces with a flat endothelial or biliary epithelial lining and serous fluid content (Figure 54-10). The stroma is myxomatous and bland. At the interface with the remaining parenchyma, bile ducts proliferate actively. Seven reports document simultaneous occurrence or subsequent development of undifferentiated or embryonal hepatic sarcoma in mesenchymal hamartoma.[109–115] The frequently observed translocation

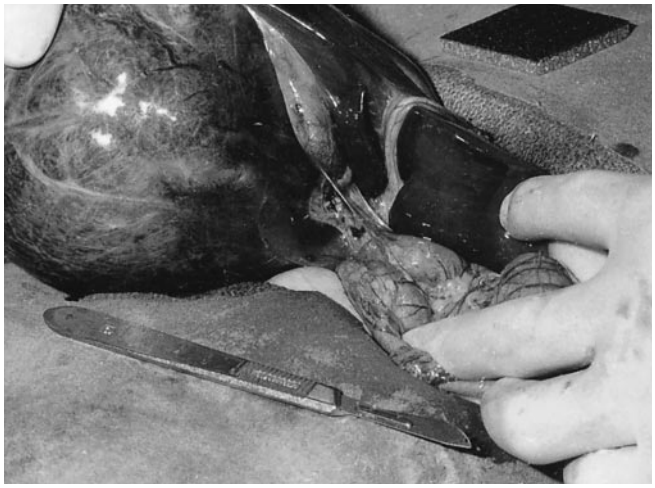

FIGURE 54-8 Mesenchymal hamartoma at surgery. An 11-month-old boy presented with a distended abdomen. Ultrasonography revealed a multilocular cystic mass. It protruded from the right lobe inferiorly and was completely resectable.

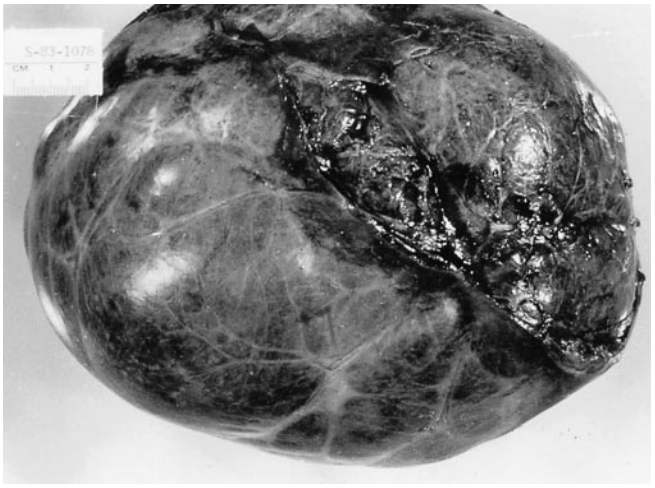

FIGURE 54-9 Mesenchymal hamartoma after resection. The huge bulging mass weighed 1,250 g and consisted mainly of tense cysts. The patient had about 35% of the liver left and recovered uneventfully.

involving chromosome 19q 13.4 in mesenchymal hamartomas has not been described in sarcomas, however. The treatment of unresectable cystic lesions by drainage and marsupialization is undesirable in light of this association.

Malignant mesenchymomas were so named because of the multiple derivatives of stromal cells they contain, including myxoid, chondroid, muscular, bony, and fibrous tissues. Since the report by Stocker and Ishak,[116] most authors have referred to the lesion as an undifferentiated or embryonal sarcoma, even though many of the tumors have indeed had regions of fibrous histiocytoma, liposarcoma, and even benign pericytoma.[117] Half of the cases have presented in children between the ages of 6 and 10 years. Embryonal undifferentiated sarcoma tends to be huge and unresectable at presentation. The liver is found to be replaced by a variegated, hemorrhagic, cystic mass of gray-white soft tissue (Figure 54-11). Microscopically, the undifferentiated aspect is characterized by huge bizarre cells having prominent glycoprotein inclusions associated with small nondescript cells and abundant myxoid stroma (Figure 54-12). One case has occurred in a family with Li-Fraumeni syndrome, in which germline mutations of *p53* have been found.[118] Comparative genomic hybridization of sarcomas has revealed multiple amplifications and deletions, none of which involve the locus affected in mesenchymal hamartoma.[119] The prognosis of these lesions was generally very poor, but reports from Germany,[120] Italy,[121] and the United States[122] indicate that these sarcomas may respond to intensive chemotherapy, with cisplatinum, doxorubicin, and ifosfamide in most regimens.

Rhabdomyosarcomas of the biliary tract tend to form polypoid masses of soft, gelatinous, pink-gray tissue that

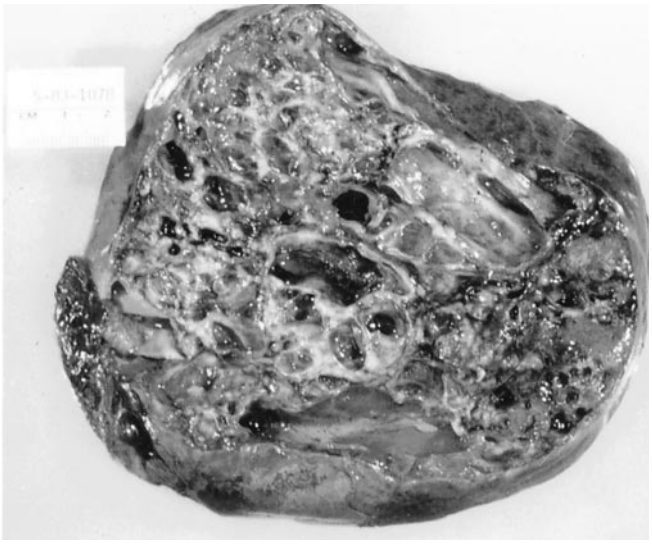

FIGURE 54-10 Mesenchymal hamartoma after sectioning. Multiple cysts filled with serous fluid are separated by myxomatous stroma.

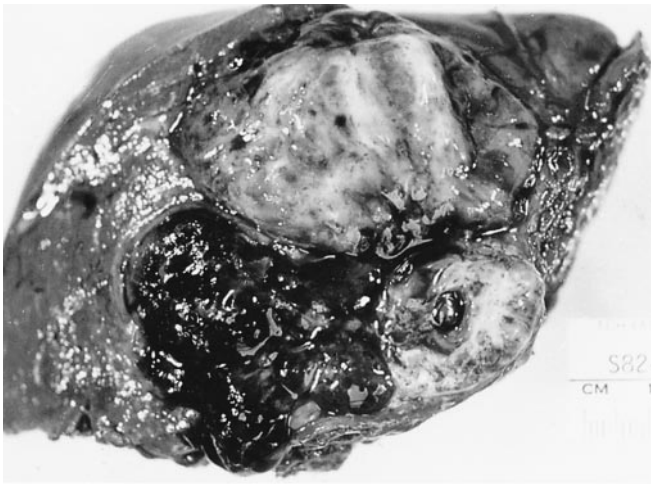

FIGURE 54-11 Undifferentiated sarcoma replaces most of the right lobe in a 6-year-old boy. A multinodular fleshy mass with large areas of hemorrhage and cystic degeneration, it gives the impression of encapsulation but, in fact, insinuates into the surrounding host liver.

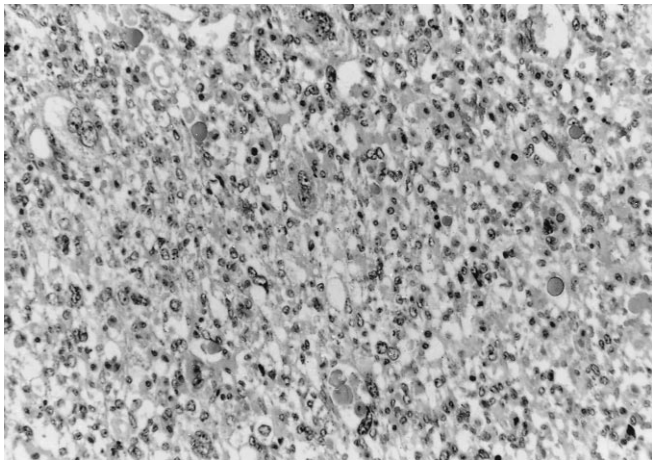

FIGURE 54-12 Undifferentiated sarcoma. Scattered among numerous small and nondescript cells are several giant cells with multilobate vesicular nuclei having prominent nucleoli. Large globular inclusions of glycoprotein are regularly present in the cytoplasm of such cells and sometimes spill out into the adjacent loose ground substance. Portions of such tumors have differentiated into fibroblasts and fibrohistiocytic, lipoblastic, and chondroid cells (hematoxylin and eosin; ×160 original magnification).

tend to obstruct bile flow. The cells are generally primitive embryonal forms with rare, ill-defined muscle filaments. All bile ducts, from ampulla and gallbladder, can be affected. Patients have ranged in age from infancy to the teens. Two of the eight cases reported by Geoffray and colleagues were resectable, but both recurred.[123] In the series of Mihara and colleagues, six of nine patients responded well to vigorous chemotherapy and radiotherapy.[124]

Rhabdoid tumors of the liver were mentioned in the discussion of hepatoblastoma. One such tumor was found in a 14-day-old boy with a primitive neuroectodermal tumor of the cerebrum that contained similar cells,[125] just as has been observed for renal rhabdoid tumors.

A 9-year-old girl with acquired immune deficiency syndrome (AIDS) is the twentieth and youngest child with leiomyosarcoma arising in the liver.[126] No human immunodeficiency virus (HIV) DNA was detected in her tumor. Unlike most such tumors in immunodeficient children,[127] this one was negative for Epstein-Barr virus. Granular cell myoblastomas, which actually arise from Schwann cells, are benign tumors that can arise in the bile ducts. Eighty-seven percent of the cases in the United States have been in the black population. Two have been described in teenagers.[128]

Bile Duct Epithelial Tumors

Biliary cystadenomas with mesenchymal stroma are benign tumors in young women but have malignant potential in middle-aged women. Cholangiocarcinomas have occurred in three adults with congenital hepatic fibrosis, the mild form of infantile polycystic disease.[129] We have observed cholangiocarcinoma in a 1-year-old boy with bile salt excretory protein deficiency. Carcinomas have been observed in the remnants of choledochal cysts, so surgeons have learned to excise the affected region.[130] Two cases of cholangiocarcinoma have been reported in young women with chronic ulcerative colitis and sclerosing cholangitis. One patient was 17 years old.[131]

Teratomas

Primary teratomas are rare and usually affect females; half contain undifferentiated elements. When yolk sac tissue (endodermal sinus tumor) is present or the tumor is wholly yolk sac in type, α-fetoprotein levels can be strikingly increased. It was extremely high (77,000 μg/L) in a 17-month-old child with adjoining benign cystic teratoma and mixed hepatoblastoma and whose preoperative imaging studies identified only the benign tumor.[132] Ninety percent of teratomas are in infants. Treatment is surgical.

Inflammatory Myofibroblastic Tumor, Lymphoma, and Leukemia

Inflammatory myofibroblastic tumor was originally called inflammatory pseudotumor or plasma cell granuloma. Anthony and Telesinghe described 17 patients with liver involvement, 6 of whom were less than 12 years old.[133] They presented with fever, abdominal pain, and/or vomiting. Jaundice was evident in four patients. Weight loss and diarrhea were also noted. There were solitary masses in eight patients and multiple nodules in four. The clinical impression and initial pathologic diagnosis may be confused with malignancy, but the histologic features of dense plasma cell infiltrates associated with active fibroplasia are indications of a chronic inflammatory process. The etiology is unknown, but some tumors have regressed with steroid therapy. On the other hand, the finding of hyperdiploidy in four of nine inflammatory pseudotumors, three of which recurred or metastasized,[134] suggested a neoplastic process. Rearrangements of the *ALK* gene at chromosome 2p23 have been observed in several inflammatory myofibroblastic tumors but not specifically in those involving the liver.[135] Portoenterostomy has been required for biliary obstruction in some cases. Transplant was needed for an 8-year-old girl whose hilar region was so extensively involved as to preclude resection.[136] Primary lymphomas of the liver of children are very rare and have monomorphous infiltrates without fibrosis.[137] Involvement of the liver in various forms of leukemia is common but only rarely of clinical significance. An exception is the megakaryoblastic leukemia of infancy, which can present with hepatomegaly and abnormal liver function tests prior to peripheral blood manifestations.[86] Liver biopsy shows diffuse infiltration of sinusoids by blast cells whose nature may not immediately be apparent. Platelet glycoprotein 2b immunostaining will be diagnostic. Diffuse scarring is prominent and a useful clue to the diagnosis.

Non-neoplastic Hepatic Masses

Non-neoplastic tumors include parasitic cysts, biliary and simple cysts, and nodular regenerative hyperplasia. All but the latter tend to present as masses and rarely because of jaundice. Most are diagnosed readily by a combination of imaging techniques, particularly ultrasonography. Nodular regeneration may present with signs of portal hypertension and is sometimes associated with collagen-vascular dis-

ease. The diagnosis is difficult even on biopsy, but neoplasia can be excluded. Two cases of maldeveloped fetuses with nodular regeneration have been described.[138]

Secondary Neoplasia of the Liver

Neuroblastoma is the most common solid tumor metastasis in children. The primitive cells infiltrating sinusoids may be mistaken for hepatoblastoma in routine microscopic studies of small biopsies. Other malignancies spreading to the liver include Wilms tumor, rhabdomyosarcoma, Ewing sarcoma, intra-abdominal small cell desmoplastic tumor, and ovarian germ cell tumors. Among the 123 cases submitted for review to CCG and POG pathologists in the hepatoblastoma/hepatocarcinoma 1986 to 1989 intergroup study, 13 incorrectly diagnosed cases (10.6%) included examples of each of the above.[86]

TREATMENT

The primary goal in treating liver neoplasms is complete surgical removal. The tendency of tumors to reach very large size before discovery and an anatomy that allows interlobar spread are handicaps. For benign tumors, such as vascular hamartoma, focal nodular hyperplasia, and adenoma, extensive surgery may be unnecessary. Many benign lesions have responded to medical management or arterial embolization, and many are stable indefinitely.[103] Therefore, vigorous efforts are made to reach a diagnosis preoperatively and intraoperatively. When that is not possible or when malignancy is suspected, surgical resection is attempted. Newer imaging modalities have been helpful in delineating the extent of involvement preoperatively[70] and intraoperatively.[69] When initial surgery for a malignancy is deemed to be too risky, preoperative chemotherapy has proven effective in shrinking many hepatoblastomas and some sarcomas to the point of resectability. Beginning in 1981, Gauthier and colleagues have routinely used doxorubicin in combination with vincristine, cyclophosphamide, 5-fluorouracil, or cisplatinum, achieving resectability in over 80% of cases.[139] The POG was able to achieve resectability 29 of 37 (79%) hepatoblastomas with cisplatinum, vincristine, and 5-fluorouracil.[140] Seventy-seven percent of those resected had no evidence of disease at 13 to 54 months, a result identical to that observed among 26 patients who had primary resections. These good results have led some centers to treat all new cases with chemotherapy, sometimes without even a biopsy if the imaging studies and serum α-fetoprotein point to neoplasia.[141,142] This approach is deemed undesirable for three reasons.[143] The first is based on the concept of "favorable" histology discussed (above and) below. Primary resection of a stage I tumor permits a thorough histologic study. If such a tumor has favorable histology, toxic chemotherapy can be minimized or even eliminated. Second, in both the latest CCG[144] and POG[140] trials, the major surgical complications after chemotherapy (hemorrhage or bile duct injury) were significantly greater than those occurring after primary resections (CCG, 25% versus 8%; POG, 23% versus 0%). Finally, small cell undifferentiated histology in otherwise typical, completely resected hepatoblastomas was associated with recurrence and fatality in at least 6 of 10 patients.[86,91] This unfavorable histology requiring alternative adjuvant therapy can easily be missed in a small biopsy.

The same chemotherapeutic regimens used preoperatively for unresectable tumors are then employed postoperatively. Nine patients with hepatoblastoma so treated by Gauthier and colleagues were cured,[139] and there have been case reports of pulmonary metastases being eliminated by such intense regimens. Recently, irinotecan has been added for unresponsive hepatoblastomas by the US Children's Oncology Group. The incidence of severe hematologic toxicity in the CCSG–Southwest Oncology Group study of 57 patients with hepatic malignancies given combination chemotherapy was 50%, and 3 children died of the complication.[145]

When completely resected lesions (stage I), which comprise 15 to 50% of different series, have been reviewed according to histologic subtype, the "pure" or "predominantly" fetal tumors were found in several series to have a good prognosis even without adjuvant therapy.[82,87] An analysis of 168 hepatoblastomas collected by the US Intergroup Study found 28 of 90 fetal cases to be resectable, and 87% of them survived 48 months.[85] In those series, the fetal cases were not further classified into "well-differentiated with low mitotic rate," a requirement that Weinberg and Finegold emphasize is necessary for a "favorable" designation.[3] Only 15% of 333 hepatoblastomas in the Intergroup,[85] Armed Forces Institute of Pathology,[146] and POG studies through 1989[140] were primarily resectable, but 92% (47 of 51) were disease free beyond 2 years. Embryonal tumors behaved less well in the early Intergroup series, with 20 of 50 cases being resectable and 63% of those surviving 48 months. All received combination chemotherapy. Tumors with a macrotrabecular growth pattern were uniformly unresectable, but 9 of 18 survived 48 months, which also is much more favorable than in all other reports to date. Just one of the small cell undifferentiated tumors was resectable, but all 10 children with small cell undifferentiated histology died within 24 months.[86] The POG and CCSG have since treated nine stage I well-differentiated fetal hepatoblastomas with doxorubicin for 4 months, with no recurrences in 5 years.[87] Patients with more advanced disease and unfavorable histologic patterns were treated with cisplatinum, vincristine, and 5-fluorouracil or cisplatinum and doxorubicin by continuous infusion; both groups had excellent results for stage I and II tumors and 64% event-free survival even for stage III. The former regimen had lesser toxicity.[87]

HCCs are resectable only 10 to 20% of the time. Nine of 46 HCCs in the latest intergroup study were resectable. Eighty-eight percent of those patients survived 5 years without disease on the same regimens as above. Neither regimen was beneficial for the higher stages of disease.[147] A better result was reported from Johns Hopkins University School of Medicine, where external beam radiation (2,100 cGy), chemotherapy, and radioiodinated antiferritin antibody were combined.[148] Forty-eight percent of the patients survived 5 years without evidence of tumor. Starzl and colleagues have performed liver transplants in selected

cases with modest success.[149] Many of the failures were in the precyclosporine era, so early death owing to graft rejection or other complications limited evaluation. Starzl and colleagues reported transplant success in three of six patients with unresectable FLCs.[149] On the other hand, they also concluded that aggressive resection was preferable because only one of eight patients with a subtotal hepatectomy suffered a recurrence. A more recent review of the University of Pittsburgh School of Medicine experience with orthotopic liver transplant for liver cancers includes six children with hepatoblastomas, of whom five are alive with no evidence of disease at 1.9 ± 0.5 years, and nine with HCCs, with four being disease free for 1.2 years.[150] One additional patient with HCC who had upper abdominal exenteration is alive 14 months later. Six transplanted patients died of malignancy, five with recurrences and one of lymphoma. More recent transplant data indicate improved transplant expectations for HCC provided that tumors were not multicentric, larger than 5 cm, and did not exhibit vascular invasion, metastasis, or positive margins at initial surgery.[151] There is active discussion among surgeons caring for children with hepatic neoplasms about the potential benefits of primary transplant versus attempted resections in anatomically problematic cases.

CONCLUSION

The likelihood of new metabolic interventions to prevent hepatic malignancies, such as that for tyrosinemia, is currently highly speculative. However, rapidly increasing knowledge about molecular interactions of growth control and gene expression, as in the APC–β-catenin pathway, offers great promise for targeting loci that might be sensitive to manipulation, perhaps by specific inhibitory RNA, for example. Until these measures became clinically applicable, however, the potential benefits of careful periodic screening with the existing tools of ultrasonography and serum α-fetoprotein in susceptible populations such as survivors of extreme prematurity, chronic cholestasis, and the glycogenoses seem worthy of general application.

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CHAPTER 55

GENETIC AND METABOLIC DISORDERS

1. *Carbohydrate Metabolism*

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An infant is brought into the office or seen as an inpatient consultation. You ask yourself, "Is this conglomeration of clinical features the result of a defect of hepatic carbohydrate metabolism? If it is, how do I make the diagnosis? How do I manage the infant in the short term? What are the long-term consequences of this disease state? And how do I explain the disease to the family?" The intention of this chapter is to assist in answering these questions.

A brief review of normal hepatic glucose, fructose, and galactose metabolism will allow the individual metabolic defects that have been identified in humans to be placed within a physiologic context. Each enzyme defect is explored in terms of clinical presentation, genetics, pathogenesis and clinical consequences, treatment, and prognosis.

Apart from the classic pathways of carbohydrate metabolism (ie, glycolysis/gluconeogenesis), other pathways involve carbohydrate moieties. Examples include defects of glucuronidation, as in Crigler-Najjar syndrome, in which glucuronide conjugation with bilirubin is interrupted; defective glycosylation of intracellular proteins, as in I-cell disease, in which mannose 6-phosphate fails to be added to proteins intended for lysosomal compartmentalization; and defective glycosylation of glycoproteins, as occurs in the carbohydrate-deficient glycoprotein syndromes. Defects in these pathways may have hepatic implication but lie outside the scope of this chapter.

INITIAL MANAGEMENT OF SUSPECTED CASES OF INBORN ERRORS OF CARBOHYDRATE METABOLISM

The first suspicion that a defect in carbohydrate metabolism may be underlying a child's illness arises from the understanding of the presenting features of this group of conditions. Clinical features that may be associated with disorders of hepatic carbohydrate metabolism are shown in Table 55.1-1. Initial investigations may include those suggested in Table 55.1-2. In many circumstances, an infant may be acutely unwell and in need of care prior to the results of diagnostic tests being available. The following steps should be taken while awaiting the results of diagnostic investigations:

- Measure the blood glucose and correct hypoglycemia with intravenous (IV) glucose solutions aiming at 8 to 9 mg of glucose/kg/min, an infant's normal rate for hepatic glucose synthesis. Avoid protein and lipid input until more diagnostic information is available (there may be an inborn error of metabolism other than one affecting carbohydrate metabolism).
- Collect blood and urine cultures followed by empiric broad-spectrum antibiotics. *Escherichia coli* sepsis is a particular association with galactosemia. Rule out disseminated viral syndromes, particularly herpesviruses.
- Measure prothrombin time and partial thromboplastin time and consider correcting coagulopathy if present.
- Manage any other features of liver failure.
- Provide general supportive measures such as rehydration and management of acidosis.

The approach to investigation and management of infants with potential metabolic defects has been excellently reviewed in a number of publications.[1–3]

TABLE 55.1-1 POSSIBLE PRESENTING FEATURES OF DEFECTS OF CARBOHYDRATE METABOLISM

Neonate	Hypoglycemia, vomiting, diarrhea, lethargy, poor feeding, sepsis syndrome, lactic acidosis, jaundice, hemolysis, hypotonia, seizures, liver failure
Infant	Hypoglycemia or hypoglycemic seizure, failure to thrive, episodic vomiting, hepatomegaly, abnormal liver function tests, chronic liver disease, cataracts
Child	Hepatomegaly, failure to thrive, short stature, anomalous eating behavior/sugar avoidance, developmental delay

TABLE 55.1-2 SUGGESTED INITIAL INVESTIGATIONS

Blood/plasma/serum samples
Electrolytes
Liver function tests
Coagulation studies (prothrombin time/partial thromboplastin time)
Blood gases
Blood glucose
Insulin level
Lactate and pyruvate
Free fatty acid and 3-OH-butyrate
Urate
Triglycerides and cholesterol
Phosphate and magnesium
Red blood cell galactose 1-phosphate uridyl transferase activity
Sugar chromatography
Urine sample
Ketones
Reducing substances

Many of these investigations will be most informative if collected at the time of hypoglycemia. Therefore, when suspecting an inborn error of carbohydrate metabolism in a sick infant, it is important to attempt to collect the samples immediately at presentation because the opportunity for "safe" hypoglycemia may not present itself for some time. In other cases, a controlled fast should be arranged. In many cases, this list will be in addition to specific investigations of infantile liver disease and/or a full metabolic workup depending on specific clinical features.

NORMAL CARBOHYDRATE METABOLISM IN HUMANS

Glucose is the main energy source for most tissues in the human body; therefore, the liver's role in maintaining glucose homeostasis is one of fundamental importance. Glucose derived from dietary intake is intermittent, and it is the responsibility of the liver to maintain the circulating glucose pool within relatively confined limits during fasting and following a feed. To do this, the liver needs to be able to take up and store excess carbohydrate to be released as glucose at times of need. Glucose can be generated either through the pathways of gluconeogenesis or by the degradation of stored glycogen. Although there are gluconeogenic tissues other than liver, such as muscle and kidney, the only cell type capable of exporting free glucose in significant amounts is the hepatocyte. Regulation of glucose uptake and release is under hormonal control. At times of high dietary glucose intake, insulin increases and promotes the uptake of glucose and the laying down of glycogen by the liver cells. As the time from the last feed increases, insulin levels fall, glucagon levels increase, and

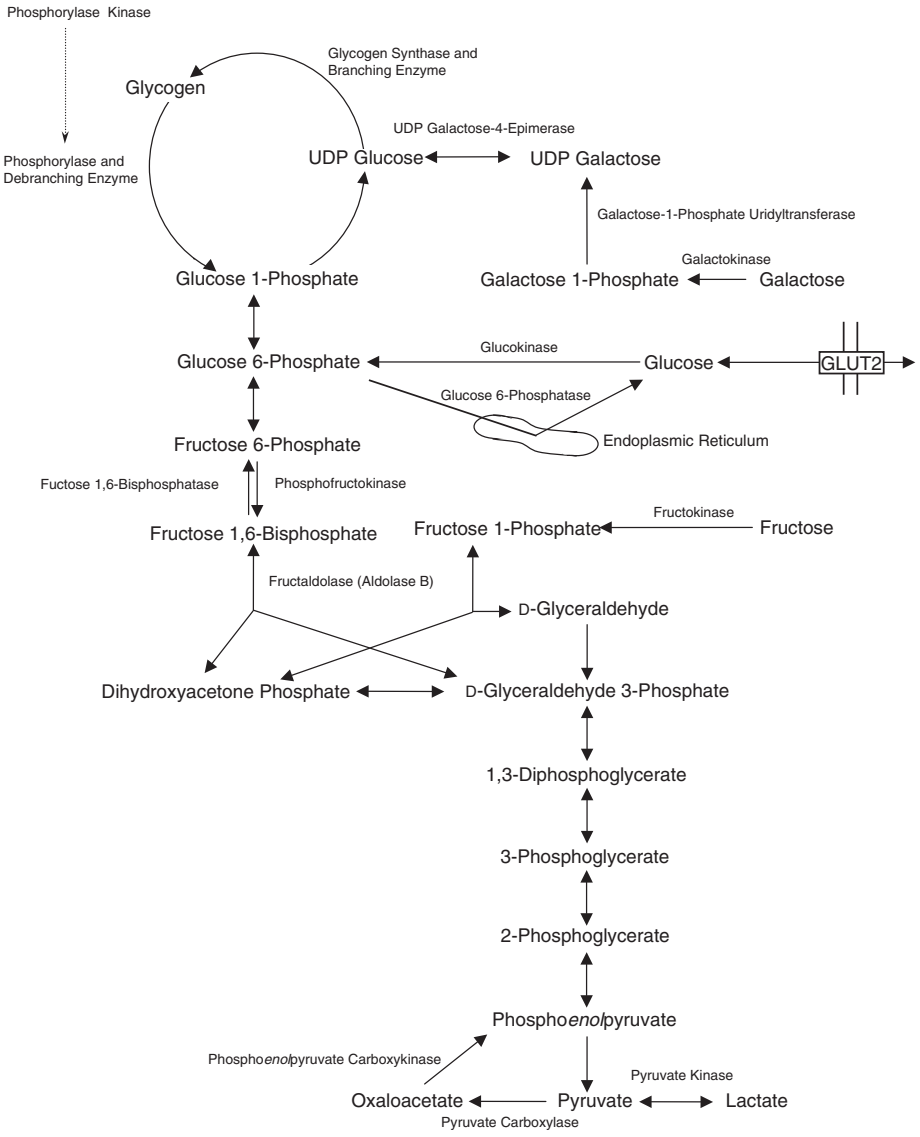

FIGURE 55.1-1 Major pathways of normal hepatic carbohydrate metabolism. Enzymes known to be affected in inborn errors of hepatic carbohydrate metabolism are shown. GLUT = glucose transporter; UDP = uridine diphosphate.

glucose synthesis and release by the liver cells increase. Hepatocytes will also synthesize and release glucose in response to epinephrine as part of the "fight or flight" response. The ability of hepatocytes to generate free glucose is due to the expression of glucose 6-phosphatase, the enzyme required for the dephosphorylation of glucose 6-phosphate. The pathways of normal carbohydrate metabolism are outlined in Figure 55.1-1.

The majority of dietary carbohydrates are absorbed in the form of glucose, galactose, and fructose. Glucose and galactose primarily enter the hepatocytes via the facultative glucose transport (GLUT) protein GLUT2. The GLUT proteins, a family of transmembrane monosaccharide transporters, are present in all human tissues. GLUT isoforms vary by tissue type, with hepatocytes expressing primarily GLUT2. The 12 transmembrane domains are thought to form a hydrophilic tunnel through the hydrophobic lipid cell membrane. Alteration in protein configuration, triggered by the concentration gradient, exposes the specific monosaccharide-binding site either inside or outside the membrane, allowing movement of glucose in both directions. GLUT proteins transport glucose only into most cells because, unlike hepatocytes, they lack net synthesis of glucose. Exceptions to the one-way transport of glucose include, in addition to the hepatocyte, enterocytes and kidney tubular cells. Fructose transport is less clearly understood. All three sugars ultimately enter the common pathway of glycolysis/gluconeogenesis following phosphorylation by specific kinases.

To enable a rapid and consistent generation of free glucose during periods of fasting, a form of storage is essential. This is primarily in the form of glycogen, a branched-chain glucose polymer. Many other tissues in the body have the enzymes required for synthesis and breakdown of glycogen, but most glycogen is found in liver and muscle. In muscle, glycogen is stored as an energy source to be used at times of high energy expenditure by muscle fibers themselves, whereas liver exports glucose to maintain circulating glucose pool and supply the other tissues of the body. Glycogen can be seen on electron microscopy in the form of β particles in muscle and liver and also as larger α particles in liver. The chemical differences to account for the morphologic difference between α and β particles have not been explained. The β particle consists of one molecule of glycogen with the proteins required for glycogen metabolism and some minor constituents, such as glucosamine and inorganic phosphate, the function of which is not clear.

The initiating event in glycogen synthesis is autoglycosylation of the protein glycogenin.[4] This protein not only acts as the substrate to which a glucose residue is covalently bound, it also actively catabolizes the reactions for the polymerization of the growing glucose chain up to a length of 6 to 10 glucose residues. At this stage, further chain lengthening is controlled by glycogen synthase, using uridine diphosphate (UDP) glucose and catalyzing the formation of 1,4-α linkages with the terminal glucose residue of the growing polymer. This produces straight-chain lengthening; the characteristic branching of these chains is catalyzed by another enzyme, amylo-(1,4),(1,6)-transglucosidase ("branching enzyme"). Branching enzyme disrupts 1,4-α bonds, at least six glucose residues from the terminal residue, and reforms a branch point with 1,6-α linkages.

Under hormonal control, glycogen metabolism is able to switch rapidly from net synthesis to net breakdown. Insulin induces glycogen synthesis, but both glucagon and epinephrine can override this mechanism and induce glycogen breakdown to increase glucose production. The hormonal signals have opposing effects on the phosphorylation of key enzymes for both synthesis and breakdown of glycogen. Following a feed, under the influence of elevated insulin levels, glycogen synthase is activated by dephosphorylation, whereas glycogen phosphorylase (responsible for glycogen degradation) is inhibited. In the fasting state, with glucagon predominating, the enzymes are again phosphorylated, which reduces the activity of glycogen synthase, and glycogen phosphorylase is activated.

Glycogen degradation requires two enzymes. Phosphorylase cleaves the 1,4-α link of terminal glycosyl units, releasing glucose 1-phosphate. Phosphorylase is activated by the action of phosphorylase kinase. This cytosolic protein kinase is itself activated by phosphorylation, via cyclic adenosine monophosphate–dependent protein kinase, and by binding ionic calcium. Phosphorylase continues degrading glycogen, one residue at a time, until only four glycosyl residues remain beyond a branch point. At this stage, debranching enzyme is needed for further degradation of the glycogen molecule. Debranching enzyme uniquely possesses two independent catalytic sites on a single polypeptide strand. Initially, the three terminal residues beyond the branch point are moved to the end of another chain using transferase activity. The actual debranching activity (amylo-1,6-glucosidase) then hydrolyzes the 1,6-α branch point, releasing a single molecule of glucose (Figure 55.1-2).

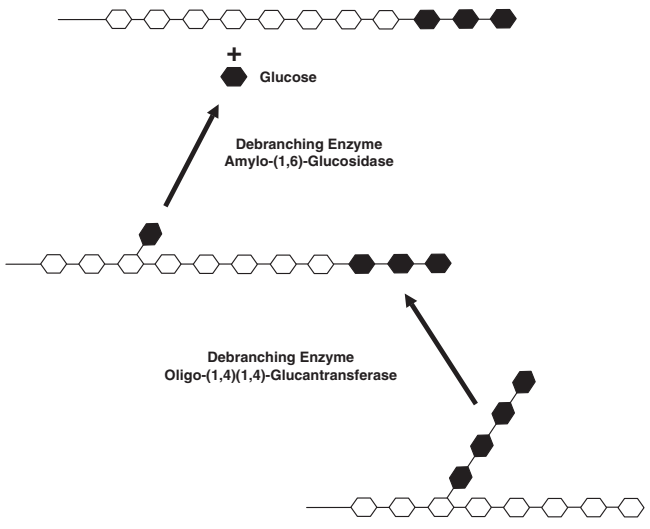

FIGURE 55.1-2 Action of debranching enzyme. Debranching enzyme has two catalytic sites on a single protein strand. When the action of glycogen phosphorylase has reduced a glycogen side chain to just four glycosol residues, the transferase activity of debranching enzyme transfers the three terminal residues to the end of an adjacent branch. The final residue is released as a single molecule of free glucose by glucosidase activity of the debranching enzyme.

Conversion of glucose 1-phosphate to glucose 6-phosphate enables the production of free glucose by the glucose 6-phosphatase system. Unlike other enzymes involved in glycolysis or gluconeogenesis, which are located in cytosol, glucose 6-phosphatase activity is found in the endoplasmic reticulum. This compartmentalization implies the need for a glucose 6-phosphate transporter, as well as the catalytic enzyme itself. These two enzymes, along with a putative phosphate transporter and a glucose transporter, are the proposed components of the "translocase-catalytic unit model" of the glucose 6-phosphatase system (Figure 55.1-3). To date, only the glucose 6-phosphatase and glucose 6-phosphate translocase units have been cloned and characterized. Glucose 6-phosphate can also be catabolized via the glycolysis pathway. This proceeds via triose phosphate to pyruvate and lactate or to acetyl coenzyme A (CoA) and entry into the tricarboxylic acid (TCA) cycle for oxidative phosphorylation and energy production.

In addition to glycogen breakdown, free glucose can also be synthesized via the gluconeogenic pathway. This is essentially the reverse of the glycolytic pathway, using all of the reversible enzyme steps from pyruvate back to glucose 6-phosphate. There are two steps in the glycolytic pathway that are irreversible, and alternate enzymes are used to bypass these steps. The first enzyme of the gluconeogenic pathway is pyruvate carboxylase, which catalyzes the conversion of pyruvate to oxaloacetate. This, in addition to phospho*enol*pyruvate carboxykinase (PEPCK), is essential for bypassing the irreversible action of pyruvate kinase. Pyruvate carboxylase is an intramitochondrial biotin-containing protein and a key regulator of gluconeogenesis. The conversion from oxaloacetate to phospho*enol*pyruvate is catalyzed by PEPCK.

The second irreversible enzymatic reaction in glycolysis involves phosphofructokinase, the conversion of fructose 1-phosphate to fructose 1,6-bisphosphate. Reversal of this, that is, splitting fructose 1,6-bisphosphate to fructose 1-phosphate and ionic phosphate, involves a Mg-dependent enzyme, fructose 1,6-bisphosphatase. The determination of overall flux in the direction of gluconeogenesis or glycolysis involves another isomeric form of fructose diphosphate. Fructose 2,6-bisphosphate inhibits the activity of fructose 1,6-bisphosphatase and activates phosphofructokinase, increasing flux through the glycolytic pathway. Fructose 2,6-bisphosphate synthesized from glucose is present in increased concentrations in the fed state and thus blocks gluconeogenesis.

DEFECTS OF HEPATIC CARBOHYDRATE METABOLISM

Inborn Errors of Glycogen Metabolism

Glycogen Storage Disease Type I: Glucose 6-Phophatase Deficiency (von Gierke Disease). The usual presentation of glycogen storage disease (GSD) I is an infant of a few months with a protuberant abdomen, short stature, and fasting hypoglycemia.[5,6] An overview of hepatic defects of glycogen metabolisms is given in Table 55.1-3. Frequent breast- or bottle-feeding of newborns often protects them during early infancy. A neonatal presentation is recognized with severe metabolic decompensation, hypoglycemia, and lactic acidosis.[7] Such a fulminant presentation may be fatal even with aggressive management.

At the time of presentation, an infant may be noted to have the characteristic "doll's" facies with big cheeks owing to excessive subcutaneous fat deposition. The protuberant abdomen is due to massive hepatomegaly, with-

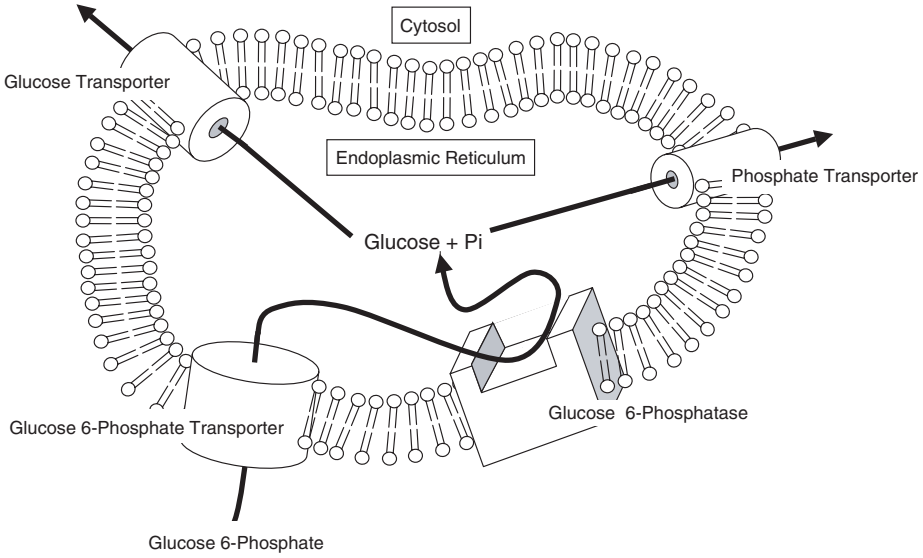

FIGURE 55.1-3 Translocase-catalytic model of the glucose 6-phosphatase system. Glucose 6-phosphatase is transported into the lumen to where the catalytic activity of the enzyme system resides. Free glucose and inorganic phosphate produced by the actions of glucose 6-phosphatase need to be removed from the endoplasmic reticulum because they inhibit the further action of glucose 6-phosphatase. The phosphate transporter remains to be fully characterized and the glucose transporter remains hypothetical at present. Pi = inorganic phosphate.

TABLE 55.1-3 GLYCOGEN STORAGE DISEASES

	EPONYM	ENZYME DEFECT	PRINCIPAL TISSUES AFFECTED
GSD I	von Gierke disease		
	a	Glucose 6-phosphatase	Liver, kidney
	b	Glucose 6-phosphate transporter	Liver, kidney, neutrophils
	c	Phosphate transporter	
	d	Glucose transporter	
GSD II	Pompe disease	Acid α-glucosidase	Heart, muscle
GSD III	Forbes or Cori disease	Debrancher enzyme	
	a		Liver, muscle
	b		Liver
GSD IV	Andersen disease	Brancher enzyme	Liver, heart
GSD V	McArdle disease	Muscle phosphorylase	Muscle
GSD VI	Hers disease	Hepatic phosphorylase	Liver
GSD VII	Tarui disease	Phosphofructokinase	Muscle
GSD IX (VIII McKusick)		Phosphorylase kinase	Liver
FBS	Fanconi-Bickel syndrome	GLUT 2	Liver, kidney
GSD 0		Glycogen synthase	Liver

GLUT = glucose transporter; GSD = glycogen storage disease.

out splenomegaly. Kidney enlargement is also present but is usually noted only on ultrasonography. The characteristic biochemical features of GSD I are fasting hypoglycemia with elevated plasma lactate, urate, and triglycerides. Cholesterol is also elevated, but not to the same extent as triglycerides. Mucosal bleeding or excessive bruising may be noted owing to platelet dysfunction as a consequence of hyperlipidemia.

Liver histology shows swollen hepatocytes with apparent cell wall thickening owing to peripheral displacement of organelles by the stored glycogen, which produces an appearance likened to plant cells. The excessive cytoplasmic glycogen stains with periodic acid–Schiff (PAS) and is readily digested by diastase. Microvesicular fat is almost invariably seen in the biopsy, but there is little in the way of inflammatory activity or fibrosis. These changes, although characteristic, are not pathognomonic, and differentiating the type of GSD on histologic criteria is not reliable.

A German pathologist, Edgar Otto Conrad von Gierke, first described GSD I in 1929.[8] Later it was demonstrated that the disease was due to the lack of activity of hepatic glucose 6-phosphatase activity.[9] There were, however, many patients who shared the phenotype but who still had glucose 6-phosphatase activity on frozen liver specimens. These patients were said to have GSD Ib. The hypothesis that this subtype was due to a defect in microsomal membrane transport was eventually proven with the identification of glucose 6-phosphate translocase (see Figure 55.1–3).[10–12] A third subtype, GSD Ic, has been shown to be genetically separate from types Ia and Ib and is thought to be a defect in a microsomal phosphate/pyrophosphate transporter.[13] Finally, it has been proposed that a microsomal glucose transporter is also part of the glucose 6-phosphatase system, and although a deficiency of this glucose transporter has been postulated (GSD Id), it has not been convincingly demonstrated.[14,15]

Both GSD Ia and GSD Ib are autosomal recessive conditions. The catalytic unit of glucose 6-phosphatase is located at chromosome 17q21 and encodes an endoplasmic reticulum membrane protein with nine transmembrane domains and a catalytic site on the luminal side of the endoplasmic reticulum membrane.[16] Over 70 separate mutations have been identified, and there is generally no genotype–phenotype correlation.[6] Glucose 6-phosphatase maintains latent activity and is dependent on transport of substrate from the cytosol into the endoplasmic reticulum lumen.[17] If the endoplasmic reticulum membrane is disrupted, either by freezing or detergent action, the catalytic activity is released and becomes apparent on enzymologic testing. The gene encoding glucose 6-phosphate translocase is located at chromosome 11q23. The protein has 10 transmembrane domains and is expressed in many human cell types, unlike glucose 6-phosphatase, which is expressed only in gluconeogenic tissues such as liver and kidney tubular cells.[18] Specifically, the glucose 6-phosphate transporter has been demonstrated in human neutrophils, where it may have a second, unspecified function, the lack of which presumably is the cause for the immune dysfunction seen in GSD Ib.[19,20]

The metabolic consequences of GSD Ia and Ib are similar, with the exception of the neutrophil dysfunction seen in GSD Ib (Figure 55.1-4).[21] Glucose 6-phosphatase is the only enzyme system capable of producing significant amounts of free glucose; therefore, the liver in GSD I is unable to maintain circulating glucose levels when the supply from a feed has been exhausted. The block in the last step of gluconeogenesis leads to excessive hepatic and renal accumulation of glycogen, which is the main cause of hepatomegaly and nephromegaly in this condition.[6] Recurrent hypoglycemia leads to increased glucagon production, which stimulates glycogen breakdown with accumulation of glucose 6-phosphate. As glucose 6-phosphate enters the glycolytic pathways, excess lactate is produced, giving rise to acidosis.

Lactate competes with urate for tubular excretion in the kidney.[5] Increased urate levels are, therefore, in part attributable to decreased urate excretion but may also be due to increased urate production. Glucose 6-phosphate may be shunted into the pentose-phosphate cycle, which stimulates synthesis of PP-ribose-P, degradation of which leads to further urate production. Finally, depletion of intrahepatic phosphate, as a result of production of glucose 6-phosphate

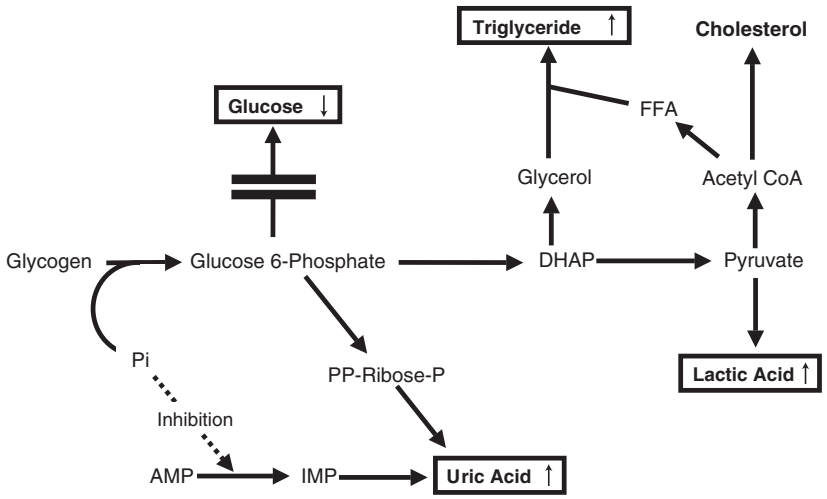

FIGURE 55.1-4 Schematic representation of the biochemical derangements responsible for the metabolic consequences of glycogen storage disease 1, namely hypoglycemia, lactic acidosis, hyperuricemia, and hypertriglyceridemia. AMP = adenosine monophosphate; CoA = coenzyme A; DHAP = dihydroxyacetone phosphate; FFA = free fatty acids; IMP = inosine monophosphate; Pi = inorganic phosphate.

from both dietary glucose and glycogen breakdown, relieves inhibition on adenosine monophosphate (AMP) deaminase, which converts AMP to inosine 5-monophosphate (IMP), which is metabolized to uric acid.[22,23]

Excess glucose 6-phosphate feeding into the glycolysis pathway increases production of glycerol and acetyl CoA and the energy precursors reduced oxidized nicotinamide adenine dinucleotide ($NADH^+$) and reduced oxidized nicotinamide adenine dinucleotide phosphate ($NADPH^+$), which provide substrates and cofactors for hepatic triglyceride production. Decreased peripheral use of fatty acids and increased lipolysis can result from low insulin levels. Bandsma and colleagues conclude that increased free fatty acid flux from adipose tissue to the liver probably makes the major contribution to hyperlipidemia and hepatic steatosis seen in GSD I.[24]

A diagnosis of GSD is usually suspected on the basis of clinical and biochemical features. Liver histology will suggest GSD but is not specific for the type. Glucagon stimulation is rarely carried out now, but in GSD I, there is little or no elevation in the plasma glucose following glucagon infusion, but lactic acid levels increase.[25] Enzyme assays on fresh (intact microsomes) and frozen (disrupted microsomes) liver biopsy specimens will diagnose and differentiate GSD Ia and Ib. Recent recommendations suggest that mutational analysis should precede liver enzymology (Table 55.1-4), with liver biopsy reserved for cases in which mutations in glucose 6-phosphatase or glucose 6-phosphate translocase genes cannot be identified but the diagnosis is still suspected.[6]

The clinical consequences of GSD I largely mirror the metabolic features discussed earlier. Recurrent hypoglycemia necessitates frequent feeding because the liver is unable to maintain glucose levels when the dietary supply of carbohydrates dissipates. Newborn infants often feed every 1 to 2 hours on demand, without significant periods of fasting, so that features of hypoglycemia may not appear. In addition, possibly because the brain can use lactate as an energy source, infants with GSD are often remarkably tolerant of hypoglycemia.[26] Hypoglycemia may first be manifest at several months of age because feeding frequency tends to decrease or at times of an intercurrent illness when dietary intake is impaired. There is an unexplained increase in fasting tolerance with age, and it may be that there is recruitment of other mechanisms for free glucose production. It is known, for example, that small amounts of free glucose can be produced by α-glucosidase, a lysosomal enzyme, deficiency of which produces GSD II, Pompe disease, and that a single molecule of free glucose is produced when debranching enzyme cleaves an 1,6-α branch point on a glycogen chain.

Untreated, massive hepatomegaly is the rule through childhood but tends to be relatively less significant in adults. There is often a modest increase in transaminases, but features of chronic liver disease are absent. Fibrosis is unusual, as is portal hypertension, but hepatic adenomas are very commonly found in untreated adults with GSD I. Adenomas in the liver are prone to bleeding and may undergo malignant transformation to hepatocellular carcinoma.[7,27]

There is slow linear growth in untreated children seen in infancy and childhood.[28] Adults tend to be short. There is frequently pubertal delay, and females have polycystic ovaries. With increasing body stores of uric acid, gout was frequently seen (before modern therapy) occurring initially at or around the time of puberty.[29] Xanthomata and lipemia retinalis may be seen at the time of presentation in infancy.[5] Massive hypertriglyceridemia is the cause proposed for the increased cases of pancreatitis seen in uncontrolled GSD I and also contributes to the platelet dysfunction responsible for the increased bleeding tendency.[30] The risk of atherosclerosis may be increased owing to the hyperlipidemia associated with GSD I.[31]

Renal dysfunction is first manifest with hyperperfusion and hyperfiltration causing an increase in creatinine clearance, but actual renal damage is heralded by microalbuminuria that may progress to frank proteinuria.[32] Chronic

TABLE 55.1-4 DIAGNOSTIC INVESTIGATIONS

DISEASE	TISSUE FOR ENZYME STUDIES	ROUTINE DNA TESTING AVAILABLE	HUMAN GENE LOCUS
GSD Ia	Liver	Yes	17q21
Ib	Liver	Yes	11q23
III	Liver and muscle	Exon 3 mutations only (ie, GSD IIIb)	1q21
IV Liver and other affected tissues	No	3p12	
VI	Liver	No	14q21–22
IX	Liver, erythrocytes, leukocytes, other affected tissues	No	Product of multiple genes
Glycogen synthase deficiency	Liver	No	12p12.2
Fanconi-Bickel syndrome	Liver, kidney	No	3q26.1–26.3
Fructosuria	Liver	No	2p23.3–23.2
Hereditary fructose intolerance	Liver	No	9q22.3
Fructose 1,6-bisphosphatase deficiency	Liver	No	9q22.2–22.3
D-Glyceric acid	Liver?	No	Unknown
Galactokinase deficiency	Erythrocytes	No	17q24
Galactose 6-phosphate uridyl transferase deficiency	Erythrocytes	Yes	9p13
UDP galactose 4-epimerase deficiency	Erythrocytes	No	1p36-35
Pyruvate carboxylase deficiency	Fibroblasts	No	11q13.4–13.5
Phospho*enol*pyruvate carboxykinase deficiency	Liver?	No	20q13.31 (soluble) Unknown (mitochondrial)

GSD = glycogen storage disease; UDP = uridine diphosphate.
Tissues primarily tested for enzyme activity in defects of hepatic carbohydrate metabolism. Few of these conditions can presently be diagnosed routinely by deoxyribonucleic acid (DNA) analysis, although the loci for most genes are known.

renal failure may ensue with hypertension, hypercalciuria, and nephrocalcinosis. Renal calculi may occur. Acidosis and renal Fanconi syndrome can accompany the renal dysfunction. Osteopenia, rickets, and fractures are also commonly seen. Histologic changes on kidney biopsy are those of focal segmental glomerular sclerosis.

In addition to the above features, there is a tendency to recurrent bacterial infections with neutropenia in GSD Ib, which may be cyclical or persistent.[21] Mucosal inflammation is common, and overt inflammatory bowel disease may be present.

It has been demonstrated that avoidance of hypoglycemia and maintenance of normoglycemia improve overall metabolic control.[33] This can be achieved with frequent enteral feeds, total parenteral nutrition, or continuous nasogastric tube feedings. The standard approach at diagnosis is now continuous overnight nasogastric feeding with glucose polymer solution or a specialized infant formula aiming to supply glucose at rates equivalent to normal hepatic synthesis, which for infants is 8 to 9 mg/kg/min and for older children is in the range of 5 to 7 mg/kg/min. During the day, frequent feeds are given. The patient should avoid galactose and fructose because these are converted to glucose 6-phosphate without contributing free glucose. In older children, the diet should be rich in complex carbohydrates. Uncooked cornstarch given every 4 hours to young children at a dose of 1 to 2 g/kg can act as a slow-release form of glucose.[34] It is slowly hydrolyzed in the gut and improves the duration of normoglycemia.[35] The period between cornstarch feeds can be extended as the child gets older, and some adults can tolerate a full 8 hours sleep on a single intake of cornstarch. Care needs to be taken when patients with GSD I are unwell. IV fluids should always contain appropriate amounts of glucose. Lactated Ringer solution should not be used. Metabolic control should be achieved and strictly maintained prior to surgery to limit bleeding problems. Successful pregnancies have been documented in women with GSD I, but strict dietary control is recommended prior to and during pregnancy.[36–38]

The use of diazoxide has been suggested to increase fasting tolerance, and Nuoffer and colleagues showed an impressive response in terms of normoglycemic control and linear growth in two patients treated with this drug.[39] Allopurinol is useful in the management of hyperuricemia, and evidence is accumulating that angiotensin-converting enzyme inhibitors may be helpful in treating the proteinuria, an early indicator of renal dysfunction.[29,40,41] With regard to the management of neutropenia and recurrent infections in patients with GSD Ib, granulocyte colony-stimulating factor has been demonstrated to increase the neutrophil counts and reduce the frequency and severity of bacterial infections.[42,43]

Orthotopic liver transplant has been carried out in patients with GSD I, usually for multiple adenomas and fear of malignant change.[44] Hypoglycemia and metabolic control are improved with successful liver transplant.[45] Hyperuricemia, lactic acidosis, and hypertriglyceridemia clear; however, it is unclear whether liver transplant influences the progression of renal disease. In those patients with end-stage renal disease, kidney transplant has been performed, but this does not improve the systemic manifestations of deranged hepatic metabolism.[46] There is one report of isolated hepatocyte transplant for this condition in an adult with poor compliance to dietary management.[47] The authors documented improved glycemic control but did not demonstrate that infused hepatocytes had, in fact, engrafted or that enzyme activity on liver biopsy had increased. Gene therapy is presently confined to laboratory studies on animal models of GSD Ia.[48,49]

Prior to modern treatment, patients frequently died soon after presentation. If they survived, they were likely to live through to adulthood with short stature and multiple complications, as discussed previously. Modern therapy, with the maintenance of normoglycemia and metabolic stability, has changed this dramatically. Although some infants succumb to acute metabolic collapse and lactic acidosis in the neonatal period, with urgent attention to glucose replacement and appropriate intensive management, these children should survive. With aggressive dietary management, overnight tube feedings, and cornstarch feeds, metabolic control can be maintained, avoiding hyperuricemia, lactic acidosis, hypertriglyceridemia, and recurrent hypoglycemia and allowing the children to achieve normal growth velocities.

Obesity can be a problem with the high carbohydrate intake, and hyperlipidemia may not be fully ameliorated.[31] Long-term sequelae such as adenomas appear to be less frequent, and adenomas already present may regress when appropriate treatment is started in teenagers and adults.[50] In spite of adequate therapy, there are suggestions that renal dysfunction may not be completely prevented; specifically, hyperfiltration does not appear to be affected even by excellent glycemic control.[5]

GSD III: Debranching Enzyme Deficiency (Cori Disease, Forbes Disease, Limit Dextrinosis). GSD III, like GSD I, commonly presents in infancy with signs and symptoms of hypoglycemia and a protuberant abdomen owing to hepatomegaly. Growth retardation is common, as is hyperlipidemia. Hypoglycemia and hyperlipidemia are frequently less severe than in GSD I and occasionally are absent completely. In addition, children may present later in childhood with isolated hepatomegaly or abnormal liver enzymes, which, in some cases, may be very high.[51] Some patients present first in adulthood with a distal myopathy.[52,53] Liver biopsy in early childhood tends to show a typical glycogenosis but is more likely to show diffuse fibrosis and less steatosis than is seen typically in GSD I. Based on clinical phenotypes, GSD III has been divided into subtypes a and b. GSD IIIa has typical liver involvement with later muscle involvement. GSD IIIb accounts for about 15% of cases, with only the liver affected by this deficiency.[5]

Snappes and Van Creveld described the first cases of this condition in 1928, and because most patients with GSD III survive into adulthood, the authors were able to demonstrate a deficiency of the debranching enzyme in the original cases 35 years later.[54] This was after Illingworth and colleagues had identified that the deficiency of this enzyme was the cause of GSD III.[55] GSD III has been identified in all races, but there is a higher incidence in Faroe Islanders (incidence 1:3,600) and North African Jews (incidence 1:5,400).[56,57] Differential translation of a single gene, located on chromosome 1p21, is responsible for the liver and muscle isoforms of this enzyme.[58,59] This gene encodes a single polypeptide that possesses two separate catalytic sites that can function independently (see Figure 55.1-2), and occasional case reports have suggested selective absence of either the glucosidase activity or the transferase activity. These cases have been termed GSD IIIc and GSD IIId, respectively.[60–62]

Glucagon stimulation gives differing results in GSD III depending on whether it is carried out in the fed or fasting state.[63] After a feed, glucagon stimulation leads to a rise in plasma glucose because hepatic phosphorylase is able to release glucose from the end-chains of glycogen. However, if the glucagon challenge takes place after an 8-hour fast, no rise in glucose is seen because the side chains have already been shortened as far as the branch points, and no further degradation can be effected in the absence of debranching enzyme. Fasting hypoglycemia results from incomplete glycogen degradation, lactic acidosis is not seen because there is no block in gluconeogenesis, and ketosis is more apparent during hypoglycemia than in GSD I.

Although hypoglycemia and hepatomegaly tend to recede with age, hepatic fibrosis and even micronodular cirrhosis can be seen in this condition.[64–66] Adenomas have been reported, but in GSD III, hepatocellular carcinoma appears to be associated only with cirrhosis.[67,68] Progressive myopathy and occasionally cardiomyopathy can occur in GSD IIIa patients, being an infrequent finding in childhood but often becoming a significant functional impairment by the third or fourth decade.[52] It is usually manifest by slowly progressive weakness and muscle atrophy. Ventricular hypertrophy is commonly seen, but frank cardiac dysfunction is the exception.[69] Polycystic ovaries have been noted in affected females; however, fertility appears to be unaffected.[70,71]

The diagnosis of GSD III is suspected on clinical and biochemical grounds, and the diagnosis is confirmed by liver enzymology, although the enzyme is expressed in other tissues such as fibroblasts. Differentiation of GSD IIIa from GSD IIIb requires a muscle biopsy. Deoxyribonucleic acid (DNA) techniques are now available for mutational analysis (see Table 55.1-4), and there appears to be a special association between exon 3 mutations and GSD IIIb.[62] More studies are needed before the type of GSD III can be ensured purely by mutational analysis.

Treatment of GSD III is similar to GSD I in that hypoglycemia needs to be effectively managed with frequent feeds, nighttime nasogastric tube feeding, and the introduction of cornstarch as the patients get older. It has been suggested that a high-protein diet can be helpful because amino acids can act as a gluconeogenic precursor and that a high-protein diet may be useful in the slowing of the progression of the myopathy even though long-term outcome studies are not available.[72] Similarly, there may be no need to restrict galactose and fructose intake because there is no impairment to their conversion to free glucose, as there is in GSD I.

Most patients with this condition survive into adulthood, usually with minimal hepatic symptoms and with an ability to tolerate a normal diet. The myopathy is slowly progressive but may become debilitating in later adulthood.

GSD IV: Branching Enzyme Deficiency (Andersen Disease, Amylopectinosis). In 1956, Andersen described a

child with progressive hepatomegaly and a hepatic storage substance similar to amylopectin, the insoluble component of plant starch.[73] In 1966, the defect in the branching enzyme was reported.[5] Although this remains the characteristic form of GSD IV, this condition has an extremely variable phenotype.[74] In addition to Andersen disease, there have been cases described with nonprogressive hepatic disease, patients with associated cardiomyopathy, or multiple system involvement including neuromuscular involvement in the form of peripheral myopathy and neuropathy with or without cardiomyopathy. A severe neonatal form with hypotonia, muscle atrophy, cardiomyopathy, arthrogryposis, pulmonary hypoplasia, and hydrops fetalis has also been attributed to mutations within the gene responsible for GSD IV. Finally, an adult-onset disease called polyglucosan body disease, a severe progressive neurologic condition with peripheral neuropathy, seizures, and dementia, has been identified to be due to a defect of branching enzyme.

GSD IV is the least common of the hepatic GSDs, and the variants are still more rare. In the classic form of this disease, the affected infant is normal at birth; however, liver disease progresses rapidly through infancy with hepatosplenomegaly, cirrhosis, and portal hypertension. Without liver transplant, death usually occurs between the ages of 2 and 5 years. Fasting hypoglycemia and massive hepatomegaly are generally absent. Liver histology differs from other forms of hepatic GSD as well, with interstitial and portal fibrosis leading to micronodular cirrhosis. Hepatocytes are enlarged and stain PAS positive but are only partially digested by diastase. On electron microscopy, aggregates reminiscent of amylopectin are visible, along with more normal-appearing glycogen particles.

The mechanism by which the abnormal unbranched glycogen leads to hepatocyte damage is not clear. It is presumably related to abnormal and insoluble glycogen inducing a foreign body reaction within hepatocytes, leading to individual hepatocyte death with an inflammatory response and fibrosis thereafter. GSD IV is an autosomal recessive condition. The gene coding for the branching enzyme has been localized to chromosome 3p12.[75] Although the enzyme is usually assayed on liver, it is also expressed in leukocytes, red blood cells, and fibroblasts (see Table 55.1-4). In addition, branching enzyme can be assayed in amniocytes and chorionic villous cells, facilitating prenatal diagnosis.[76]

There is no specific treatment for this condition. General supportive measures are recommended to maintain growth. Liver transplant has been shown to be both feasible and effective.[44] However, there are a few cases in which, after transplant, progressive cardiomyopathy has occurred, leading to death.[77,78] The situation is difficult because there is no predictor as to which patients will proceed to a progressive cardiomyopathy. Starzl and colleagues have reported patients who had evidence of cardiac involvement that regressed after liver transplant.[79] It is possible that, with further molecular studies, genotype–phenotype correlations may help to understand and predict the outcome in these patients after liver transplants.

GSD VI: Phosphorylase Deficiency (Hers Disease). Diminished phosphorylase activity in patients with excessive hepatic glycogen storage was first ascribed the term GSD VI in 1960.[5] Many of these patients have since been shown to have a defect in activation of glycogen phosphorylase rather than a defect in the gene coding for glycogen phosphorylase itself, localized to chromosome 14q21-22.[80] The number of cases with proven mutations in the phosphorylase gene is small.[81,82] Usually, GSD VI is a benign condition and affects only liver because other tissues express glycogen phosphorylases that are the products of completely separate genes. If present at all, hypoglycemia is mild and present only in infancy and early childhood. Similarly, ketosis and hyperlipidemia are minimal. Lactate and urate levels are normal. Hepatomegaly and poor growth in early childhood are the most notable features, and both tend to improve with age. In most cases, treatment is not required. If hypoglycemia is present, it can be managed with frequent feeds and nighttime tube feeding. Prognosis is excellent, and normal growth velocities can be expected beyond the first few years of life.

GSD IX: Phosphorylase Kinase Deficiency (GSD VIII according to McKusick's Online Mendelian Inheritance in Man). Of the group of mild GSDs with decreased phosphorylase activity, family studies showed that a significant proportion of these were X-linked. Defective activation of hepatic phosphorylase was demonstrated, as well as deficiency of phosphorylase kinase activity within the blood cells of these patients.[83,84] A smaller number of patients, also with measurable deficiencies of phosphorylase kinase, have an autosomal recessive mode of inheritance.[85,86] The classification of this condition (strictly a group of conditions) is a little confused; most authorities refer to phosphorylase kinase deficiency as GSD IX, but McKusick's Online Mendelian Inheritance in Man[87] categorizes it as GSD VIII.

The common presentation for this condition is that of a young child, usually not an infant, with a protuberant abdomen and moderate growth retardation. Laboratory testing may show elevated transaminases, modestly elevated cholesterol and triglycerides, and fasting ketosis. Hypoglycemia is uncommon. Like GSD VI, growth retardation is confined to early childhood. Hepatomegaly is the most dramatic finding, but this resolves with age. Curiously, the liver biopsy may show mild inflammatory changes with some fibrosis in addition to glycogen-distended hepatocytes (Figure 55.1-5). Steatosis is not usually apparent.

In the majority of cases, GSD IX is a benign condition; there are, however, a few descriptions of more significant disease with symptomatic hyperglycemia requiring dietary management as described for other GSDs.[88,89] Other patients have had more aggressive liver disease, with progression to cirrhosis, and still others have had muscle involvement.[86,90–92] The variable phenotype is largely explained by the complex nature of the phosphorylase kinase protein, which consists of multiple copies of four separate gene products: the α, β, γ, and δ subunits.[5] The α and β subunits are regulatory subunits, regulated by phos-

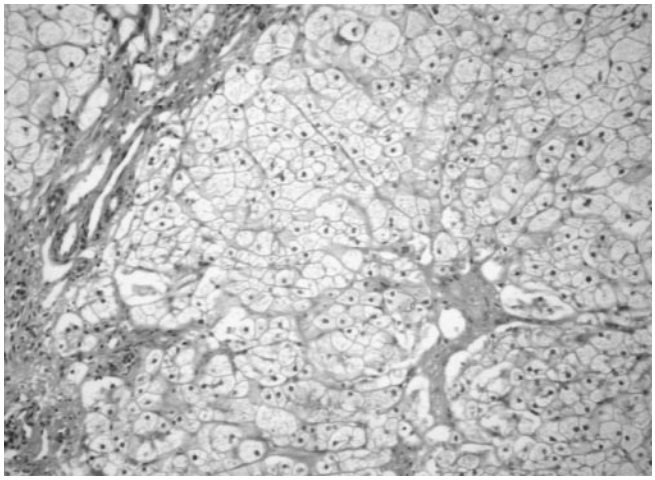

FIGURE 55.1-5 Liver histology from a 2-year-old patient with X-linked phosphorylase kinase deficiency. Note distended hepatocytes and mild periportal, pericentral, and intralobular fibrosis (hematoxylin and eosin stain; ×20 original magnification).

phorylation and dephosphorylation. There are two genes, both on the X chromosome for the α subunit, one predominantly a muscle subunit (pHka1) and the other mainly expressed in liver phosphorylase kinase (pHka2, locus Xp22.2-22.1). The β subunit is coded at chromosome 16q12-13. The γ subunit is the catalytic subunit, and, again, there are two genes on separate chromosomes for this subunit: pHkg1 (muscle) on chromosome 7 and pHkg2 (testis and liver subunit) at chromosome 16p12.1-12.2. The δ subunit is the calcium binding protein calmodulin.

Over 75% of described cases of phosphorylase kinase deficiency have defects in the α liver subunit and produce the benign hepatic form of GSD IX. Patients with β subunit defects are more likely to have evidence of muscle involvement, and cirrhosis is characteristic of the defect in the liver/testis subunit gene *PHKG2*.[85,86] Diagnosis depends on enzyme assay, which can be carried out either in red cells or liver (see Table 55.1-4). Management is generally symptomatic, but the majority of patients require no intervention. The prognosis for the X-linked glycogenosis is excellent, with hepatomegaly receding certainly by teenage years, and final adult height within the normal range. Prognosis for the variant forms is presently unclear.

Fanconi-Bickel Syndrome (Hepatorenal Glycogenosis with Renal Fanconi Syndrome). In 1949, Fanconi and Bickel described a 3-year-old boy, born to consanguineous parents from a small alpine village, with renal Fanconi syndrome and hepatomegaly.[93] He had increased liver glycogen, fasting hypoglycemia, hyperlipidemia, and renal tubular defects. Santer and colleagues reviewed this patient, with over 50 years of follow-up.[94] The same group reported knowledge of 112 patients, the vast majority being white.[95] Presentation is usually in the first year, with failure to thrive, vomiting and diarrhea, rickets and polyuria, and a protuberant abdomen. Findings include glycosuria, generalized aminoaciduria, phosphaturia, and calciuria with renal tubular acidosis. There is increased plasma alkaline phosphatase, mild to moderate fasting hypoglycemia, and hyperlipidemia; however, plasma lactate and uric acid levels are usually normal. Of particular note are postprandial hyperglycemia and hypergalactosemia. Later clinical features include a moon-shaped face, truncal obesity, retarded growth and puberty, bone problems associated with hypophosphatemic rickets, and dental caries. Cataracts from hypergalactosemia have only rarely been described, and the renal lesion is usually not progressive in nature.

The facultative glucose transporter GLUT2 was localized to chromosome 3q26.1–26.3 in 1988 and is expressed on hepatocytes, renal tubular cells, enterocytes, and pancreatic β islet cells.[95,96] It was not, however, until 1997 that a defect in GLUT2 was proposed as the possible metabolic basis for Fanconi-Bickel syndrome (FBS).[97] Mutations in the gene for GLUT2 have been demonstrated in FBS subjects, including the original patient described by Fanconi and Bickel.[98]

The features of FBS can be explained by the loss of GLUT2 function. GLUT proteins are membrane-bound monosaccharide transporters that transport sugars in an energy-independent manner. GLUT2 is responsible for transport of glucose (and galactose) into hepatocytes after feeding and export of free glucose out of hepatocytes during fasting. The hyperglycemia and hypergalactosemia seen postprandially are due to reduced uptake of these monosaccharides by the liver and may be enhanced by the poor insulin response to elevated blood glucose levels demonstrated in patients with FBS.[95] Glucose is transported inefficiently into pancreatic β cells because of the defect in GLUT2, thus failing to provoke an appropriate insulin response. Fasting hypoglycemia results from defective export of free glucose from hepatocytes when peripheral glucose supplies have been exhausted. Elevated intracellular levels of glucose stimulate increased glycogen production. Glycosuria is the result of failure to export glucose across the basolateral membranes of renal tubular cells. This again leads to an increased intracellular glucose and stimulates glycogen production. The excessive intracellular glycogen may be responsible for the other features of renal Fanconi syndrome. Finally, defective transport of glucose and galactose across enterocytes may be responsible for the diarrhea and malabsorption seen.

The treatment of patients with FBS is based on the management of renal Fanconi syndrome with maintenance of water and electrolyte balance; supplementation of vitamin D, calcium, phosphorus, and bicarbonate; and the management of the deranged glucose homeostasis. The ideal diet has not yet been decided on, but suggestions have included frequent small meals, the ketogenic diet, or uncooked cornstarch, as is used in GSD I.[95,99] On these diets, the hepatomegaly has been seen to diminish. It has also been noted that fructose metabolism does not appear to be affected by the defect in GLUT2; therefore, the use of fructose may be useful in the dietary management of patients with FBS.[100]

The prognosis for this condition appears to be generally good in terms of survival, but adults are universally short in stature. Many also have ongoing bony problems

related to rickets and osteomalacia. Hepatomegaly tends to recede after puberty.[95]

GLYCOGEN SYNTHASE DEFICIENCY

Although referred to as GSD 0, there is no excessive storage of glycogen, and, in fact, there is a demonstrable reduction in hepatic glycogen in this condition. Like many enzymes for glycogen metabolism, separate gene products account for glycogen synthase activity in muscle and liver, GYS1 and GYS2, respectively.[87]

The deficiency state of GYS2 is very rare in humans.[101,102] Although Aynsley-Green and colleagues have reported apparent asymptomatic patients with this condition, on the whole, patients have presented in infancy with features of fasting hypoglycemia.[103] The characteristic biochemical features are fasting ketotic hypoglycemia with low alanine and lactate levels and postprandial hyperglycemia with increased circulating lactate levels. There is no hepatomegaly in this condition because there is no glycogen storage, and hyperlipidemia does not occur. Liver biopsy is required for enzymologic diagnosis (see Table 55.1-4). The gene locus is chromosome 12p12.2, and mutational analysis has been conducted on affected subjects.[104] With avoidance of symptomatic hypoglycemia, the prognosis is good.

INBORN ERRORS OF FRUCTOSE METABOLISM

Fructosuria

Essential fructosuria is due to deficiency of fructokinase, which converts free fructose to fructose 1-phosphate.[105] It was first revealed in a patient checked for glycosuria while being investigated for possible diabetes.[106] Fructose, although containing no aldehyde group, becomes a reducing sugar in basic solution and will give a positive test when urine is checked for reducing substances but gives a negative reaction on glucose oxidase stick testing. Fructokinase deficiency is an autosomal recessive condition, but it is likely that many cases go undetected because of its entirely benign nature.

Hereditary Fructose Intolerance

In contrast to the previous condition, hereditary fructose intolerance (HFI) is a potentially life-threatening condition. The defect is due to absence of aldolase B (fructose 1,6-bisphosphate aldolase) from the liver, kidney, and small intestine. Other tissues in the body express aldolase A or C. This enzyme catalyzes the conversion of fructose 1,6-phosphate and fructose 1-phosphate to triose phosphates.[106]

Patients with this condition remain entirely healthy provided that they do not ingest significant amounts of fructose or sucrose. The infant is protected from harm by breastfeeding, and modern infant formulas no longer contain fructose or sucrose as a sweetener. Problems may occur on weaning to solid foods, particularly with the introduction of fruit and baby food sweetened with sucrose. Symptoms include poor feeding, vomiting, failure to thrive, and abdominal pain. If intake of fructose persists, acute hypoglycemia, bleeding, hepatomegaly, and trembling or jerking can occur. This may proceed to metabolic collapse with liver and kidney failure and, ultimately, death. With acute illness, laboratory findings are consistent with liver failure, lactic acidosis, hyperuricemia, and proximal renal tubular dysfunction. The histology of the liver at this stage shows isolated hepatocyte necrosis with intralobular and periportal fibrosis and diffuse fatty change.

If the diagnosis is not made, but the patient survives, the course may be intermittent and chronic.[106] As these children grow, they develop a dramatic aversion to sweet foods, and sometimes their odd eating habits are the reason for medical attention. Dentists have noted complete absence of caries in adult patients, and this has, on occasion, prompted the diagnosis.[107] Others have been diagnosed in adulthood after metabolic collapse, or even death, when intravenous fluids containing fructose or sorbitol have been given for routine surgeries.[108,109]

With intermittent exposure to fructose in HFI, many nonspecific symptoms may occur, such as poor feeding, intermittent vomiting, failure to thrive, irritability or apathy, and diarrhea. Chronic liver disease can also occur with hepatomegaly and cirrhosis. Symptoms are related to the fundamental toxicity of fructose. It has been demonstrated in animals and humans that large infusions of fructose are, in themselves, toxic and produce many of the features seen in patients with hereditary fructose intolerance, particularly increased circulating lactic acid and hyperuricemia.[110] The mechanism for this commences with the rapid and irreversible conversion of free fructose to fructose 1-phosphate by fructokinase.[111] Further metabolism of fructose 1-phosphate to D-glyceraldehyde and dihydroxyacetone phosphate is prevented. Production of large amounts of fructose 1-phosphate effectively sequestrates inorganic phosphate, causing a drastic reduction in cytoplasmic phosphate concentrations. This leads to a depletion of adenosine triphosphate (ATP) because of irreversible deamination of AMP to IMP. Further mitochondrial regeneration of ATP is prevented by the lack of inorganic phosphate. Elevation in Mg^{2+} seen in acute episodes is related to the fact that a large quantity of Mg is bound to ATP, and with the loss of ATP, Mg^{2+} is released.[106] The overall effect of this is a loss of cellular energy supply, and studies using ^{31}P magnetic resonance spectroscopy are providing direct proof of this mechanism.[112,113] Hypoglycemia during acute exposure to fructose is due both to deranged gluconeogenesis because of the increased fructose 1-phosphate levels and to failure of glycogenolysis because inorganic phosphate deficiency inhibits glycogen phosphorylase. Hypoglycemia can be reversed by galactose infusion, implying that there is no inhibition of the glucose 6-phosphatase pathway. Glucagon cannot correct hypoglycemia during these episodes because of the deranged gluconeogenic pathways.[114]

Aldolase B gene is located on the long arm of chromosome 9 (9q22.3).[115] The incidence of this condition in Switzerland, from where many cases have been described, is estimated at about 1 in 20,000.[116] However, the incidence in other populations is unknown and is complicated by the fact that many affected individuals may live a long life without the diagnosis being made.

The diagnosis of HFI is dependent on the physician having a high degree of awareness of this condition. A careful nutritional history will solidify the suspicions, and the urine can be checked for non–glucose-reducing substances; however, absence does not rule out the diagnosis. On initial suspicion, all dietary fructose, sucrose, and sorbitol should be excluded from the diet. Infants presenting with severe toxic episodes may need intensive support for an extended period. Once recovered, the child should be maintained on a fructose-free diet for several weeks to ensure recovery and improved growth. A fructose tolerance test can then be carried out.[117] A small dose, standardized at 200 mg/kg, is infused while monitoring laboratory parameters such as glucose, plasma phosphate, uric acid, lactate, and Mg^{2+}. A characteristic response is an increase in plasma urate and magnesium with a corresponding fall in phosphorus and glucose. Aldolase B can be assayed on liver biopsy, and DNA mutational analysis is now also available.[118,119]

Treatment involves removal of all known dietary sources of fructose, sucrose, and sorbitol, which are found naturally in fruits, vegetables, and honey, as well as in processed food. Fructose is so widespread that patients should be very open about their intolerance so as not to be inadvertently exposed. This is especially true in any contact with medical professionals. Medicines may be sweetened with sucrose. IV fluids or parenteral nutritional supplements using fructose or sorbitol have been advocated in the past but should be avoided in all patients. When recovery occurs, it is complete, with reversal of hepatic histologic changes and renal dysfunction. Growth and development become normal.

Fructose 1,6-Bisphosphatase Deficiency

Fewer than 100 cases of fructose 1,6-biphosphatase deficiency have been reported. Affected individuals may present with neonatal jaundice, but, more commonly, episodic bouts of hyperventilation around the time of weaning from frequent milk feeds are seen.[120] The hallmark is ketotic hypoglycemia, lactic acidosis on fasting, and elevated uric acid. Metabolic collapse may be rapidly lethal, and fasting or infections often trigger late episodes.

After stabilization and avoidance of dietary fructose, an IV fructose tolerance test is abnormal, but unlike HFI, there is no aversion to sweet foods and no proximal renal tubular signs. Liver biopsy shows steatosis but no fibrosis. Again, this enzyme can be assayed in liver biopsy. The condition is inherited as autosomal recessive, and the gene has been localized to the almost identical locus to aldolase B on the long arm of chromosome 9. Treatment consists of IV glucose for the acute episode and long-term avoidance of fasting. Fructose should be limited, but complete avoidance is probably unnecessary. When the diagnosis is made and treated appropriately, the course is said to be favorable.[106]

D-Glycericaciduria

This extremely rare condition is due to the absence of D-glycerate kinase, which is required in the pathway of metabolism of D-glyceraldehyde, which, in turn, is derived from the metabolism of fructose 1-phosphate or glycerol. The presence of D-glyceric acid in urine is associated with hyperglycinemia, metabolic acidosis, and neurologic features that include hypotonia, seizures, severe developmental delay, and spastic quadriplegia. However, other cases have been relatively asymptomatic.[121,122] Dietary restriction of fructose has been suggested as management for this condition.

INBORN ERRORS OF GALACTOSE METABOLISM

Galactokinase Deficiency

The first step in the hepatic metabolism of free galactose is the conversion to galactose 1-phosphate catalyzed by galactokinase, the deficiency of which was first described in a child with bilateral cataracts.[123] This has been a consistent feature in all cases described since, and it is notable that the other features of classic galactosemia do not occur. There is no acute metabolic episode associated with this condition. Ovarian failure and growth failure do not occur. Galactokinase deficiency is not thought to pose neurodevelopment problems, although pseudotumor cerebri has been described in a few cases.[124,125] Galactokinase deficiency is probably less common than classic galactosemia, based on neonatal screening data, but its true incidence is undetermined.

The diagnosis is suspected either on the basis of neonatal screening or because of infantile cataracts and is confirmed by demonstrating deficient galactokinase activity with normal galactose 1-phosphate uridyl transferase (GALT) activity in red blood cells (see Table 55.1-4). The cause of the cataracts appears to be galactitol, the product of reduction of galactose by aldose reductase.[126] High concentrations of this enzyme occur within the ocular lens and lead to accumulation of galactitol. Galactitol is poorly mobilized from the lens media and exerts an osmotic effect.[127] Cataracts can be prevented in experimental animal models of galactokinase deficiency by inhibiting aldose reductase.[128] The management of this condition involves dietary galactose exclusion and is highly effective provided that the vision has not been permanently damaged prior to diagnosis.

GALT Deficiency

GALT deficiency is the most common defect of galactose metabolism, and the clinical picture was first described by Goppert in 1917.[129] The frequency of GALT deficiency varies in different populations, with the highest incidence in Irish travelers of 1 in 480 and an estimated prevalence overall of 1 in 62,000.[125,130] In the United States, most infants with galactosemia are now diagnosed on infant screening. Often the patients are asymptomatic at diagnosis. Some infants present acutely within days of birth with vomiting and diarrhea, irritability, or lethargy with hypotonia, and the rapid diagnosis allowed by the early neonatal screening may assist in the care of these sick infants. If galactose ingestion continues, this progresses to hemolysis with jaundice and acidosis. There may be acute metabolic collapse with liver and kidney failure leading to death. Many of the fulminant early infantile cases are related to the high incidence of bacterial septicemia, with the

causative organism most commonly being *E. coli*.[131] *E. coli* sepsis is so characteristic that any neonate so affected should be investigated for underlying galactosemia. Other infants follow a more chronic course with poor feeding and growth and progressive liver disease leading to cirrhosis. In surviving children, mental retardation is almost universal, although this is rarely at a profound level. Cataracts have been observed within days of birth but are more commonly seen later in infancy in those on an unrestricted diet.

GALT catalyzes the reaction that converts galactose 1-phosphate to UDP galactose (Figure 55.1-6). The acute metabolic syndrome is probably due to sequestration of inorganic phosphate as galactose 1-phosphate, with resulting deficiency in cellular energy owing to loss and insufficient restitution of ATP supply, possibly analogous to the demonstrated acute effects of fructose intake in patients with HFI. Galactose 1-phosphate itself inhibits a number of enzymes, in vitro, involved in glucose metabolism. The acute disturbance leads to liver disease, hemolysis, lactic acidosis and renal tubular acidosis, proteinuria, and aminoaciduria.

With the introduction of neonatal screening, individuals were identified with milder disease.[132] The "Duarte" variants identified on neonatal screening have structurally altered GALT that is functionally deficient, at least in young infants. However, after a few months, GALT activity can often be measured in the 50% range. Development in patients with a Duarte variant is normal, and they can tolerate an unrestricted diet. It had also been noted that galactosemia was commonly less severe in black individuals. Despite complete peripheral GALT deficiency, such patients are able to tolerate a normal diet. This so-called "Negro" variant has zero activity of GALT in erythrocytes but up to 10% GALT activity when measured on liver biopsy.

The GALT gene is located at chromosome 9p13. A large number of mutations have been identified (some are specifically associated with variants); the most common mutation in Caucasians causing classic galactosemia is Q188R. The "Negro" variant is associated with the most common mutation in the GALT gene found in black populations, *S135L*.

The diagnosis is frequently made on the basis of a neonatal screen. Positive neonatal screens should always be followed up with assay of red blood cell GALT activity (see Table 55.1-4). Isoelectric focusing of GALT will identify the Duarte variant, which has a faster electrophoretic mobility than normal GALT. In the unscreened population, the clinical presentation with metabolic collapse and/or *E. coli* sepsis is a clue to check for nonglucose urinary reducing substances (remember that this will become negative following the cessation of galactose-containing feeds). With clinical suspicion, milk feeds should be discontinued and IV glucose given until enzyme activity can be proven to be present.

The treatment for galactosemia is dietary galactose restriction, which, for an infant, means removal of breast milk or regular formula feeds and substitution with a galactose-free formula. The elimination of galactose will lead to a fall in red cell galactose and urinary excretion of metabolites such as galactitol and galactinate within a few days. However, red cell galactose 1-phosphate levels remain high and fall only gradually, and red cell galactose 1-phosphate levels never return fully to normal. The introduction of a galactose-exclusion diet allows recovery from the initial acute illness and prevents further acute metabolic episodes. It will reverse liver and renal dysfunction and prevent the formation of cataracts. Unfortunately, the diet as it exists presently appears to do little for the long-term complications of mental retardation or ovarian dysfunction in females.[133] In addition, growth failure, speech delay, and delayed-onset neurologic lesions are also not obviously affected by diet.[134] Waggoner and colleagues have shown that the incidence of these complications in patients who start a galactose-free diet later after a clinical presentation is not significantly different from those children on galactose elimination prior to the onset of symptoms because of neonatal screening or because of a previously affected sibling.[135]

Therefore, if galactose restriction does not alter long-term outcome, what is the pathogenesis of these long-term complications? The answers remain to be elucidated, but the debate revolves around a number of questions. The first is, Does the insult or injury occur before or after birth? Amniotic fluid of fetuses with classic galactosemia has been demonstrated to have increased levels of galactitol,

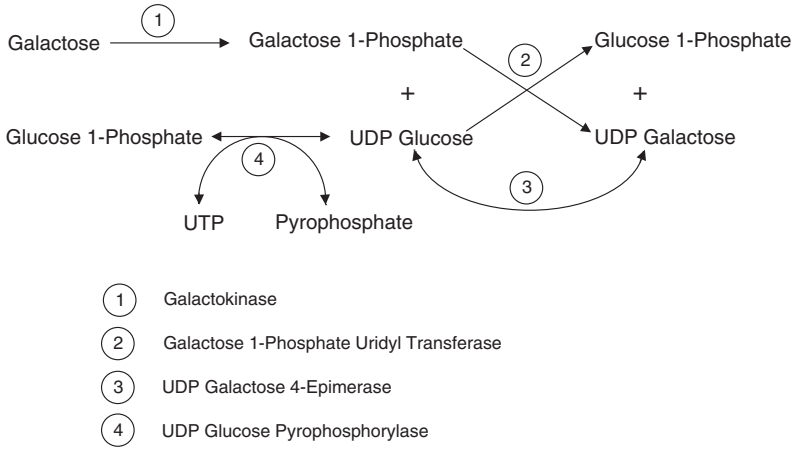

FIGURE 55.1-6 Metabolism of galactose to uridyl diphosphogalactose. UDP glucose = uridyl diphosphoglucose; UTP = uridyl triphosphate.

and cord blood has increased galactose 1-phosphate, even if the mother is on a galactose-restricted diet, suggesting in utero metabolic derangement.[136] To support the idea of postnatal injury, it has been noted in a number of studies that mental retardation measured by DQ/IQ is not a fixed defect but falls progressively with age and may not be affected by the patient's compliance with galactose restriction.[137] Similarly, ovarian failure common in affected females is not always manifest with primary amenorrhea; secondary amenorrhea may even occur after a successful pregnancy. It would seem quite possible that there is a contribution to the long-term complications of galactosemia, both from antenatal and postnatal insults.

The second question is, If the diet is adequately restricted, where does the toxic galactose come from? Are there unrevealed dietary sources of galactose, or is this some form of "autointoxication" with de novo synthesis of galactose or galactose 1-phosphate in the body? In practical terms, a completely galactose-free diet is effectively impossible. Although the vast majority of galactose in the diet comes from milk and dairy products, there are other sources of dietary galactose. Free galactose exists in some fruit and vegetables, and galactose can be derived from animal glycoproteins, galactolipids, and galactosides.[138] It has been suggested that plant oligosaccharides, which contain galactose such as raffinose and stachyose, may contribute to galactose intake. However, humans do not possess the digestive oligosaccharides to free galactose from these sugars. On the other hand, a female volunteer with classic galactosemia had no change in galactose metabolites when taking a diet rich in fruit and vegetables known to contain free galactose compared with when she adhered strictly to a galactose exclusion of less than 8 mg galactose/d.[139] This implied to the authors de novo synthesis of galactose, giving support to the "autointoxication" hypothesis. Galactose 1-phosphate can be synthesized through cleavage of UDP galactose. These metabolites are produced by the reversible actions of UDP galactose 4-epimerase from UDP glucose (see Figure 55.1-6). The flux through this pathway in the absence of GALT activity is not known.

The third question is, Are the long-term complications attributable to the toxic effects of galactose 1-phosphate and other aberrant metabolites or to defective synthesis of glycoproteins and other essential galactose-containing molecules owing to a relative depletion of UDP galactose? This, again, depends on whether the flux through the pyrophosphorylase pathway can make up for the lack of UDP galactose, which, under normal circumstances, appears predominantly to come from metabolism of free galactose. These questions are, as yet, unanswered.

Current recommendations are to maintain galactose restriction as severely as possible, especially in early infancy, while the prognosis in terms of growth and development remains guarded.

UDP Galactose 4-Epimerase Deficiency

This condition was again identified as a result of newborn screening for galactosemia. The initial patients were noted to have normal growth and development. The enzyme deficiency in these patients is restricted to circulating red blood cells and leukocytes, with normal activity of the epimerase enzyme in liver and fibroblasts.[126] This condition is entirely benign.

In contrast, a severe form has been reported in only three families, each with highly consanguineous lineages.[140] The clinical features are those of severe classic galactosemia with early metabolic dysfunction and liver disease. Galactose restriction prevents the acute syndrome but does not influence the growth and mental retardation observed. Walter and colleagues have given small amounts of lactose to these patients in an attempt to maintain essential glycoprotein synthesis because, unlike in GALT deficiency, UDP galactose cannot be generated via the pyrophosphorylase pathway (see Figure 55.1-6).[140]

INBORN ERRORS OF PYRUVATE METABOLISM

Pyruvate Carboxylase Deficiency

Pyruvate carboxylase is key to the initiation and regulation of gluconeogenesis, as well as maintaining cellular concentrations of oxaloacetate. Deficiency of pyruvate carboxylase has been identified in only a small number of patients, but there appear to be three distinct phenotypes.[141] The first group presents in infancy, with lactic acidosis, organic aciduria, elevated plasma levels of pyruvate and alanine, and severe mental retardation. Of particular note is the frequency of this condition among Canadian Natives.[142] The second group is a severe neonatal form, with macrocephaly, intractable lactic acidemia with elevated lactate-to-pyruvate ratios, hyperammonemia, hypercitrullinemia, and hepatomegaly.[143] Death usually occurs by 3 months. Finally, there are two cases described of children with intermittent acidosis but normal development.[144,145] Pyruvate carboxylase activity in fibroblasts is low in all groups of patients, but only in the severe neonatal group are cases found in which no pyruvate carboxylase activity, protein, or messenger RNA can be detected.[146] The variation in phenotype is possibly related to the amount of residual pyruvate carboxylase activity.

Lactic acidosis and elevated alanine levels are related to excess pyruvate accumulation, but the more severe symptoms of hyperammonemia and deranged lactate-to-pyruvate ratios seen in the neonatal form result from an inability to maintain cellular concentrations of oxaloacetate, which is necessary to maintain the TCA cycle and to synthesize aspartate.[141] Aspartate is an essential nitrogen donor for the urea cycle; therefore, citrulline and ammonia accumulate. Aspartate depletion also disrupts transport of reducing equivalents ($NADH^+$ and $NADPH^+$) into the mitochondria, which is reflected in the deranged lactate-to-pyruvate ratio.

Dietary treatment with correction of acidosis and addition of aspartate to supplement the cellular amino acid depletions has been tried.[147] Thiamine, a cofactor for pyruvate dehydrogenase complex, and dichloroacetate, which stimulates pyruvate dehydrogenase activity, may allow

more pyruvate to be metabolized through to acetyl CoA. Biotin, although a cofactor for pyruvate carboxylase, has not been shown to have benefit. There is one report of orthotopic liver transplant in a child with a severe form of pyruvate carboxylase deficiency, and although transplant was unable to reverse preexisting neurologic damage, much of the systemic metabolic disturbance was corrected.[148] There was some optimism that the neurologic progression of this disease may have been slowed.

PEPCK Deficiency

PEPCK, the second enzyme in the gluconeogenic pathway from pyruvate, is dependent on guanosine triphosphate for the conversion of oxaloacetate to phospho*enol*pyruvate. There are two forms of this enzyme: one soluble or cytosolic and the other mitochondrial, which are products of different genes. A number of cases of PEPCK deficiency have been described in children.[149–152] Certain cases have since been demonstrated to be due to generalized mitochondrial defects.[153] The features attributed to the cases that remain are infantile hypoglycemia, fatty infiltration of both liver and kidneys, and liver failure. The extent of the phenotype is not well defined owing to the difficulty of the enzyme assays and the extreme rarity of the cases.

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2. *Amino Acid Metabolism*

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Vomiting, irritability, and failure to thrive are frequent symptoms for which consultation with a pediatric gastroenterologist is sought. Especially when the child is an infant, inborn errors of metabolism must be included in the list of potential diagnoses. Discussed in this chapter are those errors of metabolism that occur in the use or breakdown of amino acids—frequent disorders that can result in common symptoms encountered in pediatric gastroenterology practice. Additionally, because the liver is the main site for many of these metabolic processes, other nonspecific findings, such as hepatomegaly or hepatic dysfunction, may precipitate referral erroneously for evaluation of primary liver disease. Understanding the most common conditions, their forms of presentation, and the methods with which they can be quickly distinguished and diagnosed is the main goal of this chapter.

The metabolic conditions can be categorized into (1) disorders of the aromatic amino acids, of which hereditary tyrosinemia is the most common disorder encountered by gastroenterologists; (2) disorders of branched-chain amino acids (BCAAs), including maple syrup urine disease (MSUD), isovalericaciduria (IVA), propionicaciduria (PA), and methylmalonicaciduria (MMA); and (3) the urea cycle defects, including ornithine transcarbamylase (OTC) deficiency, carbamyl phosphate synthetase (CPS) deficiency, citrullinemia, argininosuccinicaciduria, *N*-acetylglutamate synthetase deficiency, and argininemia. It should be noted that other amino acid metabolic disturbances have been identified in rare subjects; consequently, unusual patterns of metabolite profiles could be encountered for which additional consultation for diagnosis should be considered.

For each condition, the molecular basis of the disease is reviewed, the common clinical presentations are discussed, the method of diagnosis and the distinguishing patterns of metabolites detected with each condition are reviewed, and the effective therapies are covered. Additionally, it is important to understand that nonspecific amino acid disturbances occur with liver disease in general, and the bases for these changes are discussed so that they may be properly interpreted when encountered.

DISORDERED AMINO ACID PROFILES SEEN WITH HEPATIC DISEASE

Hepatic dysfunction with or without hyperammonemia, severe hepatitis, and cholestatic liver disease may cause nonspecific abnormalities in serum and urine amino acid profiles. In the setting of hyperammonemia, concentrations of glutamine, alanine, and sometimes aspartic acid are elevated. With cholestatic liver disease, severe hepatitis and hepatic dysfunction elevation of tyrosine and methionine are frequently seen as a consequence of a nonspecific reduction of tyrosine aminotransferase enzyme activity. Tyrosine aminotransferase is the rate-limiting step in tyrosine metabolism, and its activity can be reduced by as much as 50% with liver disease.

DISORDERS OF AROMATIC AMINO ACIDS

Hereditary Tyrosinemia

Disorders of aromatic amino acids are the most common of the inborn errors of amino acid metabolism owing to the high frequency of phenylketonuria in the population and frequency of alkaptonuria and tyrosinemia, types II and III. However, for the gastroenterologist, it is the disorder of tyrosinemia type I that is of major concern.

Tyrosinemia type I is a devastating disease of childhood that causes liver failure, painful neurologic crises, hepatocarcinoma, and usually death prior to the age of 10 years. It is a rare disorder in the general population, with an incidence no greater than 1 in 100,000 newborns.[1] However, because of the inconsistent and confusing nature of its clinical presentation, it is estimated that less than 50% of cases are diagnosed before their demise. Children born with tyrosinemia type I typically develop severe liver disease at less than 6 months of age and, if unrecognized or untreated, die within a few weeks to months. A more chronic form of the disorder exists in which children develop first symptoms older than 6 months and have a better short-term prognosis. These patients typically present with a Fanconi-like renal syndrome, rickets, and growth failure. They may have repetitive bouts of neurologic crises that are similar in nature to those seen in older patients with acute intermittent porphyria (changes in mental status, abdominal pain, peripheral neuropathy, respiratory depression). In all cases, the symptoms are severe, leading either to death in infancy or to chronic early childhood morbidity, with the eventual development of hepatocarcinoma and death in the later years of childhood.

Molecular Defect

Tyrosinemia type I occurs because of a deficiency of fumarylacetoacetate hydrolase (FAH),[2,3] the terminal enzyme in the tyrosine catabolic pathway (Figure 55.2-1). With the deficiency of FAH, the immediate precursor, fumarylacetoacetate (FAA), is prevented from being con-

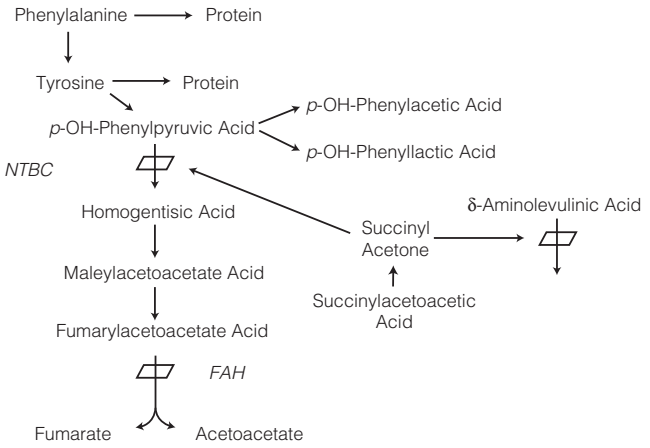

FIGURE 55.2-1 Tyrosine pathway indicating the enzymatic deficiency in tyrosinemia type I and the sites of succinylacetone inhibition. 2-(2-Nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC) acts as an inhibitor of *p*-hydroxyphenylpyruvate oxidase. FAH = fumarylacetoacetate hydrolase.

verted to fumerate and acetoacetate, and FAA is instead diverted to succinylacetoacetate and succinylacetone. These two compounds, particularly succinylacetone, are believed to be the toxic metabolites in tyrosinemia. It is known that succinylacetone interferes with two enzymatic reactions, *p*-hydroxyphenylpyruvic acid dioxygenase (*p*-HPPD) and δ-aminolevulinic acid (δ-ALA). The elevation of plasma tyrosine observed in tyrosinemia is secondary to the inhibition to *p*-HHPD by succinylacetone.

Succinylacetone is also a potent inhibitor of δ-ALA hydratase.[4] It is the inhibition of this enzyme that precipitates the acute neurologic episodes. δ-ALA dehydratase activities have been shown to be reduced in liver and circulating red cells, and δ-ALA is excreted in high levels in children with tyrosinemia type I.[5] There are also suggestions that succinylacetone may interfere with immune functions, cell functions, heme synthesis, and renal tubular function.[6–8]

Two regions of the world have a higher than expected frequency of tyrosinemia type I. In Norway and Finland, the incidence at birth is estimated to be approximately 1 in 60,000 live births, and within the Saguenay-Lac Saint-Jean region of Quebec, Canada, there is an incidence of 1 in 1,846 live births that has occurred because of a founder effect.[9] A common mutation exists within the latter population (IVS12+5 G→A). Within the United States, only a few mutations account for the majority of cases, with these mutations introduced from the populations of northern and southern Europe and from Mexico.

Clinical Features

The clinical presentation of patients with tyrosinemia can be divided into two main groups: those presenting at less than 6 months of age with severe liver involvement and those presenting after 6 months of age with either mild liver dysfunction or renal involvement, growth failure, and rickets (Table 55.2-1). Children less than 6 months of age typically have acute liver failure, with a markedly prolonged prothrombin time and partial thromboplastin time that are not corrected by vitamin K supplementation. The analysis of other clotting factors indicates the preservation of factor V and factor VIII levels but a decrease in factors II, VII, IX, XI, and XII. Serum transaminase levels may be modestly elevated, and serum bilirubin may be normal or only slightly elevated. Thus, the loss of clotting factors from synthetic function is the major abnormality in the early phases of the disorder.[11] The other striking biochemical feature is a markedly elevated α-fetoprotein that averages approximately 160,000 ng/mL at the time of diagnosis. For those children presenting in the acute phase of their illness, appropriate intervention strategies are essential to prevent their demise. This early phase can progress to loss of liver function and the appearance of ascites, jaundice, and gastrointestinal bleeding. There may be a characteristic odor of "boiled cabbage" or "rotten mushrooms." The odor and elevation of α-fetoprotein often lead to the recognition and eventual documentation of elevations of plasma methionine and tyrosine and the identification of succinylacetone in urine or plasma.

In the more chronic form of the disorder, renal involvement is the major manifestation. The renal tubular dysfunction involves a generalized aminoaciduria, phosphate loss, and, for many, renal tubular acidosis. The continued renal loss of phosphate is believed to be the mechanism for the development of rickets because abnormal calcium levels have not been systematically documented. Growth failure can be ascribed to the chronic illness from poor nutritional intake, liver involvement, or chronic renal disease.

Repeated episodes of neurologic crises often go unrecognized. Mitchell and colleagues reported that 42% of the patients in the French Canadian population had experienced these episodes.[5] They often are associated with excruciating pain and posturing and can last 1 to 7 days. They are associated with weakness or paralysis that, in some cases, may require mechanical ventilation for survival. Van Spronsen and colleagues reported in the international survey that 10% of deaths occurred during a neurologic crisis, representing a major life-threatening mechanism independent of liver failure.[10]

Diagnosis

The pathognomonic metabolite for the confirmation of the diagnosis is succinylacetone. Succinylacetone can be detected in the urine of all untreated patients with tyrosinemia type I. Organic acid analysis by gas chromatography–mass spectroscopy will identify an organic acid pattern with elevations of parahydroxyphenylacetic acid, parahydroxyphenyllactic acid, and significant elevations of succinylacetone. Succinylacetone may need to be requested specifically on laboratory request forms.

The measurement of FAH in cultured skin fibroblasts can be used as a confirmatory test or, potentially, the identification of an abnormal mutation within the FAH gene. These latter approaches are not readily available as a common clinical assay.

TABLE 55.2-1 COMPARATIVE FEATURES OF THE MOST COMMON DISORDERS OF AMINO ACID METABOLISM

DIAGNOSIS	PHENOTYPE	CLINICAL FEATURES	SERUM GLUCOSE	SERUM AMMONIA	METABOLIC ACIDOSIS	URINE KETONES	SERUM AMINO ACIDS	URINE AMINO ACIDS	URINE ORGANIC ACIDS	DIAGNOSIS
Tyrosinemia type I	Acute chronic	Acute liver failure or renal Fanconi syndrome with rickets	Normal	Normal	Absent or mild	None	Elevated methionine and tyrosine	Elevated methionine and tyrosine	Succinylacetone	Succinylacetone in urine or plasma
Maple syrup urine disease	Classic	Neonatal encephalopathy; "maple syrup" urine odor	Sometimes low	Normal	Prominent	Elevated	Markedly increased alloisoleucine, BCAAs	Elevated BCAAs	Elevated BCKAs	BCKD activity in cultured fibroblasts or lymphocytes < 2%
	Intermediate	Developmental delay, seizures	Normal	Normal	Prominent during symptoms	Elevated	Same as above	Same as above	Same as above	3–30%
	Intermittent	Episodic ataxia and lethargy	Normal	Normal	Prominent during symptoms	Elevated during symptoms	Elevated BCAA during symptoms	Elevated BCAA during symptoms	Elevated BCAA during symptoms	5–20%
Isovalericaciduria, propionicaciduria, methylmalonicaciduria	Same as above	Same as above; dehydration, hepatomegaly; "sweaty feet" odor in IVA	Variable	Elevated	Prominent	Elevated	Nonspecific elevations of glycine	Nonspecific elevations	Specific OA patterns	Cultured fibroblast enzyme activity
Ornithine transcarbamylase deficiency	Classic	Lethargy, poor feeding, coma, seizures, developmental delay	Normal	Elevated	Absent	None		Nonspecific elevation	Orotic acid elevation	Small intestine or liver biopsy enzyme activity
Carbamyl phosphate synthetase deficiency		Same as above	Normal	Elevated	Absent	None	Absent citrulline	Nonspecific elevation	Nonspecific elevation	Same as above
Citrullinemia		Same as above	Normal	Elevated	Absent	None	Elevated citrulline	Elevated citrulline	Nonspecific elevation	Cultured fibroblast or lymphocyte enzyme activity
Argininosuccinicaciduria	Neonatal	Same as above; fragile hair	Normal	Elevated	Absent	None	Elevation of glutamine and argininosuccinic acid	Elevated arginine-succinate	Nonspecific elevation	Cultured fibroblast or erythrocyte enzyme activity
	Late onset	Developmental delay, hepatomegaly, fragile hair	Normal	Episodically elevated		None	Same as above	Same as above	Nonspecific elevation	Same as above
Arginase		Developmental delay, spasticity	Normal	Moderately elevated	Absent	None	Elevated arginine	Elevated lysine, arginine, ornithine, cystine	Orotic acid elevation	Erythrocyte enzyme activity

BCAA = branched-chain amino acid; BCKA = branched-chain α-keto acids; BCKD = branched-chain α-keto dehydrogenase; IVA = isovalericaciduria; OA = organic acid.

Treatment

Prior to 1995, there was no effective medical treatment for tyrosinemia type I. Dietary interventions using an artificial formula, low in phenylalanine and tyrosine, were modestly helpful in reducing the formation of succinylacetone and were of some benefit in the more chronic forms of the disease. They were not very effective, however, in managing the acute stage of the disease of young children presenting with liver failure or in the long-term prevention of hepatocarcinoma for those children who survived their early presentation. Data supplied by van Spronsen and colleagues in 1994 showed that for those children diagnosed at less than 2 months of age, there was a 75% mortality rate by 2 years; for those diagnosed between 2 and 6 months of age, there was a 70% mortality by 6 years; and for those diagnosed older than 6 months of age, there was a 40% mortality by 10 years.[11] The only effective therapy was liver transplant to remove the genetic burden of FAH deficiency from the host.

In 1992, Lindstedt and colleagues reported preliminary data on the use of an inhibitor called NTBC [2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione] (or nitisinone; available as Orfadin from Rare Disease Therapeutics, Nashville, TN) as an effective inhibitor of parahydroxyphenylpyruvic acid oxidase for the treatment of tyrosinemia.[11] This inhibitor blocks the enzyme proximal in the catabolic pathway of tyrosine and prevents substrate from reaching the FAH enzyme. Thus, succinylacetoacetate and succinylacetone are unable to be formed, and this prevents the deleterious consequences of their existence. In studies in Europe and the United States, over 250 children with documented tyrosinemia type I have now received this compound (E. Holme, personal communication, 2001). It has reduced the mortality of children with tyrosinemia type I and has dramatically affected the natural history of the disease. Within the United States, of 63 children placed acutely on the medication, there have been 5 deaths. Each death occurred within the first 2 weeks of drug administration, all in children in acute liver failure who were unable to recover from the acute stage of their disease. For the remainder of the children, there was a dramatic improvement (C. R. Scott, unpublished data, 2004). Liver function begins to recover within a week, prothrombin and thromboplastin times return to normal values within 1 month and 2 months, respectively, high levels of α-fetoprotein return to normal levels between 6 months and 1 year, and detectable succinylacetone in the urine disappears within 1 to 2 days of initiation of oral treatment. Normal growth has been demonstrated in children following treatment for a period of 5 to 7 years (C. R. Scott, unpublished data, 2004).

Dietary intervention remains an important aspect of treatment, even with the use of nitisinone. Because nitisinone blocks *p*-hydroxyphenylpyruvic acid oxidase, tyrosine values in blood can rise to levels that cause tyrosine crystals to be deposited in tissue. Tyrosine has a low degree of solubility and readily crystallizes in tissue at concentrations that exceed its solubility. The major complication from nitisinone therapy is tyrosine crystal deposition in the cornea, which leads to photophobia and an inflammatory response in the eye. To prevent this, it is recommended that tyrosine concentrations be monitored in children on a tyrosine-restricted diet and nitisinone to maintain the tyrosine concentration at less than 500 μM. At this level, no eye complications have been observed. To achieve a satisfactory plasma concentration of tyrosine requires special formulas deficient in phenylalanine and tyrosine, coupled with a low-protein diet, not dissimilar to that given to children with phenylketonuria. For young children under the age of 6 months, this is usually not a problem; however, for older children, it may require significant nutritional skills and counseling to achieve these goals.

Monitoring of the liver for the potential development of hepatocarcinoma is essential. It is recommended that magnetic resonance images or computed tomographic scans of the liver be obtained on an annual basis. Given that the liver texture is frequently nodular, monitoring for interval changes that could suggest emerging or enlarging hepatocarcinoma, in the setting of persistently elevated α-fetoprotein, is imperative. This, coupled with monitoring of α-fetoprotein as an indicator of tumor formation, is necessary. Radiographic monitoring should continue until the α-fetoprotein has persistently returned to normal.

Liver transplant still remains an option for eventual treatment but is not as emergent as in the past. Longer-term studies are necessary at this time to make a value judgment on whether liver transplant will be necessary in a significant percentage of affected children, depending on their age of diagnosis or the eventual formation of hepatic nodules with transformation. Of the 19 patients who underwent liver transplant, only 5 patients were operated on for the possibility of hepatocarcinoma, and 4 of these were confirmed to have carcinomatous changes.

DISORDERS OF BCAAS

Maple Syrup Urine Disease (Branched-Chain Ketoaciduria)

The cases of four siblings with cerebral degeneration within the first weeks of life and death within months, all with urine smelling of maple syrup, were first reported in 1954.[12] Westall and colleagues subsequently studied another child with the same clinical features and found high levels of serum BCAAs, and coined the term "maple syrup urine disease."[13] High levels of the branched-chain α-keto acids (BCKAs) derived from the BCAA were then isolated in the urine of these patients, implicating the decarboxylation step as the blocked step in the metabolism of the α-keto acids—hence the alternate name "branched-chain ketoaciduria."[14]

Hepatic presentations may occur with MSUD and other organicacidurias with a Reye-like syndrome characterized by coma, cerebral edema, hepatomegaly, liver dysfunction, hypoglycemia, and hyperammonemia with liver histology showing macro- or microvesicular fatty infiltration.[15] Additionally, pancreatitis is sometimes observed with MSUD and other organicacidurias.[16]

MSUD is an autosomal recessive panethnic metabolic disorder caused by a deficiency in activity of the mitochondrial branched-chain α-keto acid dehydrogenase (BCKD)

complex. The worldwide frequency is approximately 1 in 185,000 live births. In select genetically isolated populations such as the Old Order Mennonite populations of Pennsylvania, however, MSUD occurs as frequently as 1 in 176 live births.[17]

Molecular Defect

MSUD results from a deficiency in the activity of the BCKD multienzyme complex (Figure 55.2-2). In normal catabolism of BCAAs, the amino acids are transported into the cell by a cytosolic membrane transporter. Inside the cell, they are reversibly transaminated by the cytosolic or mitochondrial isoforms of the BCAA aminotransferase to produce the BCKA. The BCKAs are then translocated by a specific transporter into the mitochondria, where they are oxidatively decarboxylated by the single BCKD complex. These reactions generate the corresponding branched-chain acyl CoAs, which are further metabolized to acetyl CoA and acetoacetic acid (leucine), acetyl CoA and succinyl CoA (isoleucine), and succinyl CoA (valine). These end products are used in fatty acid and cholesterol synthesis and adenosine triphosphate synthesis. In the human, the skeletal muscle is the main site of BCAA transamination and oxidation, but there is also BCKD activity in the liver, kidney, heart, brain, and adipose tissue.

Deficiency of the BCKD complex results in inadequate oxidative decarboxylation of the BCKAs, causing the accumulation of BCAAs (leucine, isoleucine, valine) and the associated BCKAs (α-ketoisocaproic acid, α-keto-α-methylvaleric acid, α-ketoisovaleric acid).

The mitochondrial BCKD is an enzyme complex macromolecule with three catalytic components: a thiamine pyrophosphate–dependent decarboxylase, a transacylase, and a dehydrogenase. Additionally, the complex has two regulatory enzymes, a kinase and a phosphatase, which control the complex's activity through reversible phosphorylation-dephosphorylation. Each component is coded for by a separate characterized gene, leading to wide genetic heterogenicity underlying the MSUD phenotype.

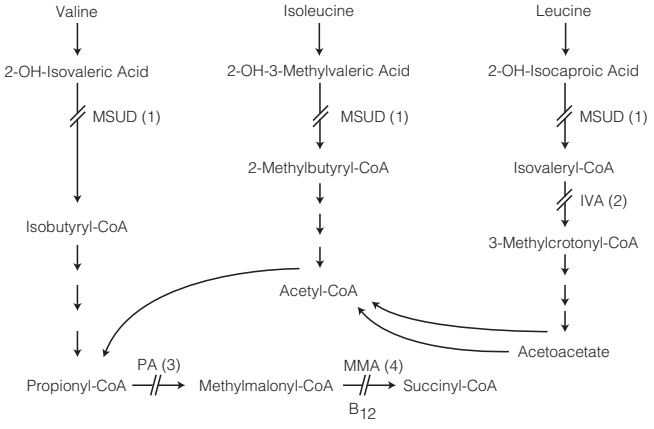

FIGURE 55.2-2 Branched-chain amino acid metabolism. IVA = isovalericaciduria; MMA = methylmalonicaciduria; MSUD = maple syrup urine disease; PA = propionicaciduria. 1 = branched-chain ketoacid decarboxylase; 2 = isovaleryl-CoA dehydrogenase; 3 = propionyl-CoA carboxylase; 4 = methylmalonyl-CoA mutase.

Clinical Features

Multiple clinical phenotypes of MSUD are described based on the biochemical and clinical features (see Table 55.2-1). Distinction based on the patient's responsiveness to thiamine is also made in practice.

Classic. The "classic" form of MSUD is both the most common and most severe form of the disease. It is characterized by the onset of encephalopathy in the neonatal period with high levels of BCAAs, particularly of leucine, in the blood, cerebrospinal fluid, and urine. The presence of alloisoleucine is also characteristic. Symptoms usually develop toward the end of the first week of life, but breastfeeding may delay the onset into the second week. Lethargy and apathy toward feeding are followed by progressive neurologic signs of alternating hyper- and hypotonia, with dystonic extension of the upper extremities and weight loss. It is at this stage that ketosis and the maple syrup or burnt sugar odor to the urine become apparent. Hypoglycemia is not a prominent feature but can be observed. Ultimately, seizures and coma followed by death will occur unless treatment is instituted. Untreated, most patients die in the first months of life from repeated bouts of metabolic crisis and ongoing neurologic deterioration precipitated by infection or other stresses. Surviving individuals may suffer from mental retardation and spasticity. Early treatment has greatly improved the complications faced by these patients, but patients are still at high risk of neurologic sequelae. Even in apparent metabolic control, transient ataxia can occur, and visual hallucinations are frequently experienced in periods of ketonemia.[18] Metabolic crises induced by physical stress can cause encephalopathy and death.[18,19] These patients usually have less than 2% of the normal BCKD activity in cultured fibroblasts or lymphoblasts.

Intermediate. These rare patients are spared the catastrophic neonatal neurologic crises but do have persistently elevated BCAAs. Neurologic impairment with developmental delay and seizures are still a risk. In these subjects, the BCKD activity is roughly 3 to 30% of normal. Few patients have been diagnosed with this form of MSUD, most in the 5-month to 7-year age range during evaluations for their neurologic abnormalities.

Intermittent. Children with this variant usually present between 5 months and 2 years of age. Earlier in life, they have normal growth and development; however, with infectious stresses such as normal childhood illnesses, they develop metabolic decompensation, exhibited most commonly as ataxia and lethargy. More subtle findings of unsteady gait or behavioral changes may progress to seizures, stupor, and coma. Although the plasma BCAAs are normal during well, nonstressed times, the classic findings of maple syrup urine odor and elevated plasma BCAAs and BCKAs occur during symptomatic periods and can be detected in serum amino acid and urine organic acid profiles. The BCKD complex activity typically ranges between 5 and 20% of normal.[17]

Thiamine-Responsive MSUD. Some individuals who have a chronic elevation of BCAAs and gradual advancement of neurologic involvement without crises (intermediate variant) will have enhanced responsiveness with thiamine administration in addition to the usual protein restriction therapy. The patient population is heterogeneous, and none have been able to be treated with thiamine alone.

Diagnosis

Detection of elevated BCKAs by gas chromatography–mass spectroscopy analysis of urine and elevated BCAAs in the blood by amino acid analysis is indicative of the disorder. The presence of alloisoleucine is pathognomonic for MSUD. Definitive diagnosis is established by a low measured activity of BCKD in cultured lymphocytes or fibroblasts.

Newborn Screening and Prenatal Diagnosis

In some states, MSUD may be detected by newborn screening. The fragmentation pattern of the protonated molecular ions of leucine, isoleucine, and alloisoleucine is measurable by tandem mass spectroscopy. Classic and intermediate variants can be detected through newborn screening; however, the intermittent variant may be missed if their levels of BCAAs in the newborn period are normal.

Prenatal diagnosis is possible by measuring the BCKD activity from cultured cells obtained through amniocentesis (amniocytes) or chorionic villus sampling. The BCKD activity from these cultured cells is in the same range as found with cultured fibroblasts and can be performed from amniotic fluid cells obtained at midtrimester (weeks 14 to 18 of gestation).

Treatment

Both removal of the toxic metabolites and minimizing catabolism and promoting anabolism are important in the initial phase of management. In the acute symptomatic situation, treatment must be instituted emergently to prevent or limit the rapid neurologic deterioration that will otherwise occur. Hemodialysis and continuous venovenous hemofiltration are the most efficient methods for BCAA and BCKA clearance from very high levels.

Dietary therapy is the mainstay of acute and chronic management. Initially, the BCAAs are totally omitted from the diet for a few days to allow for correction of their elevated levels. When enteral intake is possible, this can be achieved with BCAD 1 or 2 (Mead Johnson and Company, Evansville, IN) or Ketonex 1 or 2 (Ross Pediatrics, Abbott Laboratories, Columbus, OH), formulas that are leucine, isoleucine, and valine free (Table 55.2-2). The plasma levels of isoleucine, followed by valine, and then leucine 7 to 10 days later, will normalize with proper management. Because leucine is the most neurologically toxic metabolite, its normalization is imperative. Leucine-level normalization can be accelerated by adding isoleucine and valine into the diet to maintain their plasma concentration above normal. When parenteral nutrition is necessary, a BCAA-free L-amino acid mixture in combination with glucose, lipid, electrolytes, and vitamins can provide balanced nutrition. Again, isoleucine and valine supplementation after the first few days of therapy can expedite the return to normal levels of leucine. With either parenteral or enteral nutrition, special attention to the changing protein requirements of the subject must occur to properly adjust their dietary therapy.

During stable metabolism, the daily requirements for the BCAA vary dramatically with age, the severity of the enzyme deficiency, and the child's growth rate. The leucine requirement is highest in the first 6 months of life and then falls to stable requirements around the second and third year of life, remaining relatively stable through the first decade. Although the normal daily leucine requirement is between 300 and 600 mg, the optimal intake of this BCAA in subjects with MSUD must be individualized because their requirements may be a fraction of those for normal children. Plasma levels should be kept as close to normal as possible but less than 300 μM, with special attention to avoiding the situation of high leucine in relation to valine and isoleucine. Frequent monitoring of these levels is advisable during the first year of life.

In some subjects, pharmacologic doses of thiamine (5 mg/kg/d) for 3 weeks may improve BCAA tolerance[20] and biochemical stability. Consequently, a trial of thiamine is usually warranted.

TABLE 55.2-2 ENTERAL FORMULAS FOR METABOLIC DISEASE

METABOLIC DISORDER	DESCRIPTION	FORMULAS	
		MEAD JOHNSON NUTRITIONALS*	ROSS PEDIATRICS*†
Tyrosinemia	Phenyalanine and tyrosine free	TYR 1‡ and Tyros 2, 3200AB§	Tyrex 1 and 2
Maple syrup urine disease	Leucine, isoleucine, and valine free	BCAD 2,‖ MSUD diet‡	Ketonex 1 and 2
Propionicaciduria, Methylmalonicaciduria	Isoleucine, valine, methionine, and threonine free	OS 1 and 2#	Propimex 1 and 2
Isovalericaciduria	Leucine free		I-Valex 1 and 2
Urea cycle defects	Contains only essential and some conditionally essential amino acids	UCD 1,# WND 2§	Cyclinex 1 and 2

*"1" formulas are for infants under 1 year of age and "2" formulas are for children over 1 year of age.
†Ross products contain carbohydrate: hydrolyzed cornstarch; fat: coconut, soy, and palm oil; electrolytes, minerals, vitamins, and trace elements.
‡Contains carbohydrate: sucrose; fat: corn oil; electrolytes, minerals, vitamins, and trace elements. Formula names and formulas are subject to change.
§Contains corn syrup solids, modified tapioca starch, corn oil; electrolytes, minerals, vitamins, and trace elements.
‖Contains corn syrup solids, sugar, modified corn starch, soy oil; electrolytes, minerals, vitamins, and trace elements.
#Contains sucrose, no fat; electrolytes, minerals, vitamins, and trace elements.

In severe cases of the neonatal (classic) form of MSUD, liver transplant has been used successfully to correct the metabolic perturbations and allow an unrestricted diet with neurologic stability.[21]

Isovalericaciduria, Propionicaciduria, and Methylmalonicaciduria

Like MSUD, these organicacidurias result from defects in the catabolism of the BCAAs. They, too, can present clinically with severe neonatal crisis, intermittent and late forms, and chronically progressive forms with developmental delay, hypotonia, failure to thrive, and seizures.

Molecular Defects. *Isovalericaciduria*. The first children described with IVA were siblings with an odor of "sweaty feet" or "cheese."[22,23] IVA is now known to be an autosomal recessive inherited disorder caused by a deficiency in the mitochondrial flavoprotein isovaleryl-CoA dehydrogenase apoprotein, which transfers electrons to the respiratory chain via the electron transfer flavoprotein (see Figure 55.2-2, enzyme 2). This deficiency results in the accumulation of isovaleryl-CoA derivatives, including isovaleric acid, 3-hydroxyvaleric acid, *N*-isovalerylglycine, and isovaleryl-carnitine; the latter two forms are nontoxic, readily excreted derivatives of the toxic isovaleric acid; hence, glycine and carnitine can be used therapeutically to aid in isovaleric acid excretion.

***Propionicaciduria*.** PA is an autosomal recessive disorder with an incidence of less than 1 in 100,000 live births.[16] It is caused by a deficiency in the mitochondrial biotin-dependent enzyme propionyl-CoA carboxylase (see Figure 55.2-2, enzyme 3). This enzyme defect results in elevated levels of free propionate, propionylcarnitine, 3-hydroxypropionate, and methylcitrate in the urine and blood. More rarely combined defects that are partly responsive to biotin have been described.

***Methylmalonicaciduria*.** MMA is an autosomal recessive disorder, with an incidence of 1 in 100,000 live births, caused by a deficiency in vitamin B_{12}–dependent methylmalonyl-CoA mutase apoenzyme (see Figure 55.2-2, enzyme 4). The resultant metabolic block results in elevated levels of methylmalonyl CoA, methylmalonic acid, and, secondarily, the same elevations as seen with PA in plasma and urine. In subjects in whom elevated methylmalonic acid is found, vitamin B_{12} deficiency must be ruled out.

Clinical Features. The clinical presentations of patients with IVA, PA, or MMA follow the same patterns as those in subjects with MSUD. Patients presenting with IVA, PA, or MMA are frequently dehydrated and commonly have moderate hepatomegaly. They have a metabolic acidosis from hyperlactacidemia, elevated anion gap, and ketonuria. Hyperammonemia is uniformly present with IVA, PA, and MMA and, if severe enough, can cause a confounding picture that may lead the clinician to erroneously diagnose a urea cycle defect (Figure 55.2-3). Moderate hypocalcemia and variable glucose levels (hypo-, normal, or hyper-) are common, and cytopenias are frequent with IVA, PA, and MMA. IVA can be distinquished by its "sweaty feet" unpleasant odor.

Diagnosis and Prenatal Diagnosis. Diagnosis in IVA, PA, and MMA is based on the detection of the elevated organic acids in the plasma or urine in the pattern specific for the different disorders.

For those states or countries using tandem mass spectroscopy as a component of newborn screening, each of these organic acid disorders will be detected from submitted blood spots. An abnormal profile of acylcarnitine will detect increased concentrations of propionyl-, methylmalonyl-, or isovalerylcarnitine.

In the case of IVA, PA, and MMA, reliable and fast prenatal diagnosis is possible by the twelfth to fourteenth week of gestation by directly measuring the metabolites in the amniotic fluid. Direct enzyme analysis can also be performed from fresh or cultured chorionic villi or cultured amniocytes.

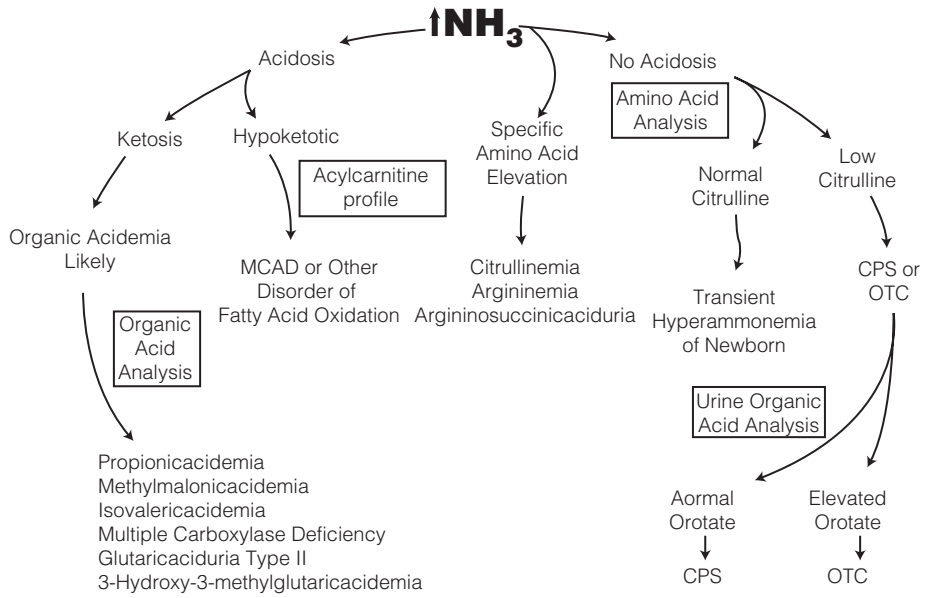

FIGURE 55.2-3 Diagnostic evaluation of the hyperammonemic infant. CPS = carbamyl phosphate synthetase; MCAD = medium-chain acyl-CoA dehydrogenase deficiency; OTC = ornithine transcarbamylase deficiency.

Treatment. The same principles as with the treatment of MSUD apply to the treatment of these disorders. Protein restriction, specifically BCAA restriction, is required. Specialty formulas with supplemental amino acids or protein as indicated are again effective (see Table 55.2-2) with attention to maintaining anabolism even in the face of metabolic crisis. In the case of IVA, leucine restriction is required. For patients with defects of PA, the administration of biotin (5 mg/d orally) may help in clinical and biochemical improvement.

In acute metabolic crisis, hemodialysis or continuous venous-venous hemoperfusion is effective, and for IVA, oral L-glycine (250–600 mg/kg/d) and intravenous L-carnitine (100–400 mg/kg/d) may assist with toxin removal.

DISORDERS OF THE UREA CYCLE

The urea cycle serves to convert the ammonia into nontoxic and readily excreted urea. Ammonia is produced continuously from degradation of the amino groups of amino acids and purines and from the amide groups of glutamine and asparagine. Although some is reused in the synthesis of nonessential amino acids and pyrimidines, the majority of ammonia is excreted as urea. The urea cycle is found in its entirety only in the liver and is composed of both cytosolic and mitochondrial enzymes. Patients with defects at each step in the cycle have been detected, resulting in disorders of the urea cycle. Although there are clinical distinctions between these conditions, they all result in significant hyperammonemia, neurologic compromise, and significant morbidity and mortality. Presentation with these disorders can occur at any time, but they typically become apparent with higher protein intake or metabolic stress from infection; hence, infancy, the toddler years, and puberty are the greatest risk periods. The distinction in infancy between the different disorders of hyperammonemia resulting in coma can be important for therapeutic intervention (see Figure 55.2-3).

The mainstay of therapy involves protein restriction, but pharmacologic interventions are also effective with many of these disorders. Despite therapy and dietary control, however, many of these conditions will result in repeated episodes of hyperammonemia and mental deficiency. Consequently, liver transplant has been offered and has been successful for many of the more aggressive conditions to ameliorate the metabolic deficiency.[24–29]

OTC Deficiency

Molecular Defect. OTC deficiency is an X-linked disorder of the mitochondrial enzyme OTC (Figure 55.2-4, enzyme 2). Enzyme activity is found exclusively in the liver, although some activity is in the small intestine, and a minimal amount is found in the brain. The enzyme catalyzes the formation of citrulline from carbamyl phosphate and ornithine. Its absence results in the accumulation of not only ammonia but also orotic acid and other pyrimidine derivatives, including uracil and uridine, which are derived from the excessive carbamyl phosphate.[30] These accumulated compounds can be detected in blood and urine.

Clinical Features. *Males.* The classic and severe form of OTC deficiency results in an enzyme activity level of less than 2% of normal and the death of 75% of the affected male infants in the first months of life.[30] Within hours or days of birth, poor feeding, lethargy, and tachypnea with labored breathing become apparent. Seizures and coma subsequently develop, and cerebral edema and death follow without treatment. In those patients who survive, severe neurologic impairment with mental retardation is common.

A milder variant occurs in males who present at a later age, usually precipitated by a protein challenge either from a dietary change, infection, or concomitant illness. These individuals generally have a higher level of enzyme activity. Hepatomegaly may be observed in these older patients.

Heterozygous Females. In females, the most common presenting symptoms are those of vomiting, feeding difficulties, headache, or tiredness after a protein-containing meal. Onset is in infancy or during the first decade most commonly. In older subjects, again, hepatomegaly may be observed.

Diagnosis. The condition should be suspected in any male infant with hyperammonemia without acidosis (see Figure 55.2-3). The presence of oroticaciduria and the absence of citrulline in plasma are diagnostic. Enzyme analysis from duodenal or rectal mucosal biopsy or liver tissue will confirm the diagnosis and determine the degree of enzyme deficiency.

Heterozygosity for OTC deficiency in females may be detected by assaying the urine for orotic acid following a protein load of 1 g/kg. The urine is collected in three 4-hour aliquots after the protein ingestion.[30,31] Loading with alanine has alternatively been used; however, false-positive results are more common.[32] The allopurinol test is also a

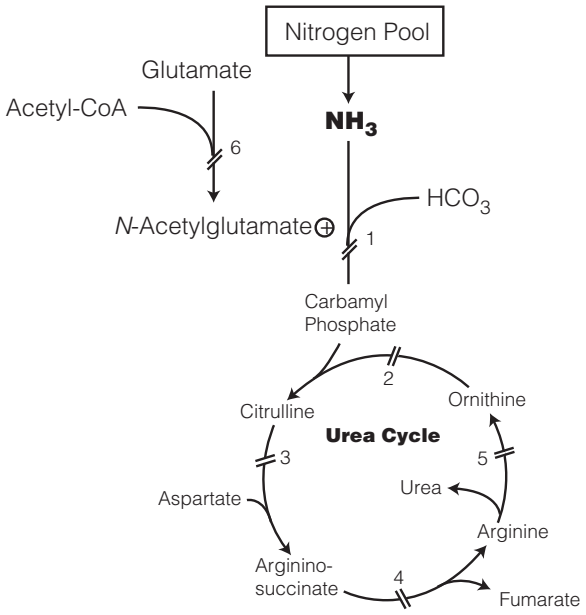

FIGURE 55.2-4 The urea cycle and associated defects. 1 = carbamyl phosphate synthetase; 2 = ornithine transcarbamylase; 3 = argininosuccinate synthetase; 4 = argininosuccinase; 5 = arginase; 6 = *N*-acetylglutamate synthetase.

common way to detect heterozygotes. Allopurinol inhibits the decarboxylation of orotidine monophosphate, a breakdown product of ototic acid. This accumulation of orotidine monophosphate can then be detected in the urine as orotic acid and orotidine.[33] In all cases, an experienced laboratory is required to perform and interpret these tests.

Treatment. The goal of treatment of patients with urea cycle defects is to eliminate the accumulated ammonia and precursors to its formation, decrease ureagenesis by a low-protein diet, and increase arginine levels (except in arginase deficiency) for the conversion of waste nitrogen into urea.

In the acute setting with hyperammonemia causing life-threatening symptoms, rapid and effective ammonia removal can be achieved with hemodialysis or continuous venous-venous hemoperfusion.[34,35] All protein intake should be stopped and an infusion of 10% glucose initiated.

The pharmacologic provision of alternative routes for waste nitrogen excretion is also effective after the acute removal of ammonia (NH_3) (Figure 55.2-5). Glycine and glutamine can be removed from the nitrogen pool by giving benzoate and phenylbutyrate, respectively. Benzoate conjugates to glycine, forming hippurate, and phenybutyrate conjugates to glutamine, forming phenylacetylglutamine. Each mole of glycine conjugated to benzoate removes one mole of NH_3, and each mole of glutamine conjugated to phenylacetate removes two moles of NH_3. Hippurate and phenylacetylglutamine are excreted in the urine without the need for conversion to urea.[30,35,36] Providing arginine as a substrate for ornithine in those without complete deficiency in OTC can help in urea synthesis and hence further enhance nitrogen excretion, but this is far more helpful in those with citrullinemia or argininosuccinicaciduria. Practically, these compounds are delivered by a loading infusion of 200 to 800 mg/kg arginine hydrochloride, 250 mg/kg sodium benzoate, and 250 mg/kg sodium phenylacetate in 20 mL/kg of 10% glucose over 1 to 2 hours intravenously. A continuous infusion of 250 mg/kg/d each of sodium benzoate and sodium phenylacetate and 200 to 800 mg/kg/d of arginine hydrochloride in 10% glucose with maintenance electrolytes is then provided until the ammonia level is normal.[30] The 10% glucose further limits hyperammonemia by minimizing catabolism and hence ammonia production. The additional use of mannitol in the setting of cerebral edema may further aid in nitrogen excretion by promoting diuresis.

The long-term management of the chronically hyperammonemic child usually requires a combination of dietary and pharmacologic manipulations. Phenylbutyrate 250 mg/kg/d (less odoriferous than phenylacetate) can be given alone or in combination with sodium benzoate (250 mg/kg/d) and arginine (200 mg/kg/d). The diet is usually restricted to protein of 700 mg/kg/d, with the essential amino acids added at 700 mg/kg/d total through a combination of regular formula and the specialty formulas (see Table 55.2-2).

CPS Deficiency

Molecular Defect. CPS I (CPS) is a liver mitochondrial enzyme that catalyzes the formation of carbamyl phosphate from ammonia using acetylglutamate as an activator and is the first step in the urea cycle (see Figure 55.2-4, enzyme 1). There also exists cytosolic CPS, designated CPS II, which is involved in pyrimidine biosynthesis and is largely responsible for the formation of orotic acid in the face of carbamyl phosphate excess with OTC deficiency. CPS I deficiency is a rare condition resulting from an autosomal recessive mutation. This mutation gives rise to a wide clinical heterogenicity in the patients affected depending on the degree of enzyme deficiency. Owing to the early block in the urea cycle caused by this deficiency, subjects have high ammonia in the face of low orotic acid, citrulline, and arginine.

Clinical Features. Children affected with CPS deficiency typically present in the first days of life after feeds are initiated. Drowsiness, poor feeding, lethargy, vomiting, and hypo- or hypertonia become apparent, followed by seizures, hypothermia, and coma. Death usually occurs in the first 2 to 18 months without treatment. Untreated survivors are typically mentally retarded, with neurologic complications.

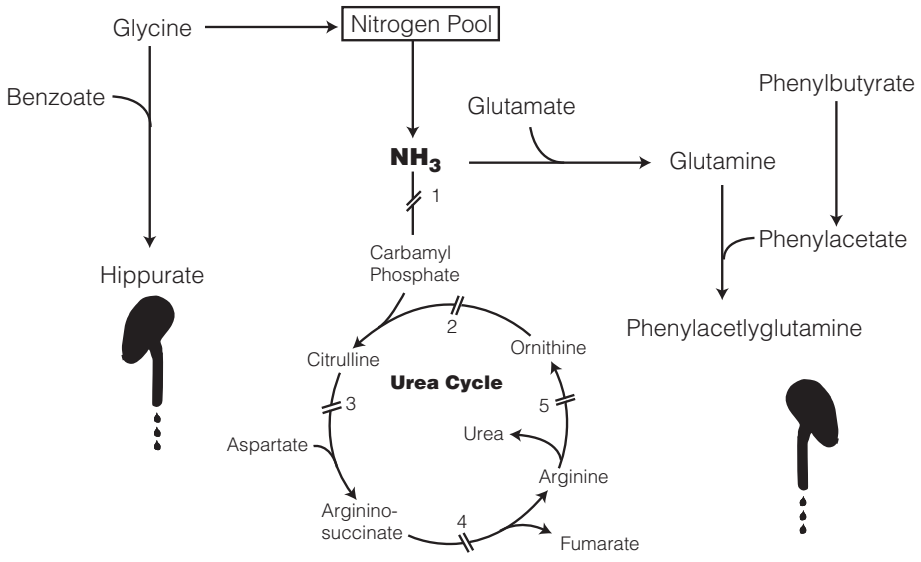

FIGURE 55.2-5 Alternative routes of waste N_2 excretion. 1 = carbamyl phosphate synthetase; 2 = ornithine transcarbamylase; 3 = argininosuccinate synthetase; 4 = argininosuccinase; 5 = arginase; 6 = *N*-acetylglutamate synthetase.

More rarely, a partial deficiency of CPS I results in older individuals having episodic lethargy and vomiting associated with high protein intake or states of catabolism, usually with progressive, severe neurologic consequences.

Diagnosis. CPS deficiency should be suspected in the young infant with hyperammonemia in the absence of a metabolic acidosis, low orotic acid, low citrulline, and low arginine. The diagnosis can be confirmed and enzyme activity measured from biopsy material from the liver, small intestine, or rectal mucosa.

Treatment. The treatment of CPS is the same as that for OTC deficiency.

Citrullinemia

Citrullinemia was first reported in 1963[37] and is now known to be secondary to deficiency of argininosuccinate synthetase, a widely expressed enzyme.

Molecular Defect. Citrullinemia is an autosomal recessive disorder resulting from a mutation in the gene for the cytosolic enzyme argininosuccinate (argininosuccinic acid) synthetase, mapped to chromosome 9 at q34.[38] This enzyme catalyzes the formation of argininosuccinic acid from citrulline and aspartic acid (see Figure 55.2-4, enzyme 3). Argininosuccinate synthetase is widely distributed and has been measured in cultured fibroblasts and in liver.

Clinical Features. The clinical presentation of patients with citrullinemia is heterogeneous. The majority of subjects present in infancy either with the classic neonatal form or later in the first year with a more subacute form. Patients with the neonatal form resemble infants with OTC deficiency. After the first few days, infants develop poor feeding, lethargy, and irritability; seizures are common, followed by coma and death within weeks, if not treated. Hepatomegaly is sometimes observed. In infants with the classic neonatal form, the enzyme activity is usually zero. The subacute form may present later in the first year with developmental delay, failure to thrive, recurrent vomiting, ataxia, or seizures. Presentations in late childhood or early adulthood have been reported in some patients.[39]

Diagnosis and Prenatal Diagnosis. Diagnosis should be suspected in the hyperammonemic infant without metabolic acidosis and with elevation of plasma and urine citrulline. The diagnosis can be confirmed and level of enzyme activity determined from cultured fibroblasts or lymphocytes.

Prenatal diagnosis is possible by analyzing the amniotic fluid for citrulline or by assay of the enzyme from cultured amniocytes or, less reliably, chorionic villi.

Treatment. The general principles of treatment are the same as in the treatment of patients with OTC deficiency. In citrullinemia, however, the administration of arginine can dramatically lower the level of ammonia by providing this compound as the backbone for urea synthesis. Furthermore, as the elevated citrulline is not thought to be the toxic metabolite (ammonia causes the toxicity), and citrulline itself is excreted in the urine, the provision of arginine allows for increased synthesis of citrulline, use of ammonia, and its subsequent excretion. Citrulline adds only one N atom to ornithine and hence is not as efficient as urea in waste nitrogen excretion but is adequate in times of anabolism. Arginine can be provided along with sodium benzoate and phenylacetate in both the loading solution and continuous infusion solution at a dose of at least 660 mg/kg.[39] Sodium bicarbonate and chloride monitoring may be necessary because arginine is supplied as arginine hydrochloride for intravenous use.

Argininosuccinicaciduria

Argininosuccinicaciduria is somewhat unique compared with the previous urea cycle defects discussed in that many patients with this disorder come to attention for chronic, more indolent complaints of alopecia or mild retardation, and most have hepatomegaly. The majority, however, do present in the neonatal period with symptoms typical of the urea cycle disorders.

Molecular Defect. This condition results from a defect in the enzyme argininosuccinate lyase (argininosuccinase), which catalyzes the conversion of argininosuccinic acid (ASA, formed from aspartate and citrulline) to fumarate and arginine (see Figure 55.2-4, enzyme 4). These patients have marked elevations in the levels of ASA in blood, urine, and cerebrospinal fluid and also may have elevations of orotic acid because this is created from the combination of aspartate and carbamyl phosphate.

Deficiency of argininosuccinate activity is inherited in an autosomal recessive manner, and the gene for this enzyme has been localized to chromosome 7.[40] The enzyme deficiency has been demonstrated in the liver, red blood cells, and cultured fibroblasts.[41]

Clinical Features. *Neonatal.* This is a relatively rare condition in which infants present in the first days of life with the classic symptoms of a urea cycle defect and similar to those with OTC deficiency. Differing features have been observed, however, including hepatomegaly and abnormally fragile hair by weeks of age.

Infantile. Patients present at months of life with failure to thrive, feeding difficulties, and, later, seizures and psychomotor retardation.

Late Onset. This is the most common form of presentation. In the second year of life, patients are noted to have developmental delay and frequently develop feeding difficulties, irritability, and seizures. Symptoms more attributable to hyperammonemia, such as episodic ataxia, lethargy, and seizures, are provoked by infection or increased protein in the diet. Many of these subjects have hepatomegaly, and roughly 50% have abnormally fragile hair. The hepatomegaly is usually associated with elevation of transaminases, but the synthetic capacity of the liver is generally preserved.[42,43]

The fragile hair is trichorrhexis nodosa, short dry hair that never needs to be cut because it breaks easily. Microscopically, the hair shafts have nodules.

Diagnosis and Prenatal Diagnosis. The diagnosis is made by demonstrating an excess of ASA in urine or plasma. The diagnosis can then be confirmed by assaying ASA lyase in red blood cells or cultured fibroblasts.[41]

Prenatal diagnosis is possible by assaying for enzyme activity in cultured amniocytes[44] or by the detection of ASA in amniotic fluid.

Treatment. Treatment is similar to that of citrullinemia. Providing arginine enables forward cycling of the urea cycle using ammonia in the formation of ASA. ASA is effectively excreted in the urine and is as effective in N removal as urea. Sodium benzoate and phenylacetate are not generally needed; however, sodium bicarbonate and chloride monitoring may be necessary because arginine is supplied as the hydrochloride for intravenous use.

N-Acetylglutamate Synthetase Deficiency

Few patients have been reported with deficiency of hepatic *N*-acetylglutamate synthetase. This mitochondrial enzyme catalyzes the combination of acetyl CoA and glutamate to form acetylglutamate, which, in turn, is required for activation of CPS I (see Figure 55.2-4, enzyme 6). The clinical phenotype and treatment are similar to those observed with CPS deficiency.

Argininemia

Argininemia is the least common of the urea cycle defects and was initially reported in 1965 and 1969.[45,46]

Molecular Defect. Argininemia is an autosomal recessive disorder caused by a deficiency of arginase, an enzyme coded for on chromosome 6 at band q23.[47] Arginase is responsible for the conversion of arginine to ornithine and urea (see Figure 55.2-4, enzyme 5). Individuals with arginase deficiency have a nearly complete absence of enzyme activity and have predictably elevated levels of arginine. They may also have elevated levels of orotic acid, however, because arginine serves an effector function toward *N*-acetylglutamate synthetase, which, in turn, stimulates CPS to form carbamyl phosphatase. Because ornithine is lacking in the face of arginase deficiency, the carbamyl phosphate flows in the pathway of pyrimidine synthesis and orotic acid is formed.

Clinical Features. Symptomatic onset is usually in infancy, with irritability, poor feeding, vomiting, lethargy, seizures, and coma. Unlike with the other urea cycle defects, however, survivors have spasticity or opisthotonos with developmental delay and may be thought initially to have cerebral palsy. Although ammonia levels elevate intermittently with infection or increased protein in the diet, the degree of ammonia elevation in this condition is not as dramatic as with the other urea cycle defects.

Diagnosis. Elevated plasma arginine levels are diagnostic, and the enzyme activity can be assayed in erythrocytes.

Treatment. The principle of treatment is to restrict arginine from the diet by providing a mixture of amino acids at an equivalent of 2 g/kg of protein, fat and carbohydrates to supply 125 kcal/kg/d, and vitamins and minerals. As the child ages, fruits, low-protein vegetables, and cereals can be introduced and then later the protein types liberalized but to never exceed 5 g/d of high biologic protein (300 mg arginine). Arginine intake must be episodically reduced in the face of infection or illness and blood levels of arginine and ammonia monitored regularly.[43]

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3. Inherited Abnormalities in Mitochondrial Fatty Acid Oxidation

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Mitochondrial fatty acid β-oxidation (FAO) plays a pivotal role in energy production and homeostasis once glycogen stores are depleted owing to fasting, during febrile illness, and owing to increased muscular activity. Mitochondrial fatty acid β-oxidation provides as much as 80% of energy for heart and liver functions at all times.[1] In the liver, the oxidation of fatty acids fuels the synthesis of ketone bodies, 3-hydroxybutyrate and acetoacetate. Ketones are used as an alternative energy source by extrahepatic organs, particularly the brain, and the oxidation of long-chain fatty acids also provides the energy required for nonshivering thermogenesis by brown adipose tissue.

The first genetic defect of FAO in humans was recognized in 1973 as a disorder of skeletal muscle presenting with exercise-induced rhabdomyolysis and myoglobinuria.[2] Many additional FAO disorders covering a wide spectrum of phenotypes have since been discovered. A growing number of clinical entities such as Reye syndrome, sudden infant death syndrome, cyclic vomiting syndrome, liver failure, and maternal complications of pregnancy have been associated with various FAO disorders.[1]

BIOCHEMISTRY OF MITOCHONDRIAL FATTY ACID METABOLISM

Lipid Mobilization and Transport

Glycogen serves as the primary energy source, but once glycogen stores become depleted, energy must be acquired by alternative means. Decreasing blood glucose concentrations cause a reduction of the insulin-to-glucagon ratio and subsequent lipid mobilization from adipose tissue. Free fatty acids are released into the plasma following the hydrolysis of triglycerides by endothelial-bound lipoprotein lipase and hepatic lipase. The most abundant species are long-chain fatty acids, in particular palmitic ($C_{16:0}$), stearic ($C_{18:0}$), oleic ($C_{18:1}$), and linoleic ($C_{18:2}$) acids.[3,4]

Long-chain fatty acids are weakly soluble in plasma and readily bind to albumin. Two models, involving both saturable (protein mediated) and nonsaturable (nonprotein mediated) components, specific for cellular long-chain fatty acid uptake exist.[5] In a non–protein-mediated model, fatty acids partition into membranes and become protonated, and the neutral molecule is translocated across the membrane into the cytosol.[6] Growing evidence also supports the presence of high-affinity tissue-specific fatty acid transporters on liver and muscle cell membranes (Figure 55.3-1).[7] A family of fatty acid transport proteins (FATPs) has been characterized in different species, and in vitro experiments demonstrated increased fatty acid import when expressed in cultured cells and reduced uptake when the *FATP1* gene was disrupted.[8,9] To date, however, mutations in the *FATP1* gene have not been associated with a disease.

Inside the cytosol, fatty acid binding proteins play a similar role as plasma albumin in the binding and transportation of intracellular fatty acids.[10] Eventually, the intracellular fatty acids are esterified by acyl coenzyme A (CoA) synthetases to fatty acyl CoAs. These can bind to ubiquitously expressed, high-affinity acyl-CoA binding proteins (ACBPs) or to the lower-affinity fatty acid binding proteins (~ 1,000-fold lower affinity than the ACBPs). Bound to ACBPs, acyl-CoA esters are delivered to carnitine palmitoyltransferase (CPT) 1.[11]

Role of Carnitine

Whereas short- and medium-chain fatty acyl-CoA esters are able to passively cross the mitochondrial membranes, long-chain fatty acyl-CoA esters are actively transported. This transport mechanism requires intracellular carnitine as a cofactor and involves three steps mediated by two CPTs (1 and 2) and a carnitine acylcarnitine translocase (CACT) (see Figure 55.3-1).[12]

Carnitine (β-hydroxytrimethylaminobutyrate) is an essential molecule in intermediary metabolism.[13] It is involved in the transfer of cytosolic fatty acids across the mitochondrial membrane for β-oxidation. Products of peroxisomal β-oxidation are also transported by carnitine to the mitochondria for further oxidation. Carnitine influences the acetyl CoA to free CoA ratio by acting as a reservoir for activated acetyl groups. Finally, carnitine influences the toxicity associated with abnormal accumulation of fatty acids and organic acids by forming carnitine esters, which can be removed from the intracellular environment.

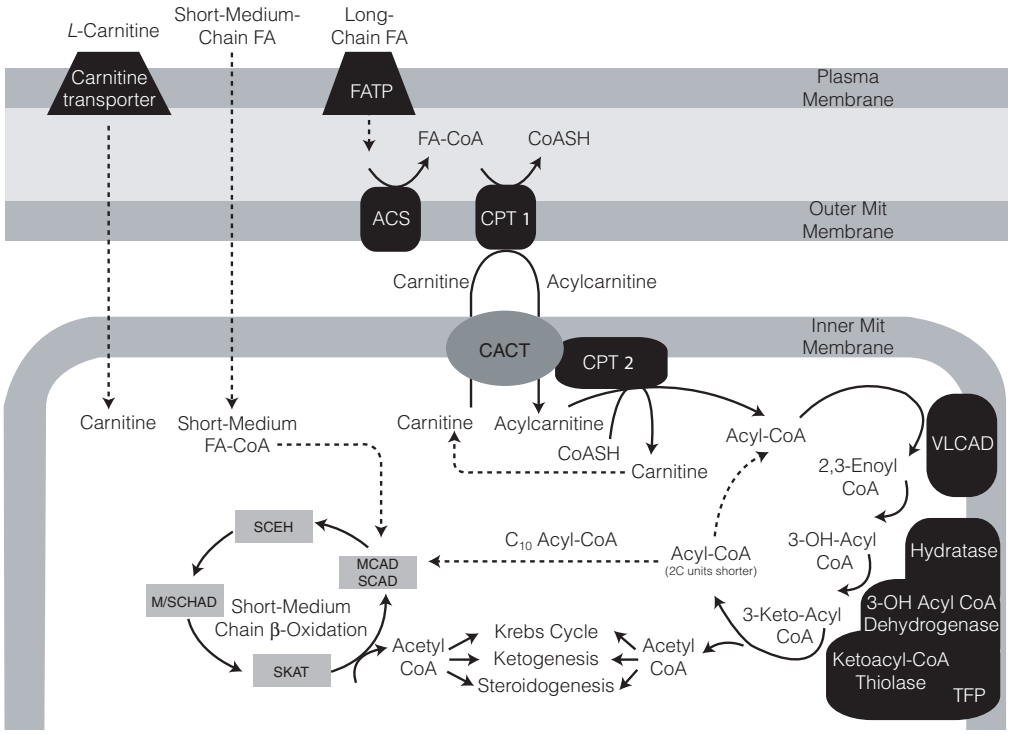

FIGURE 55.3-1 Overview of fatty acid import and metabolism. ACS = acyl-CoA synthetase; CACT = carnitine acylcarnitine translocase; CoA = coenzyme A; CoASH = unacylated coenzyme A; CPT1 = carnitine palmitoyltransferase 1; CPT2 = carnitine palmitoyltransferase 2; FA = fatty acid; FA-CoA = fatty acyl CoA; FATP = fatty acid transport protein; MCAD = medium-chain acyl-CoA dehydrogenase; Mit = mitochondrial; M/SCHAD = medium-/short-chain 3-hydroxyacyl-CoA dehydrogenase; SCAD = short-chain acyl-CoA dehydrogenase; SCEH = short-chain enoyl-CoA hydratase; SKAT = short-chain ketoacyl-CoA thiolase; TFP = trifunctional protein; VLCAD = very-long-chain acyl-CoA dehydrogenase.

Carnitine originates to about 75% from dietary intake of meat, fish, and dairy products. Under normal conditions, endogenous synthesis from lysine and methionine plays a minor role but can be stimulated by a diet low in carnitine. Carnitine is not further metabolized and is excreted in urine as free carnitine or as conjugated carnitine esters. Adequate intracellular levels of carnitine are therefore maintained by mechanisms that modulate dietary intake, endogenous synthesis, reabsorption, and cellular uptake.

Plasma Membrane Uptake of Carnitine

Only the kidney, liver, and brain have the full complement of enzymes necessary to synthesize carnitine. Other tissues depend on carnitine from the circulation. Carnitine uptake is an active process and occurs against a gradient to concentrate intracellular levels as free carnitine or carnitine esters. In humans, concentrations of carnitine in skeletal and cardiac muscle tissue exceed levels in plasma by 50-fold.

Muscle, heart, placenta, and fibroblasts have been shown to possess high-affinity carnitine transport systems with Michaelis constant (*K*m) values ranging between 6 and 60 μM. The best characterized member of this family is the organic cation transporter 2 (OCTN2) (encoded by the *SLC22A5* gene), a low-*K*m (2–6 μM), high-affinity, sodium-dependent active transporter present in muscle, heart, and renal tubule cells.[13] OCTN2 dysfunction, particularly in the kidney, results in excessive carnitine losses to the urine and consequently reduced plasma and tissue carnitine levels, affecting particularly those tissues relying heavily on ketogenesis as an energy source.

Carnitine Cycle

CPT1 is located in the outer mitochondrial membrane and converts long-chain acyl-CoA intermediates into the corresponding acylcarnitine. There are muscle and liver isoforms of this enzyme, the latter also being expressed in fibroblasts and amniocytes. CACT mediates the transfer of acylcarnitine species from the intermembrane space into the mitochondrial matrix in exchange for free carnitine, thereby maintaining the mitochondrial and cytosolic carnitine pools. CPT2 is located on the matrix aspect of the inner mitochondrial membrane and converts the translocated long-chain acylcarnitine back into its corresponding acyl-CoA ester, releasing free carnitine.

Mitochondrial β-Oxidation

Substrates for β-oxidation are fatty acyl-CoA esters, which repeatedly undergo four chain-shortening steps, each cycle resulting in the removal of a 2-carbon acetyl group (Figure 55.3-2). The four steps of each cycle require the action of four enzymatic activities, each with overlapping chain-length specificities for very-long-, long-, medium-, and short-chain acyl-CoA fatty acid substrates. They include (1) acyl-CoA dehydrogenases (ACADs), (2) 2-enoyl-CoA hydratases, (3) 3-hydroxyacyl-CoA dehydrogenases, and (4) 3-ketoacyl-CoA thiolases. The enzymes with specificity for very-long-chain and long-chain fatty acid substrates are membrane associated, whereas the enzymes recognizing the medium- and short-chain fatty acids are soluble mitochondrial matrix enzymes. The genes for most enzymes involved in FAO have been cloned and pathogenic mutations identified.[14]

Acyl-CoA Dehydrogenases. ACADs are flavin adenine dinucleotide (FAD)-requiring oxidoreductases that catalyze the first dehydrogenation step in the β-oxidation of fatty acids, resulting in the formation of a double bond between the 2 and 3 position of the fatty acyl-CoA derivative. Very-long-chain acyl-CoA dehydrogenase (VLCAD) is

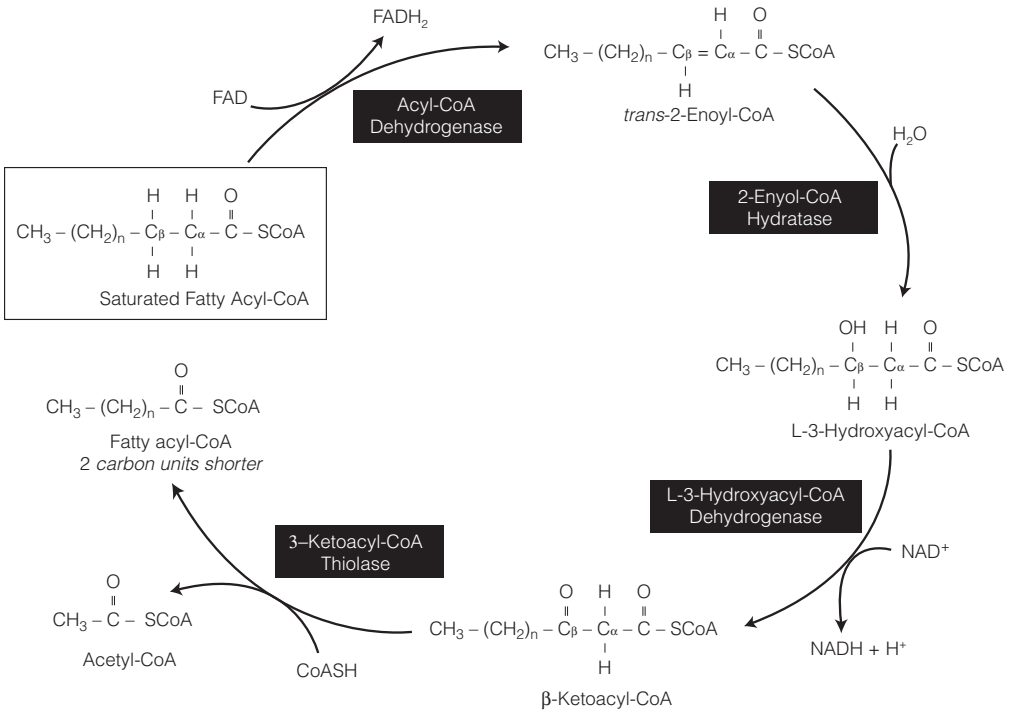

FIGURE 55.3-2 Reactions involved in β-oxidation of saturated fatty acids. CoA = coenzyme A; CoASH = unacylated coenzyme A; FAD = flavin adenine dinucleotide (oxidized form); $FADH_2$ = flavin adenine dinucleotide (reduced form); NAD = nicotinamide adenine dinucleotide (oxidized form); NADH = nicotinamide adenine dinucleotide (reduced form).

a 154 kD heterodimer associated with the matrix surface of the inner mitochondrial membrane.[15] Medium- and short-chain acyl-CoA dehydrogenases (MCAD and SCAD, respectively) are located within the mitochondrial matrix and are homotetramers with subunits ranging from 43 to 45 kD.[16,17] Chain-length specificities for the various ACADs are C_{14} to C_{20} fatty acyl CoA for VLCAD, C_{12} to C_{18} for long-chain acyl CoA dehydrogenase (LCAD), C_6 to C_{10} for MCAD, and C_4 to C_6 for SCAD.

Enoyl-CoA Hydratases. The second step of β-oxidation is catalyzed by 2-enoyl-CoA hydratase and results in the hydration of the double bond of the 2-*trans*-enoyl-CoA ester to form L-3-hydroxyacyl CoA. There are two different hydratases with different substrate specificities. The short-chain enoyl-CoA hydratase, also referred to as crotonase, acts on substrates with short chain lengths with reduced activity as chain length increases. The hydratase with specificity for longer-chain substrates is part of the trifunctional protein (TFP).

Trifunctional Protein. TFP is a hetero-octamer ($\alpha_4\beta_4$) that catalyzes three steps in the mitochondrial β-oxidation of long-chain fatty acids. Long-chain enoyl-CoA hydratase (LCEH) and long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) activities are located at the amino- and carboxy-terminal domains, respectively, of the four α subunits, whereas the four β subunits contain long-chain ketoacyl-CoA thiolase (LCKAT) activity.[18]

L-3-Hydroxyacyl-CoA Dehydrogenases. The conversion of L-3-hydroxyacyl CoA to the corresponding 3-ketoacyl-CoA derivative is mediated by L-3-hydroxyacyl-CoA dehydrogenase and occurs with the concomitant reduction of oxidized nicotinamide adenine dinucleotide (NAD^+) to reduced nicotinamide adenine dinucleotide. Long-chain 3-hydroxyacyl-CoA substrates are metabolized by LCHAD, which is part of the aforementioned TFP. The medium-/short-chain L-3-hydroxyacyl-CoA dehydrogenase (M/SCHAD) has highest activity toward 3-hydroxybutyryl CoA but will also recognize substrates with higher chain lengths with reducing activity as chain length increases.

3-Ketoacyl Thiolase. The last reaction in the β-oxidation spiral is performed in the presence of CoA and involves a thiolytic cleavage between C-2 and C-3 atoms of the 3-ketoacyl-CoA intermediate with the release of acetyl CoA and the generation of an acyl-CoA product shortened by two carbons. There are two mitochondrial thiolases. The ketothiolase that resides in the β subunit of the TFP cleaves long-chain substrates, whereas the remaining keto acyl-CoA derivatives of shorter chain lengths are substrates for medium-chain ketoacyl thiolase (MCKAT).[18]

Oxidation of Unsaturated Fatty Acids

Unsaturated dietary fatty acids such as oleic ($C_{18:1}$), linoleic ($C_{18:2}$), and linolenic ($C_{18:3}$) acids undergo β-oxidation in a manner similar to saturated fatty acids, sequentially releasing acetyl CoA with each round until the formation of intermediates, which are not recognized by the enzymes of β-oxidation (Figure 55.3-3). Unsaturated fatty acids with double bonds located at odd-numbered and even-numbered positions, respectively, generate intermediates with the double bond between the C-3 and C-4 atoms (*cis*-3-enoyl CoA) and between the C-2 and C-3 atoms (*cis*-2-enoyl CoA). Further metabolism requires modification by ancillary enzymes. The *cis*-2-enoyl-CoA esters can be hydrated and either be epimerized to form L-(+)-3-hydroxy intermediates or converted to

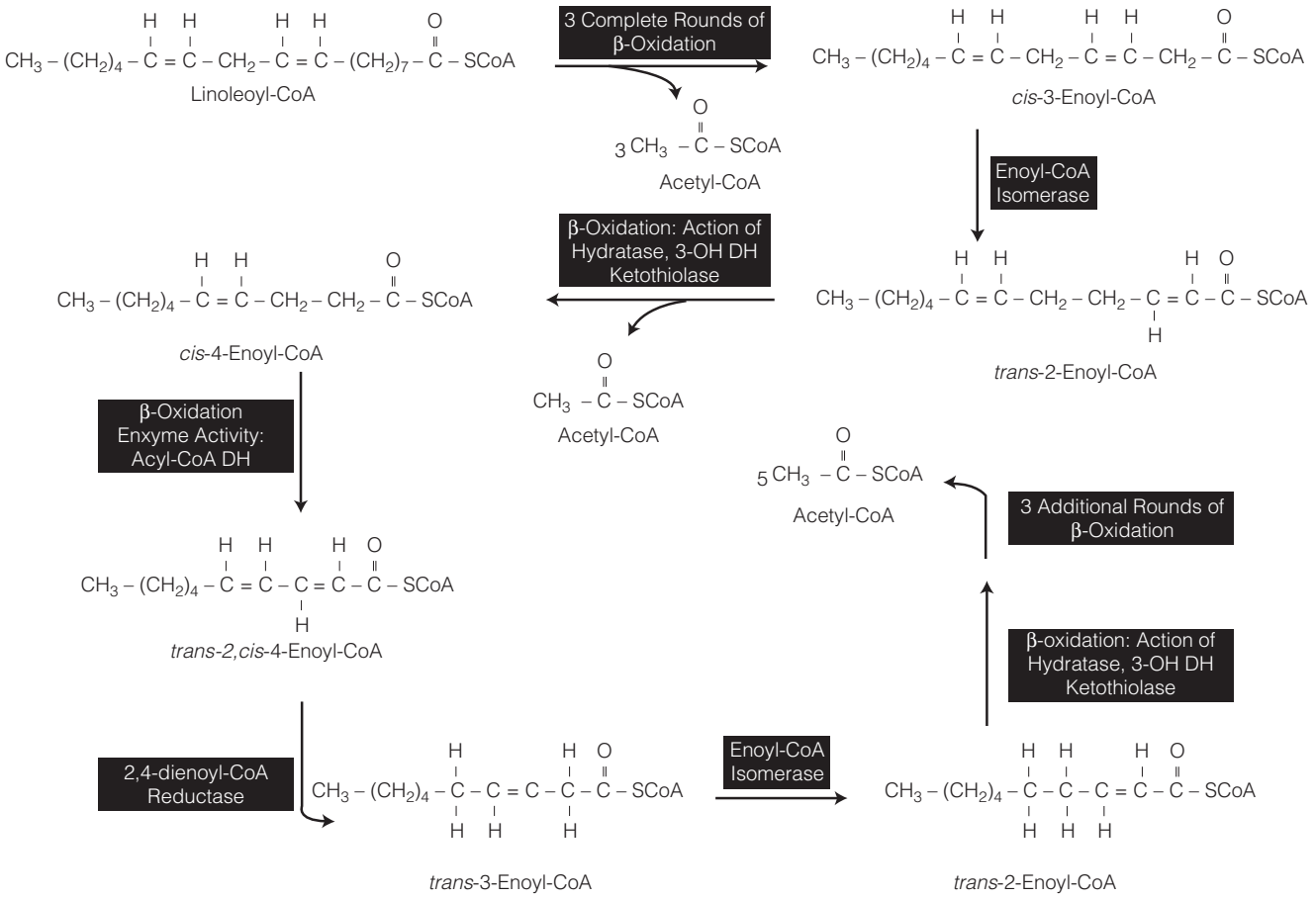

FIGURE 55.3-3 Oxidation of unsaturated fatty acids. CoA = coenzyme A; DH = dehydrogenase.

3-*trans*-enoyl-CoA esters by the action of 2,4-dienoyl-CoA reductase. Re-entry of 3-enoyl-CoA intermediates into the β-oxidation pathway is accomplished by a reaction mediated by 3,2-*trans*-enoyl-CoA isomerase, which results in a shift of the double bond from the C-3 position to the C-2 position. In this step, all 3-enoyl-CoA intermediates, occurring in either the *cis* or *trans* configuration, are converted into their corresponding 2-*trans*-enoyl-CoA intermediates, which can subsequently be metabolized by the enoyl-CoA hydratase of the β-oxidation pathway.

Only one patient has been described with 2,4-dienoyl-CoA reductase deficiency.[19] No defect of enoyl-CoA isomerase has been reported to date.

Oxidation of Odd-Chain Fatty Acids

Fatty acids with an odd number of carbons undergo β-oxidation as described above until the 3-carbon propionyl-CoA molecule is formed. Propionyl CoA is also generated by the metabolism of amino acids and is decarboxylated to methylmalonyl CoA by propionyl-CoA carboxylase. Methylmalonyl CoA is further metabolized by methylmalonyl-CoA mutase to form succinyl CoA, an intermediate of the Krebs cycle.

Ketone Body Metabolism

The three compounds referred to as ketone bodies are acetoacetate (AcAc), 3-hydroxybutyrate (3HB), and acetone. They represent the physiologic products of the metabolism of fatty acids and ketogenic amino acids. The two predominant ketone bodies are AcAc and 3HB, and they share a metabolic relationship in the mitochondria similar to that of pyruvate and lactate in the cytoplasm. 3HB is a stable, nonvolatile metabolite that arises only through the reduction of AcAc. Acetone, formed by the decarboxylation of the chemically unstable AcAc, is responsible for the fruity smell recognized in ketoacidotic individuals.

Acetyl CoA generated by fatty acid β-oxidation is converted to acetoacetyl CoA by the action of β-ketothiolase (Figure 55.3-4). This reaction marks both the first step in ketogenesis and the last step in ketolysis. This enzyme can also catalyze the conversion of the isoleucine intermediate 2-methylacetoacetyl CoA to acetyl-CoA and propionyl CoA. Human β-ketothiolase is a homotetramer composed of 41 kD subunits, and two isoenzymes have been described. The human β-ketothiolase gene is known, and pathogenic mutations have been described in deficient patients.[20]

Acetoacetyl CoA thus formed is transformed into 3-hydroxy-3-methylglutaryl (HMG)-CoA by mitochondrial HMG-CoA synthase. Two HMG-CoA synthase isoenzymes are encoded by distinct genes. One is located inside the mitochondria and is involved in ketogenesis; the other is located in the cytoplasm and plays a role in cholesterol synthesis. Patients with mitochondrial HMG-CoA synthase

deficiency have been described clinically and confirmed at the molecular level.[21]

In the last step of ketogenesis, HMG-CoA is irreversibly converted to AcAc by HMG-CoA lyase. The mitochondrial HMG-CoA lyase is a homodimer, the gene of which has been identified.[22] The HMG-CoA lyase enzyme is also targeted to the peroxisome, where its physiologic role is as yet undefined.[23]

The interconversion between AcAc and 3HB is mediated by 3HB dehydrogenase (3HBD). This protein is located on the inner mitochondrial membrane and has an absolute requirement for phosphatidylcholine as an allosteric activator. Highest activity has been described in the liver, with lower activity present in the heart, adrenal glands, and kidney.

Ketolysis involves the metabolism of AcAc and 3HB and occurs in the mitochondrial compartment of extrahepatic tissue (see Figure 55.3-4). The action of 3HBD converts 3HB to AcAc, which can subsequently be metabolized by succinyl-CoA oxoacid transferase (SCOT) to AcAc-CoA. β-Ketothiolase present in these cells converts AcAc-CoA into Ac-CoA, which subsequently enters the Krebs cycle. SCOT is a monomer that assembles into a homodimer. Although highly expressed in heart muscle, kidney, adrenal glands, and brain, SCOT is not present in liver.[24]

Electron Transfer to the Respiratory Chain Complex

The electron transfer flavoprotein (ETF) and ETF-ubiquinone oxidoreductase (ETF-QO) function together to transfer electrons from at least nine flavoprotein dehydrogenases.[25] Electrons are transferred between the two flavin moieties in the dehydrogenases and ETF. The reduced ETF is subsequently reoxidized by ETF-QO activity, with the concomitant reduction of ubiquinone.[26]

ETF is a mitochondrial matrix heterodimer consisting of α and β subunits, which are encoded by different genes.[27,28] ETF-QO is a monomer associated with the inner mitochondrial membrane. It contains two redox groups, a FAD cofactor and an iron-sulfur prosthetic group, which are thought to act as entry and exit sites, respectively, for electron transfer.[29] The gene for ETF-QO and pathogenic mutations have also been identified.[30]

PATHOPHYSIOLOGY AND CLINICAL PRESENTATION

Typically prompted by increased energy requirements during fever, fasting, or prolonged exercise, an enzyme defect in the FAO pathway leads to energy depletion owing to inadequate production of acetyl CoA, ketone bodies, and, ultimately, adenosine triphosphate in tissues with high energy demands, such as liver, heart, skeletal muscle, and brain.[1,31] Transient to fulminant liver failure, hepatic encephalopathy, dilated or hypertrophic cardiomyopathy, skeletal myopathy, and sudden, unexpected death at any age are the major clinical manifestations. The biochemical hallmark of FAO defects is hypo- or nonketotic hypoglycemia resulting from two different mechanisms: glucose depletion and secondary impairment of gluconeogenesis owing to a lack of reducing equivalents. Nonmetabolized free fatty acids are incorporated into triglycerides and can account for the observed fat storage in liver and muscle.[32]

Intracellular toxicity is triggered by the accumulation of acyl-CoA intermediates upstream of the block and the secondary depletion of carnitine and CoA. The metabolites may have toxic effects either directly (ie, membrane disruption) and/or by the inhibition of other enzymes. For example, cardiac arrhythmias are thought to be caused by long-chain acylcarnitine accumulation in mitochondrial fatty acid transport and β-oxidation defects.[33]

Complications of Pregnancy

A peculiar association between FAO disorders and severe complications during pregnancy has been well documented in women carrying LCHAD-deficient fetuses.[34,35] Preeclampsia, HELLP (hemolysis, elevated liver enzymes,

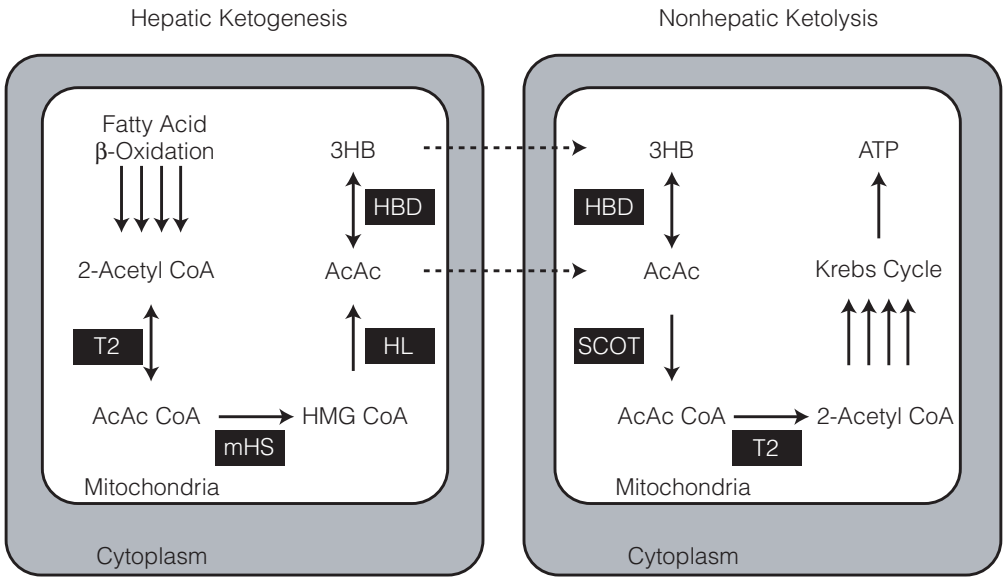

FIGURE 55.3-4 Ketone body metabolism: hepatic ketogenesis and ketolysis in nonhepatic tissue. AcAc = acetoacetate; ATP = adenosine triphosphate; CoA = coenzyme A; 3HB = 3-hydroxybutyrate; HBD = 3-hydroxybutyrate dehydrogenase; HL = HMG-CoA lyase; HMG = 3-hydroxy-3-methylglutarate; mHS = mitochondrial HMG-CoA synthase; SCOT = succinyl-CoA oxoacid transferase; T2 = β-ketothiolase.

and low platelets) syndrome, acute fatty liver of pregnancy (AFLP), and placental floor infarction are the observed maternal phenotypes (Table 55.3-1).[36] The mechanism of this association is not clearly understood. A combination of different factors might result in hepatic dysfunction during the third trimester of pregnancy.[1]

It is possible that the placenta, which shares the same genetic makeup as the fetus, is responsible for the production and accumulation of abnormal fatty acid metabolites.[37] These metabolites pass into the maternal circulation and overwhelm the β-oxidation pathway of the heterozygote mother, who has reduced capacity to oxidize long-chain fatty acids. In addition, during the latter trimester of pregnancy, β-oxidation in the mother is already challenged by the shifting of maternal metabolism toward ketogenesis. This metabolic imbalance is also harmful for the fetus, making it difficult to receive enough energy from the placenta.

Intrauterine growth retardation and premature delivery are relatively common observations. Although there is a predominant association with LCHAD deficiency, severe complications during pregnancy have been reported also in other FAO.[36]

Other Clinical Manifestations

Less frequently observed and poorly understood clinical manifestations have also been described. Hyperinsulinism, described in a case of SCHAD deficiency, supports the hypothesis of the existence of a lipid signaling pathway, strictly related to the β-oxidation spiral, which intervenes in the control of insulin secretion.[38] Hypoparathyroidism has been an occasional finding in patients affected with LCHAD or TFP deficiency.[39]

GENERAL APPROACH TO THE DIAGNOSIS

The approach to the diagnosis of patients at risk for FAO disorders should involve a combination of routine, specialized, and in vitro analyses, with careful consideration of the clinical condition at the time of sample acquisition (Table 55.3-2). When the patient is clinically stable and in an anabolic state, the concentration of key metabolites may not be significant. Patient samples collected under these conditions may not be informative because the metabolic intermediates may be absent or undetectable. The most useful specimens for evaluation are typically acquired during periods of metabolic decompensation, before the initiation of treatment. Because metabolic status has such critical consequences on the metabolite profiles, the interpretation of biochemical parameters relies heavily on the clinical and therapeutic context at sample procurement. Accordingly, it must be remembered that although metabolite analyses can be diagnostic, negative or inconclusive results in cases of high clinical suspicion should be followed up by in vitro investigations that are independent of patient status.

Routine Testing

During episodes of metabolic decompensation, plasma and urine specimens should be collected at the earliest possible opportunity. Hypoketotic hypoglycemia is a hallmark of most, but not all, FAO disorders. Routine chemistries include blood gases, electrolytes, glucose, ammonia, uric acid, liver function tests, creatine kinase, ratio of lactic to pyruvic acid, 3-hydroxybutyric and acetoacetic acids, and urinalysis. These more readily available analyses help to assess the extent of organ involvement and often allow for initiation of adequate treatment, while awaiting the results of more specialized metabolite analyses in plasma and urine. Careful consideration of the patient's personal (eg, preceding fever, fasting), pregnancy (eg, maternal liver disease during pregnancy, intrauterine growth retardation, prematurity), and family history (ie, sudden unexpected death in a sibling) may also provide important clues that should trigger inclusion of FAO disorders into the differential diagnosis.[40,41]

Specialized Metabolite Analyses

Defects associated with mitochondrial FAO result in the accumulation of fatty acids and acyl-CoA intermediates proximal to the metabolic block. These intermediates can be converted into dicarboxylic and hydroxydicarboxylic acids through peroxisomal ω- and ω-1-oxidation. Mechanisms to detoxify these accumulating metabolites and to restore the depleted pool of CoA result in the formation of acylglycine conjugates and acylcarnitine species from their respective acyl-CoA esters. Abnormal accumulation of these acyl-

TABLE 55.3-1 DISORDERS OF FATTY ACID TRANSPORT AND MITOCHONDRIAL OXIDATION ASSOCIATED WITH MATERNAL COMPLICATIONS OF PREGNANCY

	DISORDERS OF FATTY ACID TRANSPORT AND MITOCHONDRIAL OXIDATION						
MATERNAL COMPLICATION	LCHAD	TFP	CPT1	CACT	MCAD	SCAD	UNKNOWN*
Acute fatty liver of pregnancy	+++	+	(+)	–	(+)	+	+
HELLP syndrome	+++	–	–	–	+	+	+
Preeclampsia	+++	–	(+)	(+)	–	–	–
Placenta floor infarction	(+)	–	–	–	–	–	–

CACT = carnitine acylcarnitine translocase; CPT = carnitine palmitoyltransferase; HELLP = hemolysis, elevated liver enzymes, low platelets; LCHAD = long-chain 3-hydroxyacyl-CoA dehydrogenase; MCAD = medium-chain acyl-CoA dehydrogenase; SCAD = short-chain acyl-CoA dehydrogenase; SCHAD = short-chain 3-hydroxyacyl-CoA dehydrogenase; TFP = trifunctional protein; + = association reported in more than one case; (+) = association reported in one case, possibly a coincidental event; +++ = association reported in multiple cases; – = association not reported.

*Mothers of children with unspecified disorders but with clinical manifestations and strong biochemical evidence in vivo and in vitro of an underlying fatty acid oxidation disorder (P. Rinaldo et al, unpublished observations, 2003).

TABLE 55.3-2 CLINICAL PRESENTATION AND BIOCHEMICAL DIAGNOSIS OF DISORDERS OF THE CARNITINE CYCLE, FATTY ACID TRANSPORT AND MITOCHONDRIAL OXIDATION, AND KETOGENESIS

	CLINICAL PRESENTATION								BIOCHEMICAL SCREENING TESTS							NEWBORN SCREENING			
TRANSPORTER/ENZYME	FASTING HYPOGLYCEMIA	LIVER	GI	MUSCLE	HEART	CNS	SUDDEN DEATH	OTHER	URINE OA	URINE AG	PLASMA AC	FC	AC/FC	FFA	PRENATAL DIAGNOSIS*	POSSIBLE	EFFICACY OF EARLY THERAPY	RISK FOR ACUTE CRISIS	PM DIAGNOSIS†
Carnitine uptake defect	+	+	+	+	++	+	+	Hypochromic anemia	–	–	–	↓↓	–	–	+	+	++	+	+
Carnitine palmitoyltransferase 1 (liver)	+	++	+	+	+/–	+	+	Respiratory failure, renal tubulopathy	–	–	+	N–↑	–	–	(+)	+	+	+	+
Carnitine acylcarnitine translocase	+	+	+	+	++	–	+	–	–	+	N–↓	↑	–	+	+	–	+	+	
Carnitine palmitoyltransferase 2 (type I)	+	+	–	+	+	+	+	Renal abnormalities	–	–	+	N–↓	↑	–	(+)	+	–	+	+
Carnitine palmitoyltransferase 2 (type II)	+	+	–	+	+	–	+	+/–	–	+	N–↓	↑	–	(+)	+	+	+	+	
Carnitine palmitoyltransferase 2 (type III)	–	–	–	+	–	–	–	+/–	–	+	N–↓	↑	–	(+)	+	+	+	+	
Very-long-chain acyl-CoA dehydrogenase (early onset)	+	+	+	+	++	+	++	+ (acute)	–	+	N–↓	↑	+	+	+	+	+	+	
Very-long-chain acyl-CoA dehydrogenase (late onset)	+	–	–	+	–	–	–		+ (acute)	–	+	N–↓	↑	+	+				
Isolated long-chain 3-hydroxyacyl-CoA dehydrogenase	+	++	–	+	++	+	++	Peripheral neuropathy, retinopathy	+ (acute)	–	+	N–↓	↑	+	+	+	+	+	+
Trifunctional protein (α and β subunits)	+	–	–	+	–	–		Peripheral neuropathy, retinopathy	+ (acute)	–	+	N–↓	↑	+	+	+	+	+	+
Medium-chain acyl-CoA dehydrogenase	++	++	–	–	–	–	++	Asymptomatic to fulminant course	+ (acute)	+	+	N–↓	↑	+	+	+	+	+	+
Medium-/short-chain 3-hydroxyacyl-CoA dehydrogenase	+	+/–	–	+	+	–	+	Hyper-insulinism	+ (acute)	–	+	N–↓	↑	+	(+)	+	+	+	+
Medium-chain ketoacyl-CoA thiolase	+	+	+	++	–	–	–		+ (acute)	+	+	N–↓	↑	–	–	+			
Short-chain acyl-CoA dehydrogenase	+	–	+	+	–	+	–	Asymptomatic to fulminant course	+ (acute)	+	+	N–↓	↑	–	–	+	+	+	+
3-Hydroxy-3-methylglutaryl-CoA synthase	++	+	+	–	–	+	–	(+)	–	–	N	–	–	–	+	+	+		
3-Hydroxy-3-methylglutaryl-CoA lyase	++	+/–	–	–	+/–	++	+	Pancreatitis, + deafness, retinopathy	–	–	N	–	–	+	+	+	+		
Succinyl-CoA oxoacid transferase	+	–	–	–	+/–	–	–	Intermittent ketoacidosis	(+)	N	–	+	+						
Acetoacetyl-CoA thiolase	+	–	+	–	+	+	+/–	Neutropenia, thrombocytopenia	+	N	+	+							
Electron transfer flavoprotein (ETF): α or β subunit	++	+	+	+	+	+	+	Congenital anomalies	+	+	+	N–↓	↑		+	+	+/–	+	+
ETF:quinone oxidoreductase	++	+	+	+	+	+	+	Renal cysts, Congenital anomalies	+	+	+	N–↓	↑		+	+	+/–	+	+
2,4-Dienoyl-CoA reductase	–	–	++	–	–	–	–		+	N–↓	↑	–	+	?	?	+			

AC = acylcarnitines; AC/FC = acylcarnitine-to-free carnitine ratio; AG = acylglycine; CNS = central nervous system; FC = free carnitine; FFA = free fatty acids; GI = gastrointestinal involvement; N–↓ = normal to reduced; N–↑ = normal to elevated; OA = organic acid; – = not present, not described; + = common manifestation; ++ = frequent manifestation; + (acute) = present usually during episodes of acute decompensation; +/– = may or may not be present; (+) = not described but theoretically possible; ? = not known.
*Prenatal diagnosis reported by biochemical and/or molecular genetic analysis of amniotic fluid, cultured amniocytes, or chorionic villus samples.
†Postmortem diagnosis reported by biochemical and/or molecular genetic analysis of blood, bile, cultured fibroblasts, liver, or muscle.

glycine and acylcarnitine metabolites can therefore assist in the evaluation of the metabolic defect in these patients. Of note, the buildup of esterified carnitine fractions can subsequently result in secondary L-carnitine deficiency, a condition frequently observed in patients with FAO disorders.

Urinary Organic Acid Analysis. Organic acids are water-soluble compounds that have at least one carboxyl group. They are best detected in urine by gas chromatography–mass spectrometry (GC-MS).[42] Urine organic acid analysis plays an important role in the diagnosis of FAO disorders. Especially during metabolic stress, the finding of inadequate ketonuria in the presence of particular excretion patterns of medium-chain saturated and unsaturated dicarboxylic acids and acylglycines provides strong evidence for an underlying mitochondrial FAO disorder. Nonspecific and even normal organic acid profiles are typically encountered in defects of fatty acid transport, carnitine uptake, and HMG-CoA synthase.

Urinary Acylglycine Analysis. Stable isotope dilution GC-MS allows for quantitative determination of specific urinary acylglycines at concentrations below the detection limit of routine organic acid analysis. MCAD deficiency can be diagnosed reliably by the findings of elevated excretions of hexanoylglycine, phenylpropionylglycine, and suberylglycine in random specimens of asymptomatic patients.[43] Other FAO disorders with informative acylglycine profiles are SCAD deficiency, MCKAT deficiency, and glutaricaciduria type II (ETF and ETF-QO deficiencies).[44–46]

Acylcarnitine Analysis. Acylcarnitine analysis has become the most widely used tool for the investigation of FAO disorders. However, although it is capable of identifying and distinguishing most of the different FAO defects, limitations of this methodology are frequently overlooked. Because adequate tissue levels of free L-carnitine are necessary to form carnitine esters, patients with FAO disorder and severe secondary carnitine deficiency may not exhibit diagnostic or even abnormal acylcarnitine accumulations.[47] Patients with primary carnitine deficiency are also expected to have overall low concentrations of acylcarnitine species.[48] In addition, acylcarnitine analysis cannot differentiate between isomeric carnitine esters, making the performance of other biochemical investigations a necessity to provide appropriate interpretation.[49,50]

Free and Total Carnitine Analysis. Determination of total and free carnitine as well as the acylcarnitine fraction aids in the diagnosis of primary carnitine deficiency and CPT1 deficiency, which are not readily identified by the above-mentioned analyses. Furthermore, carnitine analysis is useful when deciding whether L-carnitine supplementation is warranted in disorders associated with secondary carnitine deficiency and for monitoring patients on carnitine supplementation.[51]

Fatty Acid Analysis. Quantitative determination of plasma fatty acid profiles, in particular C_8 to C_{18} species and 3-hydroxy fatty acids, also provides useful and complementary information when assessing patients suspected of having an FAO defect.[52] Abnormal profiles may be observed in patients with MCAD, LCHAD/TFP, SCHAD, and VLCAD deficiencies as well as glutaricacidemia type II.[53,54]

Biochemical In Vitro Studies

Cell-based biochemical in vitro assays are used to confirm a preliminary diagnosis suggested by clinical findings and biochemical data. With the ability to establish fibroblast cultures from small skin punch biopsies, assays were developed allowing metabolic in vitro challenges for the diagnosis of most FAO disorders in a single test.[55,56] Only those few FAO disorders not expressed in fibroblasts (ie, the muscle form of CPT1) are not amenable to this approach and require more specific enzyme investigations in other tissues or a molecular genetic approach. Assays for direct enzyme and transporter analyses are available in a few laboratories worldwide.[57]

Molecular Genetic Studies

Genes harboring disease-carrying mutations responsible for FAO disorders have been identified, and a genotype-phenotype correlation is emerging in a few disorders as well. Accordingly, mutation analysis can principally confirm a tentative diagnosis and facilitate counseling. However, such studies are available for only relatively common mutations that have been described in a few defects. In many cases, deoxyribonucleic acid (DNA) sequencing of the complete gene would be required to reach a conclusive diagnosis by identification of private mutations, which hinders the broad application of molecular genetic analysis to the diagnosis of FAO disorders.[14]

PRENATAL DIAGNOSIS

All known FAO disorders have an autosomal recessive mode of inheritance, carrying with it a recurrence risk of 25% in another pregnancy of the same couple. A rare exception was recently described in two unrelated cases with fatal TFP deficiency whose etiology was found to be associated with uniparental disomy for chromosome 2, which has a significantly lower recurrence risk.[58] Parental investigations are therefore a prerequisite for the provision of comprehensive genetic counseling. Because FAO disorders cover a wide spectrum of clinical presentations and treatability, and disease status in the fetus may even have consequences on the mother's well-being, several criteria must be considered before seeking prenatal diagnostic testing.[36]

Treatment of most FAO disorders is simple and results in a normal life when initiated early. However, an ethical issue may arise when families who suffered the tragic loss of an undiagnosed child are reluctant to accept that simple treatment is indeed available. The decision is easier for disorders in which the outcome must be considered dismal based on the absence of significant treatment options. A prerequisite for all prenatal diagnostic considerations is a related index case with a well-established diagnosis and the availability of a diagnostic test that can be performed on amniotic fluid,

amniocytes, or chorionic villi samples using any or a combination of metabolite, protein, and molecular analyses.[59–62]

With the exception of cases in which the prognosis is clearly poor or the mother's health is a significant cause for concern, it is recommended that newborns suspected of having an FAO disorder should undergo a comprehensive evaluation and be managed aggressively immediately after birth until the FAO in question has been excluded.

NEWBORN SCREENING

Newborn screening is a public health program whose mandate is the early identification of diseases in newborns in whom timely intervention could significantly improve the prognosis for their long-term health. Guthrie's innovative application of the bacterial inhibition assay to the detection of levels of phenylalanine in dried blood spots on filter paper led to the establishment of the first newborn screening assay for phenylketonuria.[63] In the following decades, additional tests were added to screening panels using similar or entirely new technology for the detection of key metabolites or enzymatic activity.

The introduction of tandem mass spectrometry (MS/MS) to newborn screening as a sensitive and specific means for the simultaneous detection of key diagnostic metabolites has significantly increased the number of metabolic diseases that could be screened for in a single process using dried blood spot specimens.[64] Currently, over 30 diseases can be screened for, and in addition to several organic acidemias and aminoacidopathies, the ability to quantify acylcarnitine species has also enabled the detection of FAO disorders.[31] However, there is no uniformity in the panel of disorders that each state screens for, with the number of diseases ranging from 4 to over 30.[65]

The prognosis for most patients with FAO disorders who are identified presymptomatically and managed appropriately is generally favorable and suggests benefit from early diagnosis and treatment.

POSTMORTEM SCREENING

The period after birth is a critical time for infants with FAO disorders, and episodes of hypoglycemia can be triggered by inadequate food intake and limited glycogen stores.[66] The severity of these episodes is variable, with symptoms resolving in response to either intravenous glucose or feeding in some patients, whereas, on the other end of the spectrum, the decompensation events may be fatal, resulting in sudden and unexpected death.[67] An estimated 5% of sudden, unexpected deaths in children less than 1 year of age and an even higher percentage of deaths observed in the 1- to 5-year age group are caused by FAO disorders.[68–70]

Postmortem studies involving histochemical and biochemical analyses of liver specimens,[70] organic and fatty acid analysis of urine and plasma specimens,[69] analysis of acylcarnitine profiles in dried blood and bile spots,[71,72] and the determination of FAO rates using cultured skin fibroblasts[73] have all been used to investigate sudden unexpected death in previously healthy patients.

An approach for the investigation of such a patient suspected of having an FAO disorder has been described.[74]

We propose a complete postmortem investigation and genetic counseling of parents who lost a child suddenly and/or unexpectedly. Blood and bile could be conveniently collected on the same filter paper card, one identical to those used for newborn screening, which can be shipped at room temperature. Liver and skin biopsies should also be collected and kept until the blood and bile samples have been analyzed. Both of these specimens should be collected to provide a better chance to detect and independently confirm the largest possible number of disorders. In cases with a higher level of suspicion, an effort should be made to collect a frozen specimen of liver and a skin biopsy. Although fatty infiltration of the liver and/or other organs (heart, kidneys) is a common finding in FAO disorders, caution should be exercised not to use steatosis as the sole criteria to indicate a possible underlying FAO disorder during the postmortem evaluation of a case of sudden death.[70] Additional risk factors include a family history of sudden death, Reye syndrome, or maternal pregnancy complication and evidence of lethargy, vomiting, and/or fasting in the 48 hours prior to death. To ensure that all possible avenues are covered, cases of alleged child abuse should also be fully investigated. The frozen liver and skin biopsy could be discarded at a later time without further testing when a credible cause of death has been established but could otherwise be crucial to reach a proper diagnosis and conclusive confirmation in vitro.

These studies are important in providing the basis for appropriate genetic counseling and even testing of family members because the identification of affected, healthy appearing siblings is not uncommon.[75]

DISORDERS OF PLASMA MEMBRANE FUNCTIONS

Long-Chain Fatty Acid Transport/Binding Defect

Two patients have been described who presented with episodes of acute liver failure and mild acute encephalopathy associated with nonketotic hypoglycemia and hyperammonemia.[76] Both of them received liver transplants, and their further development was normal. The biochemical phenotype of these patients was nonspecific, and steatosis was not observed in either patients' livers. In cultured skin fibroblasts, reduced intracellular concentration of C_{14} to C_{18} fatty acids and reduced cellular oxidation of palmitate and oleate were found. The latter finding was normalized by permeabilization of the plasma membrane with digitonin. No mutations were found by sequencing of several target genes involved in fatty acid transport. The exact defect in these patients remains to be determined.

Carnitine Uptake Defect

Carnitine uptake defect is the only cause of primary carnitine deficiency and is due to a defective plasma membrane carnitine transporter. Renal reabsorption and intestinal uptake are impaired, with subsequent tissue depletion of

carnitine. Intracellular carnitine deficiency impairs the entry of long-chain fatty acids into the mitochondrial matrix and therefore compromises FAO, particularly in tissues that are most reliant on this pathway. Patients present with progressive cardiomyopathy, episodes of hypoketotic hypoglycemia associated with encephalopathy, and hepatomegaly. Skeletal myopathy is also a common clinical manifestation. Some patients had episodes of abdominal pain and diarrhea owing to gastrointestinal dysmotility; others had hypochromic anemia.[77,78] Several cases of sudden and unexpected death at variable ages have also been reported.[67,79] Microvesicular steatosis and lipid infiltration may be seen in liver and muscle biopsies, respectively. Plasma carnitine concentrations are less than 10% of normal and typically associated with increased urinary carnitine excretion. The diagnosis is confirmed either by determination of carnitine uptake in fibroblasts or molecular genetic analysis of the gene (*SLC22A5*) encoding the sodium ion-dependent carnitine transporter OTCN2. A genotype-phenotype correlation is not apparent.[80] Presymptomatic identification through newborn screening using tandem mass spectrometry has been reported.[48] Heterozygotes for OTCN2 mutations show moderately reduced plasma carnitine levels and reduced rates of carnitine uptake,[79] increased urinary carnitine losses,[81] and predisposition to late-onset benign cardiac hypertrophy.[82]

DISORDERS OF THE CARNITINE CYCLE

CPT1 Deficiency

CPT1 exists as two tissue-specific and genetically distinct isoforms, a hepatic (CPT1A) and a muscle type (CPT1B).[83,84] CPT1A is expressed in liver and other tissues except muscle, whereas CPT1B is primarily expressed in skeletal and cardiac muscle. Accordingly, CPT1A deficiency is characterized primarily by nonketotic hypoglycemia and liver dysfunction, which may result in a fatal Reye-like encephalopathy. Several cases were reported with cardiac (cardiomegaly, arrhythmia) and renal involvement (distal renal tubular acidosis).[85,86] AFLP was reported in two subsequent pregnancies, both resulting in CPT1A-deficient offspring.[87]

The biochemical diagnosis is not straightforward. Organic acid analysis of urine collected during clinically significant episodes is not informative. Blood acylcarnitine analysis may reveal an elevation of free and acetylcarnitine in the presence of relatively low concentrations of long-chain acylcarnitines allowing the calculation of a ratio specific for CPT1 deficiency.[88] In contrast to other FAO defects, CPT1A deficiency is associated with an elevated level of plasma carnitine, in particular free carnitine. This is probably caused by the unusually high renal threshold for free carnitine and secondary increase in carnitine transport.[89] Accordingly, high plasma concentrations of total and free carnitine in a patient with a suspicious clinical presentation should lead to the inclusion of CPT1 in the differential diagnosis. CPT1A can be confirmed by enzyme assay in fibroblasts and molecular genetic analysis of the CPT1A gene. However, most mutations are private, with the exception of the G710E mutation, which is common in the Hutterite communities of North America.[90] Treatment is based on strict avoidance of fasting and subsequent hypoglycemia and a low-fat diet supplemented with medium-chain triglycerides (MCTs). Patients with CPT1B deficiency have not been reported to date.

CACT Deficiency

Patients with CACT deficiency typically present in the neonatal period with hypoketotic hypoglycemia, liver failure accompanied by hyperammonemia, and/or hypertrophic cardiomyopathy.[91] Impaired motility of the whole gastrointestinal tract has been reported in one patient.[92] Frequent feedings of a low-fat diet, with most fat consisting of MCTs, which do not require CACT to cross the mitochondrial membranes, are the mainstay of treatment. Nevertheless, despite therapeutic efforts, most patients will die following the development of cardiac arrhythmias, possibly caused by an accumulation of arrhythmogenic long-chain acylcarnitines.[33] Autopsy reveals fatty infiltration in liver, kidney, and muscle and hypertrophic cardiomyopathy.[92,93] Outcome has been more favorable for patients, with in vitro evidence of some residual enzyme activity.[91,94,95] Because CACT deficiency is identifiable through expanded newborn screening using tandem mass spectrometry, it is expected that in the future, more patients will be identified and treated before the onset of symptoms. Whether the prognosis can be improved by presymptomatic initiation of treatment, however, remains to be seen. Acylcarnitine analysis in plasma or dried blood spots reveals markedly elevated long-chain acylcarnitines, with C_{16} and C_{18} species being particularly prominent. However, because CPT2 deficiency (see below) results in virtually the same acylcarnitine profile, further biochemical testing by enzyme assay or molecular genetic analysis of the CACT gene is necessary to arrive at the correct diagnosis. Molecular analysis usually requires gene sequencing owing to significant allelic heterogeneity.[91]

CPT2 Deficiency

CPT2 deficiency can be differentiated into several clinical presentations. A relatively mild, adult-onset form of CPT2 deficiency was the first mitochondrial FAO defect to be described.[96] It is typically limited to the skeletal muscle, with episodes of myopathy and rhabdomyolysis usually triggered by prolonged exercise combined with either fasting or cold exposure. One patient had recurrent pancreatitis with intermittent myoglobinuria; another's myopathic crises appeared to be induced by valproate therapy.[97,98]

A recently proposed classification is based on organ involvement, residual enzyme activity, and genotype.[99] Accordingly, CPT2 type I is the most severe form that presents in the neonatal period and affects the liver, skeletal muscle, and heart and is associated with congenital brain and kidney abnormalities.[100,101] CPT2 type II involves the liver, skeletal muscle, and heart and presents in the first few years of life. Type III is limited to skeletal muscle, presents in early adulthood, and, relative to types I and II, is associated with the highest residual enzyme activity. Because of several patients having experienced malignant

hyperthermia during surgery, it has been suggested to include CPT2 deficiency in the differential diagnosis of this anesthetic complication.[102]

Myoglobinuria and markedly elevated serum creatine phosphokinase levels are hallmarks of CPT2 deficiency. Acylcarnitine profiles are also abnormal but identical to those observed in CACT deficiency. More specific enzyme assays or molecular genetic tests are needed to verify a presumptive diagnosis. The most common mutation in CPT2 type III is S113L, whereas 413delAG appears to be common in the Ashkenazi Jewish population.[103] Symptomatic heterozygotes for particular mutations have also been described.[104]

DISORDERS OF LONG-CHAIN FATTY ACID METABOLISM

VLCAD Deficiency

The first patients with VLCAD deficiency were described as having LCAD deficiency, based on the ETF reduction assay using palmitoyl CoA as a substrate.[105] However, once molecular genetic studies of the LCAD gene were possible and the VLCAD protein was identified, patients previously labeled as LCAD deficient were shown to have no mutations in the LCAD gene, whereas they had no detectable VLCAD protein by immunoblot analysis.[106] A patient with true LCAD deficiency has not yet been described. However, an LCAD knockout mouse model features typical symptoms of an FAO disorder, suggesting that LCAD is indeed a critical enzyme and will likely be demonstrated in humans eventually.[107]

VLCAD catalyzes the first step of the fatty acid β-oxidation spiral. The severity of the phenotype seems to correlate with specific genotypes.[108] The most severe phenotype manifests early in life and is associated with nonsense mutations, resulting in complete loss of enzyme activity. Morbidity and mortality are significant owing to involvement of multiple organ systems, in particular the liver and heart, resulting in recurrent metabolic crises and cardiomyopathy.[109] Patients carrying missense mutations or with single amino acid deletions permitting some residual enzyme activity are at high risk of metabolic decompensation with nonketotic hypoglycemia and a Reye-like syndrome triggered by increased energy demands (eg, prolonged fasting, fever).[110] The phenotype switches from a more hepatic presentation to muscle weakness as patients become older; some are able to sustain metabolic stress during childhood and do not present until adulthood with exercise-induced myopathy and rhabdomyolysis.

Laboratory hallmarks during acute episodes include hypoketotic hypoglycemia and creatine phosphokinase elevations. Urine organic acid analysis is nonspecific, with hypoketotic dicarboxylicaciduria. Acylcarnitine analysis is most informative, revealing a characteristic profile with elevated levels of saturated and unsaturated C_{14} to C_{18} carnitine esters, the predominant species being tetradecenoylcarnitine ($C_{14:1}$).[57] The diagnosis can be confirmed by specific enzyme assay or FAO in vitro probing in fibroblast cultures[111] or by molecular genetic analysis of the VLCAD gene. Because acylcarnitine analysis can be performed in newborn screening blood spots, early identification and treatment of affected patients are generally possible.[112,113]

TFP and LCHAD

The TFP is a hetero-octamer containing four α and four β subunits encoded by two different genes. The α subunit harbors the activities of the second and third steps of fatty acid β-oxidation, LCEH and LCHAD. The β subunit harbors LCKAT, which catalyzes the last step in β-oxidation of long-chain fatty acids. Although mutations in either gene may cause complete TFP deficiency, a few mutations in the α subunit cause isolated LCHAD deficiency. In keeping with the previously mentioned disorders, a genotype-phenotype correlation has also emerged for TFP deficiency, with residual enzyme activity being associated with a milder, later-onset phenotype. Patients with late-onset TFP deficiency present with a progressive peripheral neuropathy and episodic rhabdomyolysis.[114] Recently, two patients with lethal TFP deficiency owing to isodisomy for chromosome 2 were described. This finding is of great importance in particular for genetic counseling of families because the recurrence risk in future pregnancies would be significantly lower than the 25% risk typical for FAO disorders, which are generally autosomal recessive.[58]

Although the phenotype of both disorders includes all of the symptoms expected in a FAO disorder, patients with LCHAD and TFP deficiencies may also develop pigmentary retinitis and a peripheral neuropathy, the pathophysiology of which remains obscure. In addition, many patients are born prematurely and small for gestational age. The initial hypothesis that a secondary deficiency of docosahexaenoic acid may cause the retinal changes and may be treatable by dietary supplementation has not been proven conclusively.[115]

A recent study described the clinical and biochemical abnormalities in 50 patients with isolated LCHAD deficiency.[116] All patients presented by 26 months, and most became symptomatic within the first 6 months of life. Thirty-nine patients had episodes of acute metabolic decompensation, with symptoms of hepatic dysfunction (79%), coma (56%), seizures (38%), and apnea (23%). Fewer patients experienced cardiorespiratory arrest, arrhythmia, and sudden death. Hypoglycemia was a key laboratory finding in all 39 patients, and most of the patients showed decreases in total carnitine and significant elevations in plasma lactate, aspartate aminotransferase, creatine kinase, and ammonia levels. The 11 patients who did not experience an acute metabolic decompensation before diagnosis of LCHAD deficiency were also found to have a range of more chronic symptoms, which included hepatomegaly, cholestasis and hepatic dysfunction, cardiomyopathy, feeding difficulties, failure to thrive, hypotonia and lethargy, psychomotor retardation, peripheral neuropathy, and microcephaly. This spectrum of clinical signs before and at the time of diagnosis was similar for both groups of patients presenting with or without acute metabolic crisis. With more newborn screening laboratories introducing tandem mass spectrometry for acylcarnitine analysis into their programs, patients should come to attention earlier in life, and follow-

up studies will have to determine whether presymptomatic initiation of treatment is beneficial.[50,117]

Patients with isolated LCHAD deficiency have a reduction in LCHAD activity with normal LCEH and LCKAT activities. The most common cause of isolated LCHAD deficiency is the 1528G→C mutation, which changes a glutamate to a glutamine residue (E474Q) in the active site of the α subunit of the LCHAD gene. The replacement of the acidic residue with a neutral amide amino acid is thought to affect the NAD^+-binding site of the enzyme and subsequently results in an isolated reduction of LCHAD activity without affecting the activities of LCEH and LCKAT.

Another feature that was thought to be specific to LCHAD deficiency was the observation that approximately 20% of patients were born following pregnancies complicated with maternal liver disease, in particular AFLP and HELLP syndrome. However, several other FAO disorders have meanwhile been implicated as possible triggers of maternal liver disease during pregnancy.[36] The simplified hypothesis is that pathologic fatty acid metabolites accumulating in the affected fetus eventually overwhelm the obligate heterozygous mother's FAO capacity, which already is reduced in the third trimester of pregnancy.[34]

DISORDERS OF MEDIUM-CHAIN FATTY ACID METABOLISM

MCAD Deficiency

MCAD is responsible for the initial dehydrogenation of acyl CoAs with a chain length between 4- and 12-carbon atoms. MCAD deficiency is probably the best known FAO defect because it has gained prominent attention over the last decade as a disorder fulfilling all classic criteria for inclusion into newborn screening programs.[118,119] Early diagnosis is crucial because MCAD is a potentially lethal disease, documented by the fact that up to at least 20% of undiagnosed patients die during their first metabolic decompensation.[120] A common missense mutation (985A→G; K304E) has been identified in whites of Northern European descent that results in reduced production of an unstable protein. But patients homozygous for this mutation may present at any time during life—typically, when a metabolic stressor is experienced (fever, prolonged fasting) that triggers an acute manifestation. However, identification of patients by newborn screening has led to the discovery of cases with only mild biochemical abnormalities and specific genotypes involving mutations that preserve significant enzyme activity in vitro. Whether patients with such genotypes will remain asymptomatic even during times of increased energy demands remains to be determined either by long-term follow-up or by carefully conducted fasting challenges.

A defect of the MCAD enzyme leads to fasting-induced hypoglycemia and accumulation of medium-chain fatty acids. The latter are further metabolized not only to dicarboxylic and carnitine esters but also to glycine esters, which can be determined in urine by organic acid analysis or more accurately by stable isotope dilution methods and GC-MS.[43] Analysis of plasma acylcarnitines by MS/MS reveals accumulation of C_6 to C_{10} acylcarnitine species, with prominent octanoylcarnitine.[121] Detection of these metabolites is the basis for newborn screening for MCAD deficiency by MS/MS.[64,118] Affected individuals may develop secondary carnitine deficiency; thus, the characteristic metabolites may not be reliably detectable.[47] Urine acylglycine analysis should therefore be pursued in carnitine-deficient and asymptomatic patients, making obsolete previously advocated provocative tests in most cases.[43] The biochemical diagnosis of MCAD deficiency can be confirmed by measurement of the activity of the MCAD enzyme in fibroblasts and other tissues and by molecular genetic testing of the *ACADM* gene; both test methods can be used for prenatal diagnosis. MCAD deficiency has been observed in all populations but Asians.[122]

M/SCHAD Deficiency

Previously, the clinical entity now known as M/SCHAD deficiency was described as a defect of SCHAD, an enzyme of the mitochondrial short-chain fatty acid β-oxidation pathway converting L-3-hydroxybutyryl CoA to acetoacetyl CoA.[123] However, our present understanding of the protein encoded by the SCHAD gene suggests a chain-length specificity toward C_8 to C_{10} acyl CoA.[124] At the clinical and biochemical levels, a deficiency of this enzyme has been described with three different phenotypes. The first one presented with cardiomyopathy and recurrent rhabdomyolysis, culminating in death as a teenager.[125] Enzyme activity was reduced in skeletal muscle but not in skin fibroblasts; other tissues were not studied enzymatically. A second phenotype was described with a presentation similar to that seen in ketotic hypoglycemia, and enzyme deficiency was described in isolated mitochondria from cultured skin fibroblasts.[126] Other tissues were not analyzed in these patients. A third group of patients had hepatic involvement and steatosis; the catalytic activity with the C_4 substrate was deficient in liver but normal in muscle and fibroblasts.[127] Mutation analysis of the SCHAD gene in these patients has been inconclusive, suggesting the existence of another protein with high activity toward acetoacetyl CoA.[128]

Recently, however, five patients in two unrelated families were reported who presented with hypoketotic hypoglycemia associated with hyperinsulinism, reduced M/SCHAD activity in fibroblast mitochondria, and mutations in the SCHAD gene.[38,129,130] Further molecular information and detailed studies of M/SCHAD protein interactions will hopefully improve our understanding of the function, substrate specificity, and tissue expression of this peculiar enzyme.

In addition to hypoglycemia, which may be either ketotic or hypoketotic, elevated transaminases, creatine phosphokinase, and hyperammonemia may be present during acute episodes of metabolic decompensation in affected patients. Specific laboratory findings include various chain-length dicarboxylic and 3-hydroxydicarboxylicaciduria. Plasma acylcarnitine analysis may be normal or show an elevated concentration of 3-hydroxybutyryl carnitine.[38]

MCKAT Deficiency

Only one patient with this disorder has been described to date.[131] This Japanese male newborn presented at 2 days

of life with emesis, dehydration, liver dysfunction, and terminal rhabdomyolysis and died several days later. Laboratory findings included hypoglycemia, hyperammonemia, metabolic acidosis, and elevated creatine phosphokinase levels. Urine organic acid analysis showed ketotic lacticaciduria and significant C_6 to C_{12} dicarboxylicaciduria, with prominent C_{10} and C_{12} species. Whereas palmitate oxidation on skin fibroblasts was normal, octanoate oxidation was reduced to 31% of controls. An isolated deficiency of MCKAT was demonstrated in fibroblasts. This result was supported by the findings of immunoprecipitation studies, which demonstrated a 60% reduction in protein signal. A better understanding of this disorder will require the elucidation of the molecular basis and identification of additional patients.

DISORDERS OF SHORT-CHAIN FATTY ACID METABOLISM

SCAD Deficiency

Initially suggested in 1984,[132] SCAD deficiency has been reported in less than 30 patients.[1,14] The first SCAD-deficient patients reported were two newborns with unusually large urinary excretion of ethylmalonic acid (EMA). One of these patients died during the neonatal period, whereas the other suffered only one metabolic decompensation in the newborn period and remained asymptomatic thereafter.[133]

EMA is an alternative metabolite of butyryl CoA that accumulates when β-oxidation of fatty acids cannot proceed in shortening fatty acyl CoAs with four carbons owing to inactivity of SCAD. Butyryl CoA is then carboxylated by propionyl-CoA carboxylase to EMA, which is readily excreted in urine, where it can be measured by organic acid or acylglycine analysis. Butyryl CoA can also be esterified with carnitine to facilitate egress out of the mitochondria and eventually into the blood, where it is measurable as a C_4-acylcarnitine by MS/MS. An increased concentration of butyrylcarnitine in blood and excretion of EMA into urine are considered biochemical markers of SCAD deficiency, although these findings are not specific for this disorder.

A variety of symptoms have been described, the most common being developmental delay and muscle hypotonia.[134] The laboratory confirmation of SCAD deficiency is difficult and should include both biochemical and molecular genetic studies. Most conveniently, fibroblasts can be used to first assess FAO,[135,136] followed by SCAD gene sequencing. However, although several pathogenic, mostly missense mutations have been identified, molecular genetic testing for SCAD deficiency is complicated by two SCAD gene variants, 511C→T (R147W) and 625G→A (G185S), which occur in the homozygous or compound heterozygous state in 7% of the US population.[137] Among patients overexcreting EMA, these variants are even more frequent.[138,139] In addition, some patients with biochemical evidence of SCAD deficiency do not carry any other SCAD gene mutations.[134,140] In vitro studies revealed that these variants compromise SCAD activity owing to abnormal protein folding and thermolability, respectively.[139,141] All of these findings considered, these variants are believed to confer disease susceptibility in combination with other, as yet unidentified genetic or environmental factors.

DISORDERS OF KETOGENESIS

HMG-CoA Synthase Deficiency

Only six patients with HMG-CoA synthase deficiency have been described to date.[21,142–145] All patients presented with recurrent hypoketotic hypoglycemic episodes leading to coma, usually triggered by prolonged fasting owing to gastroenteritis.

This diagnosis may be underestimated because of the relatively nonspecific biochemical phenotype during an acute episode with hypoketotic hypoglycemia, dicarboxylicaciduria, and normal blood acylcarnitine profile in the presence of elevated free fatty acids.[21]

Enzymatic analysis is difficult because HMG-CoA synthase is expressed in the liver, and its substrates and products are also used or synthesized by other proteins. The human *HMGCS2* gene, however, has been isolated, and the diagnostic potential of a molecular approach has been documented by the detection of disease-causing mutations.[21,146]

HMG-CoA Lyase Deficiency

HMG-CoA lyase is the second mitochondrial enzyme that mediates ketogenesis from fatty acids (see Figure 55.3-4). It also catalyzes the final step of leucine catabolism. Approximately 60 patients described presented with acute, often lethal episodes of hypoketotic hypoglycemia and acidosis.[147] Fifty percent of the cases presented in the first weeks of life acutely, triggered by fasting or intercurrent illness. Exceptionally, the onset is after 2 years of age. The central nervous system appears to be particularly vulnerable during metabolic crises, but abnormal magnetic resonance imaging signal abnormalities in white matter were observed even in asymptomatic patients.[148] In addition to possible neurologic sequelae, pancreatitis, cardiomyopathy, and deafness associated with retinopathy have been described.[149–151]

The urine organic acid profile is characteristic, with abnormal excretion of 3-hydroxy-3-methylglutaric, 3-methylglutaconic, 3-methylglutaric, and 3-hydroxyisovaleric acids. Acylcarnitine analysis in plasma and newborn screening blood spots is remarkable for elevated 3-methylglutarylcarnitine.[152] The diagnosis should be confirmed by measurement of enzymatic activity in fibroblast cultures or molecular genetic testing. The defect appears to be relatively frequent in Saudi Arabia, where it accounts for about 16% of inherited metabolic diseases.[153]

SCOT Deficiency

Succinyl-CoA:3-oxoacid-CoA transferase is the first step of ketone body use in extrahepatic tissues, particularly the heart, kidney, and brain (see Figure 55.3-4). The typical clinical presentation includes recurrent episodes of severe ketoacidosis, with persistent ketonuria and hyperketonemia even during the fed state. In almost all described patients, the onset was in the first year of life.[154] In two cases, cardiomegaly was noted,[155] and behavioral problems have also been reported.[156]

Urine organic acid analysis reveals persistent, although nonspecific, ketonuria. A tentative diagnosis should be confirmed by enzyme assay or molecular genetic analysis.[157]

The disease is potentially fatal. Treatment relies on limitation of protein intake, provision of adequate calories, and alkaline therapy to prevent aggravation of ketosis at the onset of any intercurrent infection.[158]

Mitochondrial β-Ketothiolase Deficiency

β-Ketothiolase is a ketogenic and ketolytic enzyme that also intervenes in isoleucine catabolism (see Figure 55.3-4). Although variable, most patients present with acute ketoacidosis accompanying infections and vomiting. Dilated cardiomyopathy, neutropenia, and thrombocytopenia are also possible.[147] Neurologic complications, such as mental retardation and basal ganglia involvement, have been described in a few patients.[159,160] However, asymptomatic affected individuals have been documented in families with an affected case.[161]

Urine organic acid analysis is diagnostic with the documentation of a large excretion of 2-methyl-3-hydroxybutyric acid and tiglylglycine, reflecting a block in isoleucine catabolism. The enzymatic assay presents some technical difficulties because of the presence of three other thiolases. Significant genetic heterogeneity hinders a rapid molecular genetic approach to the diagnosis.[162]

Key biochemical findings associated with the disorders of ketone body metabolism are presented in Table 55.3-3.

OTHER DISORDERS

Glutaric acidemia Type II

Primary defects of ETF or ETF-QO result in glutaric acidemia type II. This disorder is also known as multiple acyl-CoA dehydrogenase deficiency because the reoxidation of several mitochondrial dehydrogenases is impaired owing to the inability of their cofactor, FAD, to transfer electrons to ETF. The clinical phenotype is variable, with an early-onset, severe form with or without congenital anomalies and a milder, later-onset form of the disease.[162] The milder phenotype has also been described as ethylmalonic-adipicaciduria.

The severe, neonatal-onset form with congenital anomalies is often associated with prematurity. Patients generally present with overwhelming illness within the first 2 days of life and die shortly thereafter. Hypotonia, severe hypoglycemia, metabolic acidosis, large kidneys, and renal dysgenesis have also been described. Congenital anomalies include dysmorphic facial features, rocker-bottom feet, muscular and abdominal wall defects, and genital anomalies.[163–165]

Patients with early neonatal onset without congenital abnormalities also initially present with hypotonia, metabolic acidosis, hypoglycemia, tachypnea, hepatomegaly, and the odor of sweaty feet. These patients may survive for a few months with treatment. Death is often due to severe cardiomyopathy. An even longer survival was noted in patients who initially presented with hypoglycemia in the newborn period followed by episodes of Reye-like illness.[164–168]

There is considerable variability in patients with the milder, late-onset phenotype.[165] The clinical presentation may include episodic vomiting, hypoglycemia, and acidosis. Hepatomegaly, carnitine deficiency, and lipid storage myopathy have also been described. Whereas some patients present clinically within the first few years of life,[169] others may remain symptom free until adulthood.[170]

Mutations in ETF-QO but not ETF are associated with renal cysts. Although several mutations have been described in the genes for the ETF α subunit and ETF-QO, there are no common mutations. Olsen and colleagues examined the genetic basis of nine patients with the three different clinical phenotypes of glutaric acidemia type II and showed a relationship between the genotype and the phenotype.[171] The three patients with neonatal-onset and congenital anomalies were homozygous for null mutations and demonstrated palmitate oxidation levels of less than 5% of control cell lines in metabolic flux studies. The milder clinical phenotypes are associated with mutations that result in residual enzymatic activity, which itself may be modulated by environmental factors, such as fever.

Glutaric acidemia type II has a characteristic biochemical phenotype, primarily associated with the secondarily altered metabolism of its related dehydrogenases.[172] Urinary organic acid profiles demonstrate an accumulation of EMA, glutaric acid, and 2-hydroxyglutaric acid. Isovalerylglycine and hexanoylglycine accumulation is notable, particularly by urinary acylglycine analysis, and elevated levels of butyrylcarnitine, isovalerylcarnitine, glutarylcarnitine, and several other medium- and long-chain acylcarnitine species are observed by acylcarnitine analysis in plasma and newborn screening blood spots.

2,4-Dienoyl-CoA Reductase Deficiency

There has only been one published case of 2,4-dienoyl-CoA reductase deficiency.[19] The patient was a term female who presented at 2 days of age with hypotonia. Notable

TABLE 55.3-3 DISORDERS OF KETONE BODY METABOLISM AND CHARACTERISTIC BIOCHEMICAL ABNORMALITIES

DISORDER	KETOSIS	ACIDOSIS	BLOOD GLUCOSE	BLOOD AMMONIA	PLASMA LACTATE
Ketogenesis					
HMG-CoA synthase deficiency	–	–	↓	N	N
HMG-CoA lyase deficiency	–	+	↓		↑
Ketolysis					
SCOT deficiency	+++	+	N/↓	N	N
β-Ketothiolase deficiency	+++	+	↑/N/↓	N	N

HMG-CoA = 3-hydroxy-3-methylglutaryl coenzyme A; N = normal; SCOT = succinyl-CoA oxoacid transferase; ↓ = reduced; ↑ = elevated.

physical features included microcephaly; a large face; small arms, feet, and fingers; and a small trunk. The biochemical phenotype consisted of hyperlysinemia, carnitine deficiency, and a normal urinary organic acid profile. Acylcarnitine analysis, however, revealed an abnormal accumulation of decadienoylcarnitine ($C_{10:2}$) in blood and urine. The infant was placed on a lysine-restricted diet with MCTs and carnitine supplementation. Although this diet resulted in some weight gain, at 4 months of age, the patient ultimately suffered a fatal episode of respiratory distress. Autopsy findings also demonstrated pulmonary vascular congestion and bilateral hypertrophy. It is surprising that no additional cases have been found after the initial report despite the routine application of acylcarnitine analysis in the laboratory evaluation of metabolic patients.

GENERAL APPROACH TO TREATMENT

The mainstay of treatment is the avoidance of fasting. Frequent feedings are recommended to avoid accumulation of toxic metabolites resulting from peripheral lipolysis and hypoglycemia that may occur in affected patients when hepatic glycogen stores become depleted during prolonged fasting and periods of higher energy demands.[173] Infants should be fed every 3 to 4 hours, and older patients may be given uncooked cornstarch as a source of complex carbohydrates, which are slowly released into the bloodstream, allowing longer feeding intervals and prolonged nighttime rest. The initial dose is 1.0 to 1.5 g/kg, which can be slowly increased to 1.75 to 2 g/kg by 2 years of age.[174] If this is not tolerated well, a bedtime snack rich in complex carbohydrates should be provided. In severe cases with a high risk of recurrent hypoglycemia, continuous overnight administration of carbohydrates by nasogastric or gastrostomy tube may be required.

Restriction of Fat Intake

A dietary regimen of fat restriction and high carbohydrate intake has been generally recommended for the long-term management of FAO disorders. The exact limitations, however, have not been studied systematically and vary, ranging from 10 to 35%, with a median fat restriction of 29%.[173] Depending on the severity of the disorder, a reasonable approach appears to be the reduction to 25 to 30% of total calories from fat. The substitution of MCTs as a lipid substrate has been suggested for patients with long-chain FAO defects because medium-chain fatty acids do not require the carnitine cycle to cross the mitochondrial membranes and enter the β-oxidation pathway downstream of the defective enzyme.[175] Ten to 20% of total calories should come from MCT oil in these disorders.[173,176] However, MCT oil must not be prescribed for patients with deficiencies of medium- and short-chain fatty acid metabolism and ketogenesis. Supplementation of essential fatty acids at 1 to 2% of total energy intake by the use of flaxseed, canola, walnut, or safflower oils has been suggested, particularly when MCT oil is a significant part of the diet and because deficiency of docosahexaenoic acid has been hypothesized as a possible cause for the development of pigmentary retinopathy in patients with LCHAD deficiency.[115,173,177] Fat-soluble vitamins should also be supplemented to meet Recommended Dietary Allowance standards.[173]

Carnitine Supplementation

Primary carnitine deficiency (carnitine uptake defect) is the only disorder for which the supplementation of L-carnitine is proven to be essential and lifesaving.[78] In this condition, oral doses of 100 mg/kg/d and higher are required to maintain plasma carnitine levels in the normal range. The use of carnitine in other disorders of FAO that are associated with secondary carnitine deficiency is controversial. Arguments in support of carnitine are based on its potentially beneficial role in the removal of accumulated toxic acyl-CoA intermediates and repletion of the intramitochondrial carnitine pool. However, neither protective nor deleterious effects of carnitine have been proven conclusively. Treem and colleagues reported a study of one patient with MCAD deficiency who was fasted before and on carnitine supplementation.[178] Treatment did not prolong the period of fasting tolerance or prevent the accumulation of abnormal metabolites. Furthermore, the benefit of L-carnitine supplementation has been questioned for long-chain FAO disorders in particular because the accumulation of long-chain fatty acylcarnitines may cause fatal cardiac arrhythmias.[33,179] On the other hand, L-carnitine has been proposed as an antiarrhythmic medication.[180] Den Boer and colleagues did not find evidence that carnitine supplementation had any effect on the number of metabolic decompensations in LCHAD-deficient patients.[114] In a few patients with LCHAD deficiency, a positive response to carnitine administration was reported with respect to serum carnitine values and clinical status.[181,182] Other patients with FAO disorders died while treated with carnitine,[183–185] whereas the clinical condition improved for others who did not receive carnitine.[186]

Other Treatment Modalities

Although no data regarding efficacy are available, riboflavin is sometimes prescribed (100 mg/d) for SCAD and ETF/ETF dehydrogenase deficiencies. The rationale is that riboflavin, a cofactor of dehydrogenases, may boost any residual enzyme activity. More recently, Roe and colleagues reported the use of an anaplerotic odd-chain triglyceride, triheptanoin, in three patients with VLCAD deficiency.[187] It is postulated that triheptanoin increases the concentration of oxaloacetate (derived from propionic acid) that can serve to favor oxidation of acetyl CoA by the Krebs cycle or enter gluconeogenesis. This anaplerotic effect was found to be effective in the normalization of the acylcarnitine profile and some clinical symptoms in patients with VLCAD deficiency.[187] It is unclear whether odd-chain fatty acids offer any therapeutic advantage over the widely available MCT-rich diet for patients with carnitine or acylcarnitine translocase deficiency. Cardiomyopathy and muscle weakness resolved in these patients, and rhabdomyolysis did not occur at least during the 26 months of treatment with this dietary modification. Side effects were also not observed, but a larger, longer-term study has not been reported, and

studies using many available mouse models for FAO disorders have not yet been conducted.

Future pharmacologic approaches to the treatment of FAO disorders may also include the use of fibrates, which have been shown in vitro to increase enzyme activity at least in patients with some residual enzyme activity. So far, this has only been shown to be of potential benefit in CPT2–deficient fibroblast cultures.[188] The beneficial effect of increased enzyme activity appears to be related to fibrate acting as a ligand of peroxisome proliferator–activated receptor α, which induces expression of β-oxidation enzyme genes.

Management during Intercurrent Illness

The most pressing issue in a patient with an impending or present metabolic decompensation is the reversal of catabolism by provision of energy in the form of glucose. When oral intake of adequate amounts of carbohydrates cannot be achieved, intravenous glucose (8–10 mg/kg/min) should be administered. This should stimulate insulin secretion and hence suppress peripheral lipolysis. Some, particularly newborn, patients do not appear to clinically improve despite the development of even hyperglycemia. However, hyperglycemia may not be a reflection of a glucose overload but can be caused by an inadequate insulin response. In such cases, glucose should not be reduced, but insulin therapy should be carefully initiated to allow the patient's metabolism to use the administered glucose.

Once diagnosed, all patients should be provided with a frequently updated "emergency" letter to be given, if needed, to health care providers who may not be familiar with the patient's disorder. This letter should include a detailed explanation of the management of acute metabolic decompensation, emphasizing the importance of preventive measures (eg, intravenous glucose regardless of "normal" laboratory results, overnight in-hospital observation), and the telephone numbers of the patient's metabolic specialist.

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4. Bile Acid Synthesis and Metabolism

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For several decades, bile acids have been implicated in the pathogenesis of liver disease; however, their exact role in initiating or perpetuating liver injury has proven difficult to discern. Nonspecific alterations in serum, urinary, and biliary bile acid composition are found in infants and children with neonatal cholestasis. However, until recently, it was difficult to determine whether such changes were primary or secondary to the cholestatic condition. Largely as a consequence of methodologic advances,[1] specific inborn errors in bile acid biosynthesis have been recently recognized[2–5] that appear to be causal in the pathogenesis of the idiopathic and familial forms of neonatal hepatitis.[6–13] Although the exact genetic basis for these defects is still to be established, the deficiency in activity of specific enzymes involved in bile acid synthesis results in diminished production of the primary bile acids that are essential for promoting bile flow[14] and the concomitant production of atypical bile acids with the potential for causing liver injury.[15] This chapter outlines the pathways for bile acid synthesis, highlights the features of bile acid metabolism in early life, and describes the clinical and biochemical characteristics of inborn errors in bile acid synthesis.

CHEMISTRY

The bile acids are a group of compounds that belong to the steroid class.[16] Structurally, they consist of a four-ringed, cyclopentanoperhydrophenanthrene nucleus (ABCD rings) with a side chain, most commonly of five carbon atoms length, terminating in a carboxylic acid (Figure 55.4-1); they are therefore classified as acidic steroids. A great variety of bile acids can be found in biologic fluids, and significant species differences exist with regard to the synthesis and metabolism of the bile acids.[17] The vast majority of naturally occurring bile acids have the C-5 hydrogen oriented in the 5β-configuration, thereby confirming a *cis*-A/B ring structure. Bile acids with a 5α-H are referred to as *allo*-bile acids,[18] and these are found as minor metabolites in biologic fluids. In humans, the principal bile acids synthesized by the liver[3,19] have hydroxyl groups substituted in the nucleus at the carbon positions C-3, C-7, and C-12. Additional reactions involving other hydroxylations, epimerization, and oxidoreduction also take place, leading to a complex array of structures (see Figure 55.4-1). Although many of the products of these reactions may be of negligible quantitative importance in health, they are found in substantial concentrations in cholestatic syndromes, arising out of the induction of cytochrome P-450 hydroxylations.[1] During early development, alternative pathways for bile acid synthesis and metabolism become quantitatively important, as is evident from the findings of relatively high proportions of bile acids hydroxylated at the C-1, C-2, C-4, and C-6 positions of the nucleus.[20,21]

The two principal bile acids synthesized by the liver and referred to as the "primary" bile acids are cholic acid (3α,7α,12α-trihydroxy-5β-cholanoic acid) and chenodeoxycholic acid (3α,7α-dihydroxy-5β-cholanoic acid; a description of the conventions used for the systematic nomenclature of bile acids is reviewed elsewhere.[22] These bile acids are extensively conjugated to the amino acids glycine and taurine.[23] To a lesser extent, conjugation occurs with glucuronic acid to form glucuronide ethers[24] and esters[25] and with sulfuric acid to form sulfate conjugates.[26] More recently, bile acid conjugates of glucosides,[27,28] *N*-acetylglucosaminides,[29] and drugs[30] have also been recognized (see Figure 55.4-1). The diversity in bile acid structure is further increased by the fact that unsaturation (double bonds) in the steroid nucleus and side chain and substitution of oxo-groups also occurs, whereas bile

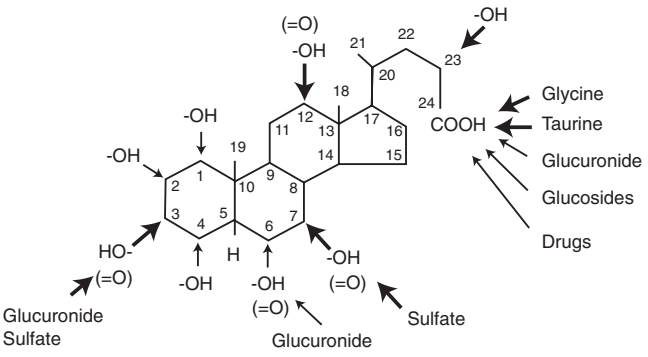

FIGURE 55.4-1 Chemical structure of the bile acid (5β-cholanoic acid) nucleus indicating the numbering system for each carbon atom and the various positions of the substituent groups for the majority of bile acids found in normal and pathophysiologic conditions. The size of the arrows indicates the relative importance of each of the substituent groups. Bile acids can additionally possess unsaturation in a number of positions in the nucleus and the side chain, but for simplicity, this is not indicated.

acids may be found with side chains longer or shorter than the usual 5-carbon side-chain length.[1]

The bile acids perform several important functions. They represent one of the major catabolic pathways for the elimination of cholesterol from the body.[3,19] From the standpoint of hepatobiliary disease, bile acids provide the primary driving force for the promotion and secretion of bile[14] and are essential to the development of the biliary excretory route for the elimination of endogenous and exogenous toxic substances, including bilirubin, xenobiotics, and drug metabolites. Within the intestinal lumen, the detergent action of bile acids facilitates the absorption of fats and fat-soluble vitamins, and the importance of this role becomes apparent in chronic cholestasis, where failure to thrive, fat malabsorption, and fat-soluble vitamin deficiency present significant clinical management problems. Indeed, the measurement of fat-soluble vitamin status is a very sensitive marker of disturbances in bile acid synthesis and secretion, as is evident from the manifestation of inborn errors in bile acid synthesis.[6,7,9–11,13]

Physiologically, the normal bile acid pool size is 2 to 4 g, but the effectiveness of this pool is increased by an efficient enterohepatic recycling (10–12 times/d) stimulated by postprandial gallbladder contraction.[31] Conservation of the bile acid pool occurs by an efficient reabsorption, principally from the small intestine, and an effective hepatic extraction from the portal venous circulation so that each day less than 5% of the pool is lost in the stool.[32] This bile acid loss is compensated for by hepatic synthesis of newly formed bile acids; therefore, in the steady state, determination of fecal bile acid excretion provides a reliable estimate of daily bile acid synthesis rates.[32]

PATHWAYS FOR BILE ACID SYNTHESIS

The biochemical pathways for bile acid synthesis in the adult have been relatively well defined and are reviewed in detail elsewhere.[3,19] Much of our understanding of these pathways results from in vitro and in vivo studies of precursor-product relationships in various animal species, most notably the rat and rabbit, and from studies of pathologic disorders affecting bile acid production. This discussion therefore serves to indicate only the salient features of the pathways. The conversion of cholesterol, a C_{27} sterol, to the two primary bile acids, cholic and chenodeoxycholic acids, requires significant alterations to the steroid nucleus (Figure 55.4-2) and side chain (Figure 55.4-3) of the molecule. These include (1) the introduction of additional hydroxyl groups at positions C-7 (for both chenodeoxycholic and cholic acids) and C-12 (for cholic acid); (2) epimerization of the 3β-hydroxyl group; (3) reduction of the Δ^5 bond; (4) reduction in length of the side chain from C_8 to C_5, with the formation of a terminal carboxylic acid; and (5) conjugation to the amino acids glycine and taurine.

Several pathways are responsible for bile acid synthesis from cholesterol.[33–35] Beginning from cholesterol, two reactions are possible: 7α-hydroxylation, initiating bile acid synthesis via the classic pathway, now referred to as the neutral pathway, or 27-hydroxylation, referred to as the acidic pathway. Although, classically, the 7α-hydroxylation pathway has been considered the major pathway for primary bile acid synthesis and cholesterol 7α-hydroxylase the accepted rate-limiting enzyme, more recently, the quantitative importance of the acidic pathway has been realized.[9,33,35–37] Nevertheless, irrespective of the route by which primary bile acids are synthesized, common reactions occur, and the enzymes catalyzing these reactions have broad specificity.[3] For the sake of simplicity, this review highlights the individual reactions involved in the classic (neutral) pathway, but it should be pointed out that these reactions do not necessarily occur in this orderly fashion. Indeed, permutations of these sequences of reactions give rise to the complex array of bile acids typically found in physiologic and pathophysiologic states, highlighting the promiscuity of these enzymes.

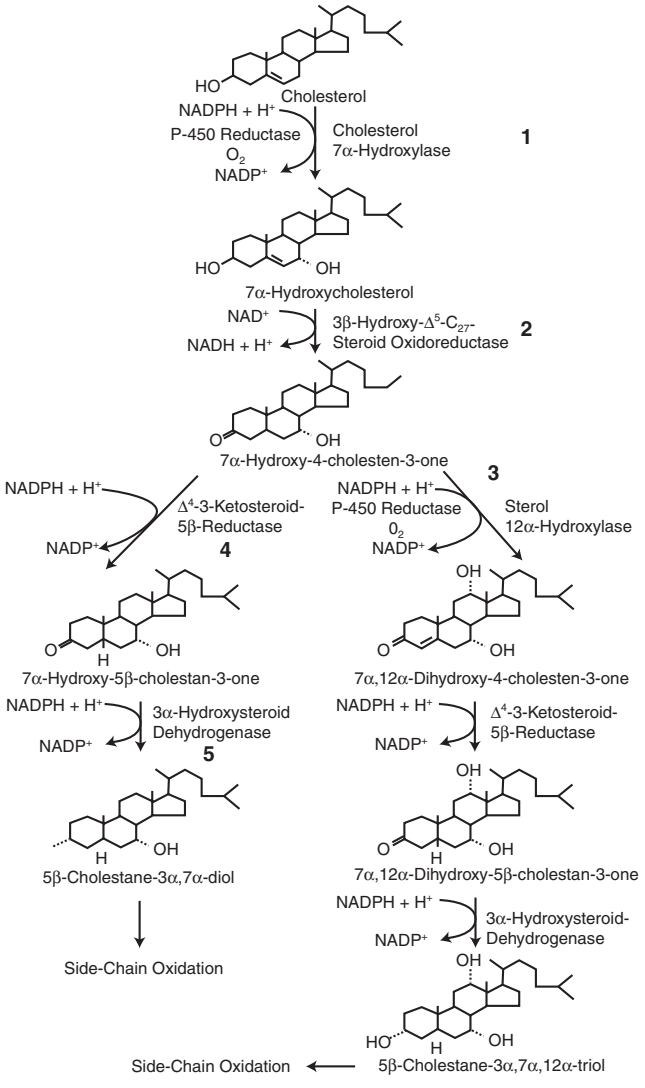

FIGURE 55.4-2 Metabolic pathway for the biosynthesis of the primary bile acids indicating the reactions involved in modifying the steroid nucleus in the conversion of cholesterol to 5β-cholestane-3α,7α,12α-triol. In the classic (neutral) pathway, these reactions were considered to precede side-chain oxidation. NADP = nicotinamide adenine dinucleotide phosphate; NADPH = reduced nicotinamide adenine dinucleotide phosphate.

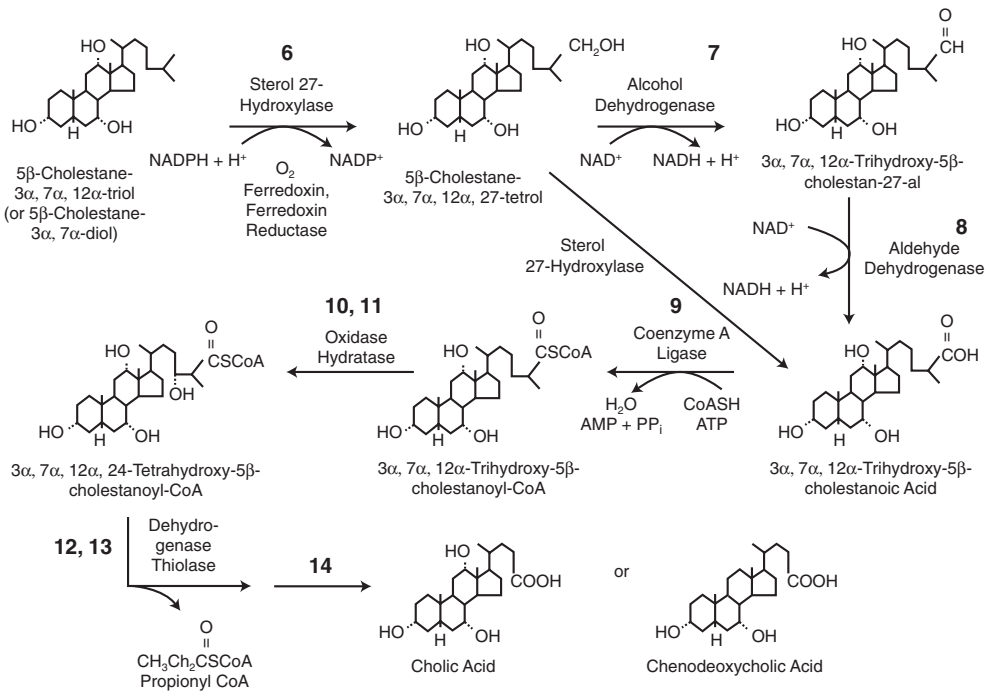

FIGURE 55.4-3 Sequence of reactions involved in the side-chain oxidation of 5β-cholestane-3α,7α,12α-triol to yield cholic or chenodeoxycholic acids, which are then conjugated with glycine and taurine. AMP = adenosine monophosphate; ATP = adenosine triphosphate; CoA = coenzyme A; NAD = nicotinamide adenine dinucleotide; NADH = reduced nicotinamide adenine dinucleotide; NADP = nicotinamide adenine dinucleotide phosphate; NADPH = reduced nicotinamide adenine dinucleotide phosphate.

In the classic pathway for bile acid synthesis from cholesterol, nine principal steps occur. All of the enzymes responsible for catalyzing these reactions are located in various subcellular fractions within the hepatocyte; consequently, there is considerable intracellular trafficking of the products of these reactions. Additionally, several of the enzymes are expressed in extrahepatic organs and tissues, including fibroblasts, macrophages, brain, lung, kidney, and vascular endothelia.[38–45] Why these enzymes are located in these tissues is unclear, but they are considered to play an important role in cholesterol homeostasis independent of their primary function, which is to catalyze reaction rates for primary bile acid synthesis.

Step 1: Cholesterol 7α-Hydroxylase

The first step in bile acid synthesis involves the introduction of a hydroxyl group at the C-7 position of the nucleus.[46] This reaction is catalyzed by a microsomal cholesterol, 7α-hydroxylase,[47] a cytochrome P-450 liver-specific enzyme with a molecular weight of 57 kD. A vast literature exists on the role of cholesterol 7α-hydroxylase in bile acid synthesis and the factors involved in its regulation that cannot be covered within the scope of this review, and the reader is directed to several excellent articles on this topic.[3,19,47,48] This step is potentially the most important because it is rate limiting for bile acid synthesis[49] and is subject to negative feedback regulation by the flux of bile acids returning to the liver. Differences exist, however, in the ability of different bile acids to regulate this enzyme.[50] For example, unlike primary bile acids, bile acids possessing a 7β-hydroxyl group (such as ursodeoxycholic acid [UDCA]) are unable to down-regulate bile acid synthesis,[51] and UDCA may even be mildly stimulatory.[52] Biliary drainage increases cholesterol 7α-hydroxylase activity approximately 10-fold in rats,[53,54] whereas taurochenodeoxycholic acid infusion restores activity to normal.[55] Likewise, cholestyramine administration increases the activity of cholesterol 7α-hydroxylase. Because bile acid synthesis is self-regulated via the activity of cholesterol 7α-hydroxylase, this provides the basis for the feeding of oral primary bile acids for the treatment of metabolic defects involving enzymes in the pathway.[56–60]

Changes in the activity of cholesterol 7α-hydroxylase parallel changes in 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity; consequently, these two key enzymes regulate the cholesterol pool size.[61] A diurnal rhythm in cholesterol 7α-hydroxylase activity occurs synchronously with the diurnal rhythm in the activity of the HMG-CoA reductase.[62–64] Bile acid synthesis increases nocturnally[65] and may be regulated by glucocorticoids.[66]

Cholesterol 7α-hydroxylase has been isolated and purified from rat liver,[67] the protein structure has been sequenced, and complementary deoxyribonucleic acids (cDNAs) have been prepared for the rat and human enzymes.[68–70] This enzyme has been shown to be exclusively of hepatic origin. With molecular tools, many studies of the gene, the messenger ribonucleic acid (mRNA), and the protein have been carried out.[71–74] These studies have also heightened awareness of the complexity in the regulation of bile acid synthesis and have led to the realization that the classic neutral pathway is not the sole contributing pathway for bile acid synthesis. When the gene for cholesterol 7α-hydroxylase is knocked out in a mouse,[37,75] most of the animals die within the first few weeks of life from liver failure and the consequences of fat-soluble vitamin malabsorption, highlighting the importance of bile acids for lipid absorption. However, if these animals are fed fat-soluble vitamins and cholic acid, they survive, the symptoms resolve,[76] and primary bile acid synthesis takes place via the developmental expression of an oxysterol 7α-hydroxylase specific to the acidic pathway.[37] Furthermore, in rats, if activity of cholesterol 7α-hydroxylase is completely

repressed by continuous infusion of squalestatin, bile acid synthesis 24 hours later is still 43% of preinfusion levels, indicating that the acidic pathway accounts for almost half of the total bile acid synthesis in the rat.[36] Our recent discovery of a genetic defect in the oxysterol 7α-hydroxylase causing fatal liver disease[9] suggests that the acidic pathway is quantitatively the most important, at least in early infancy. This contention is based on the fact that even though the patient had a normal cholesterol 7α-hydroxylase gene, there was no detectable mRNA or enzyme activity. Also, cholesterol 7α-hydroxylase activity was not detected or was very low in liver samples from infants of less than 1 year of age, confirming earlier reports that have shown cholesterol 7α-hydroxylase enzyme activity to be low or undetectable in human fetal and infant liver.[77,78] What triggers the increased activity in cholesterol 7α-hydroxylase during development is uncertain. Overall, it appears that there are significant species differences with regard to the developmental expression of the neutral and acidic pathways.

It has been shown that bile acids and their oxysterol intermediates interact with a number of nuclear orphan receptors.[79–81] Furthermore, bile acids are present in the nucleus of hepatocytes.[82] At least four such orphan receptors have been identified: liver X receptor, farnesoid X receptor (FXR), pregnane X receptor, and constitutive androstane receptor; there may be others yet to be identified.[79,80,83,84] It appears that these may be important at two levels of regulation: in the liver by transcriptional regulation of cholesterol 7α-hydroxylase[85–87] and at the intestinal level by inducing transcription of the ileal bile acid binding protein that is involved in the ileal uptake and conservation of the bile acid pool.[88] The importance of these orphan receptors in regulating bile acid synthesis and expression of bile acid transporters is illustrated from significant changes observed in studies of gene knockout models of these orphan receptors.[83,89] It is now evident that bile acid synthesis and transport may, in the future, be manipulated by the use of specific ligands for these orphan receptors to treat cholestatic liver diseases and disorders of cholesterol metabolism. Chenodeoxycholic and cholic acids have a high affinity for FXR,[81,88,90,91] and this dihydroxy bile acid is also a potent regulator of cholesterol synthesis.[88] UDCA, on the other hand, has little affinity.

Step 2: 3β-Hydroxy-C_{27}-Steroid Oxidoreductase (3β-Hydroxy-C_{27}-Steroid Dehydrogenase/Isomerase)

The conversion of 7α-hydroxycholesterol to 7α-hydroxy-4-cholesten-3-one is catalyzed by a microsomal 3β-hydroxy-C_{27}-steroid oxidase (previousy referred to as a 3β-hydroxy-C_{27}-steroid dehydrogenase/isomerase enzyme, and considerable effort has gone into understanding the mechanism of this relatively complex two-step reaction[92,93] that involves oxidation of the 3β-hydroxyl group and isomerization of the Δ^5 bond. It is possible that 7α-hydroxy-5-cholesten-3-one is formed as an intermediate, but attempts to isolate this compound have proved unsuccessful. In 1981, Wikvall isolated what appeared to be a single, highly specific enzyme of molecular weight 46 kD responsible for catalyzing this reaction in rabbits.[94] The enzyme was later isolated and purified to homogeneity from pig liver and shown to have a molecular weight of 36 kD.[95] More recently, a single gene (*HSD3B7*) on chromosome 16p11.2-12 encoding the enzyme was identified and sequenced, and a number of mutations associated with progressive familial cholestatic liver disease were described.[96,97] The enzyme only catalyzes the conversion of 7α-hydroxylated sterols or bile acid intermediates with a 3β-hydroxy-Δ^5 structure to the corresponding 3-oxo-Δ^4 metabolites. It shows no activity toward corresponding 7β-hydroxy analogs. Comparable reactions occur in the biosynthetic pathways for steroid hormones, but the enzyme active on the sterol intermediates of bile acid synthesis differs from the isozymes described for C_{19} and C_{21} neutral steroids, for which cDNAs are described.[98–101] The 3β-hydroxy-C_{27}-steroid oxidoreductase enzyme is expressed in fibroblasts,[102] although its function is unknown. Nevertheless, this does enable its activity to be determined in patients in whom there is a evidence of a deficiency in this enzyme; however, sequencing of the DNA is a preferred approach to accurately confirming a biochemical defect in this reaction step.

Step 3: 12α-Hydroxylation

The conversion of 7α-hydroxy-4-cholesten-3-one into 7α,12α-dihydroxy-4-cholesten-3-one is catalyzed by cytochrome P-450–dependent microsomal 12α–hydroxylase, which has a relatively broad substrate specificity. This reaction is responsible for diverting sterol intermediates into the cholic acid pathway. The enzyme was purified from rabbit liver and shows specificity toward 5α-cholestane-3α,7α-diol and 7α-hydroxycholesterol in rabbits.[103] The primary structure of the rabbit, mouse, and human enzymes has been deduced by molecular cloning of the cDNAs, and the enzyme has been expressed in COS cells.[104,105] There is little similarity in its peptide sequence with other cytochrome P-450 enzymes involved in bile acid synthesis, and because it shows a 43% homology with prostacyclin synthase (*CYP8A1*), it has been designated as *CYP8B1*.[105] The human gene has been localized to chromosome 3p21.3-p22 and structurally has been found to be the first cytochrome P-450 enzyme to lack introns. The enzyme is well expressed in rabbit and human liver, two species in which deoxycholic acid is quantitatively important, and to a lesser extent in rats and mice. Studies in rats have shown that the introduction of a C-27 hydroxyl group prevents subsequent 12α-hydroxylation and that thyroid hormone inhibits its activity while stimulating microsomal C-27 hydroxylase activity.[106,107] It is also transcriptionally downregulated by hydrophobic bile acids.[108] Both enzymes may therefore be of importance in regulating the synthesis of cholic acid in rats.[107] This, however, seems not to be the case for humans, in whom the introduction of a C-27 hydroxyl group has no inhibitory effect on the microsomal 12α-hydroxylase activity and thyroid hormone has only a small influence on the cholic-to-chenodeoxycholic acid ratio. These differences highlight species variations[17] that require consideration when using animal models. Other

factors influencing microsomal 12α-hydroxylase include bile acid feeding,[109,110] which has an inhibitory effect, and cholestyramine administration, which increases the ratio of cholic-to-chenodeoxycholic acid[111] owing to an interruption of the normal enterohepatic circulation of bile acids. Similarly, biliary drainage[49] and starvation[103,112] increase its activity, the latter by an increase in mRNA levels. There appears to be no correlation between 12α-hydroxylase activity and the ratio of biliary cholic acid to chenodeoxycholic acid in humans,[113] indicating that other factors, such as the extent of enterohepatic recycling, intestinal metabolism, and absorption, may be important in regulating the relative proportions of intermediates that are diverted to each pathway. Additionally, the existence of alternative pathways for bile acid synthesis, particularly chenodeoxycholic acid,[33–35] which may be under separate regulatory control, could explain this lack of relationship.

Step 4: Δ^4-3-Oxosteroid 5β-Reductase

A soluble reduced nicotinamide adenine dinucleotide phosphate (NADPH)-dependent Δ^4-3-oxosteroid 5β-reductase enzyme is responsible for catalyzing the reaction that leads to the saturation of the Δ^4-bond and the formation of the 5β-(H) configuration at the AB-ring junction[114,115] that is common to the majority of bile acids found in most animal species, including humans. The Δ^4-3-oxosteroid 5β-reductase has been purified,[116] and sequence analysis of both the human[117] and rat[118,119] cDNA encoding this enzyme indicates its molecular weight to be 37 to 38 kD, being made up of 327 amino acids. The rat and human enzymes show similar homology but differ significantly in structure from the analogous 5α-reductase enzyme responsible for the formation of *allo*-bile acids. The activity of this enzyme parallels that of cholesterol 7α-hydroxylase, as indicated from the finding that the plasma concentration of 7α-hydroxy-4-cholesten-3-one correlates with hepatic cholesterol 7α-hydroxylase activity.[120] Although this enzyme does not appear to be of regulatory importance for bile acid synthesis under normal conditions, the finding of significantly elevated levels of Δ^4-3-oxo-bile acids in severe cholestasis[121] would suggest that under pathologic conditions, it may become rate limiting for primary bile acid synthesis.

Step 5: 3α-Hydroxysteroid Dehydrogenase

Conversion of 7α-hydroxy-5β-cholestan-3-one and 7α,12α-dihydroxy-5β-cholestan-3-one by reduction into the respective 3α-hydroxy analogues takes place in the cytosolic fraction under the influence of a NADPH-dependent 3α-hydroxysteroid dehydrogenase enzyme.[114,115] This enzyme, which has broad substrate specificity, has been purified to homogeneity, and a number of cDNAs have been sequenced.[122–125] In addition to its role in metabolism, 3α-hydroxysteroid dehydrogenase was shown to be identical to the 33 kD Y′ bile acid binders involved in the intracellular transport of bile acids.[126] The enzyme is inhibited by indomethacin, and bile acid binding to this protein is a major determinant of the intracellular distribution.[127]

Step 6: Sterol 27-Hydroxylase

The mechanism by which oxidation of the C_{27} sterol side chain occurs has been the subject of extensive study. Under normal conditions, it would appear that the first step involves the introduction of a hydroxyl group at the C-27 position (see Figure 55.4-3). This reaction can take place in both the microsomal and mitochondrial fractions[3,19]; for humans, the mitochondrial C-27 hydroxylase (formerly referred to as a C-26 hydroxylase) is quantitatively more important. The mitochondrial C-27 hydroxylation has been shown to be stereospecific, involving hydroxylation of the 25-pro-*S* methyl group to yield the 27-hydroxylated product with a 25(R) configuration. On the other hand, the microsomal C-27 hydroxylation seems to involve the formation of the 25(S) product. The mitochondrial C-27 sterol hydroxylase exhibits a broad substrate specificity toward many sterols, including cholesterol and vitamin D,[128–130] but is particularly active toward 5β-cholestan-3α,7α-diol, 5β-cholestan-3α,7α,12α-triol, and 7α-hydroxy-4-cholesten-3-one.[131,132] The reaction involves a cytochrome P-450 species,[133] and the enzyme can be induced by phenobarbital treatment and by starvation. In addition to 27-hydroxylation, this enzyme is capable of catalyzing multiple oxidation reactions that give rise to 3α,7α,12α-trihydroxy-5β-cholestanoic acid (THCA).[134]

Although the microsomal C-27 hydroxylase (which is also cytochrome P-450 dependent) is of minor quantitative importance in humans, compared with the mitochondrial enzyme, it has a higher substrate specificity in the rat.[131] The microsomal fraction of rat liver, however, catalyzes the hydroxylation of the C-23, C-24 (α and β), and C-25 carbons, with the latter being as efficient as C-27 hydroxylation.[135]

It would appear that the 27-hydroxylation of cholesterol that drives the acidic pathway is catalyzed by the very same sterol 27-hydroxylase enzyme that is active on cholestane-3α,7α,12α-triol in the neutral pathway. This is in contrast to 7α-hydroxylation, in which there are separate and distinct enzymes in both pathways.[9] The sterol 27-hydroxylase is expressed in many extrahepatic tissues,[35,39,40,43,44,128,136,137] but especially in the adrenals, intestine, and lung, where its activity is highest. Its function appears to be one of facilitating the removal of cellular cholesterol for subsequent oxidation to bile acids. In the lung, where it is highly expressed, the ratio of 27-hydroxycholesterol to cholesterol is high. It appears that this organ accounts for most of the production of 3β-hydroxy-5-cholestenoic acid[38]; interestingly, removal of one lung reduces the level of this cholestenoic acid by half.

cDNAs encoding the rat, rabbit, and human C-27 hydroxylase have been isolated,[128,136,138,139] and whereas the activity and message for the sterol 27-hydroxylase have been determined in many tissues, its role in regulating bile acid synthesis is unclear, and it is evident that there are significant species differences. In the rabbit, the activity of cholesterol 27-hydroxylase is unaffected by bile acids,[140,141] whereas in rat hepatocytes transfected with the sterol 27-hydroxylase gene, bile acids repress transcription.[142] Sterol 27-hydroxylase appears to be much more important for bile acid synthesis in the mouse than in the human. When

the gene was disrupted in the mouse, the formation of bile acids was shown to be markedly reduced.[143] This is not the case in humans, in whom mutations in the cholesterol 27-hydroxylase gene account for the lipid storage disease of cerebrotendinous xanthomatosis (CTX). In CTX, bile acids are produced by a compensatory pathway involving the 25-hydroxylation pathway leading to cholic acid, but chenodeoxycholic acid synthesis is markedly impaired.[144–146]

Step 7: Formation of Cholestanoic Acids

The oxidation of the C-27 hydroxylated sterol intermediates to the respective cholestanoic (C_{27}) acids takes place in two steps[147] with the formation of an aldehyde as the intermediate. After purification of the enzymes responsible for these reactions, it was concluded that they were identical to the hepatic alcohol dehydrogenase and aldehyde dehydrogenase enzymes.[148–152] The relative importance of these enzymes in the oxidation of the side chain compared with that of the C-27 hydroxylase catalyzed reaction is unknown,[152] but the resulting product formed is the enantiomer (25R)3α,7α,12α-trihydroxy-5β-cholestanoic acid ((25R)THCA).

Step 8: Oxidation of the Side Chain

Initiation of side-chain oxidation occurs with the formation of the CoA ester of (25R)THCA, a reaction that takes place in the endoplasmic reticulum and is catalyzed by a THCA-CoA synthetase.[153,154] Racemization of (25R)THCA-CoA to (25S)THCA-CoA takes place, catalyzed by 2-methylacyl-CoA racemase,[155] and this is an obligatory step to enable subsequent peroxisomal β-oxidation to occur because only the (25S)-enantiomers are substrates for the peroxisomal branched-chain acyl-CoA oxidase that leads to primary bile acid formation. 2-Methylacyl-CoA racemase is also responsible for catalyzing the stereoisomerization of the branched-chain fatty acid (2R)pristanoyl-CoA to its corresponding (S)-isomer.[156] The gene encoding this enzyme has been sequenced, and mutations have been reported in three adult patients with sensory neuropathies[155] and in two infants with liver disease and fat-soluble vitamin malabsorption.[13] Once formed, (25S)THCA-CoA undergoes oxidation by a series of reactions analogous to those responsible for the mitochondrial β-oxidation of fatty acids, involving the formation of 24α-hydroxylated and CoA derivatives with subsequent release of propionic acid to yield the cholanoic (C_{24}) acid CoA ester.[157,158] These reactions occur in the peroxisome, although, at one time, they were thought to be of microsomal origin.[152,158] Once within the peroxisome, the rate-limiting enzyme in side-chain oxidation, THCA-CoA oxidase, yields a Δ^{24} intermediate. A cDNA encoding the THCA-CoA oxidase was recently cloned and characterized in the rat, rabbit, and human.[159–162] This enzyme is distinct from the very-long-chain fatty acid (VLCFA) CoA oxidase that performs an analogous reaction[163] but is common to the oxidation of 2-methyl branched-chain fatty acids.[156] The Δ^{24} product of this reaction is then hydrated to form 24-hydroxy-THCA-CoA, which, in turn, is dehydrogenated to yield 24-oxo-THCA-CoA.[19,156,158,163,164] These two reactions are catalyzed by a multifunctional protein,[165–167] of which two forms are known, multifunctional enzyme (MFE)-I and MFE-II, the latter being the one responsible for the degradation of bile acids and branched-chain fatty acids.[168] It is possible that the sterol carrier protein SCP-2/SCPx, which binds and complexes acyl CoAs and acyl-CoA oxidases within the peroxisome, may play a role in side-chain oxidation of bile acids, at least in the mouse, because when the gene was knocked out, an accumulation of 3α,7α,12α-trihydroxy-27-nor-5β-cholestan-24-one occurred.[169] The final step in the sequence of reactions for side-chain oxidation involves thiolytic cleavage of propionic acid, and this yields cholanoyl CoA.[170,171] The mechanisms involved in these reactions are described in more detail in a separate chapter on peroxisomes in this book.

Step 9: Conjugation of Bile Acids

The CoA derivatives of cholic and chenodeoxycholic acids are finally conjugated with the amino acids glycine and taurine. It is not known whether this reaction takes place exclusively within the peroxisome[172,173] or, as was originally thought, the cytosol, or both of these compartments.[174,175] A microsomal bile acid–CoA synthetase has been isolated,[175] which may be involved in the hydrolysis of the CoA ester and subsequent transport of cholic and chenodeoxycholic acids between these compartments.

Bile acid amino acid conjugates are formed in two consecutive enzymic reactions. The unconjugated bile acid is first converted to an acyl-CoA thioester,[174,176–178] a reaction that is catalyzed by a rate-limiting hepatic microsomal[179] bile acid CoA ligase (EC 6.2.1.7). This enzyme has also been identified in a rat kidney.[180] It has been purified and characterized from rat liver and found to have a molecular weight of 65 kD, and, more recently, its cDNA has been cloned and expressed in insect Sf9 cells.[181] It shows activity toward chenodeoxycholic and cholic acids but is inhibited by more hydrophobic bile acids, especially lithocholic and deoxycholic acids. Although the gene for the human hepatic enzyme has yet to be identified and sequenced, a gene encoding a 58 kD protein, recognized as a bile acid CoA ligase, was interestingly identified from *Eubacterium* sp strain VPI 12708.[182]

In the second reaction, the bile acid CoA thioester, catalyzed by a cytosolic bile acid–CoA:amino acid *N*-acyltransferase (EC 2.3.1.65),[183] is coupled to the amino acids glycine and taurine. This enzyme has been purified from human liver,[184] and its substrate specificity has been well characterized.[184–186] A cDNA encoding the human bile acid CoA:amino acid *N*-acyltransferase has been isolated, characterized, and expressed in bacteria,[187] as has a cDNA for the corresponding mouse enzyme, the gene for the latter being localized to chromosome 4.[188] The human cDNA encoded a monomeric protein of 46,296 D, and although there was reported close homology with the mouse enzyme[188] and with *kan-1*, a putative rat liver *N*-acyltransferase,[189] a significant species difference in substrate specificity was demonstrated. The human bile acid–CoA:amino acid *N*-acyltransferase is capable of conjugating cholic acid with both glycine and taurine, whereas

the mouse enzyme showed selectivity toward taurine only. This is consistent with the fact that the mouse is an obligate taurine conjugator of bile acids, as are the rat and the dog.[190] The human bile acid–CoA:amino acid *N*-acyltransferase was recently found to be capable of conjugating fatty acids with glycine,[191] and because it is expressed in many tissues that play no role in bile acid synthesis, it was suggested that its function in extrahepatic tissues may be one of regulating intracellular levels of VLCFA.

Bile acids with a side-chain length of four carbon atoms (nor-bile acids) and six carbon atoms (homo-bile acids) are poor substrates for the enzymes; however, cholestanoic acids (C_{27} bile acids) are conjugated efficiently with taurine, as evidenced from their conjugation patterns in patients with peroxisomal defects.[2,192] The final products of the above-described multiple reactions, glycocholic, taurocholic, and chenodeoxycholic acids, are referred to as primary bile acids and are secreted in bile. In the normal adult, the ratio of glycine to taurine conjugated bile acids is 3:1,[23] but this can be altered by an increased availability of taurine, as occurs during taurine feeding[193] or in early life,[21] when hepatic taurine stores are high[194] because of selective placental transfer.[195] Unusual amidated conjugates are formed in some species, as evidenced by the finding that 3% of the biliary deoxycholic acid of the domestic rabbit is a glycyltaurine conjugate.[196]

Other bile acid conjugates occur naturally, and these include sulfates,[26] glucuronide ethers and esters,[24,25,197–200] glucosides,[27,28,199] *N*-acetylglucosaminides,[29,201] and conjugates of some drugs.[30,202,203] These metabolic pathways serve to increase the polarity of the molecule, thereby facilitating its renal excretion, and to decrease the membrane-damaging potential of the more hydrophobic unconjugated species.[204,205] Under normal conditions, these pathways are of minor quantitative importance but are activated in early life in the diseased state, particularly cholestasis, in the presence of an increased bile acid load such as exogenous bile administration or by drug administration.

A sulfotransferase enzyme catalyzes the formation of bile acid sulfates, most commonly at the C-3 position, but C-7 and C-12 sulfates are also found.[206–208] This enzyme shows sex-dependent differences in rats[208] but not in humans. Its activity has been shown to be low in the fetus compared with the adult,[209] as is evident from the finding of relatively small proportions of bile acid sulfates in fetal bile.[21] Although sulfation of bile acids has traditionally been considered to occur in the liver, it is evident that renal sulfation is important,[210,211] and most probably accounts for the increased concentrations of urinary bile acid sulfates in cholestasis.[212]

Glucuronidation is catalyzed by a number of glucuronyl transferase isozymes[199] that give rise to glucuronide ethers (ring conjugation) and esters (side-chain carboxyl conjugates). The affinity of this conjugation system is relatively specific[213]; short-chain bile acids are preferentially glucuronidated,[214] whereas bile acids possessing a 6α-hydroxyl group form the 6-O-ethers.[200,215]

Several other conjugation pathways for bile acids have been recently recognized. Glucosides[27,28,216] and *N*-acetylglucosaminides[29,201] of nonamidated and glycine- and taurine-conjugated bile acids have been found in normal human urine,[28,217] and quantitative excretion (1 μmol/d) approximates that of bile acid glucuronides.[198,218] A microsomal glucosyltransferase from human liver has been isolated and characterized and found in extrahepatic tissues.[216] This enzyme exhibits substrate specificity toward 7β-hydroxylated bile acids,[29] which explains why *N*-acetylglucosaminide conjugates of UDCA are found in large proportions in the urine of patients undergoing UDCA therapy.[212,219]

The identification of bile acid conjugates of fluorouracil[30,203,216] demonstrates that drug interactions with hepatic conjugation enzymes can take place and may play a role in the development of drug-induced cholestasis.

Alternative Pathways for Bile Acid Synthesis

The simplified view of the pathways for bile acid synthesis described above assumes that the sequence of reactions occurs in an orderly manner, with changes to the steroid nucleus preceding side-chain oxidation. This, of course, is not the case, as is apparent from in vitro studies of enzyme kinetics using radiolabeled intermediates, which demonstrate the existence of alternative pathways for primary bile acid synthesis (Figure 55.4-4).[220–222] This is reinforced by the finding that patients with T tubes converted radiolabeled 27-hydroxycholesterol to chenodeoxycholic acid to a greater extent than 7α-hydroxycholesterol.[223] This pathway was denoted the acidic pathway[33] and is under separate regulatory control to the classic cholesterol 7α-hydroxylase (neutral) pathway. The relative importance of alternative pathways for primary bile acid synthesis, which mainly relate to initiating hydroxylation reactions on the side chain followed by 7α-hydroxylation, has become more recently appreciated.[33,35] These reactions occur in the liver and in many different extrahepatic tissues, including the brain, alveolar macrophages, vascular endothelia, and fibroblasts.[41,45,224] Their extrahepatic function may be related in some way to the regulation of cholesterol homeostasis because of their ability to generate significant amounts of oxysterols that are potent repressors of cholesterol synthesis.[35,44,225,226]

Under normal conditions, the neutral pathway is still considered to be quantitatively the most important one for cholic acid synthesis in adults. Recent studies, however, now indicate that the acidic pathway contributes significantly to overall total bile acid synthesis, especially to chenodeoxycholic acid synthesis.[33,35] It has also become evident that the acidic pathway, although being developmentally induced in the rodent,[37] is possibly the most important one for bile acid synthesis in early human life.[9] This contention is based on the finding that an infant having a mutation in the oxysterol 7α-hydroxylase gene but a normal cholesterol 7α-hydroxylase gene failed to synthesize primary bile acids[9] and instead accumulated massive quantities of 24-hydroxy-, 25-hydroxy-, and 27-hydroxycholesterol. Although the sterol 27-hydroxylase directs intermediates into the acidic pathway, it is the subsequent 7α-hydroxylation that is the most important step in this pathway because

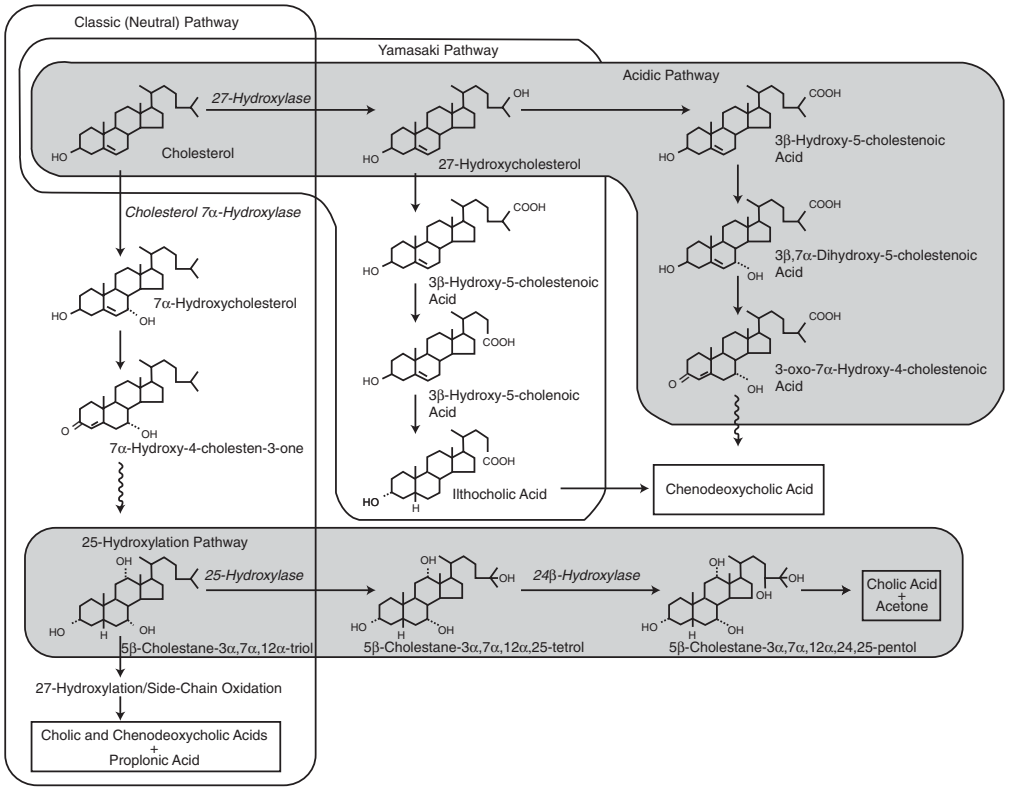

FIGURE 55.4-4 Alternative pathways for bile acid synthesis and their relationship to the classic "neutral" pathway.

it is essential to protect the liver from the toxicity of monohydroxy bile acids,[227–230] which would otherwise be formed if 7α-hydroxylation did not take place.[9]

For some time, it was evident that there were separate 7α-hydroxylases[33,231–233] and that bile acid synthesis via the acidic pathway was regulated differently and independently of the microsomal cholesterol 7α-hydroxylase.[35,50–52] The enzyme, referred to as oxysterol 7α-hydroxylase (*CYP7B1*), has a high activity in human liver,[233] but its regulation is not fully understood.[234,235] At least in the cholesterol 7α-hydroxylase (*CYP7A1*) knockout mouse, it is not affected by changes in the enterohepatic flux of cholesterol or bile acids induced by cholesterol or cholestyramine feeding.[236] It shows broad substrate specificity, being active on both 27- and 25-hydroxycholesterol[42,45,232,233] and 3β-hydroxy-5-cholenoic and 3β-hydroxy-5-cholestenoic acids.[231] The cDNA encoding the rat,[41] mouse,[235] and human[9,234] hepatic oxysterol 7α-hydroxylase (*CYP7B1*) has recently been reported. Translation of the human cDNA revealed the enzyme to have 506 amino acids, and there is a 56% and 66% homology with the rat and mouse enzymes, respectively.[9] The gene is localized to chromosome 8q21.3 and in close proximity to the gene encoding cholesterol 7β-hydroxylase (*CYP7A1*). Evidence for yet a further 7α-hydroxylase became apparent from studies of the *CYP7B1* knockout mouse,[237] which, unlike the human infant with a defect in this enzyme,[9] did not exhibit liver disease or accumulate to the same extent the oxysterols. This suggested that there was a third enzyme capable of 7α-hydroxylation, and, subsequently, *CYP39A1* was isolated[238] and shown to be active on 24-hydroxycholesterol. This enzyme is also present in humans, but its role is unclear.

The quantitative importance of the acidic pathway for bile acid synthesis has been hotly debated. When the cholesterol 7α-hydroxylase gene is knocked out in a mouse model, by 3 weeks of life, almost normal levels of bile acids are synthesized. This occurs following the developmental expression of the oxysterol 7α-hydroxylase.[37] In the rat, when cholesterol 7α-hydroxylase is chemically inhibited by continuous infusion of squalestatin, bile acid synthesis is still 43% of preinfusion levels after 24 hours.[36] These findings suggest that the acidic pathway accounts for about half of the total bile acid synthesis in the rodent. Under normal conditions, both the acidic and neutral pathways lead to the formation of cholic and chenodeoxycholic acids; however, it is suggested that 50% of the chenodeoxycholic acid synthesis is derived via this acidic pathway.[33]

As detailed above, side-chain oxidation proceeds with an initial C-27 hydroxylation and release of propionic acid; however, a pathway involving microsomal C-25 hydroxylation followed by 24-hydroxylation and release of acetone has been described. The cDNAs have been cloned of the cholesterol 25- and 24-hydroxylases,[239] and these enzymes have been found to play a key role in regulating lipid metabolism, particularly in the brain. The relative quantitative importance of this pathway to bile acid synthesis in healthy adults has been controversial.[144–146] Available evidence overwhelmingly supports 27-hydroxylation as the more important reaction initiating side-chain oxidation in normal humans.[240–242] This was confirmed in vivo by measuring the production of [^{14}C]acetone following prior labeling of the cholesterol pool with [26-^{14}C]cholesterol[145]; the 25-hydroxylation pathway accounted for less than 2% and 5% of the total bile acid synthesis in adult rats and humans, respectively.[145,146]

An alternative pathway for chenodeoxycholic acid synthesis[222] that is seemingly important in early life involves side-chain shortening prior to nuclear modifications, reactions that are initiated via a cholesterol C-27 hydroxylase.[34,210] In this pathway (often termed the Yamasaki pathway),[222] 27-hydroxycholesterol is oxidized directly to 3β-hydroxy-5-cholenoic acid, lithocholic acid, and, finally, chenodeoxycholic acid.[223,243–247] Although this pathway could be of minor importance in the adult, it may account for the increased levels of 3β-hydroxy-5-cholenoic and lithocholic acids in early life and in severe cholestatic conditions.

In many lower vertebrates, *allo*(5α-H)-bile acids are the major species of bile acids.[17,18] However, in humans, they are normally present in relatively small proportions and are generally believed to result from bacterial metabolism of 3-oxo-5β-bile acids during the course of their enterohepatic circulation. Studies of rodents have indicated that *allo*-bile acids may also be derived from 5α-cholestanol,[248,249] which can be efficiently 7α-hydroxylated in rat liver[250] and subsequently converted to 7α-hydroxy-5α-cholestan-3-one and then to 5α-cholestane-3α,7α-diol.[251] The 12α-hydroxylase enzyme shows a high specificity toward 5α-sterols,[252–254] and 5α-cholestane-3α,7α,12α-triol is readily formed from 5α-cholestane-3α,7α-diol and converted to *allo*-cholic acid in the bile fistula rat.[251]

A further mechanism for the formation of *allo*-bile acids involves their direct conversion from 7α-hydroxy-4-cholesten-3-one and 7α,12α-dihydroxy-4-cholesten-3-one by the action of an active Δ^4-3-oxosteroid 5α-reductase. The enzyme shows a three- to fourfold higher activity in female rats compared with male rats,[255] but no gender differences have been demonstrated for humans. The quantitative importance of this reaction in humans is uncertain. Large amounts of *allo*-bile acids are found in patients with a deficiency in the bile acid Δ^4-3-oxosteroid 5β-reductase.[7]

Intestinal microflora play an important role in bile acid metabolism[256] and therefore in the maintenance of the integrity of the enterohepatic circulation. Lithocholic (3α-hydroxy-5β-cholanoic) and deoxycholic (3α,12α-dihydroxy-5β-cholanoic) acids, the major bile acids excreted in feces,[32] are referred to as "secondary" bile acids. Both are formed from conjugated chenodeoxycholic and cholic acids by deconjugation and 7α-dehydroxylation, by enzymes found in a variety of organisms, such as *Bacteroides*, clostridia, bifidobacteria, and *Escherichia coli*. The mechanism by which 7α-dehydroxylation occurs has been extensively investigated by Hylemon, who purified a bile acid–CoA hydrolase from a strain of *Eubacterium* sp.[257] The dehydroxylation reaction involves a series of steps and appears to be initiated from the CoA thioester of the unconjugated bile acid but proceeds only following deconjugation.

Lithocholic acid is relatively insoluble and is consequently poorly absorbed from the intestinal lumen. It is found in relatively high proportions in meconium[258] and amniotic fluid[259,260] but is barely detectable in fetal bile.[21] In severe cholestatic conditions, deoxycholic acid levels in the serum become undetectable, and this bile acid is a useful marker of the extent of impairment of the enterohepatic circulation. Conversely, elevations in the serum unconjugated bile acid concentrations,[261] particularly secondary bile acids, reflect bacterial overgrowth of the small bowel.[262,263] Elevations in lithocholic acid sulfate, which occur in severe cholestasis,[264] demonstrate that lithocholic acid is also a primary product of hepatic synthesis and under such circumstances arises via the alternative pathways discussed previously.

BILE ACID SYNTHESIS DURING EARLY DEVELOPMENT

Knowledge of hepatic bile acid synthesis and metabolism during human development is limited and is derived largely from analysis of biologic fluids[21,265–271] and in vitro studies of the enzymes in fetal liver homogenates.[77,272–274] Ontogenic studies have been carried out in several animal species.[209,272,275–281] Detailed analytic studies of human fetal gallbladder bile[21,270] and in vitro studies of hepatic subcellular fractions[273,274] established significant qualitative and quantitative differences in bile acid synthesis and metabolism between the developing and adult liver. Because biliary excretion is the principal route for bile acid secretion, analysis of gallbladder bile permits a direct means of assessing hepatic synthesis and secretion.

The earliest studies of human fetal gallbladder bile used methodology less advanced than is currently available but nevertheless established primary bile acid synthesis to be relatively well developed during early gestation.[266,270] These early studies showed the concentration of chenodeoxycholic acid to be greater than cholic acid at midgestation, and primary bile acids were conjugated mainly with taurine. These findings were later corroborated using improved methodology, which confirmed that for humans, pathways for primary bile acid synthesis are developed as early as the twelfth week of gestation.[21] The activities of enzymes catalyzing 7α-hydroxylation, 12α-hydroxylation, side-chain oxidation, and conjugation of the primary bile acids and bile acid intermediates in homogenates of rat liver from rat embryos and suckling rats were found to increase 30-fold from day 15 after fertilization to day 5 of life.[275] Studies of preterm and older infants have found that the bile acid pool size is only one-sixth that of adults, and a rapid expansion of the pool occurs over the first year of life.[282–285] The chenodeoxycholic acid concentration in human fetal bile is relatively low in early gestation and exceeds the cholic acid concentration. This is in marked contrast to the biliary bile acid composition of the full-term infant and the adult, in whom cholic acid is the predominant bile acid.[265,267] Similar developmental differences in bile acid composition are found in amniotic fluid collected at different times of gestation.[260] There are several possible explanations for these differences: (1) cholic acid synthesis would be reduced in early life if there was an immaturity in hepatic 12α-hydroxylase activity; however, in vitro studies have established the activity of this enzyme to be relatively well developed[77,275]; (2) preferential clearance of cholic acid by metabolism to more polar C-1, C-2, C-4, or C-6 tetrahydroxylated bile acids would lead to a relative increase in the proportion of chenodeoxycholic acid; however, tetrahy-

droxy bile acids constitute less than 2% of the total biliary bile acids of the human fetus[21]; (3) chenodeoxycholic acid synthesis occurs via the C-27 hydroxylase pathway, which is under separate regulatory control and appears to be upregulated when the activity of cholesterol 7α-hydroxylase is low, as would be expected in utero. This latter explanation is most likely and would explain the increased amounts of monohydroxy bile acids found in meconium.[258]

A conspicuous feature of bile acid synthesis and metabolism during development is the relatively large proportion of a complex array of bile acids not typically found in adult bile.[20,21] Interestingly, the profile of biologic fluids of the newborn and fetus[20,21,270,286] resembles that observed for adult patients with severe cholestasis.[287,288] Analysis of human fetal gallbladder bile[21,270] and in vitro incubations of hepatic subcellular fractions with radiolabeled bile acids[273,274] has served to confirm the quantitative importance of several hepatic hydroxylation pathways, including C-6 and C-1 hydroxylation. Hyocholic acid (3α,6α,7α-trihydroxy-5β-cholanoic acid) is a major biliary bile acid of the fetus, and concentrations often exceed cholic acid concentrations,[21] whereas a series of C-1 hydroxylated isomers can also be found.[21,270]

1β-Hydroxylation has been demonstrated in vitro by human fetal microsomes,[273] and several C-1 hydroxylated bile acid isomers have been found in the urine of healthy adults[287] and infants,[286,289] in meconium,[2,20,258,268] and in biologic fluids from patients with liver disease.[288,290] A novel and prominent C-4 hydroxylation pathway was recently discovered and suggested to be unique to early human development.[21,271] 3α,4β,7α-Trihydroxy-5β-cholanoic acid was identified and found to account for 5 to 15% of the total biliary bile acids in early gestation.[271]

Newborn infants in the first few days of life excrete significant amounts of 3-oxo-Δ^4 bile acids, and this is indicative of an immaturity in bile acid synthesis.[286,291] The levels of these unsaturated bile acids typically decline rapidly in the first few months of life, but when high levels persist, a "primary" deficiency in the Δ^4-3-oxosteroid 5β-reductase enzyme should be suspected.[7] High levels of 3-oxo-Δ^4 bile acids are also associated with a severe loss of hepatic synthetic function, and differentiating the primary from the secondary deficiency in the Δ^4-3-oxosteroid 5β-reductase enzyme can be difficult.[8,121]

Secondary bile acids can be found in fetal bile but only in very small proportions. This is consistent with the lack of bacterial flora in the fetal gut and the maternal-fetal placental transport of secondary bile acids that has been demonstrated in vivo[277,278] and in vitro.[292]

The principal bile acid conjugation reaction of the fetal liver is amidation with taurine. In fetal bile, 85% of the total biliary bile acids are taurine conjugates,[21] which contrasts to the pattern for adult bile, for which the glycine-to-taurine ratio is approximately 3:1.20.[23] This reflects the increased accumulation and availability of taurine in the fetal liver[194] resulting from selective placental transport.[195]

Bile acid sulfates, which are generally increased in cholestatic conditions in adults,[264,287,293–295] are virtually absent in early gestation.[21] This probably reflects an immaturity in the bile acid sulfotransferase enzyme or may be a consequence of additional and preferential metabolism of bile acids by hydroxylation. Lithocholic acid sulfate and 3β-hydroxy-5-cholenoic acid sulfate are found in relatively large proportions in the meconium[258] and amniotic fluid[259,260,296] as a result of accumulation and sequestration during gestation.

Meconium also contains a series of short-chain monohydroxylated bile acids.[297–299] These compounds possess a steroid nucleus of 20-, 21-, and 22-carbon atoms and are predominantly found as glucuronide or sulfate conjugates.[214,299] In contrast to the monohydroxy-C_{24} bile acids, which are cholestatic, etianic acid (3α-hydroxy-5β-androstan-17β-carboxylic acid) produces a marked choleresis in the rat,[214] illustrating how relatively small changes to the structure of the steroid nucleus can cause marked differences in physiologic actions. The origin of short-chain bile acids is unknown, but their close similarity in structure to steroid hormones suggests that they may be metabolic end products of steroid hormones formed during pregnancy.

INBORN ERRORS IN BILE ACID SYNTHESIS

Disorders in bile acid synthesis and metabolism can be broadly classified as primary or secondary. Primary enzyme defects involve congenital deficiencies in enzymes responsible for catalyzing key reactions in the synthesis of cholic and chenodeoxycholic acids, and, to date, the following such defects have been described:

- Cholesterol 7α-hydroxylase (*CYP7A1*) deficiency leading to disturbances in lipid metabolism but not manifest as liver disease[300]
- 3β-Hydroxy-C_{27}-steroid dehydrogenase/isomerase (3β-hydroxy-C_{27}-steroid oxidoreductase) deficiency[6] involving the conversion of 7α-hydroxycholesterol into 7α-hydroxy-4-cholesten-3-one caused by mutations in the *HSD3B7* gene encoding this enzyme[97]
- Δ^4-3-Oxosteroid 5β-reductase deficiency[7] involving the cytosolic enzyme that catalyzes the reduction of the Δ^4-bond to give rise to a 5β-H and consequently the *cis*-configuration of the A/B rings of the bile acid nucleus
- Oxysterol 7α-hydroxylase deficiency[9] caused by a mutation in the gene encoding the enzyme catalyzing the 7α-hydroxylation of 27-hydroxycholesterol in the "acidic" pathway for bile acid synthesis
- CTX, a rare lipid storage disease[301] caused by mutations in the sterol 27-hydroxylase gene
- 2-Methylacyl-CoA racemase deficiency causing liver disease and fat-soluble vitamin malabsorption in early life[13] and late-onset sensory motor neuropathy in adults[155]
- Trihydroxycholestanoic acid CoA oxidase deficiency involving an initial step in the side-chain oxidation[10,12,302–305]
- An amidation defect involving a deficiency in the bile acid–CoA ligase, the rate-limiting enzyme for conjugation of cholic and chenodeoxycholic acids with glycine and taurine[11]

- Side-chain oxidation defect in the 25-hydroxylation pathway for bile acid resulting in an overproduction of bile alcohols[306]

Secondary metabolic defects that impact on primary bile acid synthesis include the following:

- The cerebrohepatorenal syndrome of Zellweger[307] and related disorders[308] involving enzymes responsible for β-oxidation of the side chain of cholestanoic acids, which result from abnormal peroxisomal assembly, structure, or function
- Mutations in the genes encoding organic anion transport proteins,[309,310] in particular progressive familial intrahepatic cholestasis (PFIC) type 1 or Byler disease, a familial and fatal progressive intrahepatic cholestatic syndrome
- RSH/Smith-Lemli-Opitz syndrome,[311] a disorder caused by a deficiency of Δ^7-desaturase that results in reduced cholesterol synthesis and therefore has a knock-on effect in the bile acid pathway by limiting the available supply of cholesterol

Hepatic synthesis of the primary bile acids cholic and chenodeoxycholic acids is critical to the development and maintenance of the enterohepatic circulation because of the pivotal role of bile acids in promoting the secretion of bile.[14] Progressive cholestatic liver disease is consequently a striking clinical manifestation of patients presenting with severely impaired primary bile acid synthesis, and this includes patients with both of the steroid nuclear defects and those patients with the more severe peroxisomopathies.[2,4,9,13,312] The recent identification of single-enzyme defects in side-chain oxidation and conjugation presenting as disorders of fat-soluble vitamin malabsorption or rickets[10,13] attests to the importance at the intestinal level of adequate concentrations of primary bile acids for lipid absorption.

The biochemical presentation of these bile acid synthetic defects includes a markedly reduced or complete lack of cholic and chenodeoxycholic acids in the serum, bile, and urine and greatly elevated concentrations of atypical bile acids and sterols that retain the characteristic structure of the substrates for the deficient enzyme. These signature metabolites are generally not detected by the routine or classic methods for bile acid measurement, and mass spectrometric techniques presently provide the most appropriate means of characterizing defects in bile acid synthesis. Screening procedures using liquid secondary ionization mass spectrometry (LSIMS) indicate that inborn errors in bile acid synthesis probably account for 2 to 5% of the cases of liver disease in infants, children, and adolescents, making this an important and specific category of metabolic liver disease.

PRIMARY ENZYME DEFECTS

Cerebrotendinous Xanthomatosis

CTX is a rare inherited lipid storage disease, first described by Van Bogaert and colleagues,[301] with an estimated prevalence of 1 in 70,000.[4] Characteristic features of the disease in adults include progressive neurologic dysfunction, dementia, ataxia, cataracts, and the presence of xanthomatous lesions in the brain and tendons; however, in infants, we have characterized this defect in a number of patients with neonatal cholestasis (K. D. R. Setchell, unpublished data, 2003). Biochemically, the disease can be distinguished from other conditions involving xanthomatous deposits by (1) significantly reduced primary bile acid synthesis; (2) elevations in biliary, urinary, and fecal excretion of bile alcohol glucuronides; (3) low plasma cholesterol concentration, with deposition of cholesterol and cholestanol in the tissues; and (4) marked elevations in cholestanol. Elegant studies by Salen and colleagues demonstrated the metabolic defect to be an impairment in oxidation of the cholesterol side chain,[313,314] and chenodeoxycholic acid synthesis is reduced to a greater extent than cholic acid synthesis.[315–317] Initially, it was thought to be due to a defect in sterol 24-hydroxylase,[313] but later studies indicated the primary defect to be a deficiency in the mitochondrial sterol 27-hydroxylase (Figure 55.4-5).[318] The following evidence supports this contention: (1) the mitochondrial fraction of the liver from a patient with CTX was shown to be completely devoid of sterol 27-hydroxylase activity[318]; (2) in liver homogenates, the amount of 5β-cholestane-3α,7α,12α-triol, the substrate for this enzyme, was 50-fold higher than normal[318]; (3) 27-hydroxycholesterol in the serum of patients with CTX is markedly reduced or undetectable[319]; (4) intravenous administration of radiolabeled precursors showed that only precursors with a C-27 hydroxy group were converted to cholic acid[318]; (5) the increased amounts of bile alcohol glucuronides synthesized in this defect are polyhydroxylated in the side chain and mainly at positions other than the C-27 carbon.

To explain the findings of greatly increased amounts of 5β-cholestane-3α,7α,12α,25-tetrol, Salen initially proposed a deficiency in microsomal 24(S)hydroxylation; this reaction normally yields 5β-cholestane-3α,7α,12α,24,25-pentol.[316] Studies using this radiolabeled cholestane-pentol showed that it was converted to cholic acid, indicating an alternative pathway to the classic C-27 hydroxylation pathway for cholic acid synthesis,[144,313] but the quantitative importance of this pathway in health has since been established to be relatively minor.[145,146] Furthermore, if the primary defect in CTX was a deficiency in 24(S)-hydroxylase, this would not explain the greatly reduced synthesis of chenodeoxycholic acid,[320] which, in humans, is synthesized in significant amounts via the C-27 hydroxylation pathway. A deficiency in sterol 27-hydroxylase, on the other hand, would lead to elevations in 5β-cholestane-3α,7α-diol and 7α-hydroxy-4-cholesten-3-one[321]; thus, these intermediates are available for 12α-hydroxylation and preferential conversion to cholic acid via the C-25 hydroxylation pathway.[322] Interestingly, microsomal 12α-hydroxylase activity has been shown to be threefold higher in patients with CTX.[317] Evidence reinforcing a sterol 27-hydroxylase deficiency as the primary enzyme defect in CTX was established following the cloning of the cDNA for

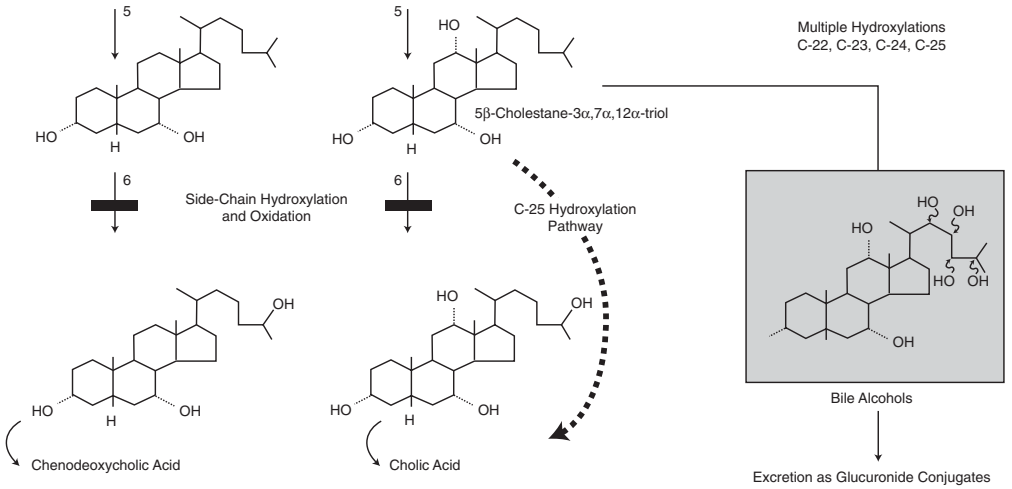

FIGURE 55.4-5 Biochemical defect in the sterol 27-hydroxylase deficiency of cerebrotendinous xanthomatosis.

this enzyme.[128,136,138,139] Using this probe, the mRNA was isolated from fibroblasts of two CTX patients, and the corresponding cDNA was synthesized by reverse transcription. Point mutations in the gene located on the long arm of chromosome 2 were identified and expressed in COS cells, and the resulting sterol 27-hydroxylase enzyme was found to be inactive.[139] These molecular studies clearly establish the primary defect in CTX to be due to a deficiency in the mitochondrial C-27 hydroxylase. In recent years, several types of mutations have been identified in CTX patients from different countries, and these include insertion, deletion, and point mutations.[323–344]

Impaired oxidation of the cholesterol side chain results in accelerated cholesterol synthesis and metabolism that leads to greatly increased production and excretion of bile alcohol glucuronides,[314,345–349] which can be readily detected in urine by fast atom bombardment ionization mass spectrometry (FAB-MS) (Figure 55.4-6).[2,350] These bile alcohols have the common 5β-cholestane-3α,7α,12α-triol nucleus, with additional hydroxyl groups in the side chain, mainly at the C-22, C-23, C-24, and C-25 positions (see Figure 55.4-5). The major bile alcohol excreted in bile and feces is the 5β-cholestane-3α,7α,12α,25-tetrol,[314,347,351,352] whereas the more polar 5β-cholestane-3α,7α,12α,23,25-pentol predominates in urine.[348,351,353] It has been suggested that the difference in these patterns could be due to more efficient renal excretion of the more polar pentol or a result of renal C-23 hydroxylation of 5β-cholestane-3α,7α,12α,25-tetrol.[351,352]

The elevation in 5α-cholestan-3β-ol (cholestanol) in the nervous system of CTX patients first observed by Menkes and colleagues[354] and the high plasma concentrations of this sterol[316] are unique features of the disease. An elevated plasma cholestanol-to-cholesterol ratio has been proposed to be diagnostic[355] but is not specific because elevations in this ratio also occur in liver disease. The origins of the increased cholestanol may be from elevations in the precursor sterol 4-cholesten-3-one; hepatic microsomes prepared from CTX patients have been shown to produce three times more 4-cholesten-3-one than similar preparations from healthy controls.[356] Using pulse-labeling techniques, Salen and colleagues showed that 4-cholesten-3-one would yield labeled cholestanol, whereas the corresponding 7α-hydroxyl intermediate was converted to bile acids.[356] An alternative pathway for the formation of cholestanol, not involving 7α-hydroxyl intermediates, was proposed implicating hepatic, rather than intestinal, 7α-dehydroxylation with the production of cholest-4,6-dien-3-one intermediate.[357,358] Evidence to support this pathway is the finding of increased levels of 7α-hydroxy-4-cholesten-3-one and cholest-4,6-diene-3-one in CTX and the observation that cholestyramine treatment, which stimulates cholesterol 7α-hydroxylase activity, increases cholestanol output, whereas the opposite response occurs during chenodeoxycholic acid feeding. The neurologic dysfunction observed in CTX appears to be a consequence of cholestanol deposition in the tissues, and because the sterol 27-hydroxylase is found in extrahepatic tissues, it is possible that some of the manifestations of the disease may be the result of nonhepatic perturbations in metabolism. Recent studies in a rat model indicate that cholestanol induces apoptosis of cerebellar neurons, and it was suggested that this could induce cerebellar ataxia in CTX patients.[359] Early diagnosis of this disorder, which is readily

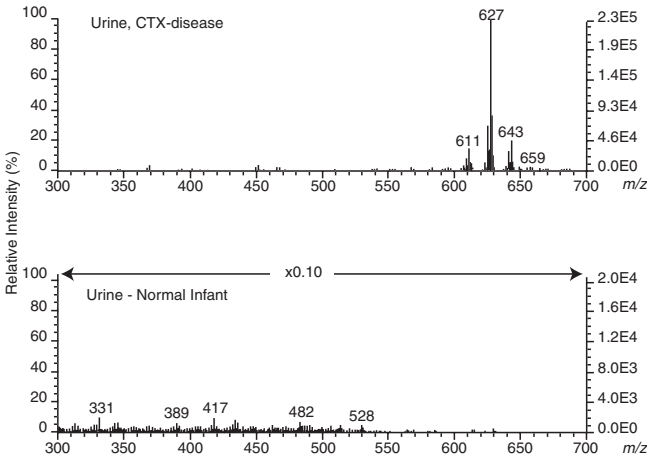

FIGURE 55.4-6 Negative-ion liquid secondary ionization–mass spectrometry mass spectra comparing the urine of a normal infant with that of a patient with cerebrotendinous xanthomatosis (CTX). The presence of increased levels of bile alcohol glucuronides is indicated by the specific ions at *m/z* 611, 627, 643, and 659.

achieved by mass spectrometry analysis of the urine, is crucial to prevent the progressive accumulation of cholestanol and cholesterol in tissues in the long term, but suspicion of a metabolic defect is not always realized because in the early years, the patients may be relatively asymptomatic. More recently, we have found a number of infants that had deficiencies in the sterol 27-hydroxylase owing to mutations in the gene encoding this enzyme but only because of a clinical presentation of elevated liver enzymes and bilirubin, which ultimately resolved by about 6 months of age presumably because the size of the cholic acid pool expanded with compensatory synthesis via the alternative 25-hydroxylation pathway. We suggest that this may be the typical early clinical presentation of CTX even though this has never been previously documented. The earliest age of diagnosis we have made was in a 1-day-old infant, which was made possible by the fact that this infant was born to a family that had a previous child with neonatal cholestasis that we had diagnosed with sterol 27-hydroxylase defect at 8 weeks of age.

3β-Hydroxy-C_{27}-Steroid Oxidoreductase Deficiency

This was the first metabolic defect to be described involving an early step in the bile acid biosynthetic pathway; the conversion of 7α-hydroxycholesterol is to 7α-hydroxy-4-cholesten-3-one, a reaction catalyzed by a 3β-hydroxy-C_{27}-steroid oxidoreductase. In response to a deficiency in this enzyme, 7α-hydroxycholesterol is metabolized by the remaining reactions, and the final products of hepatic synthesis are C_{24} bile acids that retain the 3β-hydroxy-Δ^5 structure characteristic of the enzyme substrates (Figure 55.4-7).[6] The index case was identified in a fifth child born to Saudi Arabian parents who were first cousins and was the third infant to be affected by progressive liver disease from birth; the previous infants had died within the first few years of life following similar clinical histories. Subsequently, a further infant with this defect was born to a first-cousin marriage in the kindred. The 3β-hydroxy-C_{27}-steroid oxidoreductase is the most common of all of the bile acid synthetic defects described thus far.

Although the clinical presentation of this disorder is somewhat heterogeneous, all patients generally present with progressive jaundice, elevated transaminases, and a conjugated hyperbilirubinemia.[6,58,59] Clinical features include hepatomegaly, with or without splenomegaly, fat-soluble vitamin malabsorption, and mild steatorrhea, and in most instances, pruritus is absent. The liver histology shows a generalized hepatitis, the presence of giant cells, and evidence of cholestasis.[6,58,360–362] Although the earliest cases were identified in infants, increasingly, idiopathic late-onset chronic cholestasis has been explained by this disorder.[58,59] In such patients, liver disease is not always evident in the early presentation, and many patients often have fat-soluble vitamin malabsorption and rickets, which are corrected with vitamin supplementation. Serum liver enzymes that are often normal in the early stages of the disease later show progressive increases. Serum bile acid concentrations when measured by conventional routine methods are normal or low and incompatible with the severity of liver dysfunction. However, urinary and serum bile acid concentrations are always elevated when determined by more specific techniques.

Of significance is the finding of a high association of this disease with a normal γ-glutamyl transpeptidase (Figure 55.4-8).[58,59,363] This is also a feature of patients with other conditions of familial progressive intrahepatic cholestasis or Byler disease,[364–367] but differential diagnosis of the two disorders can be readily made on the basis of the serum primary bile acid concentration, which, in the latter, is markedly elevated. Measurement of serum bile acids can be useful in establishing a diagnosis of inborn errors in bile acid synthesis and should be included in the workup of the patient with idiopathic cholestasis.

Definitive diagnosis of the 3β-hydroxy-C_{27}-steroid oxidoreductase deficiency presently requires mass spectrometric analysis of biologic fluids and is readily accomplished by LSIMS, formerly referred to as FAB-MS,[2,4,350] or by electrospray and tandem mass spectrometry.[368–371] LSIMS analysis of the urine permits the detection of the sulfate and glycosulfate conjugates of the 3β-hydroxy-Δ^5 bile acids that are the signature metabolites of this bile acid defect (Figure 55.4-9). Additionally, sulfate conjugates of tetrahydroxy-

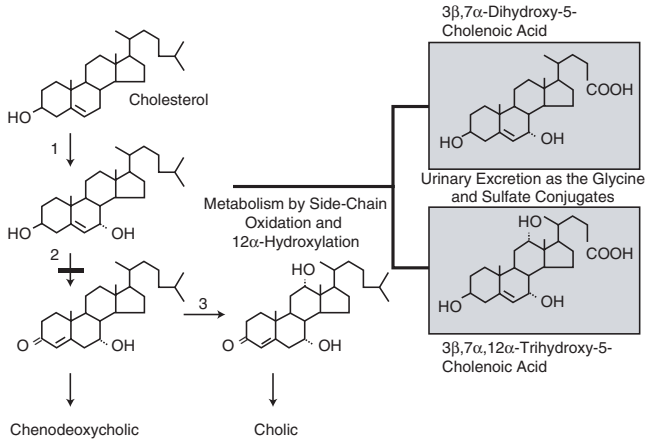

FIGURE 55.4-7 Biochemical defect in the 3β-hydroxy-C_{27}-steroid oxidoreductase deficiency.

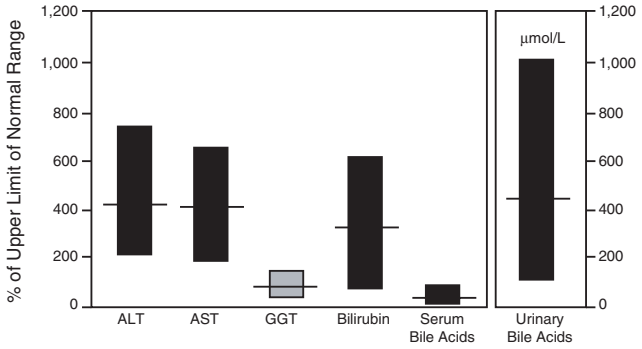

FIGURE 55.4-8 Summary of the mean and range of serum biochemistries in patients (n = 20) with the 3β-hydroxy-C_{27}-steroid oxidoreductase deficiency. ALT = alanine transaminase; AST = aspartate transaminase; GGT = γ-glutamyl transpeptidase.

and pentahydroxy-bile alcohols with a 3β,7α-dihydroxy-Δ^5 and 3β,7α,12α-trihydroxy-Δ^5 nucleus are also found in significant amounts in the serum and urine.[60]

Primary bile acids are not found in the urine but may be present in small amounts in the bile because of the action of a bacterial 3β-hydroxysteroid dehydrogenase/isomerase during the enterohepatic recycling of the atypical bile acids. This may explain the longer survival of these patients compared with patients with other defects in bile acid synthesis. Interestingly, 3β-hydroxysteroid dehydrogenase/isomerase isozymes are also involved in catalyzing analogous reactions in the pathways for steroid hormones, but steroid hormone synthesis and metabolism are unaffected in these patients. This is because the enzyme, which was recently purified, uses only intermediates that have a 3β-hydroxy-Δ^5 structure and is inactive on C_{19} and C_{21} steroids.[95]

Expression of the 3β-hydroxy-C_{27}-steroid oxidoreductase in fibroblasts affords a means of further establishing a deficiency in the activity of this enzyme. In contrast to healthy controls, patients with this defect have undetectable enzyme activity in fibroblasts, whereas the heterozygous genotypes have low or subnormal levels of activity.[102] However, this approach is somewhat redundant because molecular techniques that have led to the cloning of the *HSD3B7* gene encoding 3β-hydroxy-C_{27}-steroid oxidoreductase now permit the accurate genetic basis of the defect.[96] Using this approach to confirming the biochemical diagnosis of this enzyme deficiency in 15 patients from 13 kindreds, 12 different mutations were found to account for the disease.[97] The mechanism of cholestasis and liver injury is speculated to be the result of the failure to synthesize adequate amounts of primary bile acids that are essential to the promotion and secretion of bile and the increased production of unusual bile acids with hepatotoxic potential. The monohydroxy bile acid 3β-hydroxy-5-cholenoic acid has been shown to be markedly cholestatic in the rat and hamster,[229] and although 3β,7α-dihydroxy-5-cholenoic acid did not cause cholestasis in this latter species,[247] this may be explained by its metabolism to chenodeoxycholic acid. Recent studies using rat liver membrane vesicles have demonstrated that the taurine conjugate of 3β,7α-dihydroxy-5-cholenoic acid inhibits adenosine triphosphate–dependent bile acid transport at the canalicular plasma membrane and is not transported across this membrane.[15,372] These findings serve to explain the failure to find significant levels of bile acids in the bile of patients with the 3β-hydroxy-C_{27}-steroid dehydrogenase/isomerase defect and substantiate our initial theory that this is a cause of cholestasis.

Δ^4-3-Oxosteroid 5β-Reductase Deficiency

Application of LSIMS for urine analysis led to the discovery of a defect in the Δ^4-3-oxosteroid 5β-reductase, which catalyzes the conversion of the intermediates 7α-hydroxy-4-cholesten-3-one and 7α,12α-dihydroxy-4-cholesten-3-one to the corresponding 3-oxo-5β(H) intermediates (Figure 55.4-10).[7] The defect was initially identified in monochorionic male twins born with a marked cholestasis; a previous sibling with neonatal hepatitis had died of liver failure at 4 months of age. The clinical presentation of this defect is similar to that of patients with the 3β-hydroxy-C_{27}-steroid dehydrogenase/isomerase deficiency; however, in contrast, the γ-glutamyl transpeptidase is usually elevated, and the average age at diagnosis is lower in the Δ^4-3-oxosteroid 5β-reductase. The Δ^4-3-oxosteroid 5β-reductase has since been found in a number of patients presenting with neonatal hemochromatosis.[8] Liver function tests in these infants showed elevations in serum transaminase levels, marked conjugated hyperbilirubinemia, and coagulopathy. Liver biopsies[362,373] revealed marked lobular disarray as a result of giant cell and pseudoacinar transformation of hepatocytes, hepatocellular and canalicular bile stasis, and extramedullary hematopoiesis. On electron microscopy, bile canaliculi were small and sometimes slit-like in appearance and showed few or absent microvilli containing electron-dense material.[7]

Diagnosis of this defect is possible by LSIMS and gas chromatography–mass spectrometry (GC-MS) analysis of the urine. LSIMS spectra reveal elevated amounts of bile acids with molecular weights consistent with taurine con-

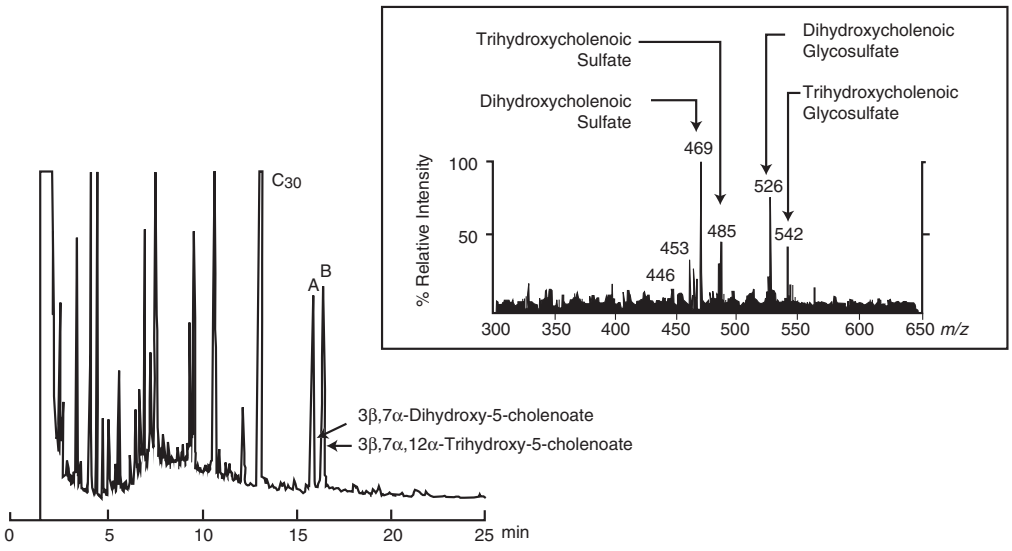

FIGURE 55.4-9 Negative-ion liquid secondary ionization–mass spectrometry mass spectrum and gas chromatography analysis of a typical urine from a patient with a 3β-hydroxy-C_{27}-steroid oxidoreductase deficiency.

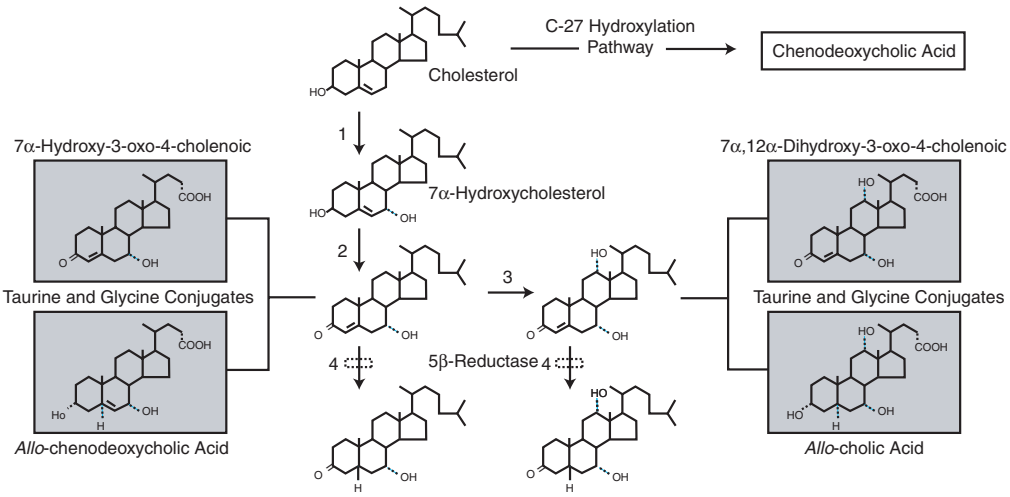

FIGURE 55.4-10 Biochemical defect in the Δ^4-3-oxosteroid 5β-reductase deficiency.

jugates of hydroxyoxocholenoic and dihydroxyoxocholenoic acids. GC-MS analysis following extraction, solvolysis, hydrolysis, and derivatization of bile acids[374] is essential to confirm the predominance of the major metabolites, 3-oxo-7α-hydroxy-4-cholenoic and 3-oxo-7α,12α-dihydroxy-4-cholenoic acids. Urinary bile acid excretion is generally elevated and consistent with a cholestatic condition. Quantitatively, the Δ^4-3-oxo bile acids comprise more than 75% of the total urinary bile acids. Gallbladder bile contains only traces (less than 2 μM) of bile acids, and because urinary excretion becomes the major route for bile acid loss, estimates of bile acid synthesis rates can be made from the daily urinary output and indicate markedly reduced total bile acid synthesis rates (less than 3 mg/d) compared with reported data for newborn infants[282] or adults.[375] In serum, relatively high concentrations of *allo*-chenodeoxycholic and *allo*-cholic acids are found, which lends support for an active hepatic Δ^4-3-oxosteroid 5α-reductase catalyzing the conversion of the Δ^4-3-oxo sterol intermediates to the corresponding 3α-hydroxy-5α(H) structures.

The Δ^4-3-oxosteroid 5β-reductase is exclusively of hepatic origin and, unlike the 3β-hydroxy-C_{27}-steroid dehydrogenase/isomerase, is not expressed in fibroblasts. Monoclonal antibodies raised against the rat cytosolic Δ^4-3-oxosteroid 5β-reductase have been used to demonstrate an absence of the 38 kD protein in a number of these patients and the formation of a truncated protein.[3] In one patient from Japan who met our previous biochemical criteria for a deficiency in this enzyme, sequence analysis of the gene revealed a single silent mutation in the coding region of the gene,[117] but the protein was normally expressed when analyzed by immunoblot of the liver homogenate using a monoclonal antibody.[376] Increased production of Δ^4-3-oxo bile acids occurs in patients with severe liver disease[121] and in infants during the first few weeks of life.[291] It is important to perform a repeat analysis of urine in the case of a suspected Δ^4-3-oxosteroid 5β-reductase deficiency because on rare occasions, a resolution of the liver disease occurs and the atypical bile acids disappear.[377] This is also the case with developmental immaturity. In general, however, it is our experience that markedly elevated levels of 3-oxo-Δ^4 bile acids are indicative of a poor clinical prognosis.

The liver injury in this defect is presumed to be the consequence of the diminished primary bile acid synthesis and the hepatotoxicity of the accumulated Δ^4-3-oxo bile acids. The lack of canalicular secretion can be explained by the relative insolubility of oxo-bile acids, and the cholestatic effects of the taurine conjugate of 7α-dihydroxy-3-oxo-4-cholenoic acid have been demonstrated in rat canalicular plasma membrane vesicles.[15] The unique morphologic findings in these patients[373] may indicate that maturation of the canalicular membrane and the transport system for bile acid secretion may require a threshold concentration of primary bile acids in early development.

Oxysterol 7α-Hydroxylase Deficiency

The recent discovery of a genetic defect in oxysterol 7α-hydroxylase[9] establishes the acidic pathway as a quantitatively important pathway for bile acid synthesis in early life. Unlike the mouse, in which this enzyme appears to be developmentally regulated,[37] or the rat, in which it is induced when there is suppression in cholesterol 7α-hydroxylase activity,[36] it would appear that in the human, the oxysterol 7α-hydroxylase may be more important than cholesterol 7α-hydroxylase for bile acid synthesis in early life. In common with the 3β-hydroxy-Δ^5 steroid-C_{27}-oxidoreductase deficiency[6] and the Δ^4-oxosteroid 5β-reductase deficiency,[7] this genetic defect presents as severe progressive cholestatic liver disease.

To date, this defect has been found in only one infant, a 10-week-old boy of parents who were first cousins, who presented with severe cholestasis, cirrhosis, and liver synthetic failure from early infancy. The patient became progressively jaundiced by 8 weeks of age and had markedly elevated serum transaminases and a normal serum γ-glutamyl transpeptidase. On examination, there was hepatosplenomegaly, and his liver biopsy revealed cholestasis, bridging fibrosis, extensive giant cell transformation, and proliferating bile ductules.[9] Oral UDCA therapy led to deterioration in liver function tests, and oral cholic acid was therapeutically ineffective. The patient subsequently underwent orthotopic liver transplant at $4^1/_2$

months of age, only to succumb to a disseminated Epstein-Barr virus–related lymphoproliferative disease.

Analysis of the urine by LSIMS revealed intense ions in the spectrum at mass-to-charge ratio (*m/z*) 453 and *m/z* 510, corresponding to sulfate and glycosulfate conjugates of 3β-hydroxy-5-cholenoic and 3β-hydroxy-5-cholestenoic acids (Figure 55.4-11). These accounted for 97% and 86% of the total serum and urinary bile acids, respectively, and primary bile acids were virtually undetectable. Monohydroxy bile acids with the 3β-hydroxy-Δ^5 structure have been previously shown to be extremely cholestatic.[229,230] Their hepatotoxicity in this patient is presumed to have been exacerbated by the lack of primary bile acids necessary for the maintenance of bile flow. Because the formation of 3β-hydroxy-5-cholenoic and 3β-hydroxy-5-cholestenoic acids occurs exclusively in the acidic pathway,[378] these findings and the observation that 27-hydroxycholesterol concentrations in serum and urine were more than 4,500 times normal support a defect in the oxysterol 7α-hydroxylase enzyme (Figure 55.4-12). Analysis of the neutral sterol fraction from serum and urine failed to demonstrate any 7α-hydroxysterols.

Molecular studies of the liver tissue established the cholesterol 7α-hydroxylase gene to be normal, but there was no measurable enzyme activity or mRNA; low or undetectable cholesterol 7α-hydroxylase activity has been previously reported for the human infant.[77] It is conceivable that gene expression may have been repressed by accumulation of the vast amounts of oxysterols. Oxysterol 7α-hydroxylase mRNA was also not present in this patient's liver tissue, and analysis of the oxysterol 7α-hydroxylase gene revealed a cytosine to thymidine transition mutation in exon 5 that converts an arginine codon at position 388 to a stop codon. The patient was homozygous for this nonsense mutation, whereas both parents were heterozygous.[9] When human embryonic 293 or Chinese hamster ovary cells were transfected with the cDNA with the R388* mutation, there was no detectable 7α-hydroxylase activity, and immunoblot analysis confirmed that the mutated gene encoded a truncated and inactive protein.

Unlike the other two nuclear defects in bile acid synthesis, the oxysterol 7α-hydroxylase deficiency is particularly severe and untreatable by primary bile acid therapy. It is possible that this cause of idiopathic liver disease may go unrecognized owing to its rapid downhill course in the early months of life. The characteristic metabolites formed in the genetic defect are some of the most cholestatic bile acids known, and, clearly, oxysterol 7α-hydroxylase is crucial for protecting the liver against the toxicity of monohydroxy bile acids produced in the acidic pathway. It is probable that our failure to find additional patients with this inborn error may be because of the devastatingly severe nature of this fatal liver disease, which does not respond to oral bile acid therapy, as in the other bile acid synthetic defects.[9]

2-Methylacyl-CoA Racemase Deficiency

2-Methylacyl-CoA racemase is a crucial enzyme that is uniquely responsible for the racemization of (25R)THCA-CoA to its (25S) enantiomer, while also performing the same reaction on the branched-chain fatty acid (2R)pristanoyl-CoA (Figure 55.4-13). Defects in this enzyme therefore have profound effects on both the bile acid and the fatty acid pathways. Mutations in the gene encoding 2-methylacyl-CoA racemase were first reported in three adults who presented with a sensory motor neuropathy[155] and later in a 10-week-old infant who exhibited severe fat-soluble vitamin deficiencies, hematochezia, and mild cholestatic liver disease in the first months of life.[13] This patient was initially and incorrectly reported in the previous edition of this book and elsewhere as having a possible THCA-CoA oxidase deficiency.[10] The infant had the same missense mutation (S52P) as that described in two of the adult patients yet was seemingly phenotypically quite different.[13] Two of the adult patients had neurologic symptoms but were asymptomatic until the fourth decade of life, whereas the other adult was described as having the typical features of Niemann-Pick type C disease at 18 months of age and presumably had some liver dysfunction. The clinical descriptions of these adult patients,[155] in particular the early history, were too scant to draw conclusions about the

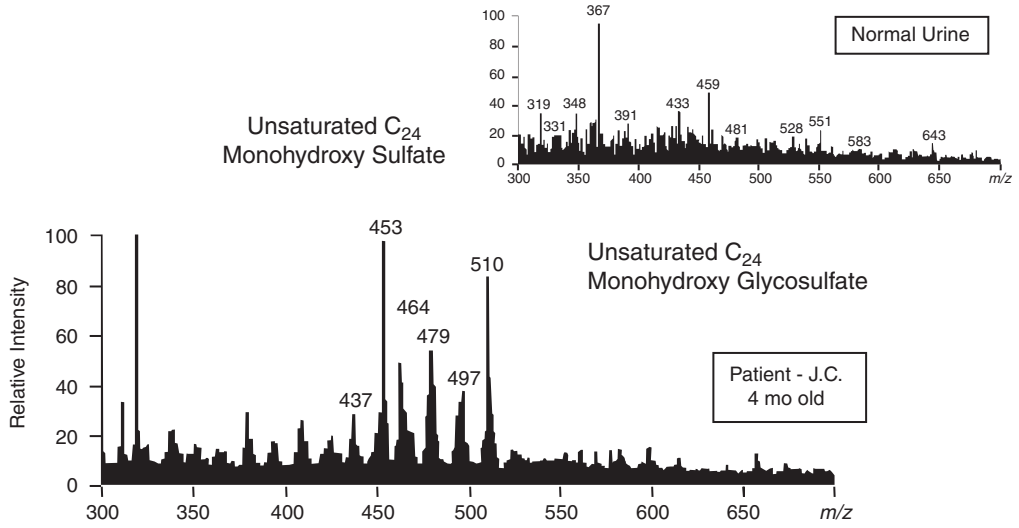

FIGURE 55.4-11 Negative-ion liquid secondary ionization–mass spectrometry mass spectra of the urine from a patient with the oxysterol 7α-hydroxylase deficiency and a normal age-matched infant. Ions characteristic of the signature metabolites of the defect are seen at *m/z* 453 and 510, corresponding to the sulfate and glycosulfate conjugates, respectively, of the monohydroxylated bile acid 3β-hydroxy-5-cholenoic acid.

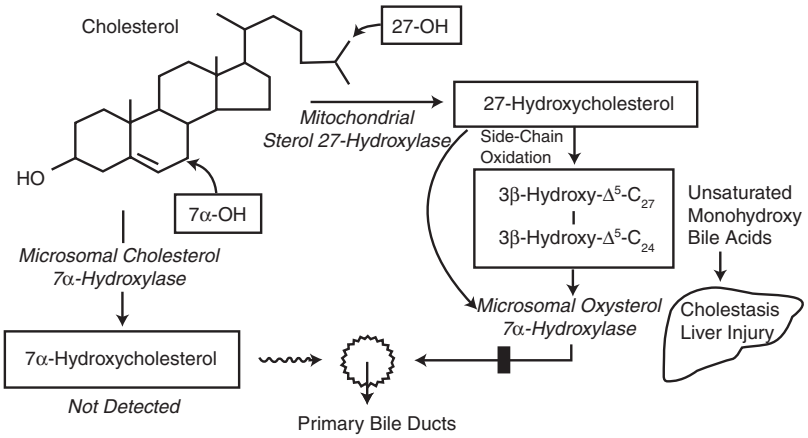

FIGURE 55.4-12 Biochemical basis for a defect in the oxysterol 7α-hydroxylase. Reproduced from Setchell KDR et al.[9]

phenotypic differences between the adult and the early presentation of the 2-methylacyl-CoA racemase. It is therefore possible that these adults could have had undocumented mild liver disease and fat-soluble vitamin absorption early in life and, if undiagnosed in infancy, would probably lead to a neuropathy owing to the tissue accumulation of phytanic and pristanic acids. Remarkable about the case of the first infant described with the 2-methylacyl-CoA racemase deficiency was the finding that the liver from a 5½-month-old sibling, who 2 years previously had died from an intracranial bleed, had been used for transplant in a child with end-stage liver disease.[13] Analysis of the urine from the recipient confirmed the same biosynthetic defect in the donor liver. Diagnosis of the defect in the infant was based on urinary, serum, and biliary bile acid analysis by FAB-MS, GC-MS, and electrospray ionization–tandem mass spectrometry, which revealed subnormal levels of primary bile acids and markedly increased concentrations of cholestanoic (C_{27}) acids, which are characteristically found as major bile acids of the alligator, other reptiles, and amphibians.[379,380] However, these cholestanoic acids were identified exclusively as the (25R)enantiomers.[13,381] The mass spectrum and GC profiles in this defect resemble closely those observed in peroxisomal disorders impacting bile acid synthesis, such as Zellweger syndrome; however, in other peroxisomopathies, both the (25S)THCA and (25R)THCA enantiomers are present, but the (25S)isomer predominates.[381] Differential diagnosis of the 2-methylacyl-CoA racemase deficiency from generalized peroxisomal disorders requires direct analysis of the urine or serum, omitting the use of destructive alkaline hydrolysis methods.[13,381] This is best achieved by high-performance liquid chromatography electrospray ionization–tandem mass spectrometry, which permits the separation and quantification of the two enantiomers of THCA,[382] which are otherwise difficult to measure by conventional techniques of bile acid analysis. Fibroblast studies can be used to further confirm a deficiency in peroxisomal 2-methylacyl-CoA racemase.[383] Incubation of cultured fibroblasts with the stereospecific (25R)THCA and (2R)pristanoic acid, the signature metabolites of this disorder, permits reduced activity of the enzyme to be established, although DNA sequencing would be the preferred approach to confirmation.

Primary bile acid therapy with cholic acid has proven effective in normalizing liver enzymes and preventing the onset of neurologic symptoms in the infant; additionally, dietary restriction of phytanic acid and pristanic acids is likely to be necessary in the long term for such patients to prevent neurologic symptoms owing to the brain accumulation of these fatty acids.

THCA-CoA Oxidase Deficiency

A number of patients have been reported to have side-chain oxidation defects involving the THCA-CoA oxidase.[12,302–305] The clinical presentation differs among these cases, and although all impact on primary bile acid synthesis, neurologic disease was the main clinical feature.[13] Whether these are primary bile acid defects or secondary to single-enzyme defects in peroxisomal β-oxidation is

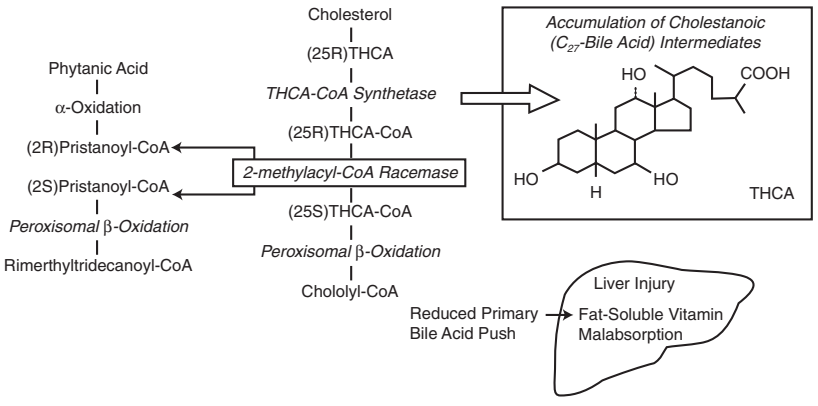

FIGURE 55.4-13 Biochemical defect in bile acid synthesis showing position of the enzyme deficiency in 2-methylacyl-CoA racemase and depicting its impact on the fatty acid and cholestanoic acid oxidation pathways. CoA = coenzyme A; THCA = 3α,7α,12α-trihydroxy-5β-cholestanoic acid.

unclear. Two distinct acyl-CoA oxidases have been identified in humans,[156] whereas the rat has three isozymes.[384] The human acyl-CoA oxidase active on bile acid C_{27} cholestanoic acid intermediates has been found to be the same enzyme that catalyzes the oxidation of 2-methyl branched-chain fatty acids.[156] The cDNA of the gene encoding this human enzyme has been cloned.[161] Of the case reports in the literature of the proposed THCA-CoA oxidase deficiency, interestingly, phytanic and pristanic acids, when measured, were elevated.[12,302–305] All had ataxia as a primary feature of the disease, with its onset occurring at about $3^1/_2$ years of age. None had evidence of liver disease. It is possible, with the exception of the patient described by Clayton and colleagues,[306] that these patients had a 2-methylacyl-CoA racemase deficiency, but the analysis of the cholestanoic acids was not sufficiently detailed to permit the diastereoisomers of THCA and 3α,7α-dihydroxy-5β-cholestanoic acid (DHCA) or pristanic acid to be measured, which would have helped in the differential diagnosis. In the case of the patient reported by Clayton and colleagues, we were able to obtain urine, serum, and bile for analysis by electrospray ionization–tandem mass spectrometry, and the major enantiomer of THCA in this patient was found to be the (25S) isomer, therefore excluding the 2-methylacyl-CoA racemase as an explanation for the clinical presentation.[306] It is evident that the phenotypic presentation of defects involving the peroxisomal apparatus can present with a wide diversity in symptoms that make it difficult to pinpoint the exact defect involved. In all suspected cases, analysis of peroxisomal enzymes, pristanic and phytanic acids, VLCFAs, and plasmalogens should be performed to complement detailed bile acid analysis.

Bile Acid CoA Ligase Deficiency and Defective Amidation

The final step in bile acid synthesis involves conjugation with the amino acids glycine and taurine.[23] Hepatic conjugation is extremely efficient, and negligible amounts of unconjugated bile acids typically appear in bile under normal and cholestatic conditions[385] and also when large doses of an unconjugated bile acid such as UDCA are administered.[386] Two enzymes catalyze the reactions leading to amidation of bile acids. In the first, a CoA thioester is formed by the rate-limiting bile acid–CoA ligase, after which glycine or taurine is coupled in a reaction catalyzed by a cytosolic bile acid–CoA:amino acid *N*-acyltransferase. A defect in bile acid amidation, presumed to involve the bile acid–CoA ligase, was described in three patients presenting with fat and fat-soluble vitamin malabsorption.[11]

The index case was a 14-year-old boy of Laotian descent who, in the first 3 months of life, presented with conjugated hyperbilirubinemia, elevated serum transaminases, and normal γ-glutamyl transpeptidase. This child also had a form of β-thalassemia. Two other patients, a 5-year-old Saudi Arabian boy and his 8-year-old sister, who were products of a consanguineous marriage, have since been diagnosed. In early life, the boy had undergone a Kasai procedure for a mistakenly diagnosed biliary atresia. The girl was asymptomatic at the time of diagnosis, and there was little clinical history available. Liver function tests were either normal or mildly elevated at the time of diagnosis. The primary manifestation of a bile acid conjugation defect is severe fat-soluble vitamin malabsorption with rickets, and in one patient, this had led to a fracture. All had subnormal levels of vitamin E, vitamin K, 25-hydroxyvitamin D, and 1,25-dihydroxyvitamin D.

The diagnosis was based on the LSIMS analysis of the urine and serum and bile, which revealed unique negative-ion spectra featuring a major peak of mass (*m/z* 407) corresponding to unconjugated cholic acid (Figure 55.4-14). In addition, ions characterizing sulfate and glucuronide conjugates of dihydroxy and trihydroxy bile acids were present. There was a complete lack of the usual glycine and taurine conjugated bile acids, and this was confirmed after chromatographic separation and GC-MS. Serum and urinary bile acids were markedly elevated and comprised predominantly cholic and deoxycholic acids. Attempts to identify bile acid–CoA esters yielded negative data, suggesting that the likely point of defective bile acid amidation was rate-limiting bile acid–CoA ligase.[174,177–179] All of these patients have been lost to follow-up, making it impossible to ascertain the molecular genetics of the defect despite the fact that the cDNAs for both conjugating enzymes have been cloned.[181,188]

The clinical presentation and biochemical features of defective amidation in these patients closely parallel the presentation and features hypothesized by Hofmann and Strandvik.[387] Whereas the previously described inborn errors in bile acid synthesis present as well-defined progressive familial cholestatic liver diseases,[6,7,9] in contrast, cholestasis does not occur in this amidation defect because the synthesis of unconjugated cholic acid provides sufficient stimulus for bile flow. The fat-soluble vitamin malab-

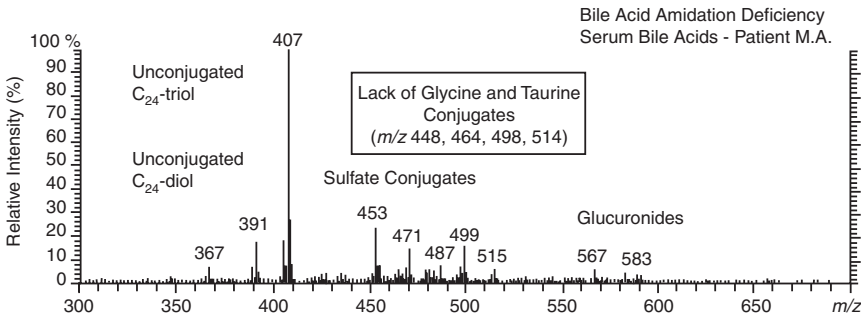

FIGURE 55.4-14 Negative-ion liquid secondary ionization–mass spectrometry mass spectrum of the serum from a patient with a defect in the bile acid-CoA ligase leading to deficient amidation of bile acids. The major ion at *m/z* 407 indicates the presence of unconjugated cholic acid.

sorption in these patients is postulated to be the result of reduced biliary secretion of bile acids and the inability to form mixed micelles because of rapid passive absorption of unconjugated cholic acid in the proximal small intestine.[388] Although these patients did conjugate bile acids with glucuronic and sulfuric acids, these conjugated bile acids are of little help in promoting lipid absorption.[389–391] Administration of primary conjugated bile acids should provide a therapeutic approach to correcting the fat-soluble vitamin malabsorption in this defect, and the recognition that genetic defects in bile acid synthesis are associated with fat-soluble vitamin malabsorption warrants a more concerted effort to explore this type of patient population.

Side-Chain Oxidation Defect in the Alternate 25-Hydroxylation Pathway

A speculative diagnosis of a defect in side-chain oxidation in the 25-hydroxylation pathway[144–146] was proposed by Clayton and colleagues for a 9-week-old infant presenting with familial giant cell hepatitis and severe intrahepatic cholestasis.[306] The rationale for the diagnosis was based on the finding of reduced cholic and chenodeoxycholic acids in the serum, concomitant with high concentrations of bile alcohol glucuronides, specifically 5β-cholestane-3α,7α,12α,24-tetrol, 5β-cholest-24-ene-3α,7α,12α,24-tetrol, and 5β-cholestane-3α,7α,12α,25-tetrol. These bile alcohols are not normally found in the plasma of infants with liver disease. Bile alcohol glucuronides were also identified as major metabolites in the urine.[306] Although the profile resembled that seen in CTX patients, it was concluded on the basis of the liver disease (not previously reported for CTX) that this represented a different side-chain defect and that it was possibly an oxidation defect downstream of the 25-hydroxylation step in this minor pathway for bile acid synthesis. The implications of the findings are that it could indicate that the 25-hydroxylation pathway, considered of negligible importance in adults,[146] may be an important pathway for infants. This is speculation, and further studies to prove the exact site of the defect are required before this is convincing. The patient was, however, treated with chenodeoxycholic acid and cholic acid, and this led to a normalization in serum transaminases and a suppression in the production of bile alcohols.

Cholesterol 7α-Hydroxylase Deficiency

Several patients have recently been identified with a homozygous mutation deletion in the *CYP7A1* gene, and when the cDNA of this mutant was expressed in vitro in cultured HEK 293 cells, cholesterol 7α-hydroxylase was found to be inactive.[300] Bile acid synthesis was reduced, and up-regulation of the alternative sterol 27-hydroxylase pathway presumably compensated for the reduced synthesis of bile acids via absent cholesterol 7α-hydroxylase activity. Three patients carrying this mutation were found to have abnormal lipids, but, in contrast to an infant identified with a mutation in oxysterol 7α-hydroxylase,[9] there was no evidence for abnormal liver function in these patients. Instead, the clinical phenotype was one of markedly elevated total and low-density lipoprotein (LDL) cholesterol and premature gallstones in two patients and premature coronary and peripheral vascular disease in one patient. The elevated serum cholesterol concentration was unresponsive to HMG-CoA reductase inhibitor therapy. Interestingly, individuals who were shown to be heterozygous for this mutation were found to have an above normal level of serum cholesterol. The phenotype of this deficiency in cholesterol 7α-hydroxylase differed significantly from that expressed in the *CYP7A1* knockout mouse model.[37] It is possible that *CYP7A1* deficiency predisposes the patient to cholesterol gallstone disease and seems to account for abnormal lipids but, surprisingly, not to liver disease. The important role of *CYP7A1* in the regulation of lipids is further supported by earlier studies showing that a number of polymorphisms in the *CYP7A1* gene were associated with abnormal LDL cholesterol concentrations in the general population.

SECONDARY BILE ACID DEFECTS

Disorders of Peroxisomal Function

Genetic defects involving peroxisomes include the cerebrohepatorenal syndrome of Zellweger[307] and related diseases. Excellent reviews describe the clinical and biochemical features of these disorders[308,392–395]; consequently, the following text focuses only on the impact of these diseases on bile acid synthesis (see Chapter 55.8, "Zellweger Syndrome and Other Disorders of Peroxisomal Metabolism").

The peroxisomopathies can be broadly subdivided into two main groups. Those syndromes in which there is a generalized impairment in numerous peroxisomal functions as a consequence of a markedly reduced or undetectable number of peroxisomes include Zellweger syndrome,[307,392,396] infantile Refsum disease,[397] neonatal adrenoleukodystrophy,[398] hyperpipecolic acidemia,[399] and rhizomelic chondrodysplasia punctata,[400] and these conditions share many similarities in their clinical presentation and neurologic manifestation. These include severe hypotonia, psychomotor retardation, hepatomegaly, simian crease, craniofacial dysmorphism, and failure to thrive. Genetic diseases involving a single-enzyme defect and a normal number of peroxisomes[401–403] include pseudo-Zellweger syndrome,[403] which shows many clinical and pathologic similarities to Zellweger syndrome.

Only those disorders with a generalized impairment in peroxisomal function have been found to have abnormal bile acid synthesis reflected by an accumulation of bile acid precursors. Although both the mitochondrial and microsomal fractions were originally shown to convert THCA into cholic acid, the peroxisomal fraction was later found to have the highest capacity for this reaction.[164] For this reason, elevated levels of trihydroxycoprostanoic (THCA) and dihydroxycoprostanoic (DHCA) acids are consistently found in the biologic fluids of patients with Zellweger syndrome, neonatal adrenoleukodystrophy, pseudo-Zellweger syndrome, and infantile Refsum disease (Figure 55.4-15). Interestingly, these long-chain C_{27} bile acids are not found in rhizomelic chondrodysplasia punctata,[400] and to our knowledge, there appear to have been no studies of bile acid metabolism in hyperpipecolic acidemia[399] and acatalasemia.[404] The presence of

other bile acid precursors is not uncommon, and it is possible that earlier descriptions of increased proportions of bile acid precursors in children with intrahepatic biliary atresia may have been due to the failure to recognize milder variants of Zellweger syndrome.[405,406] The in vivo and in vitro capacity of the liver to convert bile acid precursors into cholic and chenodeoxycholic acids by patients with Zellweger syndrome was studied by Kase and colleagues.[164,242,407] Tritiated 7α-hydroxy-4-cholesten-3-one was rapidly converted to DHCA and THCA but only slowly converted to cholic and chenodeoxycholic acids, with only 10% conversion after 48 hours, whereas cholic acid and chenodeoxycholic acid pool sizes and synthesis rates were markedly reduced. These data confirmed a defect in side-chain cleavage of the cholestanoic acid precursors and highlighted the important role of the peroxisome in bile acid synthesis.[408] Frequently, levels of DHCA are lower than THCA, which may be accounted for by its rapid transformation by 12α-hydroxylation to THCA.[409] Despite the reportedly low bile acid synthesis rate,[242] many studies have shown normal or increased serum levels of primary bile acids in patients with peroxisomal disorders. This may be a consequence of impaired hepatic uptake of bile acids because of generalized hepatic dysfunction.

In addition to DHCA, THCA, and varanic acid (the C-24 hydroxylated derivative of THCA), other atypical bile acids have been identified in Zellweger syndrome. C_{29} dicarboxylic acid is a major component of the serum (see Figure 55.4-15),[410,411] and although not always present, it can account for up to 40% of the total serum bile acids in Zellweger syndrome and infantile Refsum disease.[192,242,410,412–414] The biosynthetic pathway leading to the production of this unusual bile acid is uncertain. Administration of tritiated 5β-cholestane-3α,7α,12α-triol and THCA to a patient with Zellweger syndrome showed only a slow conversion to the C_{29} bile acid; however, its accumulation in serum may be accounted for by its relatively poor renal clearance and biliary excretion. Monohydroxy C_{27} bile acids also found in the serum of patients with Zellweger syndrome include 3α-hydroxy-5β-cholestanoic and 3β-hydroxy-5-cholestenoic acids.[410,415] In contrast to other cholestatic conditions, only low concentrations of 3β-hydroxy-5-cholenoic acid have been reported in the serum and urine of three patients with Zellweger syndrome.[410]

Perhaps not surprisingly, in view of the predominance of 1β- and 6α-hydroxylation pathways in early life,[21,270] the urine of these patients usually contains large proportions of 1β- and 6α-hydroxylated tetrahydroxycholestanoic acids that are mainly conjugated with taurine.[192] These more polar metabolites arise from the accumulated THCA and DHCA, and this metabolic pathway consequently facilitates the urinary excretion of these metabolites. These specific urinary metabolites are of diagnostic significance for the Zellweger and pseudo-Zellweger syndromes and can be recognized by LSIMS from the intense ion of *m/z* 572 corresponding in mass to the taurine conjugated tetrahydroxycholestanoic acids.[192]

Of the well-defined single-enzyme defects, X-linked adrenoleukodystrophy,[416–418] involving the VLCFA acyl-CoA synthetase, and pseudoneonatal adrenoleukodystrophy,[419] a defect in the VLCFA-CoA oxidase, both show normal bile acid synthesis and highlight the fact that there are separate isozymes for this common reaction sequence. Pseudo-Zellweger syndrome, a defect of the thiolase,[170,412] and a deficiency in the multifunctional protein[420] are conditions that present with abnormal bile acid synthesis.[421] A number of isolated examples of patients in whom there is impaired β-oxidation of bile acids have been reported, but the exact defects were not defined. With improvements in methodologies[422] and the advantage of molecular probes to identify the enzymes and sequence genes, it is expected that further defects will be delineated.

Defects in Canalicular Bile Acid Transport Proteins

In the last decade, tremendous strides have been made in the understanding of the mechanisms behind bile formation and the transport of organic anions across the hepatocyte.[423,424] Full details of these advances are in Chapter 55.6, "Billary Transport," and in an excellent summary by Trauner and colleagues.[425] It is outside the scope of this

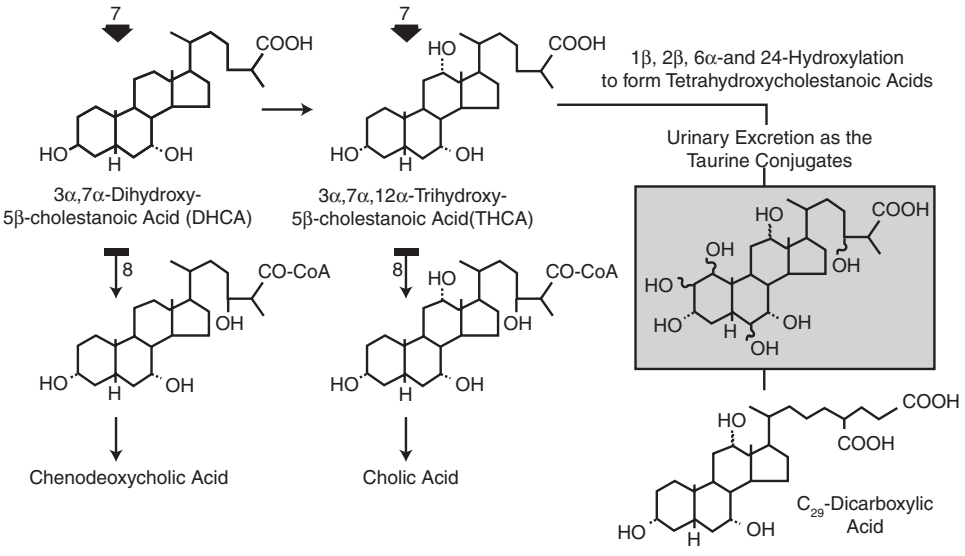

FIGURE 55.4-15 Biochemical defect in the cerebrohepatorenal syndrome of Zellweger.

review to detail the voluminous work on transport proteins and their role in cholestasis, and the reader is directed to an excellent summary by Trauner and colleagues.[425] Bile acids play a critical role in providing the major driving force for the promotion and secretion of bile, and defects in bile acid synthesis will inevitably cause cholestasis. In recent years, familial cholestasis has also been associated with mutations in the genes encoding the transport proteins for organic anions.[310,426,427] In the context of bile acid synthesis, patients with Byler disease (now classified as PFIC type 1) are of interest. This is an autosomal recessive familial progressive intrahepatic cholestasis[364,428] that has been shown to be due to a mutation in the *FIC1* gene encoding a P-type adenosine triphosphatase.[309] Some of the clinical features of PFIC are shared with bile acid synthetic defects, most notably the low γ-glutamyl transpeptidase.[59,367,429] However, they are easily differentiated because, unlike primary bile acid synthetic defects, patients with PFIC type 1 do synthesize primary bile acids, albeit in suboptimal levels. A striking feature of the bile from patients with PFIC type 1 is the negligible amount of chenodeoxycholic acid present, even though cholic acid is secreted, indicating impaired canalicular secretion of this dihydroxy bile acid.[430] In contrast, serum and urinary bile acids are composed of large proportions of chenodeoxycholic acid and its 6α-hydroxylated metabolite hyocholic acid.[367,429,431] 6α-Hydroxylation of bile acids is not, however, specific for PFIC type 1 because hyocholic acid can be found in biologic fluids from patients with other cholestatic diseases and is a characteristic of early development. Activation of this P-450 enzyme is presumed to occur in response to increases in intracellular chenodeoxycholic acid concentrations. Biochemical confirmation of this specific bile acid transport defect is by analysis of duodenal or biliary bile for the ratio and concentration of the primary bile acids.

RSH/Smith-Lemli-Optiz Syndrome

Since publication of the previous edition of this book, there has been an expansive literature on the RSH/Smith-Lemli-Optiz syndrome covering clinical features and variants, biochemical presentation, pre- and postnatal diagnosis, and the molecular genetics of this syndrome. It is beyond the scope of this review to detail all of these studies, and the reader is directed to the articles cited here and references therein. RSH/Smith-Lemli-Optiz syndrome is an autosomal recessive disease with an estimated frequency of 1 in 20,000 to 40,000.[4] The clinical characteristics of this syndrome are highly variable but include dysmorphism, microcephaly; poor growth; limb abnormalities; cardiac, renal, and endocrine abnormalities; cataracts; mental retardation; and early death.[311,432–434] It is considered the second-most common genetic defect in the North American white population. Biochemically, the condition is characterized by markedly reduced plasma cholesterol concentrations and elevated concentrations of 7-dehydrocholesterol and isodehydrocholesterol, and these Δ^7 sterols, which are normally not present, are the major neutral sterols of tissue, plasma, and feces.[435–437] The condition is accounted for by a defect in the 7-dehydrocholesterol Δ^7-reductase (7-DHC), an enzyme that catalyzes the final reaction in the formation of cholesterol.[438] 7-DHC has the highest activity in the adrenal glands and the liver.[439] The defect can be reproduced in an animal model using a drug (BM 15.766), a competitive inhibitor of 7-DHC.[440] The 7-DHC gene has been cloned for the human[439,441,442] and rat[443] enzymes and is localized on chromosomes 11q13 and 7F5, respectively. It encodes a 55 kD protein,[439] and a number of missense, nonsense, and splice-site mutations have been described.[432 ,444] Although this is a primary defect in cholesterol biosynthesis, it impacts on bile acid synthesis because the available supply of cholesterol is reduced, and Δ^7 sterols are poor substrates for 7α-hydroxylation; consequently, bile acid synthesis is markedly reduced.[436]

Diagnosis and Treatment of Inborn Errors in Bile Acid Synthesis

A battery of techniques is available for the measurement of bile acids in biologic fluids, and these have been compiled and extensively reviewed in the book series *The Bile Acids*[1] and elsewhere.[445–447] Technologic advances have meant that techniques such as paper and thin-layer chromatography have largely become superseded by extremely sensitive and specific assays. Immunoassays[448] are commonly used in routine laboratories because of their high sensitivity, precision, and suitability for handling large numbers of samples but presently lack the specificity for detecting specific inborn errors in bile acid metabolism. High-performance liquid chromatography is a useful tool[449,450] but, owing to its limited sensitivity, particularly with ultraviolet detection, is best suited to the analysis of the principal amidated species of biliary bile acids and has limited value in measuring the lower concentrations of bile acids in serum. Improvements in sensitivity have been achieved by pre- or postcolumn reactions,[447,449] coupling with thermospray,[450] or electrospray ionization–mass spectrometry.[451,452]

Accurate identification of inborn errors in metabolism requires techniques that afford detailed metabolic profiles, and, for the moment, GC-MS continues to be the principal confirmatory analytic tool.[446,453,454] Because of the high cost, technical difficulty, and time-consuming nature of bile acid analysis by GC-MS, the technique is outside the scope of most routine clinical laboratories. For this reason, the diagnosis of patients with inborn errors in bile acid synthesis has proven difficult and probably accounts for the low reported incidence of such metabolic defects.

Perhaps the most significant advances in mass spectrometry in recent years have been the introduction of FAB-MS and electrospray mass spectrometry, both of which are referred to by the generic term LSIMS. These techniques greatly simplified and extended the scope of mass spectrometry so that many nonvolatile compounds can be analyzed rapidly and directly in biologic samples or simple crude extracts, thereby circumventing the need for extensive and time-consuming sample pretreatments. Intact bile acid conjugates are ideally suited to LSIMS, and negative ionization mass spectra of steroid and bile acid conjugates can be generated from microliter volumes of urine and blood.[2,192,350,370,446,451–456]

In healthy individuals, urinary bile acid excretion is of negligible quantitative importance; consequently, the mass spectrum obtained is unremarkable, showing only background ions from the matrix and the presence of some steroid hormone metabolites. During cholestasis, urinary bile acid excretion increases and bile acid conjugates can be readily detected by the presence of single intense ions corresponding to the pseudomolecular ($[M\text{-}H]^-$) ions. With cholestasis, and in the absence of an inborn error in bile acid synthesis, the ions corresponding to the glyco- and tauroconjugates of the primary bile acids appear in the mass spectrum, and the intensity of the ions is proportional to the degree of cholestasis.[2] When bile acid synthesis is impaired, a unique mass spectrum is obtained, revealing ions corresponding in mass to the accumulated intermediates and/or metabolites with structural characteristics of the substrates proximal to the enzyme block. Positive identification of these bile acids generally requires GC-MS analysis after prior hydrolysis of the conjugates and preparation of volatile derivatives, and this is a time-consuming technique. Positional or stereoisomers of bile acid conjugates can be differentiated if reaction chemistry is carried out in a tandem mass spectrometer, and useful collision-dissociated spectra can be obtained.[369,457–460] The potential for rapid screening of bile acid defects has been realized with the electrospray ionization–mass spectrometry, and bile acid metabolites can be detected in dried blood spots obtained from newborns for the Guthrie test.[370] This approach allows fast throughput of samples for screening, but definitive diagnosis of suspected inborn errors in bile acid synthesis is still likely to be complemented with GC-MS and, for the moment, will be restricted to specialist laboratories. Now that many of the genes encoding the enzymes involved in bile acid synthesis have been cloned, the application of molecular techniques to sequence DNA from patients identified by mass spectrometry as having bile acid synthetic defects is an important complementary tool and should prove of value in prenatal diagnosis in these familial diseases.

Early diagnosis of inborn errors in bile acid synthesis is important because, untreated, these conditions are inevitably fatal. The reduced or total lack of synthesis of primary bile acids, coupled with the overproduction of large amounts of atypical bile acids and sterols that have intrinsic hepatotoxicity,[15,228,372] results in a clinical course leading to fibrosis, cirrhosis, and liver failure in most patients with the steroid nuclear defects.[6,7,9] The possibility of bile acid synthetic defects in older children, and even some adults with idiopathic forms of liver disease, should also be considered given that many cases of 3β-hydroxy-C_{27}-steroid oxidoreductase have been found in older children and teenagers presenting with late-onset chronic cholestasis.

We now appreciate that the inability to synthesize primary bile acids may clinically manifest as a fat-soluble vitamin malabsorption syndrome that can cause rickets in the absence of symptomatic liver disease. Again, treatment with primary bile acids is important in these conditions because it will facilitate the absorption of vitamins A, D, E, and K while also staving off cholestatic liver disease in the longer term. The rationale for using primary bile acids, rather than UDCA, which is commonly prescribed for cholestatic liver diseases, is threefold. Primary bile acids provide a stimulus for bile flow and will generate a choleresis. Primary bile acids, unlike UDCA, down-regulate cholesterol 7α-hydroxylase, the rate-limiting enzyme for bile acid synthesis, thereby limiting further production and accumulation of hepatotoxic atypical bile acids. Also, primary bile acids will facilitate the absorption of fats and fat-soluble vitamins by providing adequate intraluminal bile acid concentrations.

The earliest experience with feeding a primary bile acid was for the treatment of CTX,[56,57] even though this is not a condition that is manifest as liver disease. Long-term treatment with chenodeoxycholic acid (750 mg/d) normalized plasma cholestanol concentrations,[56,461] markedly reduced the urinary excretion of bile alcohols,[2,57,348] and improved the clinical condition.[461–463] Similar suppression of endogenous synthesis of cholestanol and bile alcohols occurs with cholic acid and deoxycholic acid administration.[57] UDCA, which is unable to down-regulate cholesterol 7α-hydroxylase, is ineffective in CTX.[57,461] The improvement in the biochemical and clinical status of CTX patients treated with chenodeoxycholic acid is the result of marked suppression in endogenous bile acid synthesis mediated by the negative feedback on hepatic cholesterol 7α-hydroxylase and HMG-CoA reductase, the latter enzyme being rate controlling for cholesterol synthesis. Treatment of these patients may be more effective if bile acid is combined with an HMG-CoA reductase inhibitor because this combination has a greater effect in lowering plasma cholestanol.[464,465]

Oral bile acid therapy was found to be an effective means of treating patients with the 3β-hydroxy-C_{27}-steroid oxidoreductase (Figure 55.4-16) and the Δ^4-3-oxosteroid 5β-reductase deficiencies.[58,60,466] The first patient diagnosed with the 3β-hydroxy-C_{27}-steroid oxidoreductase deficiency was treated with chenodeoxycholic acid (125–250 mg/d), with remarkable results.[60] Serum liver enzymes and bilirubin normalized, and there was an improvement in clinical symptoms. However, one concern with chenodeoxycholic acid therapy, particularly for patients with liver disease, is that it can cause increases in serum transaminases and symptoms of diarrhea, as was documented when it was used for gallstone dissolution. For patients with preexisting liver disease, chenodeoyxcholic acid alone is a less desirable option. We subsequently chose to treat patients with cholic and ursodeoxycholic acids in combination or with cholic acid alone. The therapeutic dose of bile acid is somewhat empiric and has been based on the ability to significantly suppress the continued production of the atypical bile acids that are monitored by LSIMS. In general, the dose administered is in the range of 10 to 15 mg/kg body weight/d. UDCA has proven helpful for some patients with the 3β-hydroxy-C_{27}-steroid oxidase deficiency, lowering serum transaminases and improving liver histology.[59] However, it does not suppress the synthesis of atypical 3β-hydroxy-Δ^5 bile acids, which, over the long term, is important given that these bile acids are cholestatic and interfere with canalicular bile acid transport.[15,372] When UDCA was

used in combination with cholic acid, it was our experience that the effectiveness of cholic acid in down-regulating endogenous bile acid synthesis was reduced, and this we believe is because UDCA during its enterohepatic recycling competitively inhibits the ileal uptake of cholic acid. Based on our experiences to date, we recommended that these patients be treated with cholic acid alone.

We have learned that it is almost impossible to completely shut down hepatic bile acid synthesis by feeding orally primary bile acids. Nevertheless, significant down-regulation occurs, sufficient to markedly reduce the signature atypical metabolites of the inborn error. Concomitant with this effect is an improvement in liver function tests, clinical symptoms, and liver histology.[373] In several patients, significant morphologic changes in the fine ultrastructure were noted. Electron micrographs of the canaliculi of patients with the Δ^4-3-oxosteroid 5β-reductase indicate significant abnormalities, including a loss of the usual microvillus structure and electron-dense material within and around the canaliculi. After bile acid therapy, electron microscopy showed a normalization in morphology with a disappearance of the electron-dense material,[373] suggesting that a threshold level of primary bile acids may be essential for normal morphologic development of canalicular structure.

The success of this therapeutic approach for patients with these two defects[58–60,466] is evident from the few treatment failures, and several patients have avoided the need for orthotopic liver transplant even though they were waitlisted for a donor liver. One notable failure was the treatment of the only patient found to have a mutation in the oxysterol 7α-hydroxylase gene.[9] Cholic acid therapy was unable to down-regulate the synthesis of the oxysterols and hepatotoxic 3β-hydroxy-Δ^5-monohydroxy bile acids, and this patient eventually underwent transplant.[9] Although cholic acid treatment should be considered potentially beneficial for patients with an oxysterol 7α-hydroxylase deficiency because it will provide a pool of primary bile acid, it is crucial to concomitantly inhibit the sterol 27-hydroxylase to protect the liver from the toxicity of the monohydroxy bile acids that are synthesized.

Oral bile acid therapy has been used to treat the liver disease associated with some of the peroxisomal disorders affecting side-chain oxidation of bile acids. Its success, however, has been hampered by the multiorgan involvement of these conditions, but it can be helpful in managing some of the symptoms of the disease. Peroxisomal proliferating drugs such as clofibrate[467,468] have proved to be of no therapeutic value. The progressive liver disease in peroxisomopathies is probably the result of the increased synthesis and accumulation of C_{27} bile acids combined with reduced primary bile acid synthesis. Infusion of tauro-THCA in rats, for example, has been shown to induce red cell hemolysis and to produce hepatic lesions showing mitochondrial disruptions similar to those found in patients with Zellweger syndrome.[469] Down-regulation in endogenous synthesis of C_{27} bile acids, as occurs with primary bile acid administration, was found to improve serum liver enzymes and bilirubin in one patient with Zellweger syndrome, even though the patient eventually succumbed with pulmonary failure.[470] Liver histology showed a reduction in the extent of bile duct proliferation and inflammation, and a significant improvement in neurologic symptoms occurred after initiating bile acid therapy with cholic acid.[470] It is probable that cholic acid may be of greater benefit to patients with single-enzyme defects involving bile acid synthesis by preventing liver disease in the longer term. One patient with the 2-methylacyl-CoA racemase deficiency has now been on therapy for 7 years, and this has served to correct the abnormally low serum fat-soluble vitamin levels.

Finally, what can be offered to patients with a bile acid conjugation (amidation) defect?[11] In these cases, they are able to make unconjugated bile acids, mostly cholic acid, yet they fail to absorb fat-soluble vitamins. Restoring the conjugated bile acid pool seems logical, and this is possible by administration of a conjugated bile acid such as taurocholate or glycocholate. Alternatively, cholylsarcosine may also be helpful because this has been shown to improve fat absorption in a patient with short-bowel syndrome.[471] The limited availability of these primary bile acids and their conjugated forms remains a major problem that needs to be addressed for the clinic.

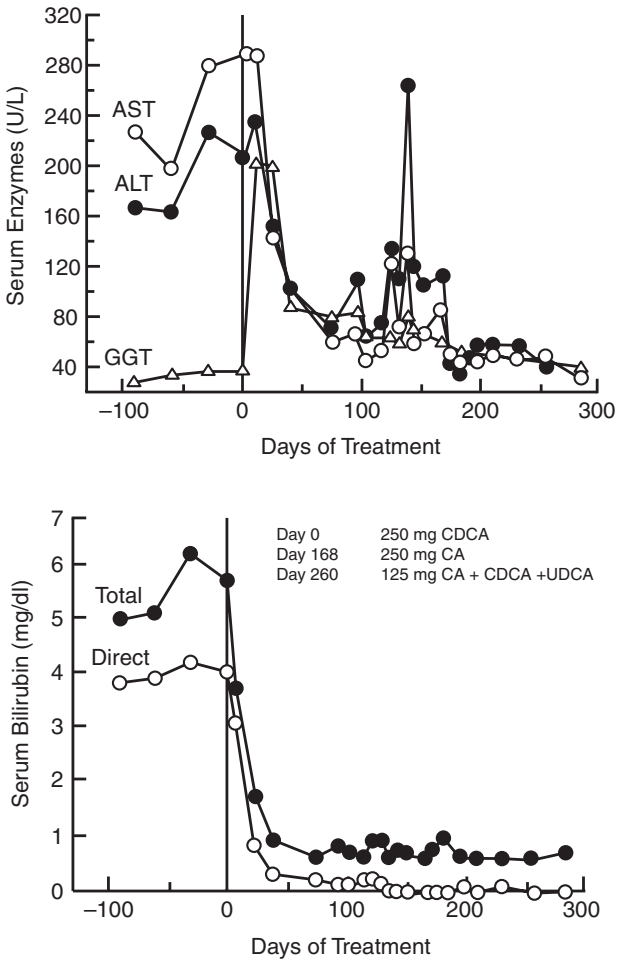

FIGURE 55.4-16 Effect of bile acid therapy on serum liver enzymes and bilirubin concentrations in a patient with a 3β-hydroxy-C_{27}-steroid oxidoreductase deficiency. ALT = alanine transaminase; AST = aspartate transaminase; GGT = γ-glutamyl transpeptidase.

SUMMARY

Inborn errors in bile acid synthesis represent a specific category of metabolic liver disease. These disorders have a significant effect on gastrointestinal physiology and function because of the key role that bile acids play in maintaining the enterohepatic circulation and in facilitating the absorption of fats and fat-soluble vitamins. At the Cincinnati Children's Hospital Medical Center, almost 4,000 cases of idiopathic liver disease have been screened in the last 15 years for bile acid synthetic defects, and more than 100 patients have been identified with defects, accounting for 2 to 3% of the cases of unexplained liver disease in infants and children. Early diagnosis is important because the liver disease and fat-soluble vitamin malabsorption associated with these inborn errors can be successfully treated medically, thereby avoiding the only alternative of orthotopic liver transplant in what are otherwise progressive and fatal conditions when undiagnosed or untreated.

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5. Bilirubin Metabolism

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Many diseases are associated with elevations of the serum bilirubin level. This chapter focuses on conditions in which hyperbilirubinemia results from a primary abnormality in the metabolism of bilirubin, whereas other liver disease is absent (Tables 55.5-1 and 55.5-2). These conditions must be distinguished from many other conditions in which hyperbilirubinemia is a secondary phenomenon caused by such things as primary liver disease, hematologic abnormalities, or infection. To understand and remember these primary defects in the metabolism of bilirubin, it is helpful to approach them from the viewpoint of the normal route by which bilirubin is metabolized and cleared from the circulation. Thus, this chapter begins by presenting the three hepatic steps necessary for clearance of bilirubin: (1) hepatocyte uptake of bilirubin from the sinusoid, (2) enzymatic conjugation of bilirubin within the endoplasmic reticulum of the hepatocyte, and (3) secretion of bilirubin conjugates out of the hepatocyte via the canalicular membrane. Defects in these metabolic steps result in the disorders described in this chapter. Recent reviews exist.[1–3]

BILIRUBIN MEASUREMENT

Serum bilirubin measurements are very common, being made in 61% of term newborns.[4] The two components of total serum bilirubin routinely measured in the clinical laboratory are conjugated bilirubin ("direct" reacting in van den Bergh test in which color develops directly without adding methanol) and unconjugated bilirubin ("indirect"). Together these components comprise the total bilirubin. Although the terms direct and conjugated bilirubin are often used as synonyms, this is not always quantitatively correct because the direct fraction includes both conjugated bilirubin and delta bilirubin.[5] Delta bilirubin results spontaneously when the conjugated bilirubin is elevated and a covalent bond is formed with albumin. Delta bilirubin is metabolized with albumin (half-life approximately 22 days), thus potentially prolonging jaundice when other liver function tests are improving. Serum bilirubin measurement has long been known to have significant interlaboratory variability,[6,7] and a variety of methods exist.[5,8,9] The Jendrassik-Grof procedure is very popular, although it is not without problems.[10] Two newer methods use high-performance liquid chromatography (HPLC)[11] and multilayered slides.[12] Analysis with HPLC is the acknowledged "gold standard" but too costly for a routine clinical laboratory.[5] Analysis with automated multilayered slide technology (Kodak Ektachem/Vitros, Johnson & Johnson Clinical Diagnostics, Rochester, NY) is currently used in some clinical laboratories and allows measurement of specific conjugated and unconjugated bilirubin fractions

TABLE 55.5-1 COMPARISON OF DISORDERS OF UNCONJUGATED HYPERBILIRUBINEMIA

	GILBERT SYNDROME	CRIGLER-NAJJAR TYPE 1	CRIGLER-NAJJAR TYPE 2
Prevalence	3%	Rare	Rare
Inheritance	Autosomal dominant or recessive	Autosomal recessive	Autosomal recessive, rarely dominant
Genetic defect	*UGT1A1* gene	*UGT1A1* gene	*UGT1A1* gene
Hepatocyte defect site	Microsomes ± plasma membrane	Microsomes	Microsomes
Deficient hepatocyte function	Glucuronidation ± uptake	Glucuronidation	Glucuronidation
BUGT activity	5–53% of controls	Severely decreased	2–23% of controls
Hepatocyte uptake	Decreased in 20–30%	Normal	Normal
Serum total bilirubin level (mg/dL)	0.8–4.3	15–45	8–25
Serum bilirubin decrease with phenobarbital (%)	70	0	77
HPLC serum bilirubin composition:			
Fraction (Normal %)			
Unconjugated (92.6)	98.8	~ 100	99.1
Diglucuronide (6.2)	1.1	0	0.6
Monoglucuronide (0.5)	0	0	0
Bile bilirubin conjugates:			
Fraction (Normal %)			
Diglucuronide (~ 80)	60	0 to trace	5–10
Monoglucuronide (~ 15)	30	Predominant if measurable	90–95
Other routine liver function tests	Normal	Normal	Normal
Prognosis	Benign	Kernicterus common	Occasional kernicterus

BUGT = bilirubin uridine diphosphate glucuronosyltransferase; HPLC = high-performance liquid chromatography.

TABLE 55.5-2 COMPARISON OF DISORDERS OF CONJUGATED HYPERBILIRUBINEMIA

	ROTOR SYNDROME	DUBIN-JOHNSON SYNDROME
Prevalence	Rare	Rare
Inheritance	Autosomal recessive	Autosomal recessive
Genetic defect	Unknown	MRP2 (cMOAT) gene
ABCC2		
Hepatocyte defect site	GST	Apical canalicular membrane
Deficient hepatocyte function	Intracellular binding of bilirubin and conjugates	Canalicular secretion of bilirubin conjugates
Brown-black liver	No	Yes
Serum total bilirubin level (mg/dL)	2–7	1.5–6.0
Serum conjugated bilirubin (%)	> 50	> 50
Other routine liver function tests	Normal	Normal
Oral cholecystogram	Usually visualizes	Usually does not visualize
^{99m}Tc- HIDA cholescintigraphy		
Liver	Poor to no visualization	Intense, prolonged visualization
Gallbladder	Poor to no visualization	Delayed or nonvisualization
Sulfobromophthalein clearance test	Serum sulfobromophthalein levels elevated (delayed clearance)	Serum sulfobromophthalein levels normal at 45 min but elevated at 90–120 min
Indocyanine green clearance test	Delayed clearance	Normal
Response to estrogens or pregnancy	No change	Increased jaundice
Total urinary coproporphyrin excretion (isomers I + III)	2.5–5 times increased	Normal
Urinary coproporphyrin isomer I composition (%) (normal = 25%)	Usually < 80% of total	> 80% of total
Prognosis	Benign (asymptomatic)	Benign (occasional abdominal complaints; probably incidental)

ABCC2 = ATP-binding cassette, subfamily C (CFTR/MRP), member 2 (cMOAT); cMOAT = canalicular multispecific organic anion transporter; GST = glutathione *S*-transferase; MRP2 = multidrug resistance–associated protein 2; ^{99m} Tc-HIDA = technetium 99m hepatobiliary iminodiacetic acid.

without inclusion of delta bilirubin. Noninvasive point of care methods for transcutaneous bilirubin measurement continue to develop. At present, two such methods are available in the United States: BiliCheck (Respironics, Pittsburgh, PA)[13] and Jaundice Meter (Minolta/Air Shields, Air-Shields Vickers, Hatboro, PA).[14]

BILIRUBIN CLEARANCE

Hepatocyte Uptake

The architecture of the liver is important for hepatic uptake of bilirubin. Cords of hepatocytes are arranged radially so that adjacent sinusoids border all hepatocytes and allow uptake of bilirubin by individual hepatocytes. Portal venous pressure rather than arterial pressure generates slow sinusoidal flow of blood. Albumin-bound bilirubin from the plasma passes into the tissue fluid space (space of Disse) between the endothelium and the hepatocyte. This is facilitated by the lack of basal laminae, which are found in other organ capillary systems.[15,16] The pores of the endothelium allow direct contact with the plasma membrane of the hepatocyte.

A schematic illustration of hepatic bilirubin metabolism is shown in Figure 55.5-1. Bilirubin dissociates from albumin[17] and can enter the hepatocyte via a membrane receptor-carrier. Carrier-facilitated transport into the hepatocyte has been demonstrated for organic anions such as bilirubin, sulfobromophthalein (Bromsulfphthalein), and indocyanine green.[18] Recently, bilirubin has also been shown to be able to pass through membranes by simple passive diffusion.[19] Bilirubin, sulfobromophthalein, and indocyanine green are believed to share the same hepatocyte receptor-carrier because they exhibit competitive inhibition when injected simultaneously. Subsequent intrahepatic metabolism of these anions is quite different and therefore cannot explain their competitive inhibition: bilirubin undergoes microsomal glucuronidation, sulfobromophthalein undergoes cytosolic conjugation with glutathione, and indocyanine green is excreted directly without biotransformation. Rat hepatocyte data suggest that the receptor-carrier is a dimeric protein with a subunit molecular weight of 55,000.[20–22] Additional supportive data regarding this receptor-carrier include antibody studies showing the expected plasma membrane location[21] and demonstrating blockage of uptake.[22]

Differences in protein binding inside and outside the hepatocyte necessitate carrier-mediated transport of bilirubin. Outside the hepatocyte, bilirubin is bound to albumin (affinity constant ~ 10^8; concentration 0.6 mM).[23] Inside the hepatocyte, bilirubin is bound to glutathione *S*-transferase B (GST; also known as ligandin or the Y protein; affinity constant ~ 10^6; concentration 0.04 mM).[24,25] GST consists of a family of proteins that can function both as enzymes and as intracellular binding proteins for nonsubstrate ligands such as bilirubin.[26] GST binds both bilirubin and bilirubin conjugates and decreases reflux from the hepatocyte back into plasma.[27]

Conjugation

Inside the hepatocyte, bilirubin enters the endoplasmic reticulum (microsomes), where it is conjugated with glucuronic acid.[28] Uridine diphosphate glucuronic acid (UDPGA) is the glucuronic acid donor. Conjugation disrupts the intracellular hydrogen bonding (Figure 55.5-2A) by forming an ester linkage with one or both of the propionic acid side chains on the

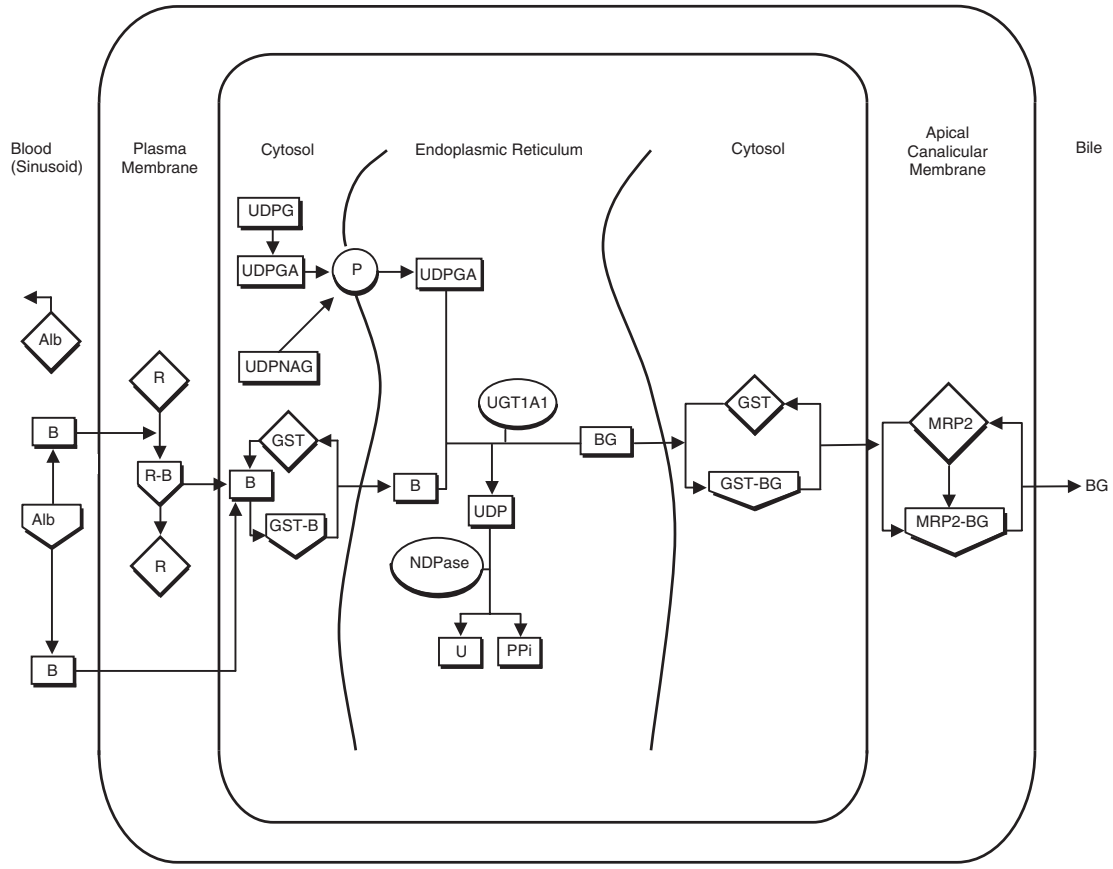

FIGURE 55.5-1 Schematic representation of hepatic bilirubin (B) metabolism. Alb = albumin; BG = bilirubin glucuronides (mono- and di-); GST = glutathione *S*-transferase (ligandin); MRP2 = multidrug resistance–associated protein 2; NDPase = nucleoside diphosphatase; P = permease; PPi = inorganic pyrophosphate; R = membrane carrier; U = uridine; UDPG = uridine diphosphate glucose; UDPGA = uridine diphosphate glucuronic acid; UDPNAG = uridine diphosphate *N*-acetylglucosamine; UGT1A1 = bilirubin uridine diphosphate glucuronosyltransferase 1A1, the isoform responsible for bilirubin conjugation.

B and C pyrrole rings of bilirubin (Figure 55.5-2B). Bilirubin uridine diphosphate glucuronosyltransferase (BUGT; Online Mendelian Inheritance in Man [OMIM] *191740; < http://www.ncbi.nlm.nih.gov/Omim/>) is the enzyme responsible for this esterification. Other glucuronosyltransferase isoforms catalyze the conjugation of thyroxine, steroids, bile acids, and xenobiotics.[29–33] The lipid environment of BUGT is important in determining enzyme activity. In vitro assays of BUGT can use different methods to perturb this lipid environment and thus greatly affect measured BUGT activity. A permease has been hypothesized to facilitate the transport of UDPGA from the cytosol across the lipid layers to the interior of the endoplasmic reticulum, where BUGT is located. The existence of a permease has been proposed because UDPGA is the preferred donor for bilirubin conjugation, despite the observation that uridine diphosphate glucose (UDPG) is present in the cytosol at higher concentrations.[34] Uridine diphosphate *N*-acetylglucosamine can increase in vitro BUGT activity threefold and is therefore considered to be a physiologic regulator of BUGT.[35] It is speculated that the mechanism for this involves facilitation of the permease UDPGA transporter.[36] Following conjugation, UDP can be converted to uridine and inorganic pyrophosphate by a nucleoside diphosphatase in the interior of the endoplasmic reticulum,[37] thus preventing the reverse reaction.

The specific isoform responsible for bilirubin conjugation is UGT1A1 (trivial name HUG-Br1, EC 2.4.1.17).[38] This is part of the UDP glycosyltransferase superfamily of enzymes encoded by the *UGT1* gene complex on chromosome 2 (Figure 55.5-3).[39] The *UGT1* gene encodes several isoforms and has a complex structure consisting of four common exons (2–5) and 13 variable exons encoding different isoforms.[40] During transcription, messenger ribonucleic acid from each variable exon 1 is spliced to exon 2 and the intervening ribonucleic acid is excised. The variable exons impart substrate specificity, whereas the common exons determine the UDPGA binding site and the membrane spanning region of the enzyme. More than 30 different *UGT1* mutant alleles have been described that cause Gilbert syndrome (GS) and Crigler-Najjar syndrome types I and II.[41] UGT1A1 catalyzes the formation of both bilirubin mono- and diglucuronides.[42–46] In normal human adults, bilirubin conjugates are excreted in the bile mainly as bilirubin diglucuronides (~ 80%) (Figure 55.5-4) with lesser amounts of bilirubin monoglucuronides (~ 15%) and very small amounts of unconjugated bilirubin and other bilirubin conjugates (eg, glucose, xylose, and mixed diesters).[47–52]

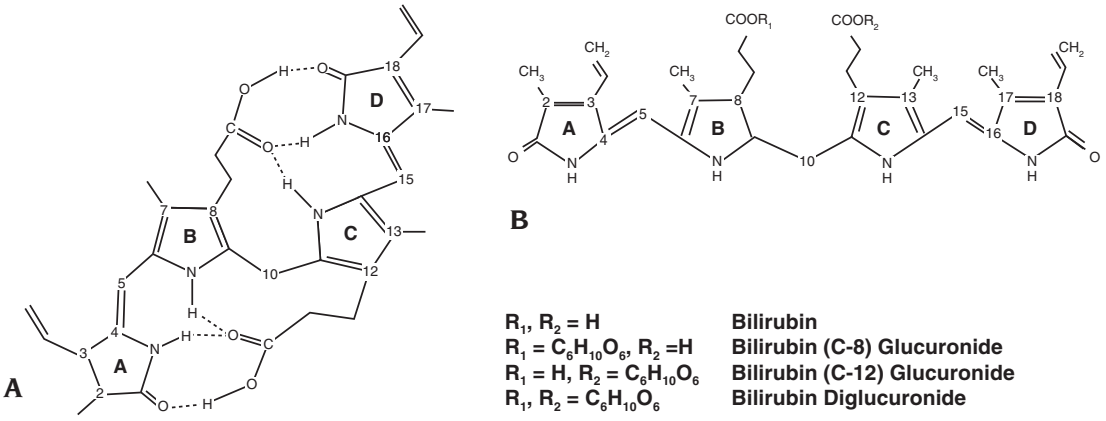

FIGURE 55.5-2 4Z,15Z-bilirubin IX-α depicted with standard carbon atom numbering demonstrating (*A*) normal internal hydrogen bonding and (*B*) conjugation sites. Substitutions (R_1 and R_2) indicate mono- and diglucuronides, which interrupt the internal hydrogen bonding, thus increasing the solubility of the bilirubin conjugates.

Secretion of Bilirubin Conjugates

Following conjugation, bilirubin conjugates are excreted against a concentration gradient from the hepatocyte through the canalicular membrane into the bile. Data from purified rat liver canalicular membrane vesicles indicate that bilirubin diglucuronide transport through the canalicular membrane is carrier mediated, electrogenic, and stimulated by bicarbonate.[53] Similar data suggest that bilirubin glucuronides are transported across the canalicular membrane by both adenosine triphosphate (ATP)-dependent and membrane potential–dependent transport systems, and in the normal rat, these systems are additive.[54] The ATP-dependent transporter responsible for bilirubin glucuronide passage from the hepatocyte through the canalicular membrane is canalicular multispecific organic anion transporter (cMOAT). cMOAT is a member of the ATP-binding cassette (ABC) transporter superfamily and is homologous to the multidrug resistance–associated protein 2 (MRP2).[55,56] cMOAT/MRP2 is involved with ATP-dependent transport across the apical canalicular membrane of a variety of endogenous compounds and xenobiotics,[57] including both bilirubin mono- and diglucuronide.[58] cMOAT/MRP2 has previously been described as the non–bile acid organic anion transporter, the glutathione *S*-conjugate export pump, or the leukotriene export pump.[59] Genetic mutations that alter these ABC transporters cause diseases, which include cystic fibrosis, hyperinsulinemia, adrenoleukodystrophy, multidrug resistance,[60] and, as is discussed later in this chapter, Dubin-Johnson syndrome (DJS). This mechanism can be saturated with increasing amounts of bilirubin or bilirubin conjugates.[61–63] Many other organic anions (eg, sulfobromophthalein, indocyanine green) are believed to share this same canalicular membrane excretion mechanism.[64] Simultaneous infusions of sulfobromophthalein and indocyanine green will decrease the maximal canalicular excretion of bilirubin and vice versa.[65,66] Bile salts do not use the same canalicular excretion mechanisms. In DJS, biliary excretion of conjugated bilirubin and sulfobromophthalein is decreased, although bile salt excretion is not impaired.[67] However, infusion of bile salts does increase the maximal excretion of bilirubin conjugates so that bile salt and bilirubin conjugate excretion by the canalicular membrane are not completely independent.[68] A similar effect is also seen with phenobarbital.[69] Conversely, the maximal excretion of bilirubin conjugates can be decreased by cholestatic agents such as estrogens and anabolic steroids.[70,71]

GILBERT SYNDROME

Clinical Presentation

GS (OMIM #143500) was first described in 1901 by Gilbert and Lereboullet.[72] GS is a hereditary, chronic or

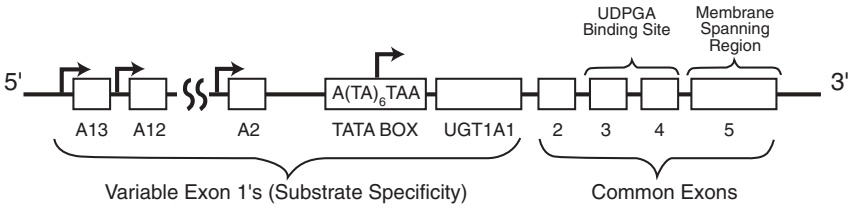

FIGURE 55.5-3 The human uridine diphosphate glucuronosyltransferase-1 (*UGT1*) gene. During transcription messenger ribonucleic acid from each variable, exon 1 (A1–A13) is spliced to exon 2, and the intervening ribonucleic acid is excised, resulting in a variety of isoforms. The variable exons impart substrate specificity, whereas the common exons determine the uridine diphosphate glucuronic acid (UDPGA) binding site and the membrane spanning region of the enzyme. The *UGT1A1* gene that results from this splicing encodes bilirubin glucuronosyltransferase.

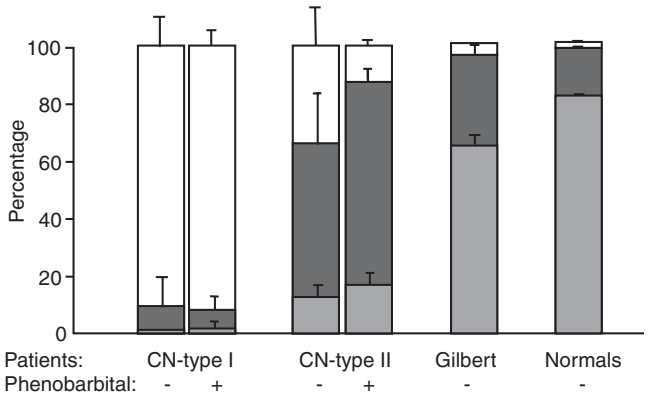

FIGURE 55.5-4 Bile pigment composition in bile from normal (n = 8), Crigler-Najjar (CN) syndrome type I (n = 3), CN type II (n = 3), and Gilbert syndrome (n = 16) patients, receiving (+) or not receiving (–) phenobarbital. Relative bile pigment composition is indicated in the vertical columns as a percentage of the total ± SD. Shading: white = unconjugated bilirubin; intermediate = bilirubin monoconjugate; dark = bilirubin diconjugate. Reproduced with permission from Sinaasappel M and Jansen PL.[49]

recurrent, mild unconjugated hyperbilirubinemia with otherwise normal liver function tests.[2,73,74] The serum unconjugated bilirubin level usually ranges from 1 to 4 mg/dL (17 µmol/L = 1 mg/dL). Often patients are first identified by an elevated serum bilirubin on routine blood chemistry or mild jaundice (perhaps only scleral icterus) during a period of fasting associated with viral illness or religious activities[75] or anorexia nervosa.[76] Icteric plasma from a blood donor may suggest GS.[77] Alternatively, hyperbilirubinemia post-transplant of an apparently healthy donor liver may be a sign that the donor had GS.[78–81] GS is generally associated with no negative implications for health or longevity, despite the large variety of symptoms reported by GS patients.[82,83] These symptoms include vertigo, headache, fatigue, abdominal pain, nausea, diarrhea, constipation, and loss of appetite. The possible relationship of these symptoms to GS has been evaluated in a group of 2,395 Swedish subjects.[84] The only symptom that was more common in the GS group was diarrhea in male subjects aged 57 to 67 years. The authors suggested that this was most likely type 1 error because of the large number of comparisons made and concluded that there was no higher prevalence of symptoms associated with GS. There are limited reports suggesting that GS is a risk factor for chronic fatigue syndrome.[85,86]

Large surveys of normal individuals found approximately 3% of the population with serum bilirubin levels greater than 1.0 mg/dL.[87,88] Although one survey noted a bimodal distribution of serum bilirubin concentrations with antimodes of 1.4 mg/dL in male patients and 0.7 mg/dL in female patients,[87] other studies have not observed this. If these levels were used to define the upper limit of normal for serum bilirubin concentration, the incidence of GS would be approximately 6% of the population, and the sex distribution would be close to 1:1. If 1.4 mg/dL is used as the upper limit of serum bilirubin concentration for both sexes, then there is a strong male predominance (approximately 4:1). This finding might be related to the observation that female subjects clear bilirubin better than male subjects.[89] GS may be inherited in either an autosomal dominant[90–93] or recessive[94] fashion.

GS is rarely diagnosed before puberty, although it is a congenital disorder. Hormonal changes of puberty have been suggested as one explanation. Steroid hormones can suppress hepatic bilirubin clearance.[71] Increased estrogen levels of pregnancy are associated with impaired clearance of exogenous bilirubin.[95] Gonadectomy has been shown to alter BUGT activity.[96] Odell speculated that some infants with nonhemolytic neonatal jaundice are manifesting GS.[97] Use of genetic markers (see below) has allowed investigation of the role that GS plays in neonatal jaundice. Individuals carrying such markers have been shown to have a more rapid rise in their jaundice levels during the first 2 days of life,[98] a predisposition to prolonged or severe neonatal hyperbilirubinemia,[99–101] and variably increased jaundice when the GS polymorphism is coinherited with hematologic abnormalities such as glucose-6-phosphatase dehydrogenase (G6PD) deficiency,[102,103] β-thalassemia,[102,104,105] hereditary spherocytosis,[106] ABO incompatibility,[107] and sickle cell disease.[108] Kaplan and colleagues have shown that infants with G6PD deficiency do not have an increased incidence of hyperbilirubinemia (compared with G6PD normals) unless they also carry the UGT1A1 promoter polymorphism[103]; homozygotes for this polymorphism have a significantly higher incidence of hyperbilirubinemia than do heterozygotes (Figure 55.5-5). Thus, numerous studies indicate that GS, as detected by *UGT1A1* analysis, is one of the many factors related to neonatal jaundice. GS may also be a factor related to the jaundice associated with pyloric stenosis.[109] GS is associated with an increased incidence and/or earlier diagnosis of gallstones when coinherited with hematologic abnormalities such as congenital dyserythropoietic anemia,[110] thalassemia,[111] or hereditary spherocytosis.[112]

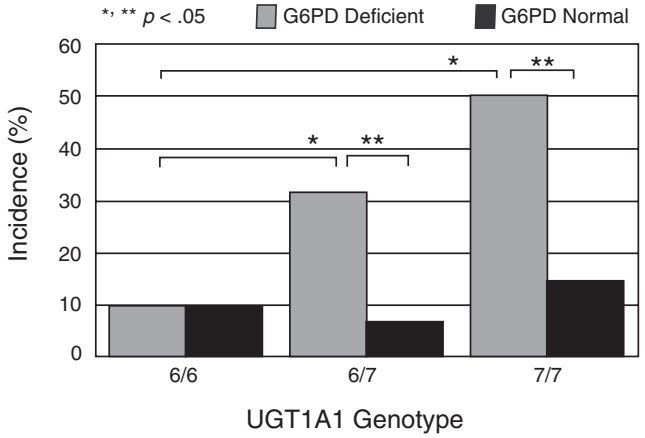

FIGURE 55.5-5 Incidence (percentage) of hyperbilirubinemia (serum total bilirubin ≥ 257 µmol/L) in glucose-6-phosphatase dehydrogenase (G6PD)-deficient neonates and normal controls, stratified for the three genotypes of the bilirubin uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1) promoter. Reproduced with permission from Kaplan M et al.[103]

Pathophysiology

GS is a heterogeneous group of disorders sharing a significant (≥ 50%) decrease in hepatic BUGT activity.[113–116] There are at least four subtypes of GS based on the plasma clearance of sulfobromophthalein and indocyanine green, which share the same hepatocyte uptake receptor-carrier.[117,118] In GS type I, clearance of sulfobromophthalein and indocyanine green is normal. In GS type II, sulfobromophthalein clearance is delayed, but indocyanine green clearance is normal. Because sulfobromophthalein uptake is normal in type II, delayed clearance must be related to subsequent intrahepatic metabolism or canalicular excretion.[119] In GS type III, clearance of both sulfobromophthalein and indocyanine green is delayed. The delay in the initial rate of disappearance from the plasma suggests a defect in uptake at the hepatocyte plasma membrane.[119] In GS type IV, indocyanine green uptake is delayed, but sulfobromophthalein uptake is normal. Thus, GS may include delayed uptake of bilirubin into the hepatocyte, delayed biotransformation, or both abnormalities.[52,120–122] Immunohistochemical staining for BUGT shows a clear reduction throughout the hepatic lobule in specimens from individuals with GS when compared with normals.[123]

The elucidation of the structure of the *UGT1* gene that encodes human bilirubin, phenol, and other UDP-glucuronosyltransferase isozymes[39] led to the discovery of *UGT1A1* mutations or polymorphisms associated with GS.[124] In white populations, the homozygous finding of an additional TA repeat in the promoter region or so-called TATA box (ie, $[TA]_7TAA$, rather than $[TA]_6TAA$) of the *UGT1A1* gene has been shown to be a necessary, although not sufficient, condition for GS.[125–127] This homozygous polymorphism occurs in 10 to 13% of whites.[125,128] Approximately 42% are heterozygous for the $(TA)_7$, and these individuals have significantly higher serum bilirubin levels than individuals with the wild-type six repeats ($[TA]_6$).[125] Hepatic UDP glucuronosyltransferase activity appears to be inversely related to the number of TA repeats in whites.[129] In Asian populations, the $(TA)_7TAA$ mutation is relatively rare,[130] but several different *UGT1A1* mutations have been associated with GS.[93,131,132] These Asian mutations involve exons of the *UGT1A1* gene rather than the previously described promoter region. One of the most common mutations in Asians, a Gly71Arg mutation in exon 1, has also linked GS and severe or prolonged neonatal hyperbilirubinemia.[100,101,133] It has recently been reported that although within whites, the promoter TA repeat number and bilirubin level are strongly positively correlated, in other ethnic groups (eg, Africans, where two other variants $[TA]_5$ and $[TA]_8$ have been identified), there is a negative correlation.[134] Thus, the ethnic implications of these genetic polymorphisms of the *UGT1A1* gene require further analysis.

Despite the universal BUGT decrease in GS, the correlation is poor between measured hepatic enzyme activity and serum bilirubin concentration.[135] This may be due to the increased bilirubin production associated with the decreased red cell half-life seen in up to 40% of patients with GS.[92,122] Although some conditions with increased bilirubin production (eg, sickle cell disease) result in BUGT induction, this is not the case in GS.[136] Even phenobarbital produces little BUGT induction in GS.[114] Hepatic BUGT activity in GS significantly overlaps with that seen in Crigler-Najjar type II (CNII),[116] although activity in GS is usually higher. If an individual has one allele producing nonfunctional UGT1A1 (Crigler-Najjar) and the other structurally normal allele possesses the GS promoter polymorphism, the resulting hyperbilirubinemia would be expected to be more severe than that found in GS but less severe than that found in Crigler-Najjar I (CNI).[137]

Consistent with the decreased hepatic BUGT activity, bile from GS individuals contains decreased bilirubin diglucuronides and increased bilirubin monoglucuronides compared with normals (see Figure 55.5-4).[49,52,116,138] This finding is similar to biliary bile pigment profiles seen in infants.[139] Phenobarbital normalizes the bile pigment profile in duodenal fluid,[52] lowers plasma bilirubin levels, and increases hepatic clearance of bilirubin.[140,141] Clofibrate and glutethimide also normalize serum bilirubin levels but do not normalize the distribution of bilirubin conjugates in duodenal bile.[142] In animals, clofibrate is an inducer of BUGT but does not affect sulfobromophthalein uptake or GST.[143]

A characteristic but poorly understood finding in GS is the exaggerated rise in serum bilirubin associated with fasting.[144–147] Normal individuals can double their serum bilirubin level when fasting, but in GS, a more pronounced increase occurs. Because indocyanine green clearance is not affected, this is not related to decreased hepatic blood flow.[145] Fasting reduces BUGT activity[147] and increases heme oxygenase (the enzyme responsible for bilirubin production) activity,[148] but these effects are not believed to explain the fasting hyperbilirubinemia of GS.[140,149] Intraluminal noncalorie food bulk can blunt the bilirubin rise.[150]

Diagnosis and Treatment

Generally, a diagnosis of GS can be made when there is a mild fluctuating unconjugated hyperbilirubinemia; otherwise, liver function tests are normal, and there is no hemolysis. Hemolysis can add confusion because it can result in similar findings, and it is not unusual in GS. Hence, other diagnostic tests are sometimes helpful.

One diagnostic test used with GS involves measuring the rise in serum bilirubin following intravenous administration of nicotinic acid.[151] Nicotinic acid is usually administered to adults in a dose of 50 mg over 30 seconds,[152–154] although similar results were seen with 300 mg injections.[155] Nonconjugated serum bilirubin is then measured every 30 to 60 minutes for the next 4 to 5 hours. Nicotinic acid produces a rise in serum unconjugated bilirubin in both normals and those with GS. However, in GS, the bilirubin rise is higher, and clearance is delayed longer.[151,155–158] Nicotinic acid causes increased osmotic fragility and hemolysis of red blood cells with splenic sequestration. Induced splenic heme oxygenase rapidly converts heme to bilirubin.[157] Nicotinic acid–induced hemolysis produces a rise in serum iron that is similar in healthy controls and those with GS.[158] Hence, the prolonged serum bilirubin levels are related to delayed hepatic

clearance of bilirubin. Nicotinic acid infusion can be a better diagnostic method for GS than a 400 kcal fast because delayed bilirubin clearance can be seen after nicotinic acid in GS subjects who otherwise had normal serum bilirubin levels.[152] The nicotinic acid test cannot differentiate GS from chronic liver disease.[159]

Rifampin, given to fasting or nonfasting adults in one oral dose of 900 mg, increases total serum bilirubin levels in normal subjects and subjects with GS, although there is an exaggerated rise in those with GS (fasting: > 1.9 mg/dL rise in bilirubin concentration 2 to 6 hours after rifampin; nonfasting: > 1.5 mg/dL rise 4 to 6 hours after rifampin).[160] This exaggerated rise in serum bilirubin enabled differentiation of 10 normal subjects and 15 GS patients with high sensitivity and specificity. This finding could not be explained by hemolysis, although haptoglobin levels were significantly lower in GS patients, compatible with baseline hemolysis.

Alkaline methanolysis and thin-layer chromatography have been used to diagnose GS by accurately separating and measuring total serum bilirubin as conjugated and unconjugated fractions.[161,162] In GS, only approximately 6% of the total serum bilirubin was conjugated compared with approximately 17% in normal subjects or those with chronic hemolysis and 28% in those with chronic persistent hepatitis. Fasting increased the serum total bilirubin level but did not change the percentage of conjugates in GS. An overlap of only three individuals was seen among the 77 subjects with GS and 60 normal subjects.[162] Other studies support these findings.[163] HPLC of serum showed similar findings with significantly decreased bilirubin monoglucuronides (1.1% vs 6.2% in normals) and increased unconjugated bilirubin (98.8 vs 92.6 in normals).[164]

Monaghan and colleagues have suggested GS genetic screening for the *UGT1A1* TA repeat as a simple, useful additional test in the investigation of very prolonged neonatal jaundice in North American, African, and European populations and for the Gly71Arg mutation in Asians.[99] Although genetic testing may be helpful in individual patients, the screening value of such a genetic test cannot be fully determined until accurate data regarding the prevalence and penetrance of the GS genotype are known.[165] Thus, genetic testing for GS cannot be routinely recommended.[165]

GS is generally believed to have no significant negative health implications. Drug metabolism studies have revealed no major dangers,[163,166] although there appears to be an increased incidence of slow acetylators,[167,168] lorazepam clearance is 20 to 40% decreased,[169] and drug-mediated toxicity is a concern.[170] Concurrent genetic deficiencies in other xenobiotic pathways may put individuals with GS at increased risk of drug toxicity to such compounds as acetaminophen[171,172] or CPT-11 irinotecan, a cancer chemotherapeutic agent metabolized by UGT1A1.[173] No specific treatment is necessary for GS, although phenobarbital has been shown to lower serum bilirubin levels.[174] If the well-documented antioxidant effect of bilirubin[175] provides a biologic advantage,[176] then the mild hyperbilirubinemia of GS might actually be a significant benefit against such things as ischemic heart disease,[177–179] in which free radicals are involved in pathogenesis. Schizophrenic patients with GS have significant enlargement of many components of their cerebrospinal fluid spaces compared with schizophrenic patients without GS and normal controls, although the significance of this remains unknown.[180]

CRIGLER-NAJJAR SYNDROME

Clinical Presentation

Crigler and Najjar, in 1952, reported seven infants with congenital familial nonhemolytic jaundice who developed severe unconjugated hyperbilirubinemia shortly after birth and died from kernicterus within months.[181] These infants were from three related families. Serum bilirubin levels reached 25 to 35 mg/dL despite a lack of hemolytic disease and otherwise normal liver function tests. Liver histology showed only deposition of bile pigments. Many subsequent reports have documented kernicterus as the main risk for patients with the Crigler-Najjar syndrome.[182] An excellent review of the neurologic perspectives of Crigler-Najjar syndrome has recently been published.[183] Although some patients survive into the second decade with normal development,[184] the possibility of developing late kernicterus is always a concern, even in adulthood.[185–188] Serum bilirubin levels vary from approximately 15 to 45 mg/dL. A Web site addressing many aspects of Crigler-Najjar syndrome has been developed (<www.crigler-najjar.com>).

Arias and colleagues, in 1969, reported a second, more frequent type of severe nonhemolytic hyperbilirubinemia.[189] The previous syndrome was termed CNI (OMIM #218800), whereas the new findings were termed CNII or Arias syndrome (OMIM #606785).[189] In CNII, hyperbilirubinemia is less severe, varying from approximately 8 to 25 mg/dL. Hence, kernicterus, although reported,[190,191] is less common in CNII.

Pathophysiology

Both CNI and CNII are generally inherited in an autosomal recessive manner, although one case of autosomal dominant inheritance of CNII has been reported.[192] CNI and CNII result from mutations to the *UGT* gene complex,[40] of which more than 50 different mutations have been described.[193] Patients with one normal allele demonstrate normal metabolism of bilirubin.[194] The genetic details determine the severity of clinical disease. In CNI, there is a complete absence of functional UGT1A1, whereas in CNII, UGT1A1 activity is markedly reduced.[195] In CNI, 18 of 23 described mutations of the *UGT1* gene are found in the common exons 2 to 5 and thus affect many UGT1 enzymes.[40,196] Intronic mutations causing CNI have also been reported.[197] However, in CNII, four of nine known mutations are found in exon 1A1. There is some overlap in classification of mild CNII and GS (eg, Gly71Arg), which relates to differences in definitions based on serum bilirubin levels.[40] The TATA box TA_7 repeat mutation seen in GS can be seen along with other mutations resulting in either CNI or CNII.[198] Thus, various homozygous, heterozygous, or compound heterozygous mutations of the *UGT1A1* gene can lead to varying degrees of reduction in hepatic bilirubin UGT activity and thus to varying degrees of clinical

hyperbilirubinemia, including severe CNI, intermediate CNII, and milder GS.[199]

In both CNI and CNII, assays of liver tissue from affected patients demonstrate negligible or very low BUGT activity.[49,50,116,186,189,200,201] Thus, liver biopsy is not helpful in differentiating these two disorders. This inability to conjugate bilirubin results in a profound block in bilirubin excretion. Study of the resected livers from four patients with CNI undergoing liver transplant showed that there was heterogeneity of the ability to glucuronidate various substrates other than bilirubin.[202] There is considerable overlap of hepatic BUGT activity between CNII and GS (see Table 55.5-1).[116] Consistent with the presumed autosomal inheritance of CNI, family studies have shown partial deficiencies in the glucuronidation of salicylate and menthol among siblings, parents, and grandparents.[203,204]

CNI and CNII are differentiated by the response to drugs that stimulate hyperplasia of the endoplasmic reticulum. In CNII, phenobarbital or diphenylhydantoin caused a significant decline in the serum bilirubin level, increased hepatic clearance of radiolabeled bilirubin,[141,189,190,205–208] and increased biliary levels of bilirubin diglucuronides (see Figure 55.5-4).[49,191] In a study of five CNII patients, phenobarbital caused a decrease in serum bilirubin ranging from 2.1 to 12.1 mg/dL (27–72%) with pre- and postphenobarbital serum bilirubin levels ranging from 7.8 to 16.9 and 4.7 to 10.1 mg/dL, respectively.[49] Summarizing data from seven earlier studies[141,189,190,205–208] regarding the response of CNII patients to oral phenobarbital treatment revealed that 11 female patients and 13 male patients had a total serum bilirubin of 15.7 ± 13.8 (mean ± SD) prior to phenobarbital. After phenobarbital (90–390 mg/d or alternatively 4 mg/kg/d), the serum bilirubin decreased 12.0 ± 4.0 mg/dL (77 ± 13%). The lowest total serum bilirubin following phenobarbital therapy was 5.9 mg/dL. Drugs have no significant effect on serum bilirubin or biliary bilirubin conjugates in CNI (see Figure 55.5-4).[49,185] Thus, response to phenobarbital is the accepted criterion by which CNI and CNII are differentiated.[209] Bile analysis has also been suggested as another method to differentiate CNI and CNII.[49] In CNI, bile contains insignificant bilirubin conjugates (< 10%), and unconjugated bilirubin predominates. In CNII, bile contains predominantly bilirubin monoglucuronides (> 60%).[116]

Two cousins with Crigler-Najjar syndrome have been described, which raises the possibility of a new variant of this syndrome (CNIII).[210] This new variant resembled CNI with no biliary excretion of bilirubin mono- or diglucuronide, but there was excretion of mono- and diglucoside conjugates of bilirubin. It has been speculated that type III patients lack the long proposed permease,[36] which has been hypothesized to transport UDP-glucuronic acid to the luminal side of the endoplasmic reticulum, where BUGT is located. This absence is suggested to result in UDP-glucose being used for bilirubin conjugation.

Diagnosis and Treatment

Evaluation for Crigler-Najjar syndrome invariably begins during the first few days of life, when serum bilirubin levels exceed 20 mg/dL. The conjugated fraction will not be elevated except possibly for the factitious elevation, which is sometimes seen when the total serum bilirubin is very high.[211] Evaluation of such infants should eliminate the more common causes of jaundice, including hemolysis, hypothyroidism, and infection. Formula feedings will help identify those infants with jaundice related to human milk. During these early days of life, Crigler-Najjar patients will require prompt and intense phototherapy and possibly exchange transfusion to avoid kernicterus and bilirubin encephalopathy.[212] Jaundice will be persistent and problematic. At present, there is no widely available simple clinical test to confirm a diagnosis of Crigler-Najjar syndrome. Crigler-Najjar syndrome can be excluded by finding significant amounts of bilirubin conjugates in neonatal stools. This HPLC analysis must be done on stools collected prior to establishment of sufficient intestinal bacterial flora to convert all bilirubin conjugates to urobilinoids.[182] HPLC analysis of duodenal bile will show that in CNI, there are negligible bilirubin di- or monoglucuronides, whereas in CNII, these conjugates are present but in low concentration.[40,49] However, this bile analysis led to misdiagnosis in six of nine infants examined during the first 3 months of life.[213] Bile can easily be collected with a pediatric Enterotest capsule (HDC Corporation, San Jose, CA).[138] This approach can potentially avoid a liver biopsy for confirmation of negligible BUGT activity using an in vitro assay. The ratio of serum bilirubin conjugates (as determined by alkaline methanolysis with thin-layer chromatography or HPLC) to total bilirubin, although abnormally low, does not allow differentiation of Crigler-Najjar patients from those with GS.[162] Deoxyribonucleic acid (DNA) analysis can be very helpful in establishing the correct diagnosis, although this is not routinely available.[214]

A world registry of patients with CNI aimed at developing management guidelines has been published.[215] Phenobarbital (4 mg/kd/d in infants) should be used when there is concern about CN. Within 48 hours, CNII patients can demonstrate a significant decrease in the serum bilirubin levels (as detailed above) and an increased biliary excretion of bilirubin di- and monoglucuronide,[50,191] whereas CNI patients will show no significant response. Occasionally, CNII patients do not respond to the first trial of phenobarbital therapy, but subsequent trials months later will demonstrate the significant decrease in serum bilirubin level.[209] Doses of phenobarbital as low as 0.6 mg/kg/d have been reported to completely normalize serum bilirubin in one patient. However, despite the decrease in serum bilirubin in response to phenobarbital, CNII patients will usually continue to manifest a significant hyperbilirubinemia (approximately 5–15 mg/dL). Phototherapy for 6 to 12 hours daily has been the primary modality to keep serum bilirubin levels below 20 mg/dL during the first several months of life[216] because CNI patients can excrete all bilirubin photoisomers.[217] CNI patients will require lifelong treatment with phototherapy until more definitive therapy such as liver transplant. Phototherapy has been found to be least intrusive when given at night, and improvements have been made in effectiveness and comfort.[218] Although phototherapy is very helpful

in infancy, in adolescence, social inconvenience and compliance problems can bring increased risk of kernicterus.[215]

Oral administration of agents that bind bilirubin can be helpful. Options include agar, cholestyramine, and calcium phosphate.[219–222] These agents block the enterohepatic circulation by binding to bilirubin, which has reached the intestinal lumen owing to phototherapy or through direct intestinal permeation.[223] Agar varies significantly in bilirubin binding affinity among various preparations and batches.[186,219,224] Cholestyramine prompts concerns about cost, taste, and bile salt depletion and fat malabsorption. Plasmapheresis can be very helpful after the first year of life as it has been shown to rapidly decrease serum bilirubin levels.[186,225] Peritoneal dialysis and exchange transfusion have not been helpful in this setting.[185] Repeated intramuscular injections of tin-protoporphyrin, a heme oxygenase inhibitor that blocks bilirubin formation, have been used in a CNI patient, with data suggesting a decreased need for phototherapy.[226] Two patients with CNI were treated with tin-mesoporphyrin to block bilirubin formation, daily phototherapy, and intermittent plasmapheresis over a 400-day period.[227] They developed an iron deficiency anemia believed to be due to the porphyrin therapy,[228] but tin-mesoporphyrin (2–4 μmol/kg)[229] is suggested to offer a promising, although still experimental, additional therapy for controlling episodes of acute, severe jaundice. Drugs that bind to albumin and can potentially displace bilirubin should be avoided at all times.[230]

Auxiliary liver transplant would be feasible for CNI patients because their liver function is normal, except for bilirubin glucuronidation. This option has only recently become clinically available.[231–233] Thus, more commonly, orthotopic liver transplant,[234–241] including living-related liver transplant,[242] represents the only true cure for the hyperbilirubinemia of CNI. The timing of transplant should precede irreversible neurologic injury. When infants are small, sleeping under a phototherapy unit plus the medical measures noted above can be effective in managing hyperbilirubinemia and avoiding kernicterus. However, there is always the risk of intercurrent illness, which can precipitate worrisome hyperbilirubinemia. As children grow, phototherapy and other medical therapies may decrease in effectiveness. Thus, the risk of kernicterus must be balanced against the risks associated with liver transplant, including lifelong immunosuppression. This can be a very difficult decision. The effectiveness and ease of medical management of hyperbilirubinemia are important considerations, as are the long-term success and complication rate of the specific transplant center involved. Bilirubin levels must be followed and extreme hyperbilirubinemia avoided at any age because the development of kernicterus is not restricted to the neonatal period. Transplant of other BUGT-containing tissues (eg, segments of small intestine,[243,244] kidneys[245]) remains experimental. Successful cloning of the gene responsible for bilirubin glucuronosyltransferase activity offers the hope of future gene therapy to correct this deficiency.[246–250] Gene therapy,[251–254] encapsulated hepatocytes,[255] and gene repair have shown success in lowering serum bilirubin levels in Gunn rats,[256] the congenitally jaundiced model for CNI.[257] In a 10-year-old girl with CNI, isolated hepatocytes equivalent to approximately 5% of the normal host hepatocyte mass were infused through the portal vein, survived for more than 11 months, and lowered serum bilirubin levels from approximately 25 mg/dL to 10 to 15 mg/dL while still receiving 6 to 8 hours of phototherapy per day.[258] More recent isolated hepatocyte transplant for the correction of another inborn error of metabolism (ornithine transcarbamylase deficiency) resulted in temporary improvement, which was lost after 11 days presumably because of rejection of the transplanted cells because of insufficient immunosuppression.[259] Isolated hepatocyte transplant remains experimental.[257]

ROTOR SYNDROME

Clinical Presentation

Rotor syndrome (RS; OMIM *237450) is a familial disorder, first described in 1948, that involves chronic elevation of both the conjugated and unconjugated serum bilirubin fractions.[260,261] Fractionation of the serum total bilirubin shows that half or more is conjugated, and total bilirubin levels range from 2 to 7 mg/dL but occasionally may reach 20 mg/dL.[262] Liver functions tests are otherwise normal, and there is no evidence of hemolysis. Both light and electron microscopy reveal normal liver histology. Oral cholecystograms reveal normal gallbladder opacification. This disorder can present in early childhood[263] or in infancy if associated with other hematologic diseases[264] and manifests no gender predisposition. Family studies suggest an autosomal recessive mode of inheritance.[260,261,265]

Pathophysiology

The primary abnormality in RS is a deficiency in the intracellular storage capacity of the liver for binding anions.[266,267] This can be demonstrated by constant infusions of sulfobromophthalein and indocyanine green.[268] Patients with RS demonstrate a delayed plasma clearance of both sulfobromophthalein and indocyanine green, and heterozygotes show delayed sulfobromophthalein clearance with values intermediate between normal subjects and those with homozygous RS.[266] GST serves as an intracellular carrier protein of certain organic molecules, acting as an intracellular equivalent to albumin in blood plasma.[269] Patients with RS have been shown to have a deficiency of hepatic GST,[270,271] resulting in impaired uptake of bilirubin within the cytosol. This appears to be due to mutations in the *hGSTA1-1* gene.[271] In addition, because bilirubin conjugates are bound to GST while awaiting excretion from the hepatocyte via the canalicular membrane,[272] deficient intracellular storage would result in leakage of bilirubin conjugates back into the circulation, with subsequent serum elevations of both conjugated and unconjugated bilirubin.

Urinary excretion of coproporphyrin is important in RS. In normal healthy individuals, only the I and III isomers of coproporphyrin are excreted in the urine. In RS, total urinary coproporphyrin excretion (isomers I + III) is markedly increased, and, usually, isomer I comprises less than 80% of the total. Heterozygotes demonstrate urinary

coproporphyrin values, which are intermediate between normal subjects and homozygotes.[273] Urinary excretion of coproporphyrin is believed to be increased because biliary excretion of coproporphyrin is impaired, similar to findings in other liver diseases.[274]

Diagnosis and Treatment

In all individuals with elevation of both conjugated and unconjugated serum bilirubin fractions along with otherwise normal liver function tests, a diagnosis of RS should be considered. The diagnosis can be confirmed by measuring urinary coproporphyrin levels, which are 2.5 to 5 times higher than normal levels.[273] Of the total urinary coproporphyrin isomers (I + III), isomer I constitutes less than 80% of the total in RS.[275] Technetium 99m hepatobiliary iminodiacetic acid cholescintigraphy has also been shown to be useful to diagnose RS and demonstrates poor to no visualization of the liver.[276,277]

Patients with RS require no specific therapy and are asymptomatic. Although jaundice is a lifelong finding, it is not associated with morbidity or mortality.

DUBIN-JOHNSON SYNDROME

Clinical Presentation

DJS (OMIM #237500) was first described in 1954[278] and involves elevation of both the conjugated and unconjugated serum bilirubin fractions.[279] Fractionation of the serum total bilirubin shows that half or more is conjugated, and total bilirubin levels usually range from 1.5 to 6 mg/dL, although they have been reported to be as high as 25 mg/dL during intercurrent illness.[280] Patients with DJS report vague abdominal complaints, although this is not believed to reflect serious pathology. Hepatomegaly is sometimes seen, but liver function tests are otherwise normal, including bile acids,[281] and there is no evidence of hemolysis.[278,279,282] Although this syndrome occurs in both sexes, males predominate and present at an earlier age. It occurs in all races; however, Iranian Jews have an increased incidence.[283,284] It is usually diagnosed after puberty, although cases have also been reported in neonates,[285–288] at which time, cholestasis can be significant.[287,289–292] DJS is inherited as an autosomal recessive trait, with heterozygotes manifesting normal serum bilirubin levels.[283,293,294] This syndrome is far more common than RS, and jaundice can be worsened by pregnancy and oral contraceptives.[295] Often patients with DJS do not visualize the gallbladder with an oral cholecystogram.[279]

Brown to black discoloration of the liver is a striking characteristic of this syndrome. This pigment is located in the lysosomes.[296] The exact identity of this pigment is still uncertain. Once thought to be lipofuscin, more recent data provide conflicting evidence for a relationship to melanin[297–299] or polymerized epinephrine or other metabolites,[300,301] which accumulate in the lysosomes. It is hypothesized that these pigments accumulate in the liver because of impaired secretion of various metabolites from the hepatocyte into the bile.[301] This pigment has been shown to disappear from the liver during acute viral hepatitis, with subsequent reappearance.[302] Other than this striking pigmentation, the liver histology is normal.

Pathophysiology

The primary defect in DJS is deficient hepatic excretion of non–bile salt organic anions at the apical canalicular membrane by the ABC transporter originally known as cMOAT (OMIM 601107)[303,304] but now also called MRP2.[305,306] The gene encoding this protein is designated *ABCC2*. cMOAT/MRP2 is encoded by a single-copy gene located on chromosome 10q24.[307] Mutations of this gene have been shown to produce a highly defective cMOAT/MRP2, which is nonfunctional or absent from the apical membrane and associated with DJS.[60,303,304,308–311] Similar findings made in the homologous cMOAT/MRP2 gene of two rat models of hyperbilirubinemia (GY/TR$^-$ and Eisai) have been very helpful in understanding DJS in humans.[60,312]

Although hepatic sulfobromophthalein clearance tests are no longer performed, they clearly demonstrate the effect of deficient transport via the canalicular membrane, which is characteristic of DJS.[313] Initially, the clearance rate of intravenously administered sulfobromophthalein from the circulation is rapid and results in sulfobromophthalein retention that is often normal at 45 minutes. However, a subsequent rise in serum sulfobromophthalein concentration occurs at 90 and 120 minutes because the conjugated sulfobromophthalein cannot be excreted and thus refluxes out of the hepatocyte back into the circulation.[313–316] Data suggest that sulfobromophthalein hepatic storage is normal, but there is a 90% decrease in the sulfobromophthalein excretory transport maximum.[295,315] Other substances (eg, indocyanine green, rose bengal, and dibromosulfophthalein) have also been shown to have a decreased excretory transport maximum, although these substances do not require hepatic biotransformation and do not show the late rise in plasma levels during clearance tests.[317] Hence, in DJS, deficient excretion of bilirubin glucuronides at the canalicular membrane, in the presence of otherwise normal intrahepatic metabolism, results in reflux of conjugated bilirubin back into the circulation.

Urinary excretion of coproporphyrins is important in patients with DJS.[318,319] DJS patients have an increase in the urinary excretion of coproporphyrin I with a concomitant decrease in the excretion of coproporphyrin III. This results in a total coproporphyrin excretion (I + III) that is normal or only slightly increased but that consists of greater than 80% coproporphyrin I (normal 25%).[319–322] In heterozygotes, the coproporphyrin I-to-III ratios are intermediate between normal subjects and homozygotes,[319,321,322] although there is some overlap between them. The explanation for these findings regarding urinary coproporphyrin excretion is unclear, and several pathogenic mechanisms have been suggested.[319] Fecal coproporphyrin levels are normal.[319] Healthy neonates have been shown to have impressive elevations of urinary coproporphyrin levels, with more than 80% isomer I on the first 2 days of life[323]; however, by day 10, levels fell to overlap normal adult values.

Diagnosis and Treatment

A diagnosis of DJS should be considered in all individuals with an elevation of conjugated bilirubin in the serum along with otherwise normal liver function tests. Measurement of urinary coproporphyrin isomers I and III can confirm the diagnosis. The total coproporphyrin level will be approximately normal, but more than 80% will be isomer I. This finding is pathognomonic for DJS when congenital erythropoietic porphyria[324] or arsenic poisoning[325] has been excluded. Although an oral cholecystogram may fail to visualize the gallbladder, ultrasound examination will show a normal biliary tree. Cholescintigraphy demonstrates prolonged intense visualization of the liver with delayed appearance of the gallbladder and only faint or nonvisualization of the biliary ducts.[277,326,327] Computed tomography of the liver has shown increased attenuation in one report.[328] Because cMOAT/MRP2 transport of leukotrienes into bile is defection in DJS, there is increased excretion of leukotriene metabolites into urine, and this has been suggested to be a new approach to the noninvasive diagnosis of this disease.[329]

No specific therapy is needed for patients with DJS. Although jaundice is a lifelong finding, it is not associated with morbidity or mortality. Avoidance of oral contraceptives has been recommended[330] because this can increase jaundice. Anticipatory guidance regarding pregnancy[295] is appropriate. Increased fetal wastage has been reported in one study.[331] In one case report of neonatal DJS with severe cholestasis, phenobarbital significantly decreased serum levels of bilirubin and bile acids,[289] although chronic phenobarbital therapy is not recommended.[1]

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6. Biliary Transport

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Cholestasis, which is a major manifestation of a wide range of pediatric liver diseases, is ultimately the result of specific or nonspecific abnormalities in biliary transport. These abnormalities can be the result of genetic defects in specific hepatic-based transporters or can be part of a series of molecular responses to a pathophysiologic process. Examples of the former include genetically determined defects in the expression of the canalicular bile acid transporter or the canalicular phospholipid flippase. An example of the latter is the cascade of responses that follow gram-negative sepsis. This chapter describes the current knowledge of disorders of biliary transport and how pathologic changes in these systems lead to pediatric liver disease. Current understandings of the natural history of, diagnostic approaches to, and treatment of these newly described diseases are reviewed.

BILIARY TRANSPORT

Bile flow is dependent on a series of vectorial transport proteins that are arrayed on the basolateral and canalicular membranes of hepatocytes (Figure 55.6-1). In addition, bile flow also requires an ordered hepatocellular processing of bile components. Transport processes in the intestine, kidney, pancreas bile duct, and gallbladder have significant effects on bile formation. This highly complex process is subject to a number of levels of regulation and consists of a diversity of genetically distinct elements. Bile itself consists of multiple individual components, including bile salts, bilirubin, phospholipids, cholesterol, and a large variety of endogenous and xenobiotic organic compounds. Vectorial flow of each of these components may be under separate genetic and regulatory controls. As such, it is not surprising that a wide variety of cholestatic liver diseases may exist, each with a separate genetic basis and each with distinct clinical characteristics and therapeutic approaches. A brief review of hepatocyte transport of bilirubin, bile acids, and organic anions is presented here (see Chapter 5.1, "Bile Formation and Cholestasis," for a comprehensive review of bile formation), especially as it pertains to the biliary transport disorders listed below. The interested reader is also directed to recent comprehensive reviews of biliary transport and analyses of modifications of bile by bile ductular epithelium.[1–5] Given the interrelation of disease and physiology in this rapidly developing field, details of the discovery of the various transporters are described in the following sections related to specific biliary transport diseases.

Highly efficient mechanisms for the basolateral extraction of bile components from serum into hepatocytes have developed partly in an effort to minimize the concentrations of these potentially toxic compounds in the systemic circulation. At least three different transporters have been shown to be potentially involved in basolateral transport of bile acids. The first and potentially most physiologically significant of these is the Na^+-dependent taurocholate cotransporting polypeptide (NTCP). This 48 kD protein is a hepatocyte-specific integral membrane protein.[6,7] It was the first of the bile acid–specific transport proteins to be cloned. Its discovery revolutionized the current approach to studying the physiology and pathophysiology of bile formation. In addition to NTCP, two other proteins have been shown to transport bile salts: the organic anion transport protein (OATP[8]) and microsomal epoxide hydrolase.[9] The physiologic significance of these latter two proteins in hepatic extraction of bile salts is currently not clear. OATP may be more important in hepatic extraction of organic anions such as bilirubin.[10]

The mechanisms involved in the transcellular movement of bile components from the basolateral to the canalicular membrane are not well understood. It is not certain whether this process involves specific binding proteins or specialized vesicles. Intracellular binding proteins for bile components have been described, although their physiologic necessity is not known. Bile acids have been shown to bind to dihydrodiol dehydrogenase, whereas bilirubin binds to ligandin.[11] Several lines of investigation have suggested that bile components are associated with or found within intracellular vesicles. As such, it is possible that molecular motor systems using

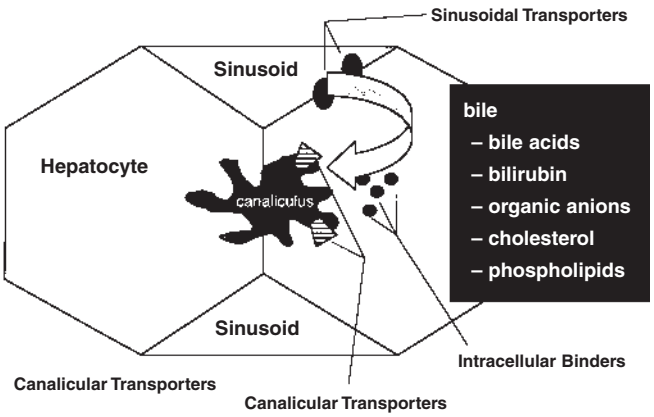

FIGURE 55.6-1 Diagram of mechanisms of bile flow. Cartoon depiction of hepatocyte couplet with vectorial flow of bile from sinusoidal blood to bile canaliculus via basolateral and canalicular transporters.

microfilaments and/or microtubules may play a role in promoting transcellular bile flow. These processes have been very difficult to assess using currently available experimental methods. Like the biliary transport proteins, it is very likely that studies of naturally occurring defects in these proteins will clarify this field. Bile acid conjugation is an additional intracellular event that influences bile flow. Conjugated bile acids are more water soluble and thus are more easily excreted.

Canalicular transport of bile components is generally recognized to be rate limiting for bile flow, and, as such, these transporters have been under active investigation by a number of laboratories. In addition, impairment of the function of any of these transport proteins has the potential to lead to the hepatocellular accumulation of toxic compounds and therefore directly lead to liver disease. A variety of studies indicated that many of these transport processes were adenosine triphosphate (ATP) dependent; thus, the ATP-binding cassette (ABC) proteins became likely candidates as transporters. A combination of basic laboratory investigations, animal models, and genetic analysis of cholestatic liver diseases (see individual sections below) ultimately identified three important ABC proteins as canalicular biliary transport proteins (Table 55.6-1). Specific and genetically distinct proteins have been identified that mediate ATP-dependent transport of bile acids (bile salt export pump [BSEP]/sister of P-glycoprotein [SPGP]), organic anions (multidrug resistance–associated protein 2 [MRP2]/canalicular multispecific organic anion transporter [cMOAT]), and phospholipids (multidrug resistance protein 3 [MDR3]) from the hepatocyte into the bile canaliculus. ATP hydrolysis is critical for each of these transport functions because the transport process takes place against a very steep concentration gradient (ie, concentrations in bile are much greater than within the hepatocyte).

NOMENCLATURE

The nomenclature that currently exists in the field of biliary transport disorders is complex, confusing, and likely to undergo significant change. In this chapter, older terminology, in particular that uses progressive familial intrahepatic cholestasis (PFIC), is generally replaced by specific reference to the defective gene or protein. This has only recently become possible because the molecular identification of the defects involved in the various forms of PFIC have been elucidated.[1–3] The future utility of these historical designations may no longer be apparent. Many of the patients who have these diseases do not have other family members who are affected by the disease; thus, the familial portion of the designation becomes limiting. As the full range of the clinical phenotype of these various disorders becomes known, a more specific and helpful system of nomenclature may be devised.

DEFECTS IN CANALICULAR BILE ACID TRANSPORT

The pivotal role of canalicular transport of bile acids in the formation of bile has made this transport process a focus of active investigation in the pathophysiology of pediatric cholestatic liver disease. In spite of the fact that it has been expected that a defect in canalicular transport of bile acids would lead to severe cholestatic liver disease, discovery of this abnormality had been very difficult. Technical problems in the analysis of canalicular transport processes have been in large part responsible for delays in these discover-

TABLE 55.6-1 SUMMARY OF BILIARY TRANSPORT DISEASES

DISEASE	*SPGP/BSEP* DEFICIENCY	*MDR3* DEFICIENCY	DUBIN-JOHNSON SYNDROME	BYLER DISEASE
PFIC designation	PFIC2	PFIC3	None	PFIC1
Common name	BSEP	MDR3	MRP/cMOAT	FIC1
Defective transport gene	*ABCB11*	*ABCB4*	*ABCC2*	*ATP8B1*
Chromosomal localization	2q24	7q21	10q24	18q21-q22
mRNA size (kb)	5.5	4.1	6.5–9.5	7.0
Protein size (kD)	170	170	200	145
Tissue distribution	Liver	Liver	Liver >> intestine	Intestine > liver
Transporter substrate	Bile acids	Phospholipids	Organic anions	Aminophospholipids?
Animal model	Mouse knockout[27]	Mouse *mdr2* knockout[31]	Rat TR$^-$ or Eisai[58]	Mouse Byler equivalent[83]
Alanine aminotransferase level	++	++	nl	+
Bilirubin level	+ -> +++	+ -> +++	++	++ -> nl -> +++
Cholesterol and γ-GTP levels	nl	++	nl	nl
Serum bile acid level	+++	++	nl	+++
Histologic findings	Giant cell transformation	Ductular prolifer Portal fibrosis/cirrhosis	Melanin-like pigment in hepatocytes	Bland intracanalicular cholestasis; diminished canalicular γ-GTP
Special diagnostic tests	Biliary bile acids	Lipoprotein X; biliary phospholipids	Urine coproporphyrin I	Electron microscopy
Treatment	Liver transplant; biliary diversion?	Liver transplant	None	Partial biliary diversion; ileal bypass
Key references	17, 20	42	54, 61	66, 70, 78

BSEP = bile salt export pump; cMOAT = canalicular multispecific organic anion transporter; GTP = glutamyl transpeptidase; MDR = multidrug resistance; mRNA = messenger ribonucleic acid; MRP = multidrug resistance–associated protein; PFIC = progressive familial intrahepatic cholestasis; SPGP = sister of P-glycoprotein.

ies. Efflux transport processes, by their very nature, are much more complex to study than uptake mechanisms.[12] Accurate analysis of efflux is critically dependent on uniform loading of either cells or vesicles, which can be nearly impossible to perform. The alternative of examining reverse transport into cells or vesicles is subject to a number of artifacts. Isolation of relatively purified inside-out canalicular membrane vesicles permitted more exact analysis of canalicular transport via uptake.[13] It was not until recently that it was determined that the primary driving force for canalicular transport of bile acids was related to the hydrolysis of ATP.[14–16] This finding suggested that the canalicular bile acid transporter might belong to a superfamily of proteins referred to as ABC transporters. These transport proteins include a number of critically important proteins that, when defective, lead to a range of diseases, including cystic fibrosis, Wilson disease, adrenoleukodystrophy, Tangier disease, and Dubin-Johnson syndrome. The ABC transporters are integral membrane proteins, which typically have two cytoplasmic consensus sequences for ATP binding. Degenerate oligonucleotide primers were designed for these binding domains, and reverse transcriptase–polymerase chain reactions were used to clone the rat canalicular bile acid transporter.[17] The cloned gene was very similar to a previously cloned pig gene referred to as the *SPGP*.[18] Expression of the protein product of the *SPGP* complementary deoxyribonucleic acid (DNA) in two separate systems, *Xenopus laevis* oocytes and *Sf9* insect cells, resulted in augmentation and acquisition, respectively, of ATP-dependent taurocholic acid transport activity. Northern blotting and immunoelectron microscopy indicate that the expression of this gene is hepatocyte specific and is localized to the canalicular membrane. Overall, these studies therefore indicated that the *SPGP* was a canalicular BSEP.

The clinical relevance of the discovery of BSEP became apparent as a result of genetic studies that were conducted nearly simultaneously. Homozygosity linkage studies mapped the genetic defect in a subset of Saudi Arabian patients with progressive familial cholestasis to chromosome 2q24.[19] Further refinement of the PFIC2 locus was performed using a larger group of patients with progressive familial cholestasis and ultimately led to the identification of the human correlate of *BSEP* as the defective gene in *PFIC2*.[20] Ten different mutations in *BSEP* were initially described (Figure 55.6-2). Four were nonsense mutations leading to a truncated protein, whereas the remaining six were missense mutations involving amino acid residues in key portions of the predicted bile acid transporter structure.

The clinical presentations of defects in *BSEP* are being defined, although the full range of presentations is not yet well understood.[20–24] Cholestasis often is manifest by pruritus and jaundice early in life, typically before 1 year of age. The inability of infants to scratch may translate into irritability as a first feature of this disorder. Biochemical findings are consistent with progressive intrahepatic cholestasis and include markedly elevated serum bile salts. There are typically variable elevations in serum bilirubin and aminotransferase levels with normal γ-glutamyl transpeptidase and normal cholesterol levels (see Table 55.6-1). Liver biopsies performed in the first 2 years of life are notable for giant cell hepatitis and cholestasis. The electron microscopic appearance of bile is reported to be distinct from that seen in familial intrahepatic cholestasis type 1 (FIC1) disease (see below). Immunohistochemical analysis of hepatic γ-glutamyl transpeptidase has been preliminarily reported to reveal strong staining at the bile canaliculus in contrast to the diminished staining observed in FIC1 disease (see below).[25] Some defects in BSEP lead to absent expression of its protein, which potentially can be demonstrated by immunohistochemistry.[26] Prospective and/or blinded analysis of these findings will be needed to confirm them as potential diagnostic criteria. Given the key role of BSEP in bile formation, it is not surprising that the clinical progression of liver disease in these patients is relatively rapid. Interestingly, a *BSEP* knockout mouse is

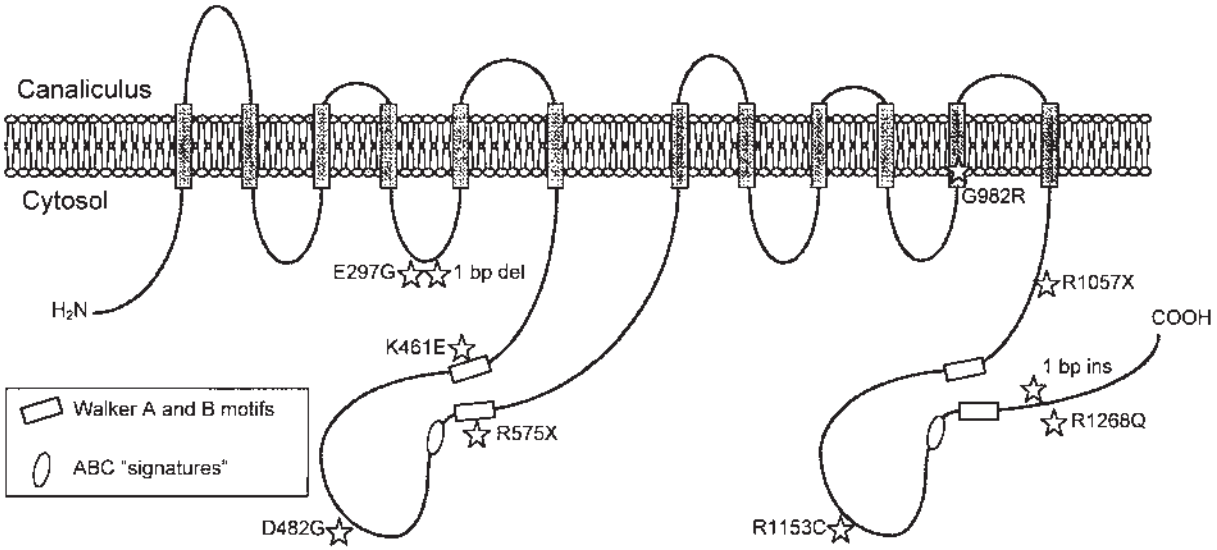

FIGURE 55.6-2 Diagram of defects in the bile salt excretory protein. Model of canalicular bile salt transporter protein embedded within the cell membrane. Specific mutations are indicated by the stars. Reproduced with permission from Thompson R.[20]

not characterized by progressive liver disease, suggesting that alterative transport mechanisms exist.[27] Because BSEP is liver specific, it is also not surprising that liver transplant appears to be curative.[17,20] A subset of patients may have mutations in BSEP that lead to a partially active protein.[23,28] These patients may have a more moderate disease course and could potentially be amenable to nontransplant surgical interventions such as partial biliary diversion or ileal bypass (see below).[29]

The diagnosis of a defect in *BSEP* should be suspected in any infant or young child with cholestasis who has normal γ-glutamyl transpeptidase and cholesterol levels. Biliary bile salt levels have been reported to be very low; unfortunately, biliary bile is often not available for routine clinical analysis. Differentiation of this disease from FIC1 disease may be problematic. It has been assumed that defects in *BSEP* have significantly more rapid progression to decompensated liver disease than seen in FIC1 disease. Careful genotype and phenotype studies are under way, and preliminary reports indicate that this assumption may not have been entirely accurate.[29] The ultrastructural appearance of canalicular bile in patients who are not receiving ursodeoxycholic acid may also be diagnostically useful.[30] Ultimately, some form of molecular diagnostic study, either analysis of hepatic protein or messenger ribonucleic acid (mRNA) expression or genomic sequencing of the *BSEP* gene, may be required to confirm a suspected diagnosis. Accuracy in assigning an appropriate diagnosis may be imperative because defects in *BSEP* appear to respond well to liver transplant, whereas FIC1 disease may not and may require a more specialized surgical approach (see below).

DEFECTS IN CANALICULAR PHOSPHOLIPID TRANSPORT

Phospholipid excretion into bile was presumed to be a passive process until the recent discovery of a P-glycoprotein, which mediates ATP-dependent phospholipid transport into bile.[31] This transport protein was somewhat serendipitously discovered in an effort to further understand the multidrug resistance proteins. One member of this class, *MDR3* (*mdr2* in mice), had been cloned and was found to be expressed in human liver, although its function was unknown.[32] Preliminary attempts to express this gene product in cell lines were uninformative with regard to its function[33]; therefore, the gene was "knocked out" of mice by homozygous targeted disruption. The resulting homozygous *mdr2*$^{-/-}$ mice have provided great insights into the physiology of bile formation and formed the basis for the understanding of a new class of cholestatic liver diseases.[31] Elevated bilirubin, alkaline phosphatase, and alanine aminotransferase were detected in the serum of these mice. Bile duct proliferation with a mixed portal infiltrate associated with loss of bile canalicular microvilli was observed in the homozygote knockout mice. Biliary bile flow and bile acid output by these mice were normal. Phospholipid, cholesterol, and glutathione secretion were all markedly reduced in bile with a graded reduction in phospholipid only in heterozygote mice. The *mdr2* gene product was therefore presumed to be involved in canalicular excretion of phospholipid.

Further analysis of the *mdr2* knockout mice provided additional insights into the function and importance of this P-glycoprotein. Long-term histopathologic studies of the *mdr2*$^{-/-}$ mice demonstrated a novel progression from a nonsuppurative inflammatory cholangitis to metastatic hepatocarcinogenesis.[34] *MDR3*, the human homologue of the mouse *mdr2* gene, was introduced as a transgene into *mdr2*$^{-/-}$ mice.[35] Fibroblasts from the transgenic mice and not the knockout mice were able to transfer phospholipid from the inner hemileaflet to extracellular acceptor liposomes. This apparent phospholipid flippase activity was found to be an ATP-dependent phenomenon that could be observed using hepatic canalicular membrane.[36] Ultrarapid cryofixation of the liver from *mdr2*$^{-/-}$ mice with subsequent analysis by electron microscopy elegantly confirmed the suspected function of the mdr2 protein to be a phospholipid flippase (Figure 55.6-3).[37] In normal bile canaliculi, phospholipid accumulation on the outer hemileaflet of the canaliculus leads to the formation of vesicles that ultimately bud off into the canalicular lumen. These vesicles primarily contain cholesterol and phospholipid. These vesicles are targeted to plasma as opposed to bile in cholestasis and result in the formation of the characteristic lipoprotein X that is seen in obstructive liver disease, especially when it is secondary to bile duct obstruction.[38] *Mdr2*$^{-/-}$ mice do not accumulate lipoprotein X in the face of extrahepatic bile duct obstruction. Absence of phospholipid flippase function results in bile characterized by significant concentrations of unmicellized bile salts. This bile therefore has high detergent capacity and is toxic to the canalicular membrane and therefore the liver. Interestingly, *mdr2*$^{-/-}$ mice can be functionally rescued by either hepatic-specific transgenic expression of the human homologue *MDR3* or by hepatocyte transplant.[39,40]

The comprehensive characterization of the *mdr2*$^{-/-}$ mouse and the elucidation of the function of the *mdr2* gene product laid the groundwork for identification of human disease related to abnormal expression of the human homologue *MDR3*. The first preliminary description of a defect in this gene product included two children who initially presented with pruritus and hepatosplenomegaly. The *MDR3* mRNA could not be detected by Northern blotting in one of these patients, and phospholipid levels in bile were markedly reduced in the second.[41] Neither of these suggestive findings could definitively demonstrate a genetic abnormality in *MDR3*.

A subsequent report definitively established defects in *MDR3* as a cause of cholestatic liver disease in two children.[42] As in the previous report, both children presented with jaundice and hepatosplenomegaly. Like the *mdr2*$^{-/-}$ mice, both children had elevated alanine aminotransferase and γ-glutamyl transpeptidase levels. Serum bile acid levels were markedly elevated in both children. Portal inflammation with bile duct proliferation and fibrosis was present despite a patent and normal extrahepatic biliary tree. Neither child was responsive to ursodeoxycholic acid therapy,

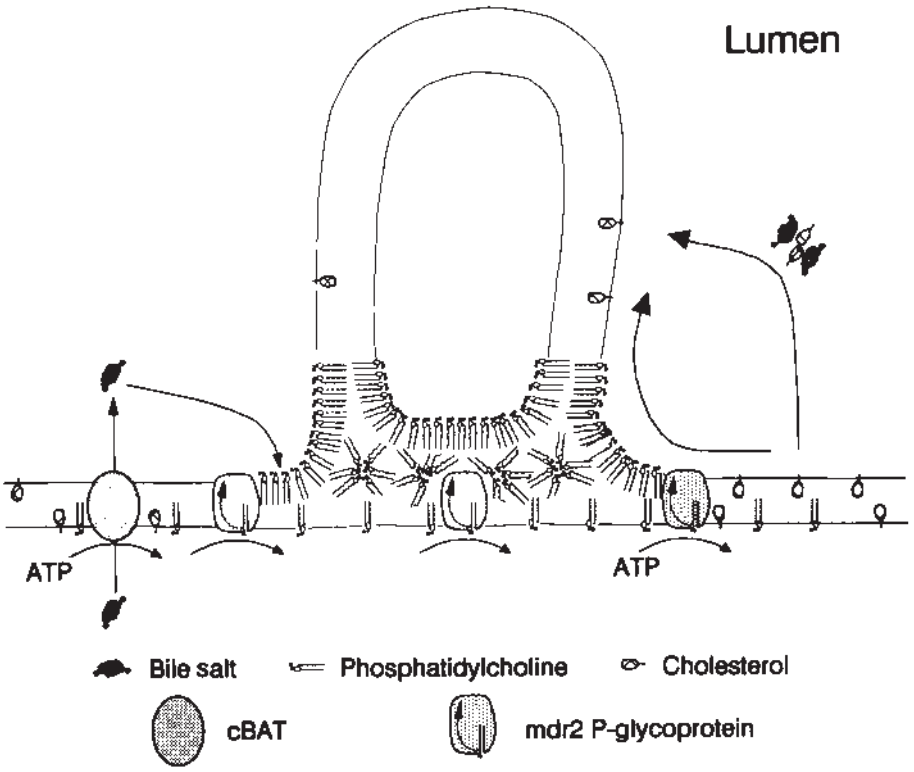

FIGURE 55.6-3 Diagram of function of the multidrug resistance 2 (mdr2) protein. In this model, the mdr2 (equivalent to human MDR3) protein is shown to function as a phospholipid flippase. Adenosine triphosphate (ATP) hydrolysis leads to the "flipping" of phospholipid from the inner to the outer hemileaflet of the lipid bilayer. Accumulation of phospholipid on the outer hemileaflet leads to the formation of vesicles, which contain cholesterol and phospholipid. cBAT = canalicular bite acid transporter (also known as BSEP). Reproduced with permission of Crawford AR et al.[37]

and complications of end-stage liver disease led to liver transplant at the ages of 3.5 and 9.0 years. Immunohistochemical analysis of the explanted liver from both children revealed an absence of staining for the MDR3 protein using a specific polyclonal antibody.[43] Reverse transcriptase–polymerase chain reaction analysis of the *MDR3* mRNA sequence in both patients identified homozygous defects that lead to premature stop codons and presumably truncated, unstable, and nonfunctional phospholipid flippase proteins. In one patient, the specific defect permitted genomic analysis of the proband and his family. The patient was found to be homozygous for the mutation at the genomic level, whereas his parents were both heterozygotes. His unaffected sister was homozygous for the wild-type gene.

The full range of the phenotypic expression of defects in the *MDR3* gene is not known and is under investigation at this time. Cholestasis of pregnancy was prevalent in one of the families of the children reported to have an *MDR3* nonsense mutation. There appears to be an association between heterozygosity for this defect and the propensity for the development of cholestasis of pregnancy.[44–46] A similar phenomenon has been observed with fatty liver of pregnancy and heterozygosity for fatty acid oxidation defects.[47] MDR3 defects have been reported in children with neonatal cholestasis, in children and adults with intrahepatic cholelithiasis, and in adults with chronic hepatitis and biliary cirrhosis.[48–52] Therefore, defects in *MDR3* should be considered in any child or adult with high γ-glutamyl transpeptidase levels and cholestasis or biliary cirrhosis of unknown etiology. Supporting clinical, biochemical, and histologic features would include onset in early childhood, significant pruritus, absent serum lipoprotein X, a normal extrahepatic biliary tree, and histologic findings, including bile duct proliferation, portal inflammation, and biliary fibrosis and cirrhosis (see Table 55.6-1). When available, analysis of phospholipid in bile can also support this diagnosis. Immunohistochemical analysis of a percutaneous liver biopsy might someday be a useful screening assay. Ultimately, analysis of genomic DNA and/or liver mRNA will be required to establish a specific genetic defect.

There are important clinical implications of a diagnosis of an MDR3 defect. At present, in its severe form, this appears to be a progressive and unremitting disease, which leads to end-stage liver disease within the first two decades of life, typically in the first decade. Neither ursodeoxycholic acid nor partial biliary diversion or ileal bypass appears to be effective in amelioriating either the pruritus or progression of this disease. Animal models of this disease indicate a potential risk for the development of liver cancer, although this has yet to be described in humans. The tissue distribution of the MDR3 protein appears to be liver specific, and, as such, liver transplant should be and has been curative.[43] Interestingly, animal studies have shown that the functional defect and presumably the disease can be ameliorated by both transgenic expression of a normal *MDR3* gene or by hepatocyte transplant.[42,53] There appears to be a subset of patients with presumably partial defects in MDR3 that are responsive to high-dose ursodeoxycholic acid.[51] Therefore, a trial of ursodeoxycholic acid is warranted in children with cryptogenic and nonobstructive liver disease characterized by high serum γ-glutamyl transpeptidase levels.

DEFECTS IN CANALICULAR ORGANIC ANION TRANSPORT

Dubin-Johnson syndrome was described in the 1950s and has been presumed to be the result of a defect in canalicular bilirubin transport.[54] Transport studies had indicated that a variety of organic anions, including conjugated bilirubin, were transported into the bile canaliculus by an ATP-dependent mechanism.[55] The identification of the specific protein involved in this transport process was aided by a naturally occurring rat species with chronic conjugated hyperbilirubinemia, the TR$^-$ rat.[56,57] Presuming that the canalicular organic anion transporter was homologous to a previously cloned human MRP1, Paulusma and colleagues screened a rat liver complementary DNA library for related gene products.[58] One of the novel isolated genes was highly expressed in liver and ultimately found to be mutated in the TR$^-$ rat. Immunoblot analysis revealed that this new protein, MRP2 (which is also referred to as cMOAT), was absent from canalicular membrane vesicles prepared from the TR$^-$ rat. Subsequent studies demonstrated a different defect in the same gene product in the Eisai hyperbilirubinemic rat.[59] The role of defects of the *MRP2* gene in Dubin-Johnson syndrome was initially complicated by the finding of absent immunostaining for MRP2 at the bile canaliculus but unexpected strong staining at the lateral or basolateral membrane of the hepatocyte.[60]

Dubin-Johnson syndrome is characterized by benign direct hyperbilirubinemia. Diagnosis is made on the finding of mild direct hyperbilirubinemia, with an absence of evidence of hepatocellular or canalicular injury. Urine typically contains an elevated percentage of coproporphyrin isomer I. Liver biopsy reveals no significant changes in hepatic architecture and characteristic melanin-like pigment in hepatocytes. When performed, the sulfobromphthalein test reveals normal hepatic uptake, delayed canalicular excretion, and a secondary peak owing to presumed reflux of dye into plasma. A number of mutations have now been described in the *MRP2/cMOAT* gene in patients with well-characterized Dubin-Johnson syndrome.[61–64] The initial confusion about the persistent basolateral staining for the transporter in these patients has been clarified with the current identification of six *MRP* genes. MRP2-specific antibodies reveal no immunologically recognizable protein in the liver from Dubin-Johnson syndrome patients.[65] The originally described basolateral staining was, in fact, cross-recognition with MRP3.

FIC1 DISEASE (BYLER DISEASE)

Byler disease has been viewed by many as the prototype for a genetic form of biliary transport disorder, yet unlike the other transport defects, its exact molecular pathophysiology remains elusive. Byler disease was first described in the 1960s as a cholestatic disease that affected members of the Amish community who were direct descendants of Jacob Byler and Nancy Kaufmann.[66,67] The disease appears to consist of two phases; the first cholestatic jaundice phase occurs in infancy. The jaundice associated with the first phase typically resolves, although the hallmark pruritus persists. The second phase of the disease includes end-stage liver disease with associated recurrent jaundice and typically becomes manifest at the end of the first decade or some time into the second decade of life. Early investigations into the pathophysiology of this disease included in vivo analysis of bile acid clearance in a patient with Byler disease.[68] Kinetic analysis of these studies pointed to a defect in hepatic excretion of bile salts, and, as such, Byler disease has always been presumed to be the result of defective biliary transport. Subsequent examination of the composition of bile in a separate set of patients supported the concept of a defect in canalicular excretion of bile salts.[69]

The clinical syndrome of Byler disease is characteristic and ultimately permitted genetic studies of a relatively homogeneous patient population. All patients have severe and, if untreated, relatively unremitting pruritus. This is in contrast to benign recurrent intrahepatic cholestasis, where the pruritus is episodic.[70] The pruritus in Byler disease is associated with markedly elevated serum bile salt levels. The biochemical pattern of the cholestasis in Byler disease is quite characteristic and includes relatively normal transaminase levels, variable bilirubin levels depending on the phase of the disease, and normal cholesterol and γ-glutamyl transpeptidase levels. In many ways, the biochemical markers are quite similar to those seen in defects in *BSEP* disease. Severe and sometimes disabling diarrhea and malabsorption may also be present. This appears to be the result of more than simple cholestasis because it may persist after successful liver transplant.[71] Wheezing and elevated sweat chloride levels have been described in some patients with Byler disease.[72,73]

The histologic findings in Byler disease have been carefully documented.[30,74] Prospective correlation of histology with genotyping is needed. Therefore, at present, it is difficult to assess whether the histologic findings that are described are relevant for PFIC in a general sense or to genetically proven Byler disease. It appears that Byler disease leads to a histologically bland form of intracanalicular cholestasis.[30] Histologic findings are clearly dependent on the age of the patient at the time of the biopsy. Early on, minimal giant cell hepatitis with mild portal inflammation is present. Bile duct paucity is not a characteristic finding, although the bile ducts themselves may be small. Over time, fibrosis in the biopsy may progress, and bile duct proliferation may be noted. A characteristic appearance of the bile on electron microscopic analysis (Figure 55.6-4) includes a coarsely particulate and amorphous granular biliary material.[30,75] Immunohistochemical analysis of liver from children with FIC1 deficiency has revealed diminished expression of γ-glutamyl transpeptidase at the bile canaliculus.[25] The specificity of these histologic and ultrastructural findings will need to be reassessed and correlated with genetic abnormalities in the *FIC1* gene (see below).

The approach to the identification of the cause of Byler disease used a genome screening technique referred to as searching for share segment.[76,77] This approach is based on the assumption that there is a common founder mutation in a population and that this mutation is the basis of a well-

characterized disease in a relatively closed community. Byler disease, as described in the Amish community, a priori appeared to fit these criteria quite well. Initially, analysis revealed that the Byler locus was found at chromosome 18q21-q22. Interestingly, this is the same locus that was identified for benign recurrent intrahepatic cholestasis. At the time of this discovery, interesting speculation took place as to the relationship between Byler disease and benign recurrent intrahepatic cholestasis. Possibilities included linked genes, a common regulatory gene, or different mutations with different functional consequences. Ultimately, more refining linkage analysis and complementary DNA library screening led to the identification of a defect in a gene labeled *FIC1* as the cause of both Byler disease and benign recurrent intrahepatic cholestasis.[78] Subsequent clinical studies have revealed that there is a clinical spectrum of disease between benign recurrent intrahepatic cholestasis and Byler disease and the designation of all of these disorders because FIC1 disease is more accurate.[79]

The current challenge is to determine the function of FIC1 and how different mutations lead to the diseases recognized as either benign recurrent intrahepatic cholestasis or Byler disease. Cloning of FIC1 led to the discovery that this gene product has a wide tissue distribution, including mRNA expression in the pancreas, small intestine, stomach, bladder, heart, placenta, lung, liver, and kidney.[78] Surprisingly, expression appears to be greatest in the pancreas and intestine. Immunohistochemistry reveals that it is expressed on the apical surface of many of these cells.[80,81] The range of tissues that express FIC1 might explain many of the systemic manifestations, including wheezing, elevated sweat chloride, and diarrhea. Recent investigations have revealed expression in bile duct epithelium, and this may be as relevant for the hepatic manifestations of the disease.[81] Computer-assisted homology analysis indicates that *FIC1* may be related to a previously cloned bovine adenosine triphosphatase II gene, which appears to have aminophospholipid transporting activity.[82] This type of transport function is critical for perpetuation of the physiologically important assymmetry of phosphatidylserine distribution within the lipid bilayer of a variety of cells. The exact interaction between FIC1 and bile acid transporters is yet to be determined. A mouse model of Byler disease is characterized by abnormal regulation of ileal bile acid transport.[83] Analysis of human ileum indicated that the ileal bile acid transporter (apical sodium dependent bile acid transporter = *SLC10A2*) is relatively overexpressed in children with absent FIC1 expression.[84] This suggests that FIC1 disease may, in part, be the result of a gain of function defect in the ileal bile acid transporter. In rats, indirect immunofluorescence and Western blotting indicate that the FIC1 protein is expressed on the canalicular surface of hepatocytes and the brush border membrane of enterocytes.[80,81] Expression in the intestine appears to be along the entire length of the small intestine. Presumably, abnormalities in FIC1 function alter the assymmetric distribution of phospholipids in the hepatic canalicular and intestinal brush border membrane and secondarily affect the normal function of critical solute transporters. The common link in these abnormalities may be a defect in the activity of the farnesoid X receptor (FXR). The FXR plays a key role in the regulation of genes involved in bile acid biosynthesis and transport.[85,86] Absence of FIC1 in the ileum of patients and in cell lines treated with FIC1 antisense oligonucleotides is associated with a reduction in FXR expression.[84] In cell lines FXR does not translocate into the nucleus when FIC1 is not expressed.[84] These studies suggest that FIC1 activity alters signal transduction pathways involved in FXR nuclear translocation.

Mutational analysis of FIC1 disease patients has revealed specific and distinct homozygous defects in patients of Amish and European origin.[78,87,88] Severe mutations have the potential to disrupt key ATP-binding sites in the FIC1 molecule. In contrast, the defects that have been identified in patients with benign recurrent intrahepatic cholestasis appear to involve less critical residues. As such, the defect in benign recurrent intrahepatic cholestasis may only partially affect FIC1 function and thus might lead to a milder phenotype. A wide variety of distinct mutations have been described in patients with either Byler disease or benign recurrent intrahepatic cholestasis (BRIC), including 13 missense mutations, 1 nonsense mutation, 3 small deletions, 1 large genomic deletion, and 5 splice-site defects.[87] A number of compound heterozygote states have been detected, most of which lead to a phenotype most consistent with BRIC. Homozygosity for one of these defective alleles (G308V) is associated with classic Byler disease, whereas compound heterozygosity of the same allele leads to BRIC. This defect has been introduced into a mouse model of this disease, which is currently under study.[89] Careful analysis of the clinical course of these patients appears to indicate that a spectrum of disease

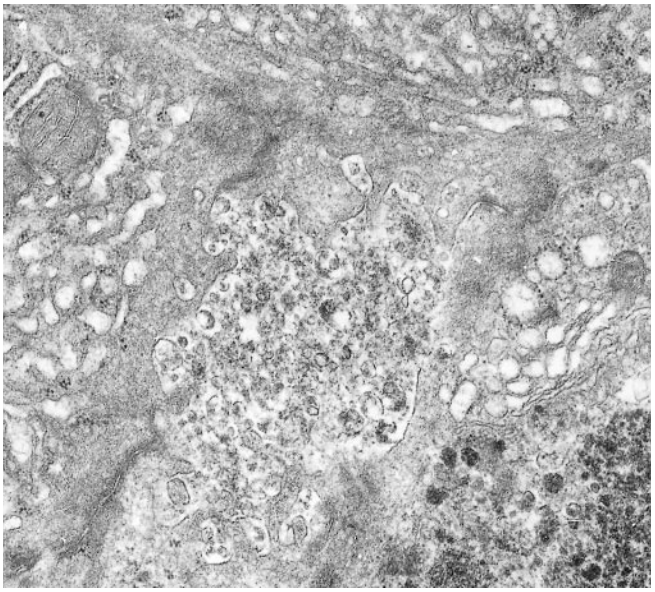

FIGURE 55.6-4 Electron microscopic appearance of Byler disease bile. Coarsely granular bile is seen in a patient with Byler disease (osmium tetroxide/uranyl acetate/lead citrate; ×31,625 original magnification). Courtesy of A. S. Knisely, Institute of Liver Studies, London, UK.

exists between BRIC and Byler disease. Clinical variability has been observed between patients with the same FIC1 genotype, suggesting that environmental influences and/or variable penetrance may exist for this disease.

Correct diagnosis of FIC1 disease has potentially very important clinical and therapeutic consequences. This diagnosis should certainly been considered in the setting of a patient with cholestasis characterized by severe pruritus who has markedly elevated serum bile salts with relatively normal cholesterol and γ-glutamyl transpeptidase. Being a member of the Amish community is highly suggestive but not diagnostic of this disorder.[90] Alternative defective genes have recently been described in Amish children. These children have compound defects involving tight junction proteins and bile acid conjugation enzymes. The slower clinical progression of FIC1 disease may help distinguish it from defects in *BSEP*. The characteristic electron microscopic appearance of bile (with the patient off ursodeoxycholic acid therapy) is highly suggestive of this diagnosis.[30] Immunohistochemical analysis of γ-glutamyl transpeptidase in liver may also be suggestive of a defect in FIC1.[25] Molecular analysis is now available on a research basis and should be sought out to help confirm a defect in *PFIC1*. Absence of intestinal FIC1 expression at the level of either protein or mRNA may be a shortcut to this diagnosis in patients with defects that lead to an unstable or absent mRNA or protein.[84]

The clinical consequences of a correct diagnosis are related to the appropriate choice of therapy. It has become apparent from clinical experience and basic investigations that *FIC1* has a wide distribution of tissue expression, and FIC1 disease, as such, is a systemic disease. Liver transplant is therefore not necessarily curative, unlike the clinical experience in cases of *BSEP* and *MDR3* deficiencies. Persistent diarrhea, graft steatosis, growth failure, and pancreatitis have all been observed after liver transplant in children with FIC1 disease.[71] Instead, surgical approaches that use depletion of the bile salt pool seem to be effective and involve significantly less risk than liver transplant. These methods include partial biliary diversion and partial ileal bypass.[91–98] Both techniques lead to wasting of bile salts and presumed depletion of toxic bile salts, although this explanation is not completely clear. Interruption of the enterohepatic circulation may ameliorate some of the problems associated with the gain of function in the ileal bile acid transporter. Both treatments have been shown to markedly improve pruritus and to stabilize or even improve both the biochemical and histologic manifestations of the disease. Clearly, at this time, these surgical approaches are the treatment of choice for compensated FIC1 disease. Better understanding of the pathophysiology of FIC1 disease might lead to alternative medical approaches, potentially including specific and highly potent bile acid–binding resins or bile acid transport inhibitors.

DISORDERS OF BILIARY TRANSPORT IN SYSTEMIC DISEASE

Jaundice and cholestasis are well-recognized features of conditions that nonspecifically affect the liver. Examples include gram-negative sepsis, administration of parenteral hyperalimentation, drug toxicity, and liver regeneration. The specific effects of these various conditions on transporters involved in bile formation can explain the development of cholestasis and might lead to specific interventions to prevent liver injury. The best understood example is the effect of gram-negative sepsis. The association between jaundice and cholestasis[99] and gram-negative sepsis has been recognized for a long time.[100,101] The molecular basis of this is now beginning to be understood. Gram-negative sepsis is associated with circulation of endotoxin, which leads to the formation of tumor necrosis factor-α and a range of inflammatory cytokines (eg, interleukins 1 and 6). Via specific interactions with *cis* elements in the promoter of the basolateral bile acid transporter, *NTCP* is down-regulated.[99,102–104] Canalicular transport of bile acids and organic anions also appears to be impaired by sepsis and is mediated by down-regulation in *MRP2/cMOAT* and *BSEP*.[105–107] The molecular effects of the administration of parenteral hyperalimentation, drug toxicities, and hepatic regeneration are currently under investigation.

CLINICAL APPROACH TO COMPLEX FORMS OF CHOLESTASIS

In the current era, the clinician is faced with an evolving and complex series of potential diagnostic and therapeutic approaches for children and adults with nontypical forms of cholestasis. An algorithmic approach to the evaluation of these nonstandard forms of cholestatic liver disease is suggested in Figure 55.6-5. The presence of pruritus and/or significantly elevated serum bile acid levels in combination with measurement of serum γ-glutamyl transpeptidase may be useful in the initial categorization of the potential molecular process underlying the particular cholestatic condition. Supplemental assays including electron microscopy, biliary bile acid and biliary phospholipid analysis, serum lipoprotein X measurement, and urine coproporphyrin analysis may support a potential molecular diagnosis. In the current era, molecular diagnostics should be strongly considered part of the comprehensive evaluation of the patient with complex cholestasis. Given the divergent potential treatments for these distinct disorders, this degree of sophisticated diagnostic testing appears warranted.

FUTURE DIRECTIONS

Our current understanding of biliary transport defects is clearly in its infancy. A variety of areas of investigation will need to be explored. New molecular mechanisms of cholestasis and genetic defects leading to biliary transport disorders are yet to be discovered. The full range of the clinical phenotypes of the currently described disorders needs to be discerned. The role of partially functioning transporters or heterozygote states in commonly occurring but poorly understood cholestatic conditions, like that seen during parenteral hyperalimentation, will need to be investigated.

Bile excretion by the liver is a critical function, and, as such, its underlying molecular mechanisms are complex.

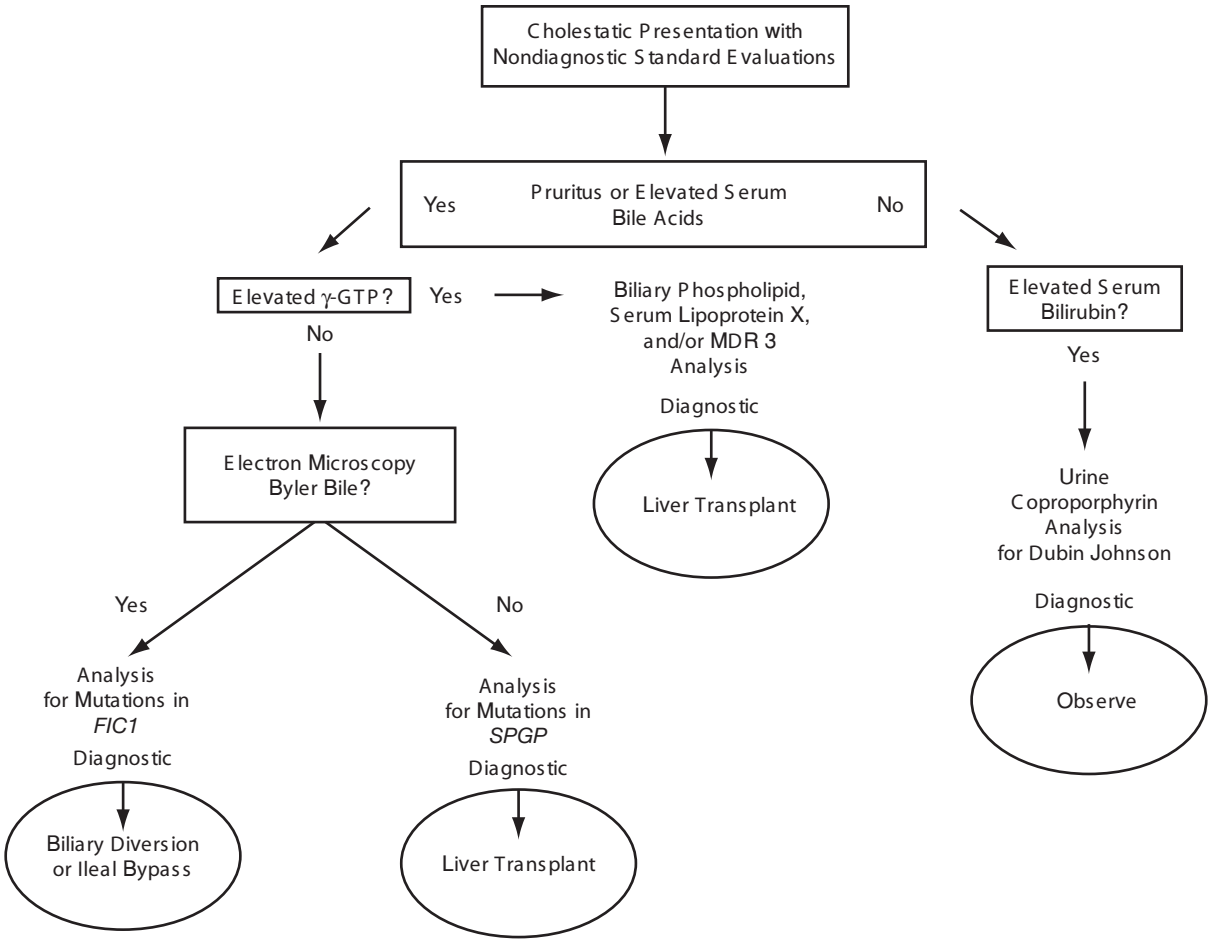

FIGURE 55.6-5 Diagnostic and therapeutic algorithm for complex cholestasis. GTP = γ-glutamyl transpeptidase; MDR = multidrug resistance; *SPGP* = sister of P-glycoprotein.

Significant achievements have been made in understanding the process of bile excretion, but a great deal remains undiscovered. Examples of areas of ongoing research include the exact pathways involved in the intracellular translocation of components of bile from the basolateral membrane to the bile canaliculus, the role of the cytoskeleton in canalicular function, the mechanisms of regulation of canalicular transport proteins, and the functional consequences of bile duct transport processes on the formation of bile. It is expected that molecular defects in each of these important pathways and mechanisms of regulation of bile formation may lead to cholestatic liver disease.

A number of cholestatic phenotypes exist for which a molecular mechanism remains elusive. A third locus for low γ-glutamyl transpeptidase cholestasis appears to exist.[108] Aagenaes syndrome is another form of inherited cholestasis that consists of periods of severe pruritus but has the additional finding of lower extremity lymphedema.[109] A syndrome of arthrogryposis, renal tubular acidosis, and cholestasis with normal γ-glutamyl transpeptidase levels has been reported in 11 kindreds, and the molecular defect has recently been described.[110,111] Rotor syndrome, like Dubin-Johnson syndrome, is characterized by persistent direct hyperbilirubinemia with no significant intrahepatic liver injury.[112] Distinguishing features include the absence of hepatic pigmentation and the urinary pattern of coproporphyrins. Neonatal sclerosing cholangitis is a relatively rare form of neonatal cholestasis and has been linked to a new and unique genetic locus (R. Thompson, personal communication, 1999).[113] Genetic, molecular, and physiologic studies of these syndromes are likely to expand our knowledge of disorders of biliary transport.

The current clinical presentations of the various disorders of biliary transport that have been described in this chapter probably do not represent the full spectrum of the clinical phenotypes that may exist. As the molecular etiology of a disease is discovered, the clinical spectrum of the disease in question changes significantly, and, in some ways, the definition of the original disease comes into question. Two recent noteworthy examples are cystic fibrosis and Alagille syndrome.[114–116] Abnormalities in the *CFTR* gene have recently been described in patients with absent vas deferens or chronic pancreatitis who do not have the usual clinical features that are characteristic of cystic fibrosis.[117–119] Similarly, isolated cardiac anomalies have been described in patients with mutations in the *JAGGED1* gene, yet these patients do not meet the standard defining criteria for a diagnosis of Alagille syndrome.[120,121] It will therefore be very important to examine the level of expression and genotype of the various newly described

transport genes in a spectrum of pediatric liver diseases in which the etiology is currently unknown.

In a related area of investigation, it will be important to carry out similar investigations of the level of expression and genotype of transporter genes in what are currently felt to be relatively well-understood disorders. Many cholestatic liver diseases have well-described phenotypes and are labeled with well-entrenched names, but have basic etiologies that are not well understood. Primary sclerosing cholangitis, total parenteral hyperalimentation, and drug-related cholestasis are potential examples. Many of the features of primary sclerosing cholangitis, especially the intrahepatic form, are akin to defects in *MDR3*. Predisposition to the development of cholestasis during total parenteral hyperalimentation may, in some cases, be related to heterozygote status for defects in one of the biliary transport genes. Alternatively, patients might be homozygotes or compound heterozygotes for mutations that lead to partial inactivation of the transporter. Similarly, drug-related cholestasis might be the result of partially dysfunctional genotypes, especially those that involve the *MRP2/cMOAT* gene. The recent description of cholestasis of pregnancy being related to heterozygote status for a defect in the *MDR3* gene is a good example of the potential implications of this process.[44]

SUMMARY

A new era in the understanding of cholestatic liver disease in children has been ushered in by the discovery of a variety of gene products that play a key role in the formation of bile. Defects in the canalicular transporters for bile acids, organic anions, and phospholipids all lead to distinct forms of cholestatic liver disease, whose onset is during early childhood. Liver transplant at present remains the best long-term approach to the treatment of defects in bile acid or phospholipid excretion. Byler disease is distinct from these disorders and involves a defect in a gene whose exact function in bile formation remains a subject of intense investigation. Clinical and laboratory studies indicate that Byler disease is a systemic disease, and, as such, liver transplant may not be curative. Partial biliary diversion or ileal bypass may be more appropriate means of treatment of this disease. Our understanding of biliary transport disorders is in its infancy, and the future holds great promise for further understanding of the mechanisms of and optimal treatment for cholestatic liver disease in children.

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7. α_1-Antitrypsin Deficiency

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The classic form of α_1-antitrypsin (α_1-AT) deficiency, homozygous for the mutant α_1-*ATZ* allele, is associated with premature development of pulmonary emphysema and, in some cases, chronic liver disease. This deficiency is the most common metabolic cause of emphysema in adults and liver disease in children and is the most common metabolic disease for which children undergo liver transplant (United Network for Organ Sharing Data Request Service, personal communication, 2000). In the most extensively studied population (the Swedish population), the incidence of the deficiency is approximately 1 in 1,639 live births.[1] Data from eight separate studies suggest that the prevalence of α_1-AT deficiency in the United States is 1 in approximately 2,000 individuals.[2] It especially affects whites of northern European ancestry.[3,4]

α_1-AT is an approximately 55 kD secretory glycoprotein that inhibits destructive neutrophil proteases, including elastase, cathepsin G, and proteinase 3. Plasma α_1-AT is predominantly derived from the liver and increases three- to fivefold during the host response to tissue injury or inflammation. It is the archetype of a family of structurally related circulating serine protease inhibitors termed serpins.

In the deficient state, there is ~ 85 to 90% reduction in serum concentrations of α_1-AT. A single amino acid substitution results in an abnormally folded protein that is unable to traverse the secretory pathway. This α_1-ATZ protein is retained in the endoplasmic reticulum (ER) rather than secreted into the blood and body fluids.

Although it does not occur until adulthood, many α_1-AT–deficient individuals develop destructive lung disease and emphysema. Most of the data in the literature indicate that emphysema results from a decreased number of α_1-AT molecules within the lower respiratory tract, allowing unregulated elastolytic attack on the connective tissue matrix of the lung.[5,6] Oxidative inactivation of residual α_1-AT as a result of cigarette smoking accelerates lung injury.[7] Moreover, the elastase-antielastase theory for the pathogenesis of emphysema is based on the concept that oxidative inactivation of α_1-AT as a result of cigarette smoking plays a key role in the emphysema of α_1-AT–sufficient individuals, the vast majority of emphysema patients.[5,8]

It has been more difficult to explain the pathogenesis of liver injury in this deficiency. The results of transgenic animal experiments have provided strong evidence that liver disease does not result from a deficiency in antielastase activity.[9,10] Most of the data in the literature corroborate the concept that liver injury in α_1-AT deficiency results from the hepatotoxic effects of retention of the aggregated mutant α_1-ATZ molecule in the ER of liver cells. Nationwide prospective screening studies done by Sveger in Sweden have shown that only 10 to 15% of the deficient population develop clinically significant liver disease over the first 20 years of life.[1,11] These data indicate that other genetic traits and/or environmental factors predispose a subgroup of deficient individuals to liver injury.

The diagnosis of α_1-AT deficiency is based on the altered migration of the abnormal α_1-ATZ molecule in serum specimens subjected to isoelectric-focusing gel analysis. Treatment of liver disease associated with α_1-AT deficiency is mostly supportive. Liver replacement therapy has been used successfully for severe liver injury. Although clinical efficacy has not been demonstrated, many patients with emphysema owing to α_1-AT deficiency are currently being treated by intravenous or intratracheal aerosol administration of purified plasma α_1-AT. An increasing number of patients with severe emphysema have undergone lung transplant. Several new pharmacologic and genetic strategies for prophylaxis of both liver and lung disease are currently under development for clinical application.

STRUCTURE OF α_1-ANTITRYPSIN

α_1-AT is encoded by a single approximately 12.2 kb gene on human chromosome 14q31-32.3.[12–15] There is a sequence-related gene 12 kb downstream from this gene.[14,16–18] Because there is no evidence that the sequence-related gene is expressed, it is considered a pseudogene. The genes for three other members of the serpin family, α_1-antichymotrypsin, protein C inhibitor, and corticosteroid-binding globulin, are also closely linked on chromosome 14.[15,19]

The α_1-AT gene (Figure 55.7-1) is organized in seven exons and six introns.[12,20] The first three exons and a short 5′ segment of the fourth exon code for 5′ untranslated regions of the α_1-AT messenger ribonucleic acid (mRNA). The first two exons and a short 5′ segment of the third exon are included in the primary transcript in macrophages but not in hepatocytes, accounting for a slightly longer mRNA. There are, in fact, two mRNA species in macrophages, depending on alternative post-transcriptional splicing pathways involving one of the first two most 5′ exons.[20,21] Most of the fourth exon and the remaining three exons encode the protein sequence of α_1-AT. There is a 72-base sequence that constitutes the 24–amino acid amino-terminal signal sequence. There are three sites for asparagine-linked carbohydrate attachment: residues 46, 83, and 247. All three are used for post-translational glycosylation. The active

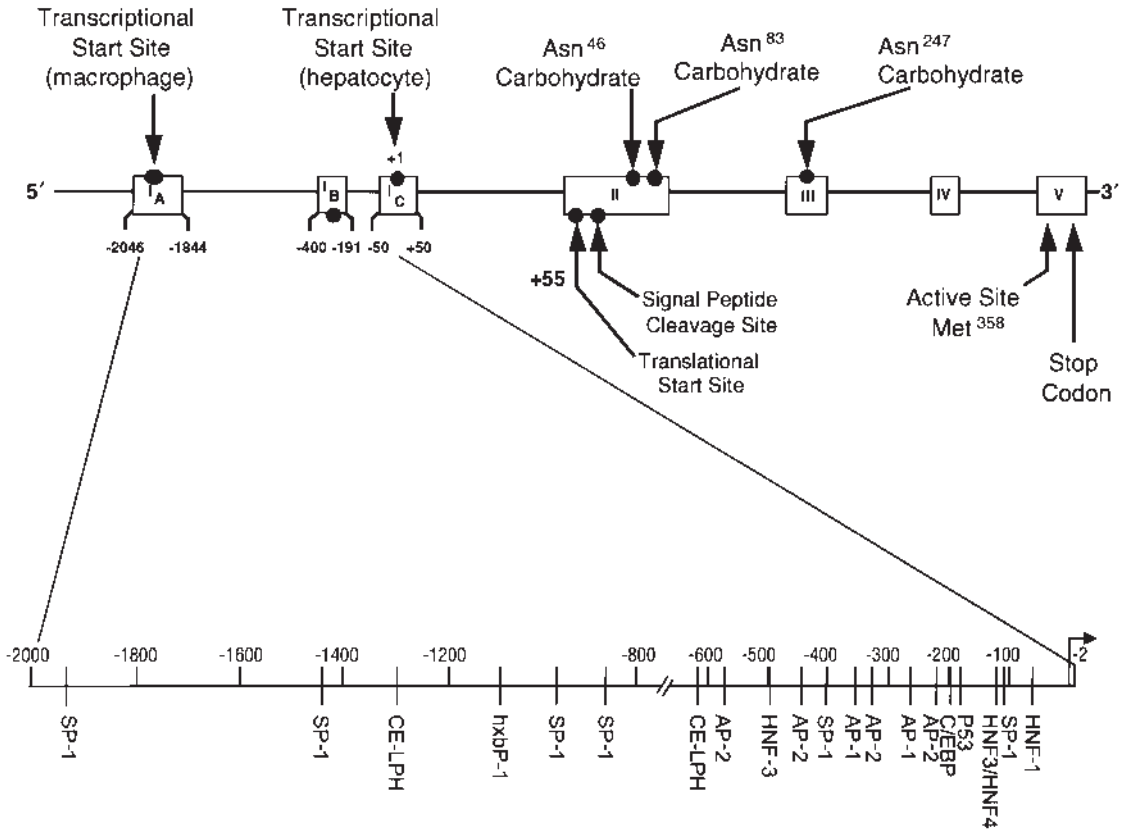

FIGURE 55.7-1 Schematic representation of the structure of the α_1-antitrypsin gene (not to scale) and map of potential regulatory elements based on its sequence.

site, so-called P_1 residue, Met 358, is encoded within the seventh exon (exon V).

The α_1-AT protein is a single-chain, approximately 52 to 55 kD polypeptide with 394 amino acids and three asparagine-linked complex carbohydrate side chains.[22] There are two major isoforms in serum, depending on the presence of a biantennary or triantennary configuration for the carbohydrate side chains.[23] X-ray crystallography studies have shown that α_1-AT has a globular shape and a highly ordered internal domain composed of two central β sheets surrounded by a small β sheet and nine α helices.[24,25] The dominant structure is the five-stranded β-pleated sheet termed the A sheet (Figure 55.7-2).

α_1-AT is the archetype of a family of structurally related proteins called serpins that includes antithrombin III, α_1-antichymotrypsin, C1 inhibitor, α_2-antiplasmin, protein C inhibitor, heparin cofactor II, plasminogen activator inhibitors I and II, protease nexin I, ovalbumin, angiotensinogen, corticosteroid-binding globulin, and thyroid-binding globulin.[25,26] These proteins share about 25 to 40% primary structural homology with higher degrees of regional homology in functional domains. Most serpins function as suicide inhibitors by forming equimolar complexes with a specific target protease. Other serpins are not inhibitory. For instance, corticosteroid and thyroid hormone–binding globulins, which are thought to represent carriers for corticosteroid and thyroid hormone, respectively, form complexes with, but do not inactivate, their hormone ligands.

A comparison of α_1-AT with other members of the serpin supergene family has generated several important concepts about the structure and function of α_1-AT. For instance, the reactive site P1 residue of α_1-AT is localized to a canonical loop that rises above the gap in the center of the A sheet (see Figure 55.7-2).[27,28] This loop may provide a certain degree of flexibility to the functional activity of the inhibitor. The reactive loop conformation of serpins is also thought to make them susceptible to proteolytic cleavage by thiolenzymes and metalloenzymes. The P1 residue itself is the most important determinant of functional specificity for each serpin molecule. This concept was dramatically confirmed by the discovery of α_1-AT Pittsburgh, a variant in which the P1 residue of α_1-AT, Met 358, is replaced by Arg 358. In this variant, α_1-AT functions as a thrombin inhibitor, and severe bleeding diathesis results.[29]

The carboxyl-terminal fragment of α_1-AT and the other serpins also bears important structural and functional characteristics. There is a much higher degree of sequence homology among serpins in the carboxyl terminus. A small fragment at this terminus is cleaved during formation of the inhibitory complex with serine protease. This carboxyl-terminal fragment possesses chemotactic activity.[30,31] Moreover, this fragment bears the receptor-binding domain for cell surface binding, internalization of α_1-AT elastase and other serpin-enzyme complexes, and activating a signal transduction pathway for up-regulation of α_1-AT gene expression.[32,33]

Protease Inhibitor System for Classification of Structural Variants of α_1-Antitrypsin

Variants of α_1-AT in humans are classified according to the protease inhibitor phenotype system as defined by agarose electrophoresis at acid pH or isoelectric focusing of plasma in polyacrylamide.[34] The protease inhibitor classification assigns a letter to variants according to the migration of the major isoform, in alphabetic order from anode to cathode or from low to high isoelectric point. For example, the most common normal variant migrates to an intermediate isoelectric point, designated M. Individuals with the most common severe deficiency have an α_1-AT allelic variant that migrates to a high isoelectric point, designated Z. Using restriction fragment length and direct deoxyribonucleic acid (DNA) sequence analysis together with isoelectric focusing, more than 100 allelic variants of α_1-AT have been reported.[35]

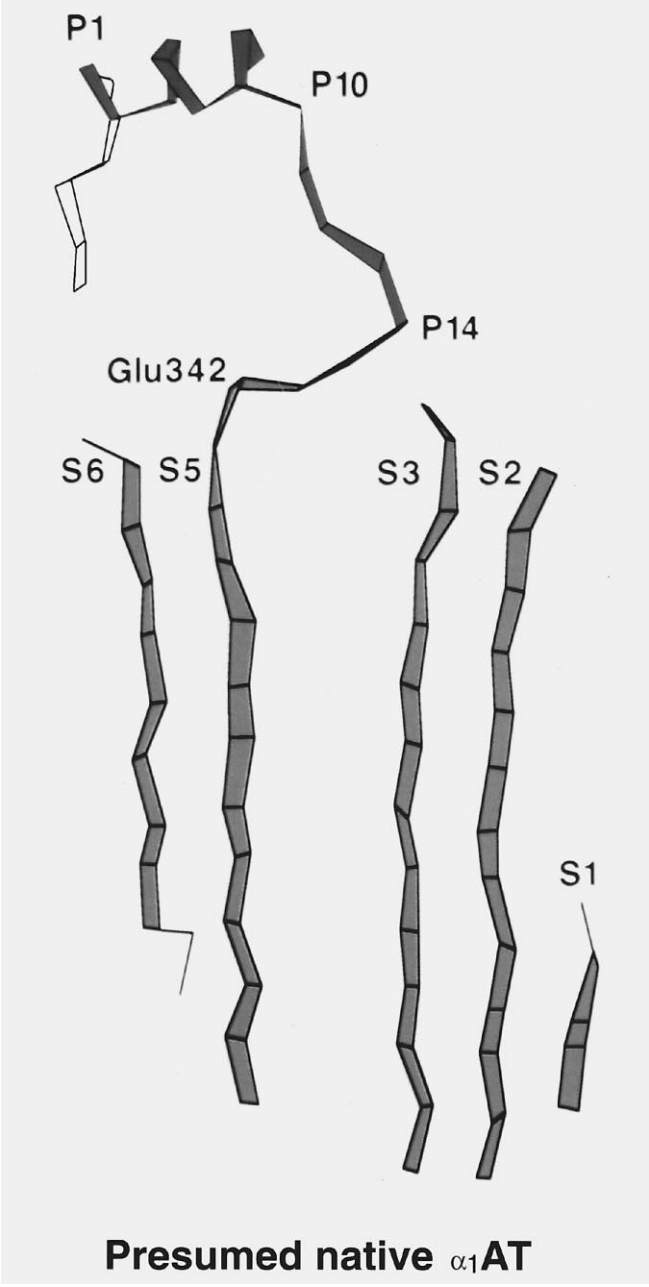

FIGURE 55.7-2 Ribbon diagram of the A sheet and reactive center loop of native α_1-antitrypsin (AT). Because native α_1-AT has not been crystallized, this ribbon diagram is generated by computer models, based on the crystal structures of cleaved α_1-AT and native ovalbumin. The reactive center loop is shown in dark gray (magenta). Residues P10 and P14 are numbered from the reactive-site methionine P1. The carboxyl-terminal fragment is shown as a white ribbon. Beta-helices of the A sheet are shown as light gray (blue) ribbons and referred to as S1, S2, S3, S5, and S6 (for color figure, see CD-ROM). The Glu 342 residue that is replaced by Lys in α_1-ATZ is designated. Adapted from Carrell RW, Evans DL, Stein DE. Mobile reactive centre of serpins and the control of thrombosis. Nature 1991;353:576.

Normal Allelic Variants. The most common normal variant of α_1-AT is termed M_1 and is found in 65 to 70% of whites in the United States.[35] A restriction fragment length polymorphism may further subdivide individuals with the classic M_1 allele.[36] The normal M_3 allele, which differs from M_1 by a single-base change,[35] is found in approximately 10% of the same population. The M_2 allele, characterized by an additional base change from the M_3 sequence, occurs in 15 to 20% of the white population.[37,38] There are many rare normal allelic variants with allelic frequencies of less than 0.1%.[38–42] In each case, these variants are associated with serum concentrations of and functional activity for α_1-AT within the normal range.[1]

Null Allelic Variants. Variants in which α_1-AT is not detectable in serum are called null allelic variants (Table 55.7-1) and, when inherited with another null variant or deficiency variant, are associated with premature development of emphysema.[43] Several types of defects, including insertions and deletions, appear to be responsible for these variants. In two cases, α_1-AT $Null_{Isola\ di\ procida}$ and α_1-AT $Null_{Reidenburg}$, there is deletion of all α_1-AT coding regions.[44,45] In two other cases, α_1-AT $Null_{Bellingham}$ and α_1-AT $Null_{Granite\ Falls}$, α_1-AT mRNA is undetectable.[46–49] Three other null alleles result in truncated proteins that are degraded in the ER—$Null_{Mattawa}$, $Null_{Hong\ Kong}$, and $Null\text{-}_{Clayton}$.[50–53] A single-base substitution has been discovered in the $Null_{Ludwigshafen}$ allele.[54] A recent study suggests that this mutant α_1-AT molecule is synthesized and secreted in transfected heterologous cells, but there is a slight decrease in its rate of secretion, and it completely lacks functional activity.[55] It is not yet known whether instability or accelerated catabolism in vivo is the explanation for the inability to detect this mutant α_1-AT molecule in serum specimens.

Dysfunctional Variants. Dysfunctional variants of α_1-AT include $\alpha_1\text{-}AT_{Pittsburgh}$.[29] There also is a decrease in serum concentration and functional activity for α_1-AT $M_{Mineral\ Springs}$.[56] For several variants that have been identified in compound heterozygotes, such as α_1-AT F,[57] α_1-AT $Null_{Newport}$, and α_1-AT $Z_{Wrexham}$,[58] it is not clear whether the variants result in normal, null, deficient, or dysfunctional changes.

Deficiency Variants. Several variants of α_1-AT associated with a reduction in serum concentrations of α_1-AT have been described and are called deficiency variants

TABLE 55.7-1 NULL VARIANTS OF α_1-ANTITRYPSIN

DEFECT			CLINICAL DISEASE		
			LIVER	LUNG	CELLULAR DEFECT
$Null_{Granite\ Falls}$	Single base deletion	Tyr 160	–	+	No detectable RNA
$Null_{Bellingham}$	Single base deletion	Lys 217	–	+	No detectable RNA
$Null_{Mattawa}$	Single base insertion	Phe 353	–	+	?IC degradation
$Null_{Hong\ Kong}$	Dinucleotide deletion	Leu 318	–	+	IC accumulation
$Null_{Ludwigshafen}$	Single base substitution	Isoleu 92-Asp	–	+	Dysfunctional protein (?EC degradation)
$Null_{Clayton}$	Single base insertion	Glu 363	–	+	?IC degradation
$Null_{Bolton}$	Single base deletion	Glu 363	–	+	?IC degradation
$Null_{Isola\ di\ Procida}$	Deletion	Exons II-V	–	+	Unknown
$Null_{Riedenburg}$	Deletion	Exons II-V	–	+	Unknown
$Null_{Newport}$	Single base substitution	Gly 115-Ser	–	+	Unknown
$Null_{bonny\ blue}$	Intron deletion	—	–	+	Unknown
$Null_{new\ hope}$	Two base substitutions	Gly 320-Glu Glu 342-Lys	–	+	Unknown
$Null_{Trastavere}$	Single base substitution	Trp 194-stop	–	+	Unknown
$Null_{Kowloon}$	Single base substitution	Tyr 38-stop	–	+	Unknown
$Null_{Saarbruecken}$	Single base insertion	Pro 362-stop	–	+	Unknown
$Null_{Lisbon}$	Single base substitution	Thr 68-Ile	–	+	Unknown
$Null_{West}$	Intron deletion	—	–	+	Unknown

EC = extracellular; IC = intracellular; RNA = ribonucleic acid.

(Table 55.7-2). Some of these variants, such as the S variant, are not associated with clinical disease.[12,59,60] Other deficiency variants are associated with emphysema, such as $M_{Heerlen}$,[61,62] $M_{Procida}$,[63] M_{Malton},[64,65] M_{Duarte},[66] $M_{Mineral\ Springs}$,[56] P_{Lowell},[42] and $W_{Bethesda}$.[67] In two persons with M_{Malton} and one with M_{Duarte}, hepatocyte α_1-AT inclusions and liver disease have been reported.[64,66,68] In one person with the deficiency variant S_{Iiyama}, emphysema and hepatocyte inclusions were reported, but this person did not have liver disease.[69]

FUNCTION

α_1-AT is an inhibitor of serine proteases in general, but its most important targets are neutrophil elastase, cathepsin G, and proteinase 3, proteases released by activated neutrophils. Several lines of evidence suggest that inhibition of neutrophil elastase is the major physiologic function of α_1-AT. First, individuals with α_1-AT deficiency are susceptible to premature development of emphysema, a lesion that can be induced in experimental animals by instillation of excess amounts of neutrophil elastase in the airways.[70] In fact, these observations have led to the concept that destructive lung disease may result from perturbations of the net balance of elastase and α_1-AT within the local environment of the lung.[5] Second, the kinetics of association of α_1-AT and neutrophil elastase are more favorable by several orders of magnitude than those for α_1-AT and any other serine protease.[71] Third, α_1-AT constitutes more than 90%

TABLE 55.7-2 DEFICIENCY VARIANTS OF α_1-ANTITRYPSIN

DEFECT			CLINICAL DISEASE		
			LIVER	LUNG	CELLULAR DEFECT
Z	Single base substitution M_1 (Ala 213)	Glu 342-Lys	+	+	IC accumulation
S	Single base substitution	Glu 264-Val	–	–	IC accumulation
$M_{Heerlen}$	Single base substitution	Pro 369-Leu	–	+	IC accumulation
$M_{Procida}$	Single base substitution	Leu 41-Pro	–	+	IC accumulation
M_{Malton}	Single base deletion	Phe 52	?	+	IC accumulation
M_{Duarte}	Unknown	Unknown	?+	+	Unknown
$M_{Mineral\ Springs}$	Single base substitution	Gly 57-Glu	–	+	No function; ?EC degradation
S_{Iiyama}	Single base substitution	Ser 53-Phe	–	+	IC accumulation
P_{Duarte}	Two base substitutions	Arg 101-His	?+	+	Unknown
		Asp 256-Val	+	+	
P_{Lowell}	Single base substitution	Asp 256-Val	–	+	IC accumulation; reduced function
$W_{Bethesda}$	Single base substitution	Ala 336-Thre	–	+	?EC degradation
$Z_{Wrexham}$		Ser 19-Leu	?	?	Unknown
F	Single base substitution	Arg 223-Cys	–	–	Unknown
T	Single base substitution	Glu 264-Val	–	–	Unknown
I	Single base substitution	Arg 39-Cys	–	–	IC accumulation; reduced function
$M_{palermo}$	Single base deletion	Phe 51	–	–	Unknown
$M_{nichinan}$	Single base deletion and single base substitution	Phe 52 Gly 148-Arg	–	–	Unknown
$Z_{ausburg}$	Single base substitution	Glu 342-Lys	–	–	Unknown

EC = extracellular; IC = intracellular.

of the neutrophil elastase inhibitory activity in the one body fluid that has been examined, pulmonary alveolar lavage fluid.

α_1-AT acts competitively by allowing its target enzymes to bind directly to a substratelike region within the carboxyl-terminal region of the inhibitor molecule. This reaction between enzyme and inhibitor is essentially second order, and the resulting complex contains one molecule of each of the reactants. A peptide bond in the inhibitor is hydrolyzed during formation of the enzyme-inhibitor complex. However, hydrolysis of this reactive site peptide bond does not proceed to completion. An equilibrium near unity is established between complexes in which the reactive site peptide bond of α_1-AT is intact (native inhibitor) and complexes in which this peptide bond is cleaved (modified inhibitor). The complex of α_1-AT and serine protease is a covalently stabilized structure that is resistant to dissociation by denaturing compounds, including sodium dodecyl sulfate and urea. The interaction between α_1-AT and serine protease is suicidal in that the modified inhibitor is no longer able to bind and/or inactivate enzyme. Studies have now shown that the irreversible trapping of target enzyme is mediated by a profound conformational change in α_1-AT such that the cleaved reactive loop, with bound enzyme, inserts into the gap in A sheet (see Figure 55.7-2).[72] Carrell and Lomas have likened the inhibitory mechanism to a "mousetrap, with the active inhibitor circulating in a metastable, stressed-form and then springing into the stable relaxed form to lock the complex with its target protease."[72] The protease is crushed and inactivated during this structural transition.[73]

The net functional activity of α_1-AT in complex biologic fluids may be modified by several factors. First, the reactive site methionine of α_1-AT may be oxidized and thereby rendered inactive as an elastase inhibitor.[74] In vitro, α_1-AT is oxidatively inactivated by oxidants released by activated neutrophils and alveolar macrophages of cigarette smokers.[75,76] Second, the functional activity of α_1-AT may be modified by proteolytic inactivation. Several members of the metalloprotease family (including collagenase and *Pseudomonas* elastase) and the thiol protease family can cleave and inactivate α_1-AT.[77] DNA released by dying cells at sites of tissue injury or inflammation has the capacity to interfere with the protease inhibitory activity of α_1-AT. [78]

Although α_1-AT from the plasma[79] or liver[80] of individuals with α_1-AT deficiency is functionally active, there may be a decrease in its specific elastase inhibitory capacity. Ogushi and colleagues have shown that the kinetics of association with neutrophil elastase and the stability of complexes with neutrophil elastase were significantly decreased for α_1-AT from deficient plasma.[81] There was no decrease in functional activity of α_1-AT from individuals homozygous for the α_1-ATS allelic variant.

Several studies have indicated that α_1-AT protects experimental animals from the lethal effects of tumor necrosis factor.[82,83] Most of the evidence from these studies indicates that this protective effect is due to inhibition of the synthesis and release of platelet-activating factor from neutrophils,[83,84] presumably through the inhibition of neutrophil-derived proteases. A recent report has suggested that α_1-AT inhibits infectivity of human immunodeficiency virus (HIV) type 1 and its production by infected cells.[85]

α_1-Antitrypsin also appears to have functional activities that do not involve the inhibition of neutrophil proteases. The carboxyl-terminal fragment of α_1-AT, which can be generated during the formation of a complex with serine protease or during proteolytic inactivation by thiol- or metalloproteases, is a potent neutrophil chemoattractant.[30,31,86] This fragment also appears to activate mononuclear phagocytes with respect to production of cytokines, active oxygen intermediates, scavenger activity, and lipid metabolism.[87]

There are several reports of α_1-AT altering immune function through effects on lymphocytes.[88,89] However, there are inherent conflicts in some of the reports, and the data have not been duplicated. There is no evidence that the immune response is systemically altered in α_1-AT–deficient individuals.

BIOSYNTHESIS AND REGULATION OF α_1-ANTITRYPSIN

The predominant site of synthesis of plasma α_1-AT is the liver. This is most clearly shown by conversion of plasma α_1-AT to donor phenotype after orthotopic liver transplant.[90,91] It is synthesized in human hepatoma cells as a 52 kD precursor, undergoes post-translational, dolichol phosphate–linked glycosylation at three asparagine residues, and undergoes tyrosine sulfation.[92–94] It is secreted as a 55 kD native single-chain glycoprotein with a half-time for secretion of 35 to 50 minutes.

Tissue-specific expression of α_1-AT in human hepatoma cells is directed by structural elements within a 750-nucleotide region upstream of the hepatocyte transcriptional start site in exon Ic (see Figure 55.7-1). Within this region, there are structural elements that are recognized by nuclear transcription factors, including hepatocyte nuclear factor (HNF)-1α and HNF-1β (–70 to –57), C-EBP (–86 to –75), HNF-4 (–134 to –100), and HNF-3 (–195 to –185).[95]

Of these factors, HNF-1α and HNF-4 appear to be particularly important for expression of the human α_1-AT gene. Two distinct regions within the proximal element bind these two transcription factors. In fact, substitution of five nucleotides at positions –77 to –72 disrupts binding of HNF-1α and dramatically reduces expression of the human α_1-AT gene in the liver of transgenic mice.[96] Substitution of four nucleotides at positions –118 to –115 disrupts the binding of HNF-4 but does not alter expression of the human α_1-AT gene in the liver of adult transgenic mice. The latter mutation does result in a reduction in the expression of human α_1-AT in the liver during embryonic development. HNF-1α and HNF-4 have a synergistic upregulating effect on expression of the α_1-AT gene in hepatocytes and enterocytes.[97]

Several elements in the upstream flanking region have enhancer activity. There is a strong enhancer element located approximately 200 nucleotides upstream of the

transcriptional start site, but the element is not specific for hepatocyte transcription.[98] This element is identical to the binding site for transcription factor AP-1. Similar AP-1 binding sequences have been identified in the 5′ flanking region of metallothioneins I and IIa, sv40, retinol-binding protein, collagenase, and stromelysin. Transcription factor AP-1 is also thought to be one of the transcription factors that mediate the effects of phorbol esters and thus of the activation of protein kinase C.[99] It represents a complex of several different proteins, including proteins encoded by the proto-oncogenes c-*jun* and c-*fos*.[100] There is a region with weak enhancer activity at residues –488 to –356,[98] but it is not clear whether this is due to transcription factor AP-1, AP-2, SP-1, HNF-3, or another transcription factor with a consensus element in this region. There are also several regions with similarity to the recognition element for transcription factor IL-6DBP (also called H-APF-2, NF-IL-6, LAP/LIP) at –195 to –189 and –169 to –164 from the hepatocyte α_1-AT cap site and –178 to –169 from the macrophage α_1-AT cap site, which may explain the effect of interleukin (IL)-6 on α_1-AT gene expression.[21]

Plasma concentrations of α_1-AT increase three- to five-fold during the host response to inflammation and/or tissue injury.[101] The source of this additional α_1-AT has always been considered the liver; thus, α_1-AT is known as a positive hepatic acute-phase reactant. Synthesis of α_1-AT in human hepatoma cells (HepG2, Hep3B) is up-regulated by IL-6 but not by IL-1 or tumor necrosis factor.[102] Plasma concentrations of α_1-AT also increase during oral contraceptive therapy and pregnancy.[103]

α_1-AT is also synthesized and secreted in primary cultures of human blood monocytes as well as bronchoalveolar and breast milk macrophages.[104] The cellular defect in homozygous α_1-AT deficiency, the selective defect in secretion of α_1-AT, is expressed in monocytes and macrophages from deficient individuals.[105] Transcription of the α_1-AT gene in macrophages starts about 2 kb upstream from the start site used in hepatocytes.[21,22,106] Although the same polypeptide is synthesized in the two cell types, slightly longer mRNA transcripts are present in macrophages (see Figure 55.7-1), depending on alternative post-transcriptional splicing of two upstream short open reading frames.[21,22]

Expression of α_1-AT in monocytes and macrophages is profoundly influenced by products generated during inflammation, such as bacterial lipopolysaccharide[107,108] and IL-6[102] (Figure 55.7-3). Bacterial lipopolysaccharide mediates a 5- to 10-fold increase in synthesis of α_1-AT in mononuclear phagocytes, predominantly increasing the translation efficiency of α_1-AT mRNA. The translational regulation of α_1-AT by lipopolysaccharide therefore involves a mechanism analogous to that of the yeast gene *GCN4* during amino acid starvation and that of the human ferritin gene in response to iron. The analogy to yeast *GCN4* is interesting in that both macrophage α_1-AT mRNA and *GCN4* mRNA have multiple short open reading frames with initiation codons in the upstream untranslated regions.[21,22,106] These sequences have been shown to control the translation of the yeast *GCN4* gene product, both under basal conditions and in response to amino acid starvation.[109]

Synthesis of α_1-AT in liver cells and mononuclear phagocytes is also regulated by a feed-forward mechanism (Figure 55.7-4). In this regulatory loop, α_1-AT–elastase complexes mediate an increase in synthesis of α_1-AT through the interaction of a pentapeptide domain in the carboxyl-terminal tail of α_1-AT with a novel cell

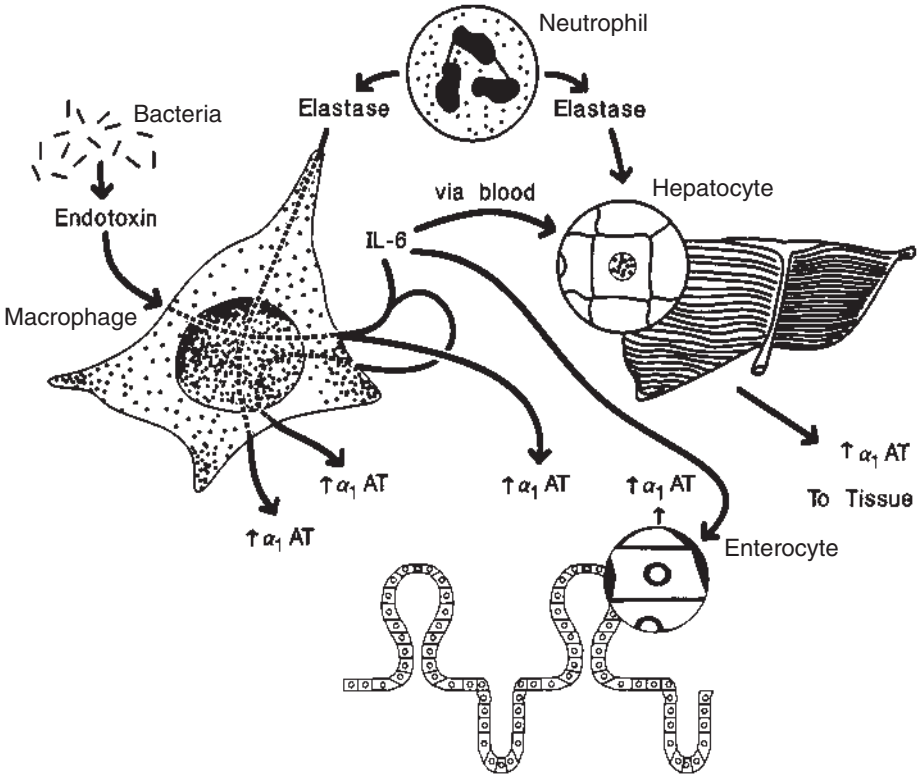

FIGURE 55.7-3 Schematic representation of the regulatory factors that affect α_1-antitrypsin (AT) expression in hepatocytes, enterocytes, and macrophages. IL-6 = interleukin-6.

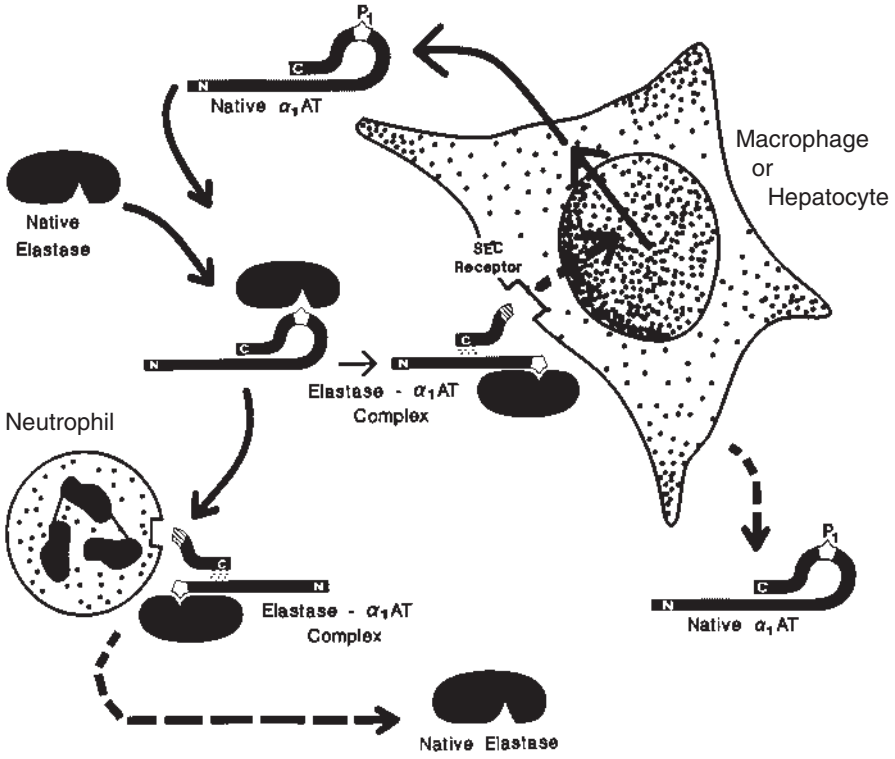

FIGURE 55.7-4 Feedback regulation of α_1-antitrypsin (AT) synthesis and neutrophil chemotactic activity mediated by the serpine-enzyme complex (SEC) receptor. Reproduced with permission from Perlmutter DH. α_1-Antitrypsin: structure, function, physiology. In: Mackiewicz A, Kusher I, Baumann H, editors. Acute phase proteins: molecular biology, biochemistry and clinical applications, Boca Raton (FL): CRC Press; 1993. p. 49–167.

surface receptor.[32,33,86,110,111] These receptor molecules are now referred to as serpin enzyme complex (SEC) receptors because they recognize the highly conserved domains of other SECs, such as antithrombin III–thrombin, α_1-antichymotrypsin–cathepsin G, and, to a lesser extent, C1 inhibitor–C1s and tissue plasminogen activator–plasminogen activator inhibitor I complexes, as well as α_1-AT–elastase complexes.[32,112] Substance P, several other tachykinins, bombesin, and the amyloid-β peptide bind to the SEC receptor through a similar pentapeptide sequence.[113] Recent studies indicate that the SEC receptor can mediate endocytosis of soluble amyloid-β peptide, but it does not recognize the aggregated form of amyloid-β peptide that is toxic to neurons and other cell types.[114] Thus, the SEC receptor may play a role in preventing amyloid-β peptide from accumulating into neurotoxic mature amyloid deposits associated with Alzheimer disease.

α_1-AT mRNA has been isolated from multiple tissues in transgenic mice,[106,115,116] but only in some cases have studies distinguished whether such α_1-AT mRNA is in ubiquitous tissue macrophages or other cell types. For instance, α_1-AT is synthesized in enterocytes and intestinal Paneth cells, as determined by studies in intestinal epithelial cell lines, ribonuclease protection assays of human intestinal RNA, and in situ hybridization analysis in cryostat sections of human intestinal mucosa.[21,117–119] Expression of α_1-AT in enterocytes increases markedly as they differentiate from crypt to villus, in response to IL-6 and during inflammation in vivo. α_1-AT is also synthesized by pulmonary epithelial cells.[120,121] Interestingly, synthesis of α_1-AT in pulmonary epithelial cells is less responsive to regulation by IL-6 than to a related cytokine, oncostatin M.[121]

Clearance and Distribution

The half-life of α_1-AT in plasma is approximately 5 days.[122–124] It is estimated that its daily production rate is 34 mg/kg of body weight, with 33% of the intravascular pool of α_1-AT being degraded daily. Several physiologic factors may affect the rate of its catabolism. First, desialylated α_1-AT is cleared from the circulation in minutes,[124,125] probably via hepatic asialoglycoprotein receptor–mediated endocytosis. Second, α_1-AT in complex with elastase or proteolytically modified is cleared more rapidly than native α_1-AT.[126] Because its ligand specificity is similar to that required for in vivo clearance of SECs, the SEC receptor may also be involved in the clearance and catabolism of α_1-AT–elastase complexes and other SECs.[33] The low-density protein receptor–related protein can also mediate clearance and catabolism of α_1-AT–elastase complexes.[127,128] Third, the rate of α_1-AT clearance may increase during the host response to inflammation.[129] There is a slight increase in the rate of clearance of radiolabeled α_1-ATZ compared with wild-type α_1-AT when infused into normal individuals, but this difference does not account for the decrease in serum levels of α_1-AT in deficient individuals.[123,125,130]

α_1-AT diffuses into most tissues and is found in most body fluids.[5] Its concentration in lavage fluid from the lower respiratory tract is approximately equivalent to its concentration in serum.[5] It is also found in feces, and increased fecal concentrations of α_1-AT correlate with inflammatory lesions of the bowel.[131] In each case, it has been assumed that the α_1-AT is derived from serum. Local sites of synthesis, such as macrophages and epithelial cells, may also make important contributions to the α_1-AT pool in these tissues and body fluids. In fact, it has been reported that fecal α_1-AT clearance is higher in patients with homozygous α_1-AT deficiency than in normal per-

sons.[132] Because the former have only 10 to 15% of the normal serum concentrations of α_1-AT, a local intestinal source for fecal α_1-AT is implicated. One possible explanation is that the bulk of α_1-AT in feces is derived from sloughed enterocytes. Increased fecal α_1-AT in those with homozygous α_1-AT deficiency would result from turnover or sloughing of enterocytes, with a greater amount of α_1-AT per cell owing to intracellular accumulation. Increased fecal α_1-AT in normal persons with inflammatory-related, protein-losing enteropathy would result from increased turnover or sloughing of enterocytes with a normal amount of α_1-AT per cell.

MECHANISM OF α_1-ANTITRYPSIN DEFICIENCY IN PIZZ INDIVIDUALS

The mutant α_1-ATZ molecule is characterized by a single-nucleotide substitution that results in an amino acid substitution of Lys for Glu 342.[133–135] There is a selective decrease in the secretion of α_1-AT, with the abnormal protein accumulating in the ER.[105,136] The defect is not specific for liver cells because it also affects extrahepatic sites of α_1-AT synthesis, such as macrophages[105] and transfected cell lines.[137–139] Site-directed mutagenesis studies have shown that this single amino acid substitution is sufficient to produce the cellular defect.[139] Once translocated into the lumen of the ER, the mutant α_1-AT protein is unable to traverse the remainder of the secretory pathway because it is abnormally folded.

Substitution of Glu 342 by Lys in the α_1-ATZ variant reduces the stability of the molecule in its monomeric form and increases the likelihood that it will form polymers by means of a "loop-sheet" insertion mechanism.[140] In this mechanism, the reactive center loop of one α_1-AT molecule inserts into a gap in the β-pleated A sheet of another α_1-AT molecule (see Figure 55.7-2). Lomas and colleagues were the first to notice that the site of the amino acid substitution in the α_1-ATZ variant was at the base of the reactive center loop, adjacent to the gap in the A sheet.[140] These investigators predicted that a change in the charge at this residue, as occurs with the substitution of Lys for Glu, would prevent the insertion of the reactive site loop into the gap in the A sheet during interaction with enzyme; therefore, the mutant α_1-ATZ would be susceptible to the insertion of the reactive center loop of adjacent molecules into the gap in its A sheet. This would, in turn, cause the mutant α_1-ATZ to be more susceptible to polymerization than the wild-type α_1-AT. In fact, their experiments showed that α_1-ATZ undergoes this form of polymerization to a certain extent spontaneously and to a greater extent during relatively minor perturbations, such as a rise in temperature. Presumably, an increase in body temperature during systemic inflammation would exacerbate this tendency in vivo. Polymers could also be detected by electron microscopy in the ER of hepatocytes in a liver biopsy specimen from a deficient individual.[140] Similar polymers have been found in the plasma of patients with the PIS_{Iiyama} and PIM_{Malton} α_1-AT variants.[141,142] The mutations in α_1-AT PIS_{Iiyama} (Ser 53 to Phe)[69] and PIM_{Malton} (Phe 52 deletion)[64] affect residues that provide a ridge for the sliding movement that opens the A sheet. Thus, these mutations would be expected to interfere with the insertion of the reactive center loop into the gap in the A sheet and, therefore, to leave the gap in the A sheet available for spontaneous loop-sheet polymerization. It is indeed interesting that hepatocytic α_1-AT globules have been observed in a few patients with these two variants. Recent observations suggest that the α_1-ATS variant also undergoes loop-sheet polymerization[143] and that this may account for its retention in the ER, albeit a milder degree of retention than that for α_1-ATZ.[144] Moreover, α_1-ATS can apparently form heteropolymers with α_1-ATZ,[145] providing a potential explanation for liver disease in patients who are compound heterozygotes for the α_1-ATS and Z alleles. In a recent study, Davis and colleagues have shown that dementia in two families is associated with mutations that cause polymerization of another member of the serpin family, neuroserpin.[146] The mutations, mechanism of polymerization, and morphology of the inclusion bodies in affected neurons are remarkably similar to those that occur in the classic form of α_1-AT deficiency

The precise mechanism by which the loop-sheet insertion develops is not yet completely understood and may be more complicated than previously thought.[141,147,148] Further studies to characterize the mechanism more precisely will undoubtedly be forthcoming.

A study by Yu and colleagues compared the folding kinetics of α_1-ATZ in transverse urea gradient gels.[149] This study shows, for the first time, that α_1-ATZ folds at an extremely slow rate, unlike the wild-type α_1-AT, which folds in minutes. This folding defect leads to the accumulation of an intermediate, which has a high tendency to polymerize, presumably by the loop-sheet insertion mechanism.

By themselves, however, these data do not prove that the polymerization of α_1-ATZ results in retention within the ER. In fact, many polypeptides must assemble into oligomeric or polymeric complexes to traverse the ER and reach their destination within the cell, at the surface of the plasma membrane, or into the extracellular fluid. If viral proteins, such as the vesicular stomatitis virus G protein or influenza virus hemagglutinin, and host proteins, such as the T-cell receptor and fibrinogen, do not assemble into oligomers, they are retained and ultimately degraded in the ER.[150]

The strongest evidence that polymerization results in the retention of α_1-ATZ in the ER has been provided by studies in which the fate of α_1-ATZ is examined after the introduction of additional mutations into this molecule. For instance, Kim and colleagues introduced a mutation into the α_1-AT molecule at amino acid 51, F51L.[151] This mutation is remote from the Z mutation, E342K, but it apparently impeded loop-sheet polymerization and prevented insertion of synthetic peptide into the gap in the A sheet, implying that the mutation led to the closing of this gap. The double-mutated F51L α_1-ATZ molecule was also less prone to polymerization and folded more efficiently in vitro than α_1-ATZ. Moreover, the introduction of the F51L mutation partially corrected the intracellular retention properties of α_1-ATZ in microinjected *Xenopus* oocytes[152] and in yeast.[153]

However, Lin and colleagues recently found that a novel, naturally occurring variant of α_1-AT, bearing the K342Q mutation that characterizes α_1-ATZ as well as carboxyl-terminal truncation, is retained in the ER for as long, or longer, than α_1-ATZ even though it does not polymerize.[154] These results could indicate that mechanisms other than polymerization determine whether mutant α_1-AT molecules are retained in the ER. An alternative possibility is that polymerization of α_1-ATZ is not the cause of ER retention but rather its result.

Despite what is stated in one review,[155] it is still not entirely clear what proportion of the newly synthesized mutant α_1-ATZ molecules is converted to the polymeric state in the ER. In one cell culture model system, Lin and colleagues found that 17.0 ± 0.9% of α_1-ATZ is in the insoluble fraction at steady state,[154] but comparable in vivo data are not yet available. It is also not known whether polymeric molecules are degraded in the ER less rapidly than their monomeric counterparts or whether polymeric molecules, when retained in the ER, are more hepatotoxic than their monomeric counterparts. Indeed, recent studies on the effect of temperature on α_1-ATZ have indicated the high degree of complexity involved in these issues. Although Lomas and colleagues showed that a rise in temperature to 42°C increases the polymerization of purified α_1-ATZ in vitro,[140] Burrows and colleagues found that a rise in temperature to 42°C resulted in increased secretion of α_1-ATZ and decreased intracellular degradation of α_1-ATZ in a model cell culture system.[156] In contrast, lowering the temperature to 27°C resulted in diminished intracellular degradation of α_1-ATZ, without any change in the small amount of α_1-ATZ that is secreted.[156] Consistent with the well-established role that temperature plays in most biochemical processes, these results suggest that changes in temperature have the potential to affect multiple steps in the pathways by which α_1-ATZ is translocated through the secretory and degradative compartments/systems, as well as affecting the relative proportions of α_1-ATZ in the monomeric and polymeric state. On the basis of these considerations, as well as long-standing clinical experience with α_1-AT–deficient children and other children with liver disease, and in the complete absence of any epidemiologic evidence, it seems unlikely that there is a simple relationship between febrile episodes and phenotypic expression of liver disease in α_1-AT–deficient patients.

To understand how polymerization of α_1-AT or alteration in folding of monomeric α_1-AT might result in retention within the ER, one needs to consider what is now known about the biology of protein secretion. Most newly synthesized secretory proteins are translocated into the lumen of the ER. Before being transported to their final destination, these nascent secretory polypeptide chains undergo a series of post-translational modifications, including glycosylation, formation of disulfide bonds, oligomerization, and folding. Moreover, transport through the secretory pathway involves interaction with resident ER proteins, termed molecular chaperones. These interactions facilitate disulfide bond formation, assembly, and folding.

Several families of ER chaperones have been identified. One has been referred to as the polypeptide chain–binding protein family and includes several heat shock/stress proteins (HSPs), GRP78/BiP and GRP94, protein disulfide isomerase, and Erp72.[157] Several calcium-binding phosphoproteins of the ER, most notably calnexin and calreticulin, have also been implicated as having molecular chaperone activity within the ER. Calnexin is an approximately 88 kD transmembrane ER-resident phosphoprotein originally discovered in association with class I major histocompatibility complex (MHC) molecules.[158,159] It is now known to facilitate the folding and assembly of many membrane and secretory glycoproteins. This chaperone activity involves a lectinlike mechanism in which calnexin binds the innermost glucose residue of the asparagine-linked oligosaccharide side chains present on most glycoproteins. The innermost glucose residue becomes accessible almost immediately after the secretory glycoprotein has undergone the initial stages of oligosaccharide side chain trimming in the lumen of the ER, including the removal of the two outermost glucose residues by the actions of glucosidases I and II (Figure 55.7-5). Once bound to calnexin, monoglucosylated glycoproteins are retained in the ER until properly folded.[160] Once folding is complete, the glycoprotein can dissociate from calnexin for vesicular transport out of the ER. Recent studies have indicated that a unique reglucosylating enzyme, uridine diphosphate–glucose:glycoprotein glucosyltransferase (UDGGT), can transfer glucose onto unfolded or denatured deglucosylated proteins in the ER.[161,162] In fact, the binding of glycoproteins to calnexin during folding in the ER is now thought to depend on a cycle of glucosidase II activity, producing the deglucosylated form of a protein, and reglucosylation by ER–luminal UDGGT, leading to regeneration of the monoglucosylated form. The glucosyltransferase acts preferentially on unfolded or denatured proteins. Thus, the repeated cycles of binding to and dissociation from calnexin are designed to maximize the possibility that a given unfolded or denatured protein will undergo proper folding for transport out of the ER.

It is now well established that the ER possesses a machinery whereby it can degrade any mutant or unassembled polypeptides that are unable to fold properly even after interaction with the ER chaperones. This machinery has come to be called the "ER degradation pathway" or the "quality control apparatus" of the ER.[163] Although the ER degradation pathway was originally thought to involve a distinct proteolytic system, it now appears to be mediated in large part from the cytoplasmic aspect of the ER by the proteasome.

Several studies have shown that α_1-ATZ is degraded in the ER and that the proteosome is a key component of the degradation pathway.[164–166] Degradation of α_1-ATZ is markedly reduced by specific proteosome inhibitors in yeast and mammalian cells.[165,166] In a mammalian cell–free system, degradation of α_1-ATZ is, at least in part, attributable to a pathway that involves interaction with the transmembrane ER chaperone calnexin, polyubiquitination of calnexin, and targeting of the α_1-ATZ–polyubiquitinated calnexin complex by the proteosome.[166] There is also evidence for the involve-

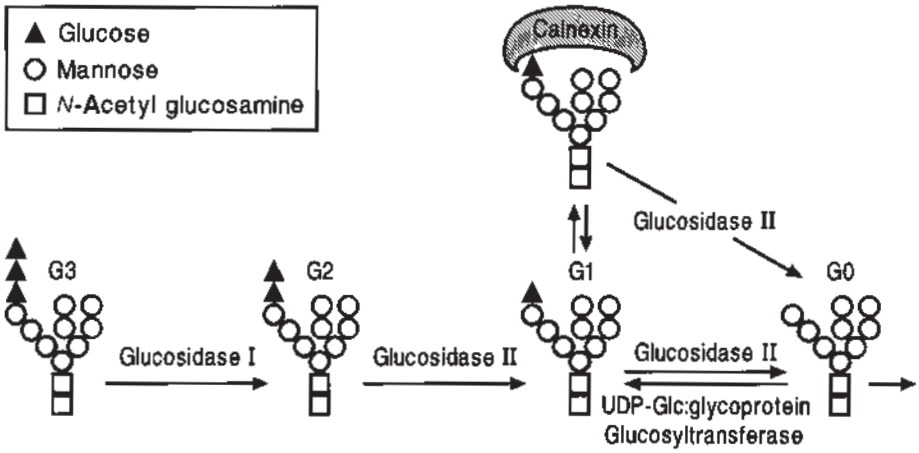

FIGURE 55.7-5 Model of glucose trimming and reglucosylation in relation to interaction with calnexin. Adapted from Hebert DN, Helenius A. Glucose trimming and reglucosylation determine glycoprotein association with calnexin in the endoplasmic reticulum. Cell 1995;81:425–33.

ment of ubiquitin-independent proteosomal and nonproteosomal pathways in degradation of α_1-ATZ in the mammalian cell–free system.[167]

As discussed below, autophagy may represent one nonproteosomal mechanism for degradation of α_1-ATZ.[168] Because this is based on the effect of chemical inhibitors of autophagy, which have other effects on cellular metabolism, definitive evidence for the role of autophagy in degradation of α_1-ATZ will require more detailed, probably genetic studies. Cabral and colleagues have recently provided evidence for a nonproteosomal degradation pathway that is sensitive to tyrosine phosphatase inhibitors.[169] In their studies, degradation of α_1-ATZ in a hepatoma cell line was not affected by proteosome inhibitors but was reduced by tyrosine phosphatase inhibitors. Although this finding was originally interpreted to suggest that there were cell type–specific differences in the role of proteosomal and nonproteosomal degradation mechanisms and that nonproteosomal degradation mechanisms were more important in hepatocytes, subsequent studies have shown that the proteosome still plays a major role in degradation of α_1-ATZ in other hepatoma cell lines.[170] This means it is more likely that nonproteosomal mechanisms, sensitive to tyrosine phosphatase inhibitors, are particularly important in specific cell lines rather than specific cell types. The relative importance of proteosomal and nonproteosomal mechanisms to the disposal of α_1-ATZ in vivo is still unknown. Despite what was stated in one review,[155] there are no data on the effect of age, inflammation, or other physiologic conditions on degradation of mutant α_1-ATZ.

The mechanism by which the proteosome gains access from the cytoplasm to α_1-ATZ on the luminal side of the ER membrane is also uncertain. Although retrograde translocation from the ER to the cytoplasm has been demonstrated for some luminal substrates of the proteosome, there is very limited evidence for retrograde translocation of α_1-ATZ. Werner and colleagues detected α_1-ATZ free in the cytosolic fraction of yeast when the proteosome was inhibited,[165] but only a small fraction of the total α_1-ATZ in the ER could be detected,[165] and there has been no other evidence for retrotranslocation. Recent studies have provided evidence for extraction of substrates through the ER membrane by the proteosome.[171] The AAA adenosine triphosphatase Cdc 48/p97 and its partners appear to play an important role in this process.[172] It is also possible that the proteasome gains access to α_1-ATZ and/or the α_1-ATZ–polyubiquitinated calnexin during the formation of autophagic vacuoles. Our recent studies have shown that retention of α_1-ATZ in the ER is associated with the induction of an autophagic response.[168] The autophagic response is thought to be a general mechanism by which intracellular organelles, or parts of organelles, are degraded. It is a highly evolutionarily conserved process that occurs in many cell types, especially during stress states, such as nutrient deprivation, and during the cellular remodeling that accompanies morphogenesis, differentiation, and senescence. Several studies have suggested that autophagic vacuoles are derived in part from subdomains of ER.[173] Autophagosomes initially form as invaginations from ribosome-free areas of the ER membrane. Together with constituents of the ER, autophagosomes engulf cytosolic constituents, including components of the ubiquitin system and the proteasome.[174,175] Thus, it is possible that degradation of α_1-ATZ is mediated by proteasomal machinery engulfed during formation of the autophagosome. Indeed, in our recent studies, an intense autophagic response was demonstrated in cell culture model systems with ER retention of mutant α_1-ATZ and in liver biopsy specimens from patients with α_1-AT deficiency.[168] Moreover, α_1-AT and calnexin were colocalized within autophagosomes as well as within the ER. Finally, degradation of α_1-ATZ in the cell culture model system is partially abrogated by inhibitors of autophagy, including wortmannin, 3-methyladenine, and LY294002. However, it is also possible that α_1-ATZ molecules taken up into autophagosomes are degraded by a nonproteasomal mechanism when the autophagosomes merge or fuse with the lyosomal pathway and that the autophagic and proteasomal pathways constitute completely independent mechanisms for degradation of α_1-ATZ.

A study by Van Leyen and colleagues suggested the possibility that a type of autophagic response termed "programmed organelle degradation" allows access of cytoplasmic proteases to both luminal and integral membrane proteins.[176] This process appears to involve the highly regulated recruitment of 15-lipoxygenase from the cyto-

plasm to the ER membrane, where it presumably oxygenates membrane phospholipids, in turn releasing proteins from the ER lumen and membrane. A mechanism like this could possibly account for the ER degradation pathway of some substrates.

PATHOGENESIS OF LIVER INJURY IN α_1-ANTITRYPSIN DEFICIENCY

There are several theories for the pathogenesis of liver injury in α_1-AT deficiency. According to the immune theory, liver damage results from an abnormal immune response to liver antigens.[177] This theory is based on the observation that peripheral blood lymphocytes from PIZZ infants are cytotoxic for isolated hepatocytes. However, this is probably a nonspecific effect of liver injury in that peripheral blood lymphocytes from PIMM infants with a similar degree of liver injury on the basis of idiopathic neonatal hepatitis syndrome are also cytotoxic for isolated hepatocytes. More recent studies have indicated an increase in the human leukocyte antigen (HLA)-DR3-DW25 haplotype in α_1-AT–deficient individuals with liver disease.[178] However, there is no difference in the expression of class II MHC antigen in the livers of these individuals compared with normal controls.[179] Moreover, an increase in the prevalence of a particular HLA haplotype in the affected population does not by itself imply altered immune function. In fact, because of the linkage dysequilibrium displayed by genes within the MHC, it is possible that increased susceptibility is caused by the products of unrelated but linked genes. For instance, the MHC contains genes for several HSPs,[180,181] which play an important role in the biogenesis and transport of other proteins through the secretory pathway.

The accumulation theory, in which liver damage is thought to be caused by accumulation of mutant α_1-AT molecules in the ER of liver cells, is the most widely accepted theory. Experimental results in transgenic mice are most consistent with this theory and completely exclude the possibility that liver damage is caused by "proteolytic attack" as a consequence of diminished serum α_1-AT concentrations.[26] Transgenic mice carrying the mutant Z allele of the human α_1-AT gene develop periodic acid–Schiff–positive diastase-resistant intrahepatic globules and liver injury early in life.[9,10] Because there are normal levels of α_1-AT and (presumably) other antielastases in these animals, as directed by endogenous murine genes, the liver injury cannot be attributed to proteolytic attack.

Some have argued that the histologic characteristics of the liver in the transgenic mouse model are not identical to those in humans. Detailed histologic characterization of the liver in one transgenic mouse model by Geller and colleagues has shown that there are focal areas of liver cell necrosis, microabscesses with an accumulation of neutrophils and regenerative activity in the form of multicellular liver plates, and focal nodule formation during the neonatal period.[182] Nodular clusters of altered hepatocytes that lack α_1-AT immunoreactivity are also seen during the neonatal period. With aging, there is a decrease in the number of hepatocytes containing α_1-ATZ globules; there is also an increase in the number of nodular aggregates of α_1-AT–negative hepatocytes and development of periosinusoidal fibrosis.[183] Within 6 weeks, there are dysplastic changes in these aggregates. Adenomas occur within 1 year, and invasive hepatocellular carcinoma is seen between 1 and 2 years of age.[183] However, the relationship between the α_1-ATZ globules and inflammation or dysplasia is not yet apparent from these animal studies. The histopathology of the α_1-ATZ transgenic mice is remarkably similar to that of hepatitis B virus surface antigen–transgenic mice; this similarity is particularly interesting because hepatitis B virus is retained in the ER, or in the ER-Golgi intermediate compartment of hepatocytes, often called "ground-glass hepatocytes."[184] It is still unclear why the liver injury in this transgenic mouse model is somewhat milder and less fibrogenic than that seen in children with liver disease associated with α_1-AT deficiency. It is possible that there are strain-specific factors that condition the response to injury in the mouse model. There are certainly host-specific factors that determine the amount of liver injury in α_1-AT deficiency (see below), and the degree of inflammation and fibrosis varies widely among our patients with liver disease from α_1-AT deficiency.

Data from individuals who have null alleles of α_1-AT and therefore negligible serum levels of α_1-AT have also been used as evidence against the "proteolytic attack" theory. These individuals do not develop liver injury, at least not enough to result in clinical detection. However, only a few individuals with null alleles have been reported; each has a different allele, and based on data in PIZZ individuals showing that only 10 to 15% of these individuals develop clinically significant liver injury, it might be necessary to evaluate 7 to 10 individuals with each null allele before detecting one with liver injury.

The recognition that several other naturally occurring variant alleles of α_1-AT associated with deficiency can undergo polymerization has provided some support for the accumulation theory. The most important of these variant alleles is the compound heterozygous α_1-ATSZ phenotype. Work by Lomas and colleagues has shown that α_1-ATS and α_1-ATZ may form heteropolymers.[145] We know from the nationwide study of α_1-AT deficiency in Sweden that the incidence of liver disease among individuals with the α_1-ATSZ phenotype is similar to that of individuals with the α_1-ATZ phenotype.[1,11] We also now know that the PIM_{Malton} allele undergoes polymerization, and liver injury has been reported in several patients with this allele.[64,68,141] However, there is a report of an individual with the PIS_{Iiyama} allele having hepatocyte α_1-AT globules but no liver injury.[69] Moreover, a recent report by Ray and Brown has indicated that $PIM_{Heerlen}$ and $PIM_{Procidia}$ undergo aggregation and that $PIM_{Mineral\ Springs}$ and $PINull_{Ludwigshafen}$ may undergo aggregation, but there are no reports of liver disease in individuals carrying these alleles.[55] However, only a few patients with M_{Malton}, S_{Iiyama}, $M_{Heerlen}$, $M_{Procida}$, $M_{Mineral\ Springs}$, and $Null_{Ludwigshafen}$ have been identified. It is also not clear how many of these patients have been thoroughly examined for liver disease. Again, on the basis of what we know about the α_1-ATZ and α_1-ATSZ phenotypes, at least

7 to 10 individuals with each of these alleles would need to be examined to detect one with liver injury.

It has been difficult to reconcile the accumulation theory with the observations of Sveger,[11] which show that only a subset of α_1-AT–deficient individuals develop significant liver damage. We have made the prediction that a subset of the deficient population is more susceptible to liver injury by virtue of one or more additional inherited traits or environmental factors that exaggerate the intracellular accumulation of the α_1-ATZ protein or that exaggerate the cellular pathophysiologic consequence of mutant α_1-AT accumulation (Figure 55.7-6). To address this prediction experimentally, the Perlmutter laboratory transduced skin fibroblasts from deficient individuals, with or without liver disease, with amphotropic recombinant retroviral particles designed for constitutive expression of the mutant α_1-ATZ gene.[185] Human skin fibroblasts do not express the endogenous α_1-AT gene but presumably express other genes involved in the postsynthetic processing of secretory proteins. The results show that expression of the human α_1-AT gene was conferred on each fibroblast cell line. Compared with the same cell line transduced with the wild-type α_1-AT gene, there was selective intracellular retention of the mutant α_1-ATZ protein in each case. However, there was a marked delay in degradation of the mutant α_1-ATZ protein after it accumulated in the fibroblasts from deficient individuals with liver disease (susceptible hosts) compared with those without liver disease (protected hosts) (Figure 55.7-7). Thus, these data provide evidence that other factors that affect the abnormal α_1-ATZ molecule, such as a lag in ER degradation, at least in part, determine susceptibility to liver disease.

The lag in ER degradation of α_1-ATZ in susceptible hosts may involve several distinct mechanisms. In one susceptible host, the retained α_1-ATZ interacts poorly with calnexin.[185] In the liver cells of this host, there is likely to be only a very little polyubiquitinated α_1-ATZ–calnexin complex that can be recognized for proteolysis by the proteasome. In several other susceptible hosts, the retained α_1-ATZ interacts well with calnexin but is degraded slowly (J. Teckman, D. Qu, D. H. Perlmutter, unpublished data, 1997). These hosts may have a defect in calnexin that prevents its ubiquitination or a defect in the ubiquitin system of the proteasome. Hosts with the latter defect would also be more likely to respond to a pharmacologic agent, such as interferon-γ,[186] that enhances the activity of the ubiquitin-dependent proteasomal system. This type of study, which involves complementation in human skin fibroblast cell lines, and recent studies involving yeast that have identified at least 30 putative recessive mutants and 7 complementation groups of strains defective in ER degradation of α_1-ATZ[187] are likely to lead to recognition of other mechanisms for excessive ER retention of α_1-ATZ.

There is still relatively limited information about the short- and long-term effects of ER retention of α_1-ATZ in model systems. In one report, the accumulation of α_1-ATZ in *Xenopus* oocytes was associated with the release of lysosomal enzymes.[188] Several recent studies have shown that ER retention of mutant α_1-ATZ provokes a rather specific cellular response with autophagy as a major feature. Autophagosomes develop in several different model cell culture systems genetically engineered to express α_1-ATZ, including human fibroblasts and murine hepatoma and rat hepatoma cell lines. Moreover, in a HeLa cell line engineered for inducible expression of α_1-ATZ, autophagosomes appear as a specific response to the expression of α_1-ATZ and its retention in the ER.[168] There is a marked increase in autophagosomes in hepatocytes in transgenic mouse models of α_1-AT deficiency and a disease-specific increase in autophagosomes in liver biopsies from patients with α_1-AT deficiency. Mutant α_1-ATZ molecules can be detected in autophagosomes by immune electron microscopy, often together with the ER molecular chaperone calnexin.

Taken together, these results have suggested that the autophagic response is induced to protect liver cells from the toxic effects of aggregated α_1-ATZ retained in the ER. We have also speculated about the role of autophagy in protecting liver cells from tumorigenesis. Several recent studies have shown that autophagic activity is decreased in tumors and that reconstitution of autophagic activity inhibits

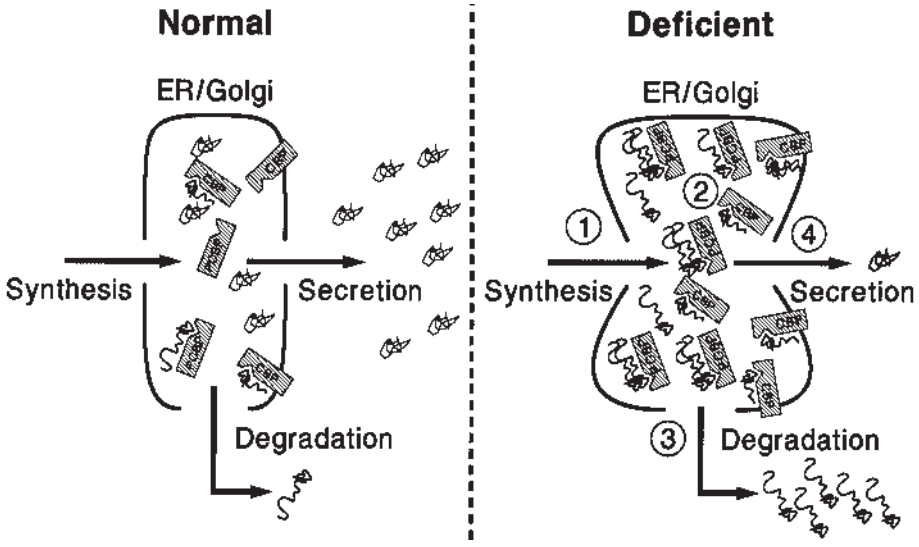

FIGURE 55.7-6 Conceptual model for liver injury in α_1-antitrypsin (AT) deficiency. CBP = calcium-binding protein; ER = endoplasmic reticulum; PCBP = polypeptide chain-binding protein. Adapted from Perlmutter DH. The cellular basis for liver injury in α_1-antitrypsin deficiency. Hepatology 1991;13:172–85.

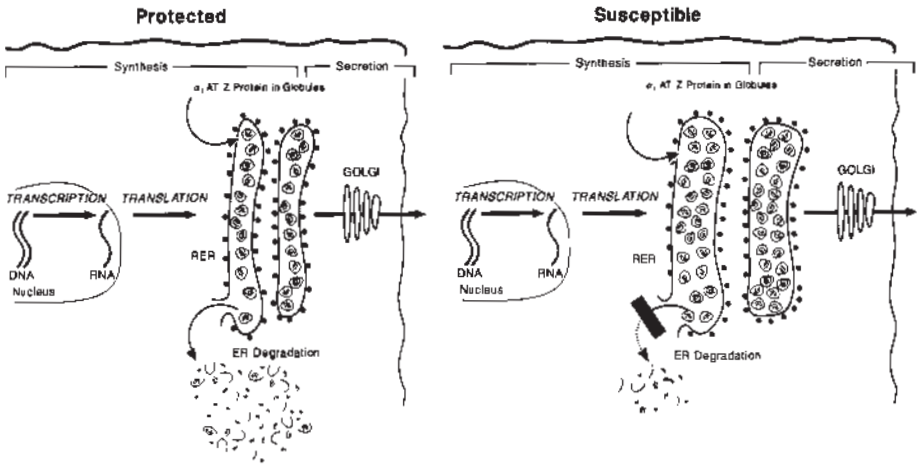

FIGURE 55.7-7 Difference in endoplasmic reticulum (ER) degradation of α_1-ATZ protein in protected and susceptible hosts. The block in ER degradation in susceptible hosts is represented by the small dark bar. Adapted from Teckman JH, Perlmutter DH. Conceptual advances in the pathogenesis and treatment of childhood metabolic liver disease. Gastroenterology 1995;108:1263–79.

tumorigenesis in vivo.[189,190] In our studies, autophagosomes are predominantly found in liver cells with dilated ER in both human and transgenic mouse liver.[168] Previous studies in transgenic mouse models of α_1-AT deficiency have shown that hepatocarcinogenesis evolves within nodular aggregates of hepatocytes that are negative for α_1-AT expression by immunofluorescent staining.[183]

It is not yet clear whether autophagy is substrate specific for α_1-ATZ or provides a more general response to aggregated proteins retained in the ER. No such cellular response has been described in studies of mutant proteins that aggregate in the cytoplasm or nucleus. Although there is some mention of autophagic vacuoles around the aggresomes that form when cystic fibrosis transmembrane regulator (CFTR) ΔF508 accumulates in the presence of proteosomal inhibitors,[191,192] the histologic pictures in cells expressing α_1-ATZ or CFTR ΔF508 are quite distinct.[168] Autophagy is not induced by tunicamycin or thapsigargin, agents that cause a generalized form of "ER stress" (N. Mitzushima, personal communication, 2002). Russell bodies, which have been described in cells retaining certain mutant immunoglobulin molecules in the ER,[193] do have many characteristics of autophagosomes. Autophagy has also been implicated in the cellular response to the mutant form of the ER membrane protein peripheral myelin protein 22, which causes a gain of function disease in Schwann cells in Charcot-Marie-Tooth disease and Dejerine-Sottas disease.[194]

Recently, we examined the autophagic response to ER retention of α_1-ATZ in vivo by testing the effect of fasting on the liver of the PiZ mouse model of α_1-AT deficiency.[195] Starvation is a well-defined physiologic stimulus of autophagy and a known environmental stressor of liver disease in children. The results show that there is a marked increase in fat accumulation and in α_1-AT–containing, ER-derived globules in the liver of the PiZ mouse induced by fasting. These changes were particularly exaggerated at 3 to 6 months of age. Three-month-old PiZ mice had a significantly decreased tolerance for fasting compared with nontransgenic C57 black mice (none of the PiZ mice tolerated a 72-hour fast even though 100% of C57BL mice survive the same duration of fasting). Although fasting induced a marked autophagic response in wild-type mice, the autophagic response was already activated in PiZ mice to levels that were more than 50% higher than those in the liver of fasted wild-type mice, and they did not increase further during fasting. These results indicate that autophagy is constitutively activated in α_1-AT deficiency and that the liver is unable to mount an increased autophagic response to physiologic stressors. From our search of the literature, the only other condition in which there is accumulation of autophagic vacuoles under homeostatic conditions is Danon disease.[196] In contrast to α_1-AT deficiency, however, autophagosomes accumulate in Danon disease because of a genetic defect in the terminal phases of autophagy, that is, the fusion of autophagic vacuoles with lysosomes and subsequent degradation within autolysosomes.[196,197]

In the course of our ultrastructural studies of the liver of the PiZ mouse and of patients with α_1-AT deficiency, we have recently been struck by the degree of mitochondrial autophagy that is induced.[198] A comparison of the liver from four α_1-AT–deficient patients with livers from eight patients with other liver diseases and four normal livers showed a marked significant increase in mitochondrial autophagy associated with α_1-AT deficiency. Even more interesting is the observation that many mitochondria that are not surrounded by autophagic vacuolar membranes are nevertheless damaged or are in various phases of degeneration in liver cells from α_1-AT–deficient hosts. This damage is characterized by the formation of multilamellar structures within the limiting membrane, condensation of the cristae and matrix, and, in some cases, dissolution of the internal structures, often leaving only electron-dense debris compressed into a thin rim at the periphery of the mitochondrion. Although this second type of damaged mitochondria appears distinct from the mitochondria that are degenerating within autophagosomes, these mitochondria are sometimes seen in close proximity to, or even fusing with, autophagic vacuoles or lysosomes.

Mitochondrial autophagy and injury are also marked in the liver of the PiZ transgenic mouse model of α_1-AT deficiency. Immunofluorescence analysis shows the presence of activated caspase 3 in the PiZ mouse liver.[198] Because cyclosporin A (CsA) has been shown to reduce mitochon-

drial injury[199] and inhibit starvation-induced autophagy,[200] we examined the effect of CsA on PiZ mice and found that it significantly reduces hepatic mitochondrial injury while eliminating activated caspase 3 and improving the animals' tolerance of starvation. These results provide evidence for the novel concept that mitochondrial damage and caspase activation play a role in the mechanism of liver cell injury in α_1-AT deficiency. Although this analysis suggests that there is mitochondrial injury that is separate from the autophagic process, the possibility that autophagy plays some role in all of the mitochondrial damage that is observed cannot be completely excluded. Thus, one model of mitochondrial damage in this deficiency holds that accumulation of α_1-ATZ in the ER is in itself responsible for mitochondrial dysfunction. Indeed, there is now ample evidence in the literature for functional interactions between mitochondria and closely apposed ER cisternae.[201,202] Recent studies show that specific signals are transmitted between these two intracellular compartments[203,204] and that mitochondrial dysfunction, including release of cytochrome *c* and caspase 3 activation, is associated with the ER dilatation and stress induced by brefeldin A, tunicamycin, and thapsigargin.[205,206] It is not yet known, however, whether mitochondrial dysfunction in the latter cases is due to ER dilatation and/or ER stress or to independent effects on mitochondria by these experimental drugs. A second possible explanation, not necessarily incompatible with the first, envisages mitochondrial dysfunction as a result of the autophagic response to ER retention of α_1-ATZ. In this scenario, mitochondria are recognized nonspecifically by the autophagic response, which is constitutively activated to somehow remove and degrade areas of the ER that are distended by aggregated mutant protein. Although our data indicate that CsA inhibits hepatic mitochondrial injury in vivo, this benefit could reflect the drug's known effects on the mitochondrial permeability transition,[199] on autophagy,[200] or on both.

The CsA findings are also noteworthy for their therapeutic implications. They indicate that CsA can prevent mitochondrial damage even under circumstances in which α_1-ATZ continues to accumulate in the ER. Thus, they provide proof in principle for mechanism-based therapeutic approaches to liver disease in α_1-AT deficiency—pharmacologic intervention directed as distal steps in the pathobiologic pathway that leads to liver injury, such as the "mitochondrial" step, without correction of the primary defect and/or the more proximal steps in the pathobiology of this liver disease.

Other cellular response pathways could potentially be activated by ER retention of mutant proteins. Work in several laboratories has shown that a novel structure called the aggresome is formed in cells when expression of misfolded membrane proteins (such as CFTRΔF508, other mutant membrane proteins, and mutant viral proteins) exceeds the capacity of the proteasome to degrade them.[191,192,207] The aggresome is a pericentriolar membrane-free cytoplasmic inclusion containing misfolded ubiquitinated protein ensheathed in a case of vimentin and perhaps other intermediate filaments. Recent studies indicate that retention of α_1-ATZ induces expansion of the ER, alteration in the structure of the ER and formation of autophagic vesicles but does not cause aggresome formation.[168]

The unfolded protein response is also induced by the accumulation of unfolded proteins in the ER. It results in the induction, or up-regulation, of a repertoire of genes that serve to protect the cell from damage by the denatured proteins.[208,209] This includes up-regulation of chaperones and enzymes in the phospholipid biosynthetic pathway to permit new ER membrane biogenesis and attenuation of the translation of most endogenous proteins, which could become denatured.

Two other signal transduction pathways activated by misfolded proteins in the ER have recently been characterized and may be relevant to α_1-AT deficiency. The ER overload pathway is a signaling pathway that appears to be distinct from the unfolded protein response and that involves activation of nuclear factor κB and release of active oxygen intermediates.[210] So far, this pathway has been described only in experimental conditions associated with ER overload of misfolded or unassembled membrane proteins. Recent studies in the Morimoto laboratory have shown that heat shock factor (HSF)2 may be activated by the accumulation of ubiquitinated proteins.[211] The downstream effect of HSF2 activation by ubiquitinated protein is induction of the classic heat shock response, including cytoplasmic and nuclear chaperones HSP90, HSP70, HSC70, HSP27, an ER chaperone GRP78/BiP, and mitochondrial chaperone HSP60.

It is not yet known whether one or all of these signaling pathways are induced by ER retention of α_1-ATZ or whether there is any alteration in their activation in the subgroup of α_1-AT–deficient patients who are susceptible to liver disease. Because these are considered response pathways that are designed to protect the cell, it is presumed that they must be overwhelmed by the concentration or intrinsic toxic potential of a particular mutant protein before cell injury occurs. However, the consequences of prolonged activation of these response pathways are entirely unknown and could potentially include cytotoxic and/or oncogenic effects.

LIVER DISEASE IN α_1-ANTITRYPSIN DEFICIENCY

Soon after homozygous PIZZ α_1-AT deficiency was described, an association with premature development of emphysema was discovered.[212] Eriksson noticed that some of the individuals with emphysema also had cirrhosis of the liver,[213] but an association between α_1-AT deficiency and liver disease was first clearly established in 1969 by Sharp and colleagues[214]; Sharp also noticed the distinctive histopathologic features of inclusion bodies in the ER of liver cells in these children.[215]

The most important study of liver disease in α_1-AT deficiency was conducted by Sveger, who prospectively screened 200,000 newborn infants in Sweden.[1] Sveger identified 127 deficient infants and has followed them since then. These infants were evaluated clinically at the

age of 6 months. Fourteen of the 127 PIZZ infants had prolonged obstructive jaundice (group I). By clinical and laboratory criteria, 9 of these infants had severe liver disease and 5 had mild liver disease. Eight other PIZZ infants (group II) had minimal abnormalities in serum bilirubin, serum transaminases, and hepatic size. Approximately 50% of the remaining infants (group III) had abnormal serum transaminases.

Sveger collated data regarding the clinical outcome for these infants at 18 years of age. Three children from the group with prolonged obstructive jaundice (group I) died from liver disease before reaching 8 years of age. One group I child died from an unrelated cause. More than 85% of the remaining PIZZ children have persistently normal serum transaminases and no evidence of liver dysfunction.

Other studies of the incidence, prevalence, or prognosis of liver disease in α_1-AT deficiency[216–226] cannot be compared with the Sveger study[1] in that these studies involve PIZZ populations in which there is a bias in ascertainment (ie, the studies include only children referred to a specialty clinic). One issue not addressed by the Sveger study is whether 18-year-old patients with α_1-AT deficiency have persistent subclinical histologic abnormalities, despite the lack of clinical or biochemical evidence of liver injury, and whether liver disease will eventually become clinically evident during adulthood.

Liver involvement is often first noticed at 1 to 2 months of age because of persistent jaundice. Conjugated bilirubin levels in the blood and serum transaminase levels are mildly to moderately elevated. Blood levels of alkaline phosphatase and γ-glutamyl transpeptidase may also be elevated. The liver may be enlarged. There is a tendency for some of the affected infants to be small for gestational age. Affected infants are usually admitted to the hospital with a diagnosis of neonatal hepatitis syndrome and are subjected to a detailed diagnostic evaluation.[214,215,227] In fact, homozygous PiZZ α_1-AT deficiency appears to be the most common metabolic disease causing neonatal hepatitis syndrome.[216,228]

Infants may also be initially evaluated for α_1-AT deficiency because of an episode of gastrointestinal bleeding, bleeding from the umbilical stump, and/or bruising.[229] Occasionally, the deficiency is identified because of hepatosplenomegaly, ascites, and liver synthetic dysfunction in early infancy; an even smaller number have severe fulminant liver failure in infancy.[230] A few infants are recognized initially because of a cholestatic clinical syndrome characterized by pruritus and hypercholesterolemia. The clinical picture in these infants resembles extrahepatic biliary atresia, but histologic examination shows a paucity of intrahepatic bile ducts. Despite what is stated in one review,[155] many infants with homozygous PIZZ α_1-AT deficiency are completely asymptomatic and often go undetected. Even when detected in the Swedish screening study, only approximately 50% of the asymptomatic infants had elevated transaminases.[1,231]

Liver disease associated with α_1-AT deficiency may also be first discovered in late childhood or early adolescence, when the affected individual has abdominal distention from hepatosplenomegaly and/or ascites or has upper intestinal bleeding caused by esophageal variceal hemorrhage. Some of these patients have a history of unexplained prolonged obstructive jaundice during the neonatal period. In others, there is no evidence of any previous liver injury, even when the neonatal history is carefully reviewed.

α_1-AT deficiency should be considered in the differential diagnosis of any adult who presents with chronic hepatitis, cirrhosis, portal hypertension, or hepatocellular carcinoma of unknown origin. An autopsy study in Sweden shows a much higher risk of cirrhosis in adults with α_1-AT deficiency than was previously suspected and shows that this deficiency has a strong association with primary liver cancer.[232]

It is still not clear which clinical manifestations or abnormal laboratory test results can be used to predict a poor prognosis for individuals with liver disease associated with α_1-AT deficiency. One study suggested that persistent hyperbilirubinemia, hard hepatomegaly, early development of splenomegaly, and progressive prolongation of prothrombin time were indicators of poor prognosis.[223] In another study, elevated transaminase levels, prolonged prothrombin time, and a lower trypsin inhibitor capacity correlated with worse prognosis.[227] However, in my experience, some children with liver disease associated with α_1-AT deficiency can lead relatively normal lives for years after the development of hepatosplenomegaly and mild prolongation of prothrombin time. Volpert and colleagues reviewed 44 patients with α_1-AT deficiency seen at St. Louis Children's Hospital since 1984 when a registry was established.[233] Seventeen of these patients had cirrhosis and/or portal hypertension. Nine of these have had a prolonged, relatively uneventful course for at least 4 years after diagnosis. Of these patients, two eventually underwent liver transplant, but seven have led relatively healthy lives for up to 22 years after diagnosis. These nine patients could be distinguished only from the remaining eight patients by overall life functioning and not by any single clinical or biochemical characteristic. Thus, the prediction of poor prognosis for liver disease associated with α_1-AT deficiency and the timing of liver transplant depend more on the overall functioning of the affected child than on the histology or laboratory data.

There is currently no evidence that the heterozygous α_1-AT MZ phenotype causes liver disease in children by itself. It is not clear whether heterozygous MZ adults are predisposed to liver injury. Early studies of liver biopsy collections suggested that there was a relationship between heterozygosity and development of liver disease.[234] A retrospective study of liver transplant recipients at the Mayo Clinic showed that they had a higher prevalence of heterozygosity for α_1-ATZ in a group of these patients without another explanation for liver disease.[235] However, these studies are biased in ascertainment and do not include concurrent prospective controls. A cross-sectional study of patients with α_1-AT deficiency in a referral-based Austrian university hospital who were re-examined with the most sophisticated and sensitive assays available suggests that liver disease in heterozygotes can be accounted for, to a

great extent, by infections with hepatitis B or C virus or by autoimmune disease.[236] Although there is an overall impression that heterozygotes for α_1-ATZ are susceptible to liver disease, the literature does not provide convincing evidence that liver injury can be explained by the α_1-AT MZ heterozygous state alone.

Liver disease has been described for several other allelic variants of α_1-AT. Children with compound heterozygosity type PISZ are affected by liver injury in a manner similar to that of PIZZ children.[1,11] There are several reports of liver disease in α_1-AT deficiency-type PIM_{Malton}.[64,68] This is a particularly interesting association because the abnormal PIM_{Malton} α_1-AT molecule has been shown to undergo polymerization and retention within the ER.[141] Liver disease has been detected in single patients with several other α_1-AT allelic variants, such as PIM_{Duarte},[66] PIW,[237] and PIFZ,[238] but it is not clear whether other causes of liver injury for which there are more sophisticated diagnostic assays (such as hepatitis C infection and autoimmune hepatitis) have been completely excluded in these cases.

The distinctive histologic feature of homozygous PIZZ α_1-AT deficiency—periodic acid–Schiff–positive diastase-resistant globules in the ER of hepatocytes—substantiates the diagnosis (Figure 55.7-8). According to some observers, these globules are not as easy to detect in the first few months of life.[239,240] The presence of these inclusions should not be interpreted as diagnostic of α_1-AT deficiency; similar structures are occasionally observed in PIMM individuals with other liver diseases.[241] The inclusions are eosinophilic, round to oval, and 1 to 40 μm in diameter. They are most prominent in periportal hepatocytes but may also be seen in Kupffer cells and cells of biliary ductular lineage.[242] There may be evidence of variable degrees of hepatocellular necrosis, inflammatory cell infiltration, periportal fibrosis, and/or cirrhosis. There is often evidence of bile duct epithelial cell destruction, and there is occasionally a paucity of intrahepatic bile ducts. Our recent study has shown that there may also be an intense autophagic reaction detected by electron microscopic examination of liver biopsies, with a full array of nascent and degradative-type autophagic vacuoles.[168]

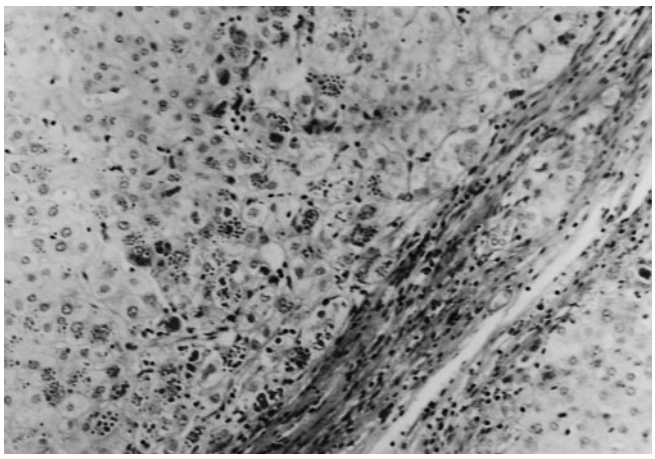

FIGURE 55.7-8 Hepatic histology in homozygous PIZZ α_1-antitrypsin (AT) deficiency. Micrograph of liver biopsy specimen in α_1-AT deficiency (periodic acid–Schiff [PAS]/diastase stain; ×40 original magnification), demonstrating the PAS+, diastase-resistant globules.

Diagnosis is established by a serum α_1-AT phenotype determination in isoelectric focusing or by agarose electrophoresis at acid pH (Figure 55.7-9). The phenotype should be determined in all cases of neonatal hepatitis or unexplained chronic liver disease in older children, adolescents, and adults. It is particularly important in the neonatal period because it may be very difficult to distinguish patients with α_1-AT deficiency from those with biliary atresia. Moreover, it is not uncommon for neonates with a PIZZ phenotype to have no biliary excretion on scintigraphic studies.[243] There is one report of α_1-AT deficiency and biliary atresia in a single patient.[244] We have had several patients with homozygous PIZZ α_1-AT deficiency and cholestasis and no biliary excretion of technetium-labeled mebrofenin, but with more prolonged observation in each of these cases, cholestasis remitted, so that it was then obvious that the patient did not have biliary atresia.

When used with isoelectric focusing, serum concentrations of α_1-AT may be helpful in distinguishing individuals who are homozygous for the Z allele from SZ compound heterozygotes, both of whom may develop liver disease. In some cases, phenotype determinations of parents and/or other relatives are also necessary to ensure the distinction between ZZ and SZ allotypes, a distinction that is important for genetic counseling. Serum concentrations of α_1-AT are occasionally misleading. For instance, concentrations may increase during the host response to inflammation, even in homozygous PIZZ individuals, giving a falsely reassuring impression.

LUNG DISEASE IN α_1-ANTITRYPSIN DEFICIENCY

The incidence and prevalence of emphysema in α_1-AT deficiency have not been studied prospectively. Autopsy studies suggest that 60 to 65% of people with homozygous PIZZ α_1-AT deficiency develop clinically significant lung injury. However, there are PIZZ smokers who do not have any symptoms of lung disease or evidence of pulmonary function abnormalities until the seventh or eighth decade of life.[245]

The typical person with lung disease is a male cigarette smoker. Onset of dyspnea is insidious in the third to fourth decade of life. About 50% of affected persons develop cough and recurrent lung infections. The disease progresses to a severe limitation of airflow. A reduction in the forced expiratory volume, increase in total lung capacity, and reduction in diffusing capacity occur. Chest radiography demonstrates hyperinflation with marked lucency at the lung bases.[246] Histopathologic studies demonstrate panacinar emphysema, which is more prominent in the lower lung.[246,247]

It is rare for emphysema to affect an α_1-AT–deficient patient during childhood. A number of patients have been described in the literature, but an alternative explanation can be offered in each of these cases. In the most convincing case, emphysema developed several years after a porta-

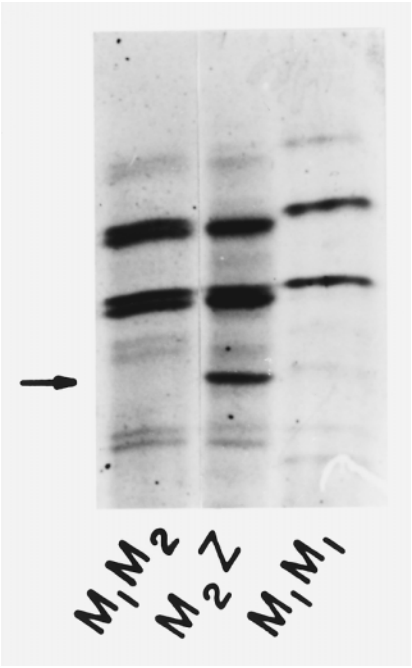

FIGURE 55.7-9 Isoelectric focusing of human serum samples for diagnosis of α_1-AT deficiency. Sera from a normal M_1M_2 individual, an M_2Z heterozygote, and a normal M_1M_1 individual were subjected to isoelectric focusing, with the anode at top and cathode at bottom. Migration of the Z allele is indicated by the arrow. The gel was provided by J. A. Pierce, St. Louis, MO. Reproduced with permission from Perlmutter DH. Alpha-1-antitrypsin deficiency. In: Snape WJ, editor. Consultations in gastroenterology. Philadelphia: WB Saunders; 1996. p. 793.

caval shunt procedure was done.[248] In three early cases, there were problems with the phenotypic diagnosis of α_1-AT deficiency.[249–251] In a more recent report, two patients had pulmonary abnormalities that could have been attributed to severe systemic illness associated with end-stage liver disease.[252] In a number of infants with α_1-AT deficiency, pulmonary function testing suggested a subtle degree of hyperinflation.[253] However, another study detected no significant difference between the pulmonary function of PIZZ children aged 13 to 17 years and that of an age-matched control group.[254] These data indicate that it is extremely rare for α_1-AT deficiency to cause emphysema in individuals less than 25 years of age.

The destructive effect of cigarette smoking on the outcome of lung disease in α_1-AT deficiency has been demonstrated in many studies. Actuarial studies suggest that cigarette smoking reduces median survival by over 20 years in deficient persons.[255] The rate of decline in forced expiratory volume is four times greater in α_1-AT–deficient persons who smoke than in α_1-AT–deficient persons who do not smoke.[256]

There is still very limited information about the incidence of liver disease in α_1-AT–deficient individuals with emphysema. In one study of 22 PIZZ patients with emphysema, there was an elevated transaminase level in 10 patients and cholestasis in 1 patient.[257] Liver biopsies were not done in this study, and such biopsies may be necessary to accurately determine the extent of liver injury in α_1-AT–deficient patients with emphysema.

TREATMENT

The most important principle in the treatment of α_1-AT deficiency is avoidance of cigarette smoking. Cigarette smoking markedly accelerates the destructive lung disease associated with α_1-AT deficiency, reduces the quality of life, and significantly shortens longevity.[255,256,258] These facts need to be presented to the families of affected pediatric patients in an unambiguous manner. Although it is not usually an issue that arises in the pediatric gastrointestinal and liver clinic, it may be necessary to carefully monitor the smoking habits of the family and, during interval visits, to re-emphasize the important effect of smoking on outcomes for deficient individuals.

There is no specific therapy for liver disease associated with α_1-AT deficiency. Therefore, clinical care largely involves supportive management of symptoms owing to liver dysfunction and prevention of complications. Although the use of ursodeoxycholic acid and colchicine has been mentioned in the literature, there is no evidence for biochemical or clinical efficacy for either drug.

Progressive liver dysfunction and liver failure in children have been treated by orthotopic liver transplant, with survival rates in one study approaching 90% at 1 year and 80% at 5 years.[259] A more recent study put 10-year actuarial survival at 68%.[260] Nevertheless, a number of PIZZ individuals with severe liver disease (even cirrhosis and/or portal hypertension) may have a relatively low rate of disease progression and may lead a relatively normal life for extended periods of time. With the availability of transplant techniques with living related donors, it may be possible to manage these patients expectantly for some time. Children with α_1-AT deficiency and mild liver dysfunction (elevated transminases and/or hepatomegaly) and without functional impairment may never need liver transplant surgery.

Most α_1-AT–deficient children with liver disease are not candidates for alternative surgical interventions. However, there are rare specific clinical situations in which a portacaval or splenorenal shunt might be considered (such as in a child with only mild liver synthetic dysfunction and mild parenchymal liver injury but severe portal hypertension). Several children with severe liver disease and α_1-AT deficiency have survived 10 to 15 years after shunt surgery before requiring orthotopic liver transplant.[261] Moreover, previous hepatobiliary surgery is not a statistically significant risk factor for poor outcome of subsequent orthotopic liver transplant.[262]

Trials of pharmacologic therapy for α_1-AT deficiency have been conducted. Patients have been given the synthetic androgens danazol or stanazolol because of the dramatic effects of the same agents in patients with hereditary angioedema,[263] which is a deficiency of the homologous serine proteinase inhibitor C1 inhibitor, and because danazol was initially found to increase serum levels of α_1-AT in PIZZ persons.[264] However, further evaluation has demonstrated that danazol increases serum levels of α_1-AT in only 50% of deficient persons, and the magnitude of the effect is small.[265] Moreover, it was not clear from any of the studies whether the effect of androgens occurred at the level of

synthesis and might also be associated with increased accumulation of α_1-ATZ in the ER, with potential hepatotoxic consequences.

Several studies have shown that a class of compounds called chemical chaperones can reverse the cellular mislocalization or misfolding of mutant plasma membrane, lysosomal, nuclear, and cytoplasmic proteins (including CFTRΔF508, prion proteins, mutant aquaporin molecules associated with nephrogenic diabetes insipidus, and mutant galactosidase A associated with Fabry disease).[266] These compounds include glycerol, trimethylamine oxide, deuterated water, and 4-phenylbutyric acid (PBA). Burrows and others recently found that glycerol and PBA mediate a marked increase in secretion of α_1-ATZ in a model cell culture system.[156] Moreover, oral administration of PBA was well tolerated by PiZ mice (transgenic for the human α_1-ATZ gene) and consistently mediated an increase in blood levels of human α_1-AT, reaching 20 to 50% of the levels present in PiM mice and normal humans. The synthesis or intracellular degradation of α_1-ATZ was not affected by PBA. The α_1-ATZ secreted in the presence of PBA was functionally active in that it could form an inhibitory complex with neutrophil elastase. Because PBA has been used safely for years in children with urea cycle disorders as an ammonia scavenger, and because clinical studies have suggested that only partial correction is needed for prevention of both liver and lung injury in α_1-AT deficiency, PBA constitutes a candidate for chemoprophylaxis of target organ injury in α_1-AT deficiency.

It also now appears that several iminosugar compounds may be potentially useful for chemoprophylaxis of liver and lung disease in α_1-AT deficiency. These compounds are designed to interfere with oligosaccharide side chain trimming of glycoproteins and are now being examimed as potential therapeutic agents for viral hepatitis and other types of infections.[267,268] We have examined several of these compounds initially to determine the effect of inhibiting glucose or mannose trimming from the carbohydrate side chain of mutant α_1-ATZ on its fate in the ER but found to our surprise that one glucosidase inhibitor, castanospermine, and two α-mannosidase I inhibitors, kifunensine and deoxymannojirimicin, actually mediate increased secretion of α_1-ATZ.[269] The α_1-ATZ that is secreted in the presence of these drugs is partially functionally active. Kifunensine and deoxymannojirimicin are less attractive candidates for chemoprophylactic trials because they delay degradation of α_1-ATZ in addition to increasing its secretion and therefore have the potential to exacerbate susceptibility to liver disease. However, CST has no effect on the degradation of α_1-ATZ and, therefore, may be targeted for development of a chemoprophylactic agent. The mechanism of action of castanospermine on α_1-ATZ secretion is unknown. An interesting hypothesis for the mechanism of action of kifunensine and deoxymannojirimicin has mutant α_1-ATZ interacting with ERGIC-53 for transport from the ER to the Golgi complex when mannose trimming is inhibited.

Novoradovskaya and others have suggested that inhibition of ER degradation of α_1-ATZ by proteasome inhibitor lactacystin and by protein synthesis inhibitor cycloheximide is associated with increased secretion of α_1-ATZ.[270] Burrows and others have been unable to confirm this result.[156] Moreover, there are now several lines of evidence indicating that there is no simple relationship between ER degradation of α_1-ATZ and its secretion such that perturbations that delay degradation are automatically accompanied by increased secretion. Some physiologic and pharmacologic perturbations are associated with delayed degradation without any change in secretion. Other perturbations increase secretion without any change in degradation. Increased temperature is associated with both delayed degradation and increased secretion.[156]

Some patients with α_1-AT deficiency and emphysema are currently receiving replacement therapy with purified or recombinant plasma α_1-AT either by intravenous or intratracheal aerosol administration.[271] This therapy is associated with an improvement in serum concentrations of α_1-AT and in neutrophil elastase inhibitory capacity in bronchoalveolar lavage fluid, without significant side effects. Although an initial study suggested that there is a slower decline in forced expiratory volume in patients on replacement therapy, this occurred only in a subgroup of patients, and the study was not randomized.[272]

Protein replacement therapy is designed only for individuals with established and progressive emphysema. It is not being considered for individuals with liver disease because there is no information to support the notion that deficient serum levels of α_1-AT are mechanistically related to liver injury.

A number of patients with severe emphysema from α_1-AT deficiency have undergone lung transplant in the past 10 years. The latest data from the St. Louis International Lung Transplant Registry show that 91 patients with emphysema and α_1-AT deficiency underwent single or bilateral lung transplant by 1993. Actuarial survival for patients in this category who underwent transplant between 1987 and 1994 is approximately 50% for 5 years. Lung function and exercise tolerance are significantly improved.[273]

Replacement of α_1-AT by somatic gene therapy has also been discussed in the literature.[271] This strategy is potentially less expensive than replacement therapy with purified protein and would alleviate the need for intravenous or inhalation therapy. Again, this form of therapy would be useful only in ameliorating emphysema because liver disease associated with α_1-AT deficiency is not caused by deficient levels of α_1-AT in the serum or tissue. Before clinical trials involving gene therapy are conducted, it would be helpful to know that replacement therapy with purified α_1-AT, as it is currently applied, is effective in ameliorating emphysema in this deficiency. Also, there are still major issues that need to be addressed before gene therapy becomes a realistic alternative.[274] Several novel types of gene therapy, such as repair of mRNA by trans-splicing ribozymes[275] and chimeric RNA/DNA oligonucleotides,[276] are theoretically attractive alternative strategies for liver disease in α_1-AT deficiency because they would prevent the synthesis of mutant α_1-ATZ protein and ER retention. In fact, a chimeric RNA/DNA oligonucleotide based on the sequence of coagulation fac-

tor IX in complex with lactose (so that it could be taken up by asialoglycoprotein receptor–mediated endocytosis) was delivered to hepatocytes with surprisingly high efficiency after intravenous administration.[276] However, efficiency has been much lower for other mutant proteins.

Recent studies have shown that transplanted hepatocytes can repopulate the diseased liver in several mouse models,[277,278] including a mouse model of a childhood metabolic liver disease termed hereditary tyrosinemia. Replication of the transplanted hepatocytes occurs only when there is injury and/or regeneration in the liver. The results provide evidence that it may be possible to use hepatocyte transplant techniques to treat hereditary tyrosinemia and perhaps other metabolic liver diseases in which the defect is cell autonomous. For instance, α_1-AT deficiency involves a cell-autonomous defect and would be an excellent candidate for this strategy.

Alternative strategies for at least partial correction of α_1-AT deficiency may result from a more detailed understanding of the fate of the α_1-ATZ molecule in the ER. First, delivery of synthetic peptides to the ER to insert into the gap in the A sheet or into a particular hydrophobic pocket of the α_1-AT molecule[279] and prevent polymerization of α_1-AT might result in the release of the mutant α_1-ATZ molecules into the extracellular fluid and might prevent its accumulation in the ER. Although it is not yet entirely clear, there is some evidence from studies on the assembly of class I MHC molecules that synthetic peptides may be delivered to the ER from the extracellular medium of cultured cells.[280] There is also evidence that certain molecules may be transported retrograde to the ER by receptor-mediated endocytosis.[281,282] Second, elucidation of the biochemical mechanism by which abnormally folded α_1-AT undergoes intracellular degradation might allow pharmacologic manipulation of this degradative system, such as enhancing proteasomal activity with interferon-γ in the subpopulation of PIZZ individuals predisposed to liver injury. Third, a competitive antagonist of binding or signal transduction by α_1-AT–proteinase complexes at the SEC receptor might prevent increases in intracellular accumulation of α_1-AT during augmentation of α_1-AT levels with protein replacement or gene replacement therapies.

Genetic Counseling

Restriction fragment length polymorphisms detected with synthetic oligonucleotide probes[283,284] and family studies[285] allow prenatal diagnosis of α_1-AT deficiency. Nevertheless, it is not clear how prenatal diagnosis for this deficiency should be used and how families should be counseled regarding the diagnosis. Data from the Sveger study indicate that 70 to 75% of persons with α_1-AT deficiency do not have evidence of liver disease at the age of 18 years and that nonsmoking PIZZ persons may not develop emphysema or even pulmonary function abnormalities until 60 to 70 years of age.[11] These data could support a counseling strategy in which amniocentesis and abortion are discouraged. The only other data on this subject suggest a 78% chance that a second PIZZ child will have serious liver disease if the older sibling had serious liver disease.[224] However, this study was retrospective and was influenced by bias in the ascertainment of patients. The issue will not be resolved until it is studied prospectively.[1,11]

Screening for α_1-Antitrypsin Deficiency

Several recent studies have suggested that population screening for α_1-AT deficiency would be efficacious. First, there is now evidence that knowledge of and counseling in the consequences of α_1-AT deficiency are associated with a reduced rate of smoking among affected adolescents.[286,287] Second, although there was some evidence for adverse psychological effects from knowledge of the deficiency by affected families,[288] more recent studies have indicated that there were no significant negative psychosocial consequences in early adulthood from neonatal screening for α_1-AT deficiency in Sweden.[289] These data should give new momentum to the reconsideration of screening programs for α_1-AT deficiency.

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8. *Zellweger Syndrome and Other Disorders of Peroxisomal Metabolism*

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Zellweger syndrome and an expanding spectrum of related peroxisomal diseases have emerged in the last 20 years as major identifiable causes of liver disease in the pediatric population. Because of the wide range of associated nonhepatic abnormalities in these disorders and the often initially silent nature of the progressive liver disease, many patients with peroxisomal diseases come to the attention of the gastroenterologist from a number of different hospital clinics, where they may have been followed for many months or years. Thus, a thorough understanding of the full spectrum of clinical and metabolic characteristics of peroxisomal disorders is essential for practicing gastroenterologists.

The cerebrohepatorenal syndrome of Zellweger is by far the best known genetic disorder of peroxisomal metabolism. Although Zellweger syndrome was first described as an autosomal recessive, multiple anomaly syndrome in 1964,[1] the discovery in 1973 that hepatic and renal cells of patients with Zellweger syndrome were devoid of recognizable peroxisomes and had dysfunctional mitochondria refocused attention on Zellweger syndrome as a possible metabolic disorder.[2] As a result, Zellweger syndrome emerged as the prototypic "metabolic malformation syndrome" and spawned the development of a new field of biochemical genetics. Indeed, more than a dozen clinical disorders have been identified or redescribed as diseases of the peroxisome. In some, such as classic Zellweger syndrome and neonatal adrenoleukodystrophy (ALD), the entire peroxisome and most of its associated biochemical functions appear to be lost or severely deficient. In others, such as X-linked ALD and several disorders of bile acid biosynthesis, only a single peroxisomal protein appears to be deficient. The different patterns of biochemical abnormalities manifest by these diverse syndromes are now understood to be caused by, principally, several different defects of peroxisomal protein importation and a variety of single enzymatic deficiencies. Overall, the discovery and biochemical characterization of these peroxisomal experiments of nature have fundamentally changed our understanding of the role of the peroxisome in human metabolism. In this chapter, the principal metabolic functions of the peroxisomes and the major clinical disorders associated with an apparent primary deficiency of peroxisomal metabolism are reviewed. Guides to the diagnosis and treatment of the peroxisomal disorders are also presented.

STRUCTURE AND FUNCTION OF NORMAL PEROXISOMES

Tissue Distribution and Characteristics of Peroxisomes

Peroxisomes are ubiquitous subcellular organelles defined by de Duve as small (0.1–1.0 μm), dense, subcellular particles bounded by a single membrane and containing the enzymatic machinery for the evolution and consumption of hydrogen peroxide.[3] Similar peroxidative organelles in plants contain the important glyoxylate cycle and related carbohydrate pathways and are known as glyoxysomes. The term *microbodies* is commonly used to refer to both organelles.[4,5]

Although large (0.5–1.5 μm) and more conspicuous peroxisomes were first identified only in hepatocytes (Figure 55.8-1) and renal proximal tubule cells, essentially all mammalian cells except erythrocytes have since been found to contain peroxisomes. These organelles range in size from smaller (0.1–0.2 μm) microperoxisomes in the brain to the larger structures found in liver and kidney. Hepatocytes and renal tubule cells have the greatest abundance of peroxisomes, which may constitute as much as 1% of the cell mass, whereas the collective volume of peroxisomes in muscle, fibroblasts, and neuronal tissue is at least an order of magnitude less.[6,7] The number of peroxisomes per cell can range from fewer than 100 to more than 1,000. In most tissues, peroxisomes appear as round or ovoid organelles with a finely granular matrix, bounded by a single membrane and stainable by a catalase-detecting reaction with diaminobenzidine. Although by electron microscopy the single peroxisomal membrane appears trilaminar, it is notably thinner than the trilaminar single membrane of lysosomes and lacks the clear zone subjacent to the lysosomal membrane. The larger peroxisomes of some species include a dense, crystalline-like "nucleoid" core containing urate oxidase. Species that lack urate oxidase, such as humans and birds, also lack peroxisomal cores. An important characteristic of hepatic peroxisomes of some species, especially rats and mice, is proliferation induced by a variety of natural and xenobiotic compounds, such as *trans*–unsaturated fatty acids, clofibrate, and thyroxine.[8,9] The proliferative action of these compounds is mediated by peroxisome proliferator-activated receptors

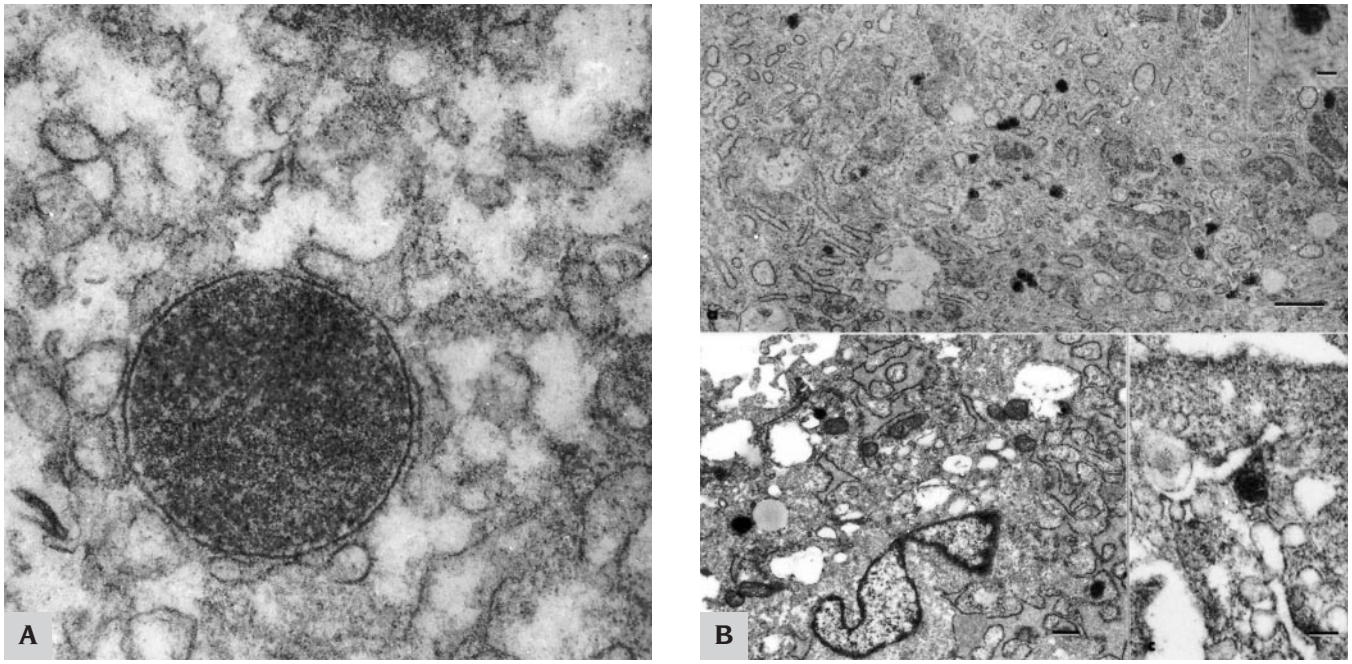

FIGURE 55.8-1 *A*, Electron micrograph of a normal human liver peroxisome showing a heterogeneous matrix surrounded by a single membrane. Human peroxisomes lack the dense "nucleoids" present in the peroxisomes of most other vertebrate species. *B*, Electron micrographs of fibroblasts incubated in a medium for the demonstration of peroxisomal catalase by the deposition of an electron-dense reaction product. *Bottom*, Normal human fibroblast containing several peroxisomes with variable staining (*inset:* magnification to show heterogeneous distribution of catalase staining). *Top*, Electron-dense small peroxisomes in the cytoplasm of fibroblasts from a patient with Zellweger syndrome. Courtesy of Sydney Goldfischer, MD.

(PPARs), which are closely related to steroid hormone receptors.[10–13] However, although peroxisomal proliferation is associated with hepatic neoplasia in rats,[14] there is little evidence for drug-mediated peroxisomal proliferation or hypolipidemic drug-mediated carcinogenesis in humans despite the presence of PPARs in human tissues.[13]

Peroxisomes appear to be independent organelles with a biogenesis separate from other subcellular organelles and compartments.[15,16] Extensive investigation of peroxisome biogenesis has refuted early hypotheses that these organelles arise from budding of the endoplasmic reticulum (ER). Gould and colleagues have proposed that there are two coexisting pathways of peroxisome biogenesis: one involving growth and division of preexisting peroxisomes and the other beginning with a preperoxisomal membrane vesicle.[16] Both models require uptake of lipid and membrane proteins as well as matrix proteins, followed by fission once a critical size is reached. Extensive genetic studies using yeast mutants, Chinese hamster ovary cell mutants, and skin fibroblasts from patients with peroxisomal biogenesis disorders have revealed the existence of at least 23 genes involved in peroxisome biogenesis.[15] The genes are referred to as *PEX* genes and their protein products as peroxins. Deficiency or mutation in 11 of the *PEX* genes is now known to result in human peroxisomal biogenesis disorders. Unlike mitochondria, there is no evidence for specific peroxisomal deoxyribonucleic acid (DNA) encoding the synthesis of peroxisomal proteins. All peroxins, as well as other membrane and matrix proteins, are encoded by nuclear genes and are synthesized on free polyribosomes.

Matrix proteins are targeted to peroxisomes primarily by one of two peptidyl targeting signals. The majority of matrix proteins have been found to contain peroxisome targeting signal (PTS) 1, a carboxy-terminal tripeptide with a suggested consensus of (S/A/C)-(K/R/H)-L-COOH.[17] Fewer matrix proteins are targeted to the organelle by PTS2, found near but not at the amino terminus, which has a consensus sequence of (R/K)-(L/V/I)-X_5-(Q/H)-(L/A).[16] The receptors for PTS1 and PTS2 proteins are encoded by the *PEX5* and *PEX7* genes, respectively.[18,19] The currently accepted model of peroxisomal matrix protein import involves numerous peroxins located in both the cytoplasm and the peroxisomal membrane.[16] After a newly synthesized matrix protein binds to its receptor in the cytoplasm, the complex is transported to the surface of the peroxisome, where it interacts with docking proteins and the import machinery. The matrix protein is imported into the peroxisome, and the receptor is returned to the cytoplasm via recycling factors. Although several peroxins have been implicated in the import of peroxisomal membrane proteins, this process remains less well characterized than matrix protein import.

Peroxisomes are mostly randomly distributed in hepatocytes but may occur closely juxtaposed to the ER, from which peroxisomes were once thought to arise by budding. In some cells, peroxisomes are seen surrounding glycogen or triglyceride deposits.[20] The close association of peroxisomes with ER is denoted structurally by a dense thickening (the "marginal plate") of the segment of the peroxisomal membrane paralleling the ER.[21,22] Moreover, in tissues with a high rate of fatty acid β-oxidation, there is a nonrandom

association of peroxisomes with mitochondria, usually separated by an intercalated bilayer of ER.[20] An extreme structural specialization of peroxisomes occurs in the cells of sebaceous glands, wherein the peroxisomal compartment exits as an extensive filamentous network believed to subserve the synthesis of the unusual waxes and etherlipids of sebum.[23] Coreless, filamentous tails and interperoxisomal connections have also been found by careful serial sectioning of rat liver[24] and may be common, if variable, features of the peroxisomal space.

Metabolic Pathways of the Peroxisome

Once thought to be vestigial, the vertebrate peroxisome is now known to contain a remarkable variety of highly specialized and essential enzymatic systems for the synthesis and catabolism of, largely, lipids and amino acids (Table 55.8-1).[5] From a clinical-biochemical standpoint, the most important of these functions are (1) β-oxidation of very-long-chain fatty acids (VLCFAs) and 2-methyl branched-chain fatty acids; (2) synthesis of sterols and bile acids; (3) synthesis of plasmalogens; (4) α-oxidation of phytanic acid, a 3-methyl branched-chain fatty acid; (5) oxidase-mediated metabolism of amino acids; and (6) catalatic and peroxidatic decomposition of hydrogen peroxide. In addition, peroxisomes appear to have a role in the synthesis of cholesterol and, in some species, in the synthesis of highly specialized biochemicals such as waxy esters and pheromones.[25] For some processes, such as β-oxidation of fatty acids, a complete pathway exists in the peroxisome, whereas for others, such as bile acid or plasmalogen synthesis, only a portion of the pathway is unique to the peroxisome.

Major Metabolic Functions of the Peroxisome

Peroxisomal Fatty Acid β-Oxidation. Although fatty acid β-oxidation was first recognized as a function of peroxisomes (more exactly, glyoxysomes) of germinating seedlings in 1969,[26] not until 1978 was a complete ensemble of β-oxidative enzymes functionally similar to those of mitochondria found in mammalian peroxisomes (Figure 55.8-2).[27] However, despite identical stereochemistry and evolutionary homology of most of the peroxisomal β-oxidation enzymes and their counterparts in mitochondria,[28] the rate-limiting enzymes and substrate specificities

TABLE 55.8-1 MAJOR METABOLIC FUNCTIONS OF THE PEROXISOME

β-Oxidation of very-long-chain fatty acids
β-Oxidation of branched-chain fatty acids
β-Oxidation of dicarboxylic acids
α-Oxidation of phytanic acid
Synthesis of ether lipids (eg, plasmalogens)
Synthesis of sterol precursors and bile acids
Synthesis of waxy esters
Amino acid transamination
Oxidation of D- and L-amino acids
Oxidation of L-α-hydroxy acids
Oxidative catabolism of polyamines
Catabolism of purines
Catalatic and peroxidatic decomposition of hydrogen peroxide

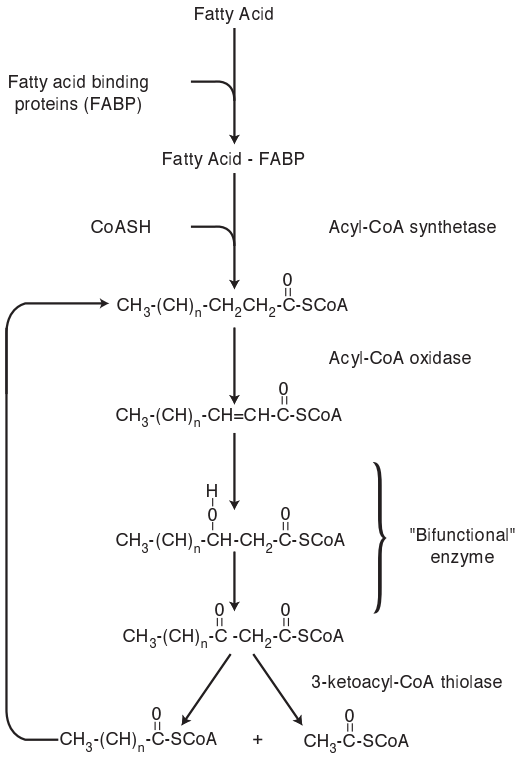

FIGURE 55.8-2 Peroxisomal pathway for β-oxidation of fatty acids. Medium-chain, long-chain, and very-long-chain fatty acids are shortened by two carbons for each cycle of β-oxidation down to an 8- or 6-carbon fatty acid. The acetate units and remnant fatty acids leave the peroxisome as carnitine esters via the action of acetylcarnitine and octanoylcarnitine transferases.

of β-oxidation in the two organelles are distinctly different. Recently, additional enzymes comprising a second, complete peroxisomal β-oxidation pathway have been identified in mammals.[29] Whereas the original peroxisomal β-oxidation pathway follows L-isomer enzymology and oxidizes straight-chain saturated fatty acids, the second, the D-specific peroxisomal pathway, catalyzes the oxidation of branched-chain fatty acids, including oxidation of the branched sterol side chain required for the synthesis of bile acids.[30] There is compelling evidence for interaction and crossover between the L- and D-specific pathways because the degradation of straight-chain VLCFAs requires enzymes from both pathways.[31]

There is considerable similarity between the enzymes and the enzymatic reactions of the two peroxisomal β-oxidation pathways. The first step in both pathways is carried out by an acyl coenzyme A (CoA) oxidase that consumes molecular oxygen and generates hydrogen peroxide. The straight-chain oxidase has broad specificity for all but short-chain (C_4–C_8) fatty acids.[32] In contrast, at least four separate acyl-CoA dehydrogenases—short-, medium-, long-, and very-long-chain acyl-CoA dehydrogenases—catalyze the degradation of straight-chain fatty acids in mitochondria. Deficiency of the straight-chain acyl-CoA oxidase in humans leads to the accumulation of VLCFAs and a distinct clinical disorder.[33,34] Known substrates for the branched-chain acyl-CoA oxidase are 2-methylacyl CoAs and include pristanic acid, a product of phytanic acid

oxidation,[35] and precursors of bile acids.[36] The second and third reactions of both peroxisomal pathways are carried out by monomeric, multifunctional proteins that contain both enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase activities.[37] In contrast, two separate proteins in mitochondria catalyze these steps. The originally identified "bifunctional enzyme," now known as either multifunctional enzyme (MFE) 1 or L-bifunctional protein (L-BP), is L-specific. However, human deficiency of the more recently described D-specific enzyme, referred to as either MFE2 or D-bifunctional protein (D-BP), causes a failure in the oxidation of VLCFAs and branched-chain fatty acids. MFE2 was found to be identical to 17β-hydroxysteroid dehydrogenase type 4 and has a domain at its carboxy terminus that resembles sterol carrier protein (SCP) 2, an important intracellular sterol and lipid-binding and transport protein.[38] The terminal β-oxidation reactions for both peroxisomal pathways are catalyzed by a 3-ketoacyl CoA thiolase, as in the mitochondrial β-oxidation system. The thiolase of the L-specific peroxisomal pathway is one of the few known PTS2-containing proteins. The thiolase of the D-specific pathway resides in the amino-terminal domain of SCPX, a 58 kD protein that is cleaved to form the two smaller functional proteins.[39]

Several other enzymes and proteins are involved in peroxisomal β-oxidation, and deficiencies of two of these are known to cause human disease. Clinically, the most important of these is the ALD protein, ALDP, a peroxisomal transmembrane transporter protein.[40] Mutations in the *ABCD1* gene that encodes ALDP lead to the accumulation of VLCFAs and the most common peroxisomal disease, X-linked ALD. Despite extensive investigation since the discovery of ALDP in 1993, the exact function of this protein remains unknown.

More recently, the importance of 2-methylacyl CoA racemase in peroxisomal β-oxidation of 2-methyl branched-chain compounds such as pristanic acid and bile acid precursors has been recognized. Only one of the two naturally occurring stereoisomers of pristanic acid or bile acid precursors, the *S*-conformers, are substrates for branched-chain acyl CoA oxidase.[41,42] Peroxisomal racemase converts compounds with an *R*-conformation at the 2-carbon to the corresponding *S*-conformer, allowing subsequent metabolism to occur. Four patients with deficiency of 2-methylacyl CoA racemase have now been identified.

From a physiologic standpoint, mitochondria are most important in the conversion of dietary fatty acids—palmitate, oleate, linoleate, and stearate—into acetyl CoA for energy metabolism, ketogenesis, and various synthetic pathways. Peroxisomes, on the other hand, appear to specialize in the β-oxidation of VLCFAs (more than 22 carbons),[43] certain unsaturated fatty acids,[44,45] dicarboxylic acids,[46] branched-chain fatty acids,[47] and a variety of xenobiotic acids, such as phenyl-substituted fatty acids.[48] The end products of β-oxidation—acetyl CoA in mitochondria versus acetylcarnitine and octanoylcarnitine in peroxisomes—as well as the fate of the extracted reducing equivalents—coupled to adenosine triphosphate (ATP) synthesis in mitochondria versus lost to hydrogen peroxide and its exergonic reactions in peroxisomes—also differ. Lastly, the total capacity of peroxisomal, but not mitochondrial, β-oxidation can be substantially amplified in some species by exposure to preferred substrates or drugs, such as clofibrate and related hypolipidemic drugs,[8,9] which also cause peroxisomal proliferation. The deficiency of this highly specialized system for β-oxidation is responsible for several of the clinically most important biochemical markers for peroxisomal disease, such as increased levels of VLCFAs.

Phytanic Acid Oxidation. Phytanic acid (3,7,11,15-tetramethylhexadecanoic acid) is a 3-methyl branched-chain fatty acid produced by oxidation of the free phytol

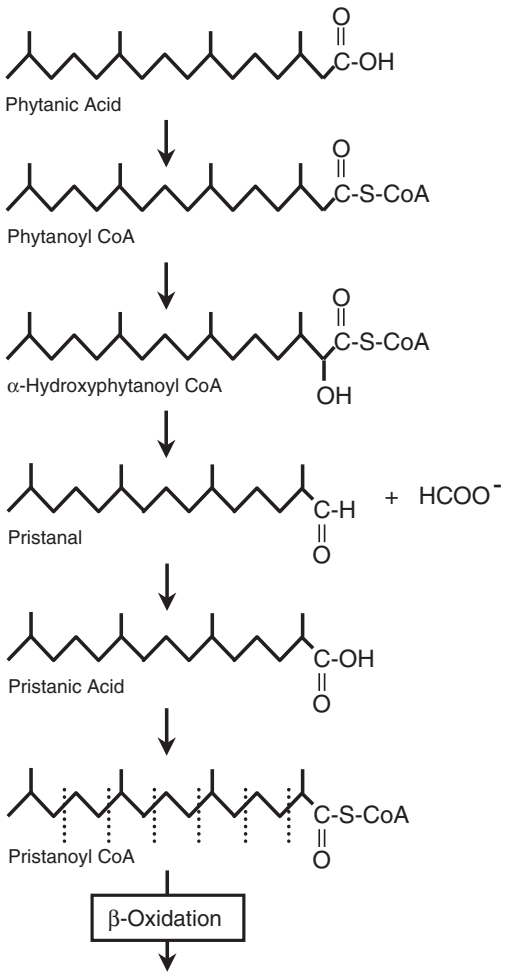

FIGURE 55.8-3 Sequence of α-oxidation of phytanic acid to pristanic acid and subsequent β-oxidation of pristanic acid to acetate and propionate units. α-Oxidation of phytanic acid, once thought to be coenzyme A (CoA) independent, requires activation of phytanate to a CoA intermediate, similar to β-oxidation in both mitochondria and peroxisomes. Pristanic acid, after reactivation to its CoA derivative, undergoes three cycles of β-oxidation in peroxisomes, alternately yielding propionyl CoA and acetyl CoA. The product of peroxisomal metabolism, 4,8-dimethylnonanoyl CoA, undergoes further degradation by mitochondrial β-oxidation. Not shown in this scheme is α-methylacyl CoA racemase, which is indispensible for converting any 2*R*-methylacyl CoAs to their 2*s* conformation.

chain of chlorophyll (Figure 55.8-3). Free phytol is concentrated in green vegetables, vegetable fats, and animal fats. However, phytanic acid formed by the action of phytol-metabolizing rumen bacteria and subsequently stored in animal and fish fats is the primary source of phytanic acid in human nutrition.[49,50] Once absorbed, dietary phytanic acid must undergo further α-oxidation in peroxisomes to remove the terminal carboxyl carbon to form pristanic acid (2,6,10,14-tetramethylpentadecanoic acid), a 2-methyl branched-chain fatty acid that is sequentially catabolized by the peroxisomal and mitochondrial β-oxidative systems.[35] Terminal Ω-oxidation activates a small portion of phytanic acid for β-oxidation from the omega end.[51]

Although it was originally thought that α-oxidation of phytanic acid began with a direct α-hydroxylation of the free acid by phytanic acid oxidase, more recent studies have established that a CoA derivative must be formed first.[52] Hydroxylation of the α-carbon by phytanoyl CoA α-hydroxylase (PAHX) yields L-2-hydroxyphytanoyl CoA, which is subsequently cleaved to formyl CoA and the aldehyde pristanal by 2-hydroxyphytanoyl CoA lyase.[53–56] Oxidation of pristanal to pristanic acid requires an aldehyde dehydrogenase, and early studies suggested that this dehydrogenase was identical to the microsomal enzyme defective in the mental retardation–ichthyosis disorder Sjögren-Larsson syndrome.[57] Most investigators now believe that a peroxisomal rather than a microsomal aldehyde dehydrogenase is responsible for the formation of pristanic acid, which, after activation to its CoA thioester, undergoes three rounds of peroxisomal β-oxidation (D-pathway) before transfer to mitochondria for further β-oxidation cycles.[35] As noted previously, pristanic acid β-oxidation requires the participation of α-methylacyl CoA racemase to convert R-pristanoyl CoA to S-pristanoyl CoA. Refsum disease has now been shown to be caused by mutations in PAHX.[58–60] Failure to import enzymes of both the α- and β-oxidation pathways in disorders of peroxisome biogenesis such as Zellweger syndrome results in the decreased ability to catabolize both phytanic acid and pristanic acid and the characteristic elevation of both phytol derivatives. Because phytanyl CoA α-hydroxylase is targeted to peroxisomes by PTS2, patients with rhizomelic chondrodysplasia punctata (RCDP) have impaired phytanic acid oxidation but normal pristanic acid oxidation.[61]

Cholesterol Biosynthesis. Hypocholesterolemia was one of the first relatively consistent biochemical abnormalities found in patients with Zellweger syndrome, suggesting that peroxisomes have a role in cholesterol biosynthesis. Sterols are synthesized by a complex series of reactions beginning with 3-hydroxy-3-methylglutaryl (HMG)-CoA and ending with the 30-carbon precursor of all other sterols, lanosterol (4,4,14-trimethylcholesta-8(9),24-dien-3β-ol). The first reaction in this complex biosynthetic pathway, the reduction of HMG-CoA to the 6-carbon acid alcohol mevalonic acid, is catalyzed by HMG-CoA reductase, which is generally accepted as the principal rate-determining step of cholesterol biosynthesis.[62] Cholesterol, a 27-carbon, monounsaturated sterol, is synthesized from lanosterol by a series of oxidations, reductions, and demethylations. Although all steps of cholesterol synthesis in mammals were once thought to take place in the ER, studies by Krisans and colleagues have established that the second enzyme of the pathway, mevalonate kinase, and at least two other enzymes required for the conversion of mevalonate phosphate to farnesyl pyrophosphate—mevalonate phosphate kinase and farnesyl diphosphate synthase—are exclusively or largely localized to peroxisomes.[63–65] SCP2, an apparent carrier protein for intracellular sterol transport, also appears to be targeted to and processed by peroxisomes.[66] In contrast, various subcellular localization studies have shown that synthesis of squalene, a nonsterol precursor of lanosterol, by squalene synthase occurs exclusively in the ER.[67] Peroxisomes also contain a form of HMG-CoA reductase that is structurally and functionally distinct from the HMG-CoA reductase in the ER.[68,69] Because the HMG-CoA reductase of the ER is clearly the apparent rate-limiting enzyme for cholesterol biosynthesis, the role of the peroxisomal isozyme is unknown at this time. In addition, although there is evidence from one laboratory that peroxisomes contain all of the enzymes necessary for the conversion of lanosterol to cholesterol,[70] the relative role of these and other peroxisomal enzymes in overall cholesterol biosynthesis and homeostasis is unclear. Nevertheless, observations that patients with Zellweger syndrome have markedly depressed serum cholesterol levels[71] and that Zellweger fibroblasts in vitro have depressed rates of cholesterol synthesis[72] suggest an important role for peroxisomes in cholesterol biosynthesis. However, not all laboratories have found that Zellweger fibroblasts have decreased rates of cholesterol synthesis. Also important to consider is that because the earlier enzymatic steps of cholesterol biosynthesis also participate in the synthesis of all isoprenoid compounds, peroxisomes must also have an important role in the synthesis of dolichols and coenzyme Q, among the many diverse products of isoprenoid biosynthesis.

Bile Acid Synthesis. Bile acids are synthesized from cholesterol by a complex series of cytochrome P-450–dependent ring hydroxylations of the cholesterol steroid nucleus, followed by a final β-oxidative cleavage of a propionate group from the C_{20}–C_{27} branched side chain of cholesterol (Figure 55.8-4). Normally, only the final end products of bile acid synthesis, cholic acid and chenodeoxycholic acid, are present in bile or other body fluids in any significant amount. However, in 1972, Eyssen and colleagues reported that the duodenal fluid of infants with a Zellweger-like syndrome contained unusually large amounts of the bile acid intermediates dihydroxycholestanoic acid (DHCA) and trihydroxycholestanoic acid (THCA).[73] Until then, DHCA and THCA had been known to be abundant acids only in the bile of certain primitive vertebrates, such as the alligator. In addition, significant levels of a previously unknown C_{29}-dicarboxylic bile acid were found in the blood of patients with Zellweger syndrome.[74] The finding of increased levels of DHCA and THCA in Zellweger syndrome and the discov-

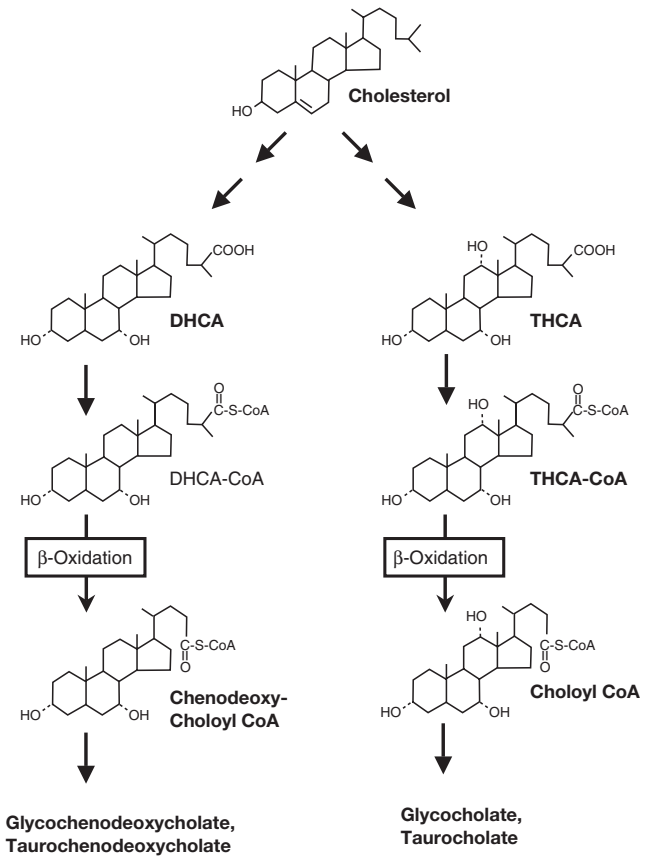

FIGURE 55.8-4 Conversion of cholesterol to bile acids via β-oxidation of the C_{22}–C_{27} side chain. After activation to their coenyzme A (CoA) derivatives, dihydroxycholestanoic acid (DHCA) and trihydroxycholestanoic acid (THCA) undergo one cycle of peroxisomal β-oxidation, yielding propionyl CoA and the CoA derivatives of chenodeoxycholic acid and cholic acid. DHCA-CoA and THCA-CoA can be conjugated to glycine or taurine by the peroxisomal enzyme bile acyl CoA:amino acid transferase. The levels of both DHCA and THCA are markedly increased in most patients with a peroxisome biogenesis disorder, such as Zellweger syndrome.

ery that hepatocytes of Zellweger syndrome are devoid of peroxisomes focused attention on the role of peroxisomes in the conversion of DHCA and THCA to their respective C_{24} bile acids, chenodeoxycholic acid and cholic acid.[36,74] Studies of the bile acids of patients with deficiency of the D-specific peroxisomal MFE2[30] also suggested involvement of peroxisomes in cholesterol side-chain cleavage.

Although bile acid ring hydroxylations take place primarily in the microsomal compartment, experimental evidence is compelling that cleavage of the cholesterol side chain, the final step in the synthesis of bile acids, occurs exclusively in the peroxisome. This conclusion has been reached both from careful subcellular fractionation studies in rat liver[36] and from the evidence that essentially all patients with Zellweger syndrome and related peroxisomal biogenesis disorders have increased levels of THCA, DHCA, and C_{29}-dicarboxylic bile acids.[75] The specific subcellular site of activation of DHCA and THCA to their CoA derivatives has not been resolved because enzymes capable of catalyzing this reaction are found both in microsomes and peroxisomes.[76,77] Naturally occurring THCA and DHCA are mixtures of *R*- and *S*-stereoisomers, but only the latter can be chain-shortened by the peroxisomal β-oxidation machinery.[41] Once inside peroxisomes, the CoA derivatives of R-THCA and R-DHCA must be converted to their respective *S*-conformers by peroxisomal α-methylacyl CoA racemase. S-C27-CoA derivatives undergo side-chain shortening via the D-specific peroxisomal β-oxidation pathway, converting DHCA-CoA and THCA-CoA to chenodeoxycholyl CoA and cholyl CoA, respectively.[29,78] These C_{24}-CoA compounds are substrates for the peroxisomal PTS1-containing enzyme, bile acyl CoA:amino acid *N*-acyltransferase, yielding glycine and taurine conjugates of the primary bile acids.[79] The mechanism by which these compounds exit the peroxisome is not understood. As discussed in Chapter 55.4, "Disorders of the Bile Acid Synthesis," in addition to peroxisomal diseases, abnormal bile acid synthesis is characteristic of a number of genetic defects of microsomal oxidases and dehydrogenases, most of which are associated with progressive congenital or postnatal liver disease.

Ether-Lipid Biosynthesis. In contrast to conventional phospholipids, which contain two fatty acyl groups ester-linked to a glycerophosphoryl backbone, plasmalogens are phospholipids with one acyl group *ester*-linked to the second carbon and an unusual, α-unsaturated long-chain alcohol *ether*-linked to the first carbon. Plasmalogens are major components of membrane structural phospholipids in all cells and constitute up to 90% of ethanolamine phospholipids in myelin.[80] Platelet activating factor (alkyl-, acetyl-glycerophosphorylcholine) also is an ether-lipid, the only one known to have a specific biochemical function.[81]

The first two steps of ether-lipid biosynthesis (Figure 55.8-5) have been shown to take place in the peroxisome.[82] Esterification of fatty acyl CoA to the sn-1 position of dihydroxyacetone phosphate (DHAP) is first catalyzed by DHAP acyltransferase,[83] a PTS1-containing protein. Subsequently, the PTS2 protein alkyl-DHAP synthase catalyzes the replacement of the sn-1 acyl group with a fatty alcohol.[83] The product of these initial reactions, 1-alkyl-glycerol-3-phosphate, is then transferred to the ER, where α,-β desaturation of the alcohol occurs and where enzymes for normal ester-lipid biosynthesis complete the formation of plasmalogens. There is also evidence that acyl CoA reductase, which catalyzes the synthesis of the long-chain alcohols incorporated into plasmalogens, is a peroxisomal enzyme and derives its reducing equivalents from reduced nicotinamide adenine dinucleotide phosphate generated through the action of a peroxisomal form of isocitrate dehydrogenase.[84]

Catabolism of Pipecolic Acid and Other Amino Acids. Pipecolic acid (2-piperidinecarboxylic acid), a cyclic imino acid and homolog of proline, is synthesized in animals via a minor pathway of lysine catabolism and then further oxidized sequentially to α-aminoadipic acid and glutaric acid (Figure 55.8-6).[85] The initial and probable rate-limiting step in the catabolism of L-pipecolic acid is catalyzed by a flavin adenine dinucleotide–dependent, L-pipecolic acid oxidase,[86] a PTS1 protein that has now been purified and

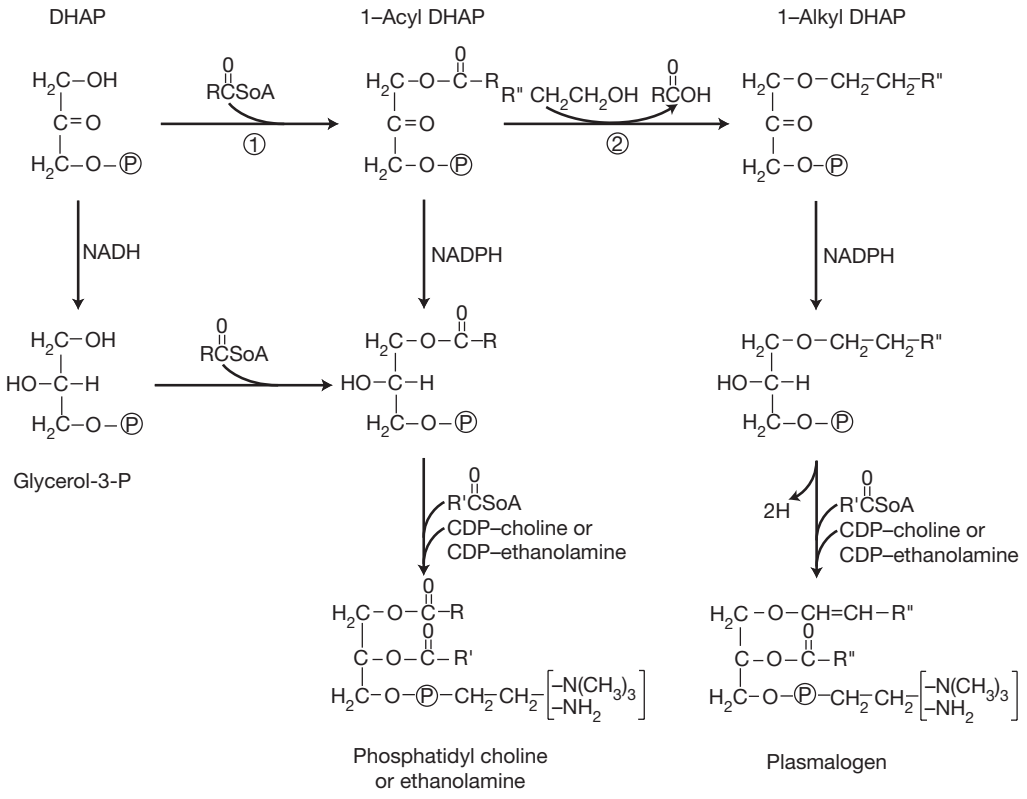

FIGURE 55.8-5 Pathway for biosynthesis of glycerol-ether lipids. Dihydroxyacetone phosphate (DHAP) acyltransferase and alkyl DHAP synthase, which catalyze the first two steps (labeled 1 and 2) in the synthesis of plasmalogens, are located in the peroxisome, whereas other reactions illustrated here take place in microsomes or mitochondria or both. CDP = cytidine diphosphate; CoA = coenzyme A; NADH = reduced nicotinamide adenine dinucleotide; NADPH = reduced nicotinamide adenine dinucleotide phosphate.

enzymatically and molecularly characterized.[87,88] Whereas both D and L forms of pipecolic acid occur in nature, only the L-isomer appears to be synthesized in animals, and only L-pipecolic acid accumulates to any significant degree in patients with peroxisomal biogenesis disorders.[89]

Although L-pipecolic acid has been shown experimentally to meet most criteria for an endogenously synthesized central nervous system (CNS) neurotransmitter and to have strong inhibitory effects on selected CNS neurons,[90] it is not clear what, if any, physiologic role pipecolic acid plays in the CNS. The rates of synthesis and oxidation of pipecolic acid, their tissue distribution, and even their subcellular localization appear to vary considerably among different vertebrate animals.[91] In most mammals, formation of pipecolic acid contributes to less than 1% of lysine degradation in the liver,[92] where the alternative saccharopine pathway of lysine metabolism (see Figure 55.8-6) appears to predominate. In contrast, conversion to L-pipecolic acid may be the major catabolic fate of L-lysine in rat brain.[93] More interesting is that whereas the peroxisome is the site of L-pipecolic acid oxidation to L-α-aminoadipic acid in humans and other primates,[94,95] only mitochondria appear to contain this activity in rabbits and rats.[95] Such differences in subcellular enzyme localization are unusual but not unprecedented and may reflect evolutionary flexibility of enzyme targeting mediated by cellular gene-splicing strategies. Interestingly, D-pipecolic acid, which is not abnormally elevated in Zellweger syndrome, appears to be oxidized only in peroxisomes in the rat and other animals.[96]

Metabolism of Hydrogen Peroxide. In mitochondria, the oxidation of a substrate by a nicotinamide adenine dinucleotide– or flavin adenine dinucleotide–dependent dehydrogenase is followed by transfer of the extracted electrons to the electron transport (respiratory) chain and then eventually to oxygen to form water. In contrast, reducing equivalents in the peroxisome are transferred directly to molecular oxygen through the action of one of the flavin-dependent peroxisomal oxidases to form hydrogen peroxide.[5] The large amounts of hydrogen peroxide generated by the many different peroxisomal oxidases would be cytotoxic without mechanisms for its safe decomposition within the peroxisome. Catalase, which is one of the most abundant proteins in liver,[97] serves this function and decomposes hydrogen peroxide by either a *catalatic* mechanism:

$$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$$

or a *peroxidatic* process:

$$H_2O_2 + RH_2 \rightarrow R + 2\ H_2O$$

Most oxidase-generated hydrogen peroxide appears to be degraded in situ by the peroxidatic mechanism.[98] Although the absolute level of catalase activity in Zellweger syndrome cells is normal, most of the enzyme is found in the cytoplasmic compartment and not in the particulate (ie, peroxisome containing) fraction.[99]

The hydrogen peroxide–generating reactions of peroxisomal oxidases are highly exergonic and, unlike mitochondrial dehydrogenation reactions, are unconstrained by respiratory control and the synthesis of ATP. This exothermic nature of peroxisomal respiration may contribute to the heat-producing capacity of specialized tissues such as brown fat, in which cold adaptation causes a marked proliferation of peroxisomes.[100]

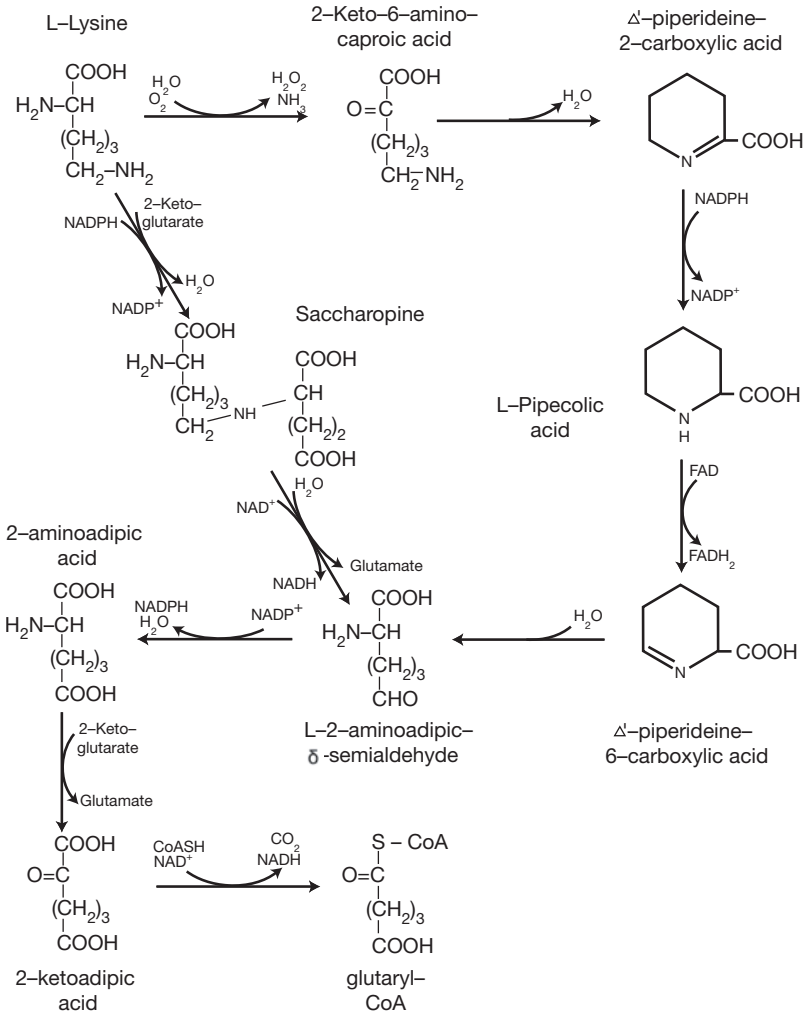

FIGURE 55.8-6 Biosynthesis of pipecolic acid and its relationship to the dual pathways for lysine catabolism to glutaryl coenzyme A (CoA). Glutaryl CoA is further catabolized both in peroxisomes and mitochondria. NADPH = reduced nicotinamide adenine dinucleotide phosphate.

PEROXISOMAL DISEASES

Disorders of Peroxisomal Biogenesis

The nomenclature of the three syndromes now classified as disorders of peroxisomal biogenesis—Zellweger syndrome, infantile Refsum disease, and neonatal ALD—reflects more the type of specialists who first described the patients than the characteristic biochemistry or pathology of these overlapping syndromes (Table 55.8-2). Indeed, evidence has appeared that, for example, patients with the clinical diagnosis of infantile Refsum disease have mutations in one of at least three different genes, all of which can also be associated with the other two clinical phenotypes of generalized peroxisomal disease.[101] Altogether, 11 different complementation groups for disorders of peroxisomal biogenesis have been defined,[16,102,103] and, among these, mutations in 10 different *PEX* genes affecting PTS1-mediated peroxisomal assembly and enzyme import have been associated with one or more of these clinical syndromes. Two of the original complementation groups, numbers 1 and 4, now associated with mutations in *PEX1* and *PEX6*, respectively, include patients of all three clinical phenotypes and account for more than 80% of patients with a disorder of peroxisomal biogenesis. Mutations in *PEX7*, which encodes the receptor for proteins containing the second peroxisomal enzyme import system PTS2, have been linked to the clinically quite different disorder RCDP.[61] These remarkable advances in our understanding of the molecular basis of peroxisomal diseases may eventually lead to a better understanding of the biochemical pathology and possibly even to therapy of these disorders. Despite our current understanding that Zellweger syndrome, infantile Refsum disease, and neonatal ALD can all be caused by different mutations in a single gene, and despite their often overlapping clinical and biochemical features, because much of the existing clinical literature views these three syndromes as separate clinical entities, they are discussed here individually.

Zellweger Syndrome. Zellweger syndrome was first delineated in 1967 as a multiple congenital anomaly syndrome by Passarge and McAdams, who suggested the descriptive term "cerebrohepatorenal" syndrome.[104] Subsequently, Opitz presented a comprehensive study of the pathology of Zellweger syndrome,[105] whereas later review articles discussed the complexity of the associated biochemical abnormalities.[71,75]

The majority of patients with Zellweger syndrome are identified as newborns or young infants, based on a relatively stereotypic phenotype (Table 55.8-3) and a variety of anatomic and histologic abnormalities (Table 55.8-4).

TABLE 55.8-2 CLASSIFICATION OF PEROXISOMAL DISEASES

DISORDERS OF PEROXISOMAL BIOGENESIS
Zellweger cerebrohepatorenal syndrome
Neonatal adrenoleukodystrophy
Infantile Refsum disease
Hyperpipecolic acidemia
Rhizomelic chondrodysplasia punctata
DEFICIENCY OF A SINGLE PEROXISOMAL ENZYME OR PROTEIN
Acyl CoA oxidase deficiency ("pseudo–neonatal adrenoleukodystrophy")
Peroxisomal multifunctional enzyme 2 deficiency
X-linked adrenoleukodystrophy
CADDS (contiguous *ABCD1 DXS1357E* deletion syndrome)
α-Methylacyl CoA racemase deficiency
Dihydroxyacetone phosphate acyltransferase deficiency
Alkyl-dihydroxyacetonephosphate synthase deficiency
Hyperoxaluria type I (alanine:glyoxylate aminotransferase deficiency)
Refsum disease (phytanyl CoA α-hydroxylase deficiency)
Glutaryl CoA oxidase deficiency
Acatalasemia

During infancy, the abnormalities that most suggest the diagnosis of Zellweger syndrome are the characteristic facial appearance (Figure 55.8-7), profound hypotonia, and absent neonatal reflexes. A typical infant with Zellweger syndrome has a high forehead with a widely open metopic suture, wide-spaced appearing and upslanting palpebral fissures, underdeveloped supraorbital ridges, triangular mouth, and apparently low-set, abnormally shaped ears. The appearance is sometimes reminiscent of Down syndrome. However, because most of the craniofacial and other dysmorphic characteristics of Zellweger syndrome are individually relatively nonspecific, the diagnosis of Zellweger syndrome is often missed at birth. Hepatocellular disease is usually less apparent during the first 3 months than later but may be evident as direct hyperbilirubinemia, hypertransaminasemia, coagulopathy, or hepatomegaly alone.[106,107] Other important and somewhat more specific clues to the diagnosis of Zellweger syndrome are glomerulocystic kidney disease,[108] abnormal calcification of the patella and other apophyseal cartilage (chondrodysplasia punctata),[109] cerebral dysgenesis,[110,111] and pigmentary retinopathy.[112] Structural abnormalities of the heart, mostly septal defects and conotruncal malformations, are also not uncommon. Seizures, which occur in over 70% of patients, are often difficult to treat. Indeed, a severe neonatal seizure disorder is one of the more common clinical problems that alone suggests the diagnosis of Zellweger syndrome. Because of the severity of the cerebral malformations, most infants with Zellweger syndrome achieve no developmental milestones and die within a few weeks or months of birth from seizures, apnea, aspiration, or pneumonia. Those patients who survive the first 6 months may show a slight degree of neurologic development and improved muscular tone but often eventually succumb to the complications of their severe neurologic disease. Rare patients in whom Zellweger syndrome has been diagnosed in the neonatal period have survived for more than 3 years.[113] Detailed compilations of the clinical characteristics of patients with Zellweger syndrome have been published by Heymans[114] and Wilson and colleagues.[115] Despite the tremendous expansion in our understanding of the biochemical and molecular bases of Zellweger syndrome and its related disorders, none of their malformative or degenerative characteristics has a clearly understood biochemical pathogenesis.

Although glomerulocystic disease of the kidney in Zellweger syndrome can be anatomically quite severe, renal glomerular or tubular insufficiency, apart from mild generalized aminoaciduria and proteinuria, is not common.[108] Similarly, the diagnostically important chondrodysplasia punctata affects mostly apophyseal cartilage, such as the patella, and does not itself cause dwarfing.[109] Other unexplained abnormalities with less obvious clinical consequences include islet cell hyperplasia, thymic hypoplasia, and siderosis.[104]

TABLE 55.8-3 MAJOR CLINICAL CHARACTERISTICS OF ZELLWEGER SYNDROME

Craniofacial	Midface hypoplasia resemblance to Down syndrome Hypertelorism, narrow papebral fissures Inner epicanthal folds, anteverted nares High narrow forehead, large fontanels, micrognathia
Skeletal	Clinodactyly, camptodactyly Equinovarus deformity, joint contractures
Neurologic	Severe hypotonia; absent Moro reflex, suck, grasp Complex seizure disorder (often neonatal) Profound psychomotor retardation Degenerative neurologic disease
Sensory	Optic atrophy, pigmentary retinopathy Cataracts, glaucoma, Brushfield spots Blindness (often congenital), nystagmus Sensorineural deafness
Hepatic	Hepatomegaly ± splenomegaly Prolonged or persistent jaundice Signs of portal hypertension Coagulopathy, biliary cirrhosis
Other	Cryptorchidism, hypospadias Patent ductus arteriosis, septal defects Single palmar creases

TABLE 55.8-4 ANATOMIC AND HISTOLOGIC ABNORMALITIES IN ZELLWEGER SYNDROME

Neurologic	Cerebral/cerebellar neuronal migration defects Microgyria, pachygyria, olivary dysplasia Septo-optic dysplasia, agenesis corpus callosum Dysmyelination, demyelination
Hepatic	Fibrosis progressing to cirrhosis Intrahepatic biliary dysgenesis and stasis Absent peroxisomes, abnormal mitochondria Iron storage (early); lipid storage (late)
Renal	Cortical glomerulocystic disease Hydronephrosis, persistent fetal lobulation
Skeletal	Chondrodysplasia punctata (nonrhizomelic) Osteoporosis, retarded skeletal maturation Bell-shaped chest (secondary to hypotonia)
Other	Pancreatic islet cell hyperplasia Thymic hypoplasia; Di George sequence

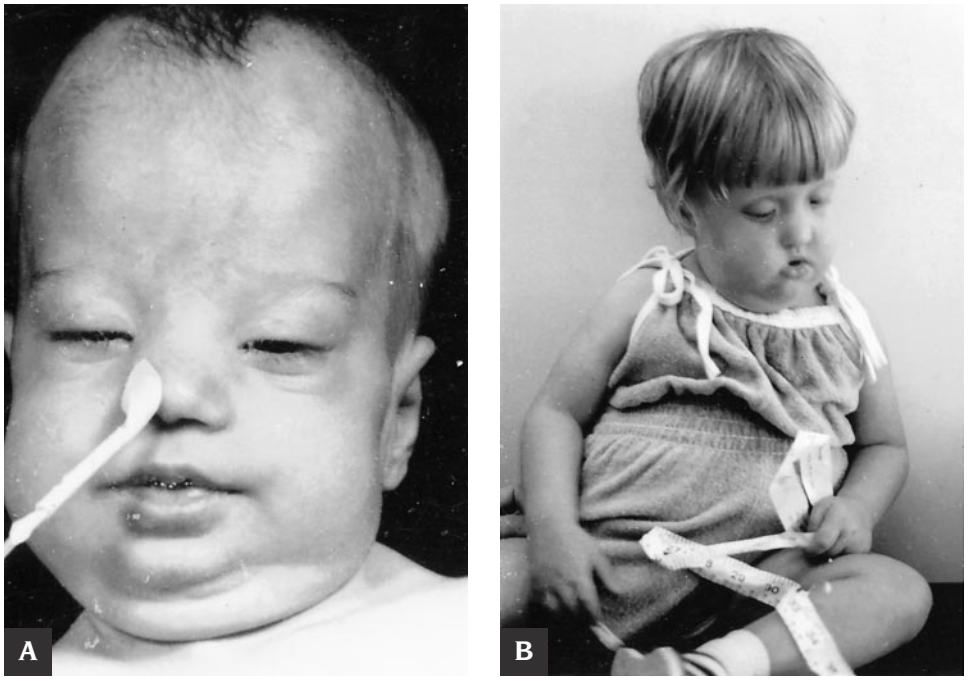

FIGURE 55.8-7 Facial appearance of two patients with Zellweger syndrome. *A*, At birth; *B*, at 3 years. Note in *B* the postural evidence of severe hypotonia.

The CNS disease of Zellweger syndrome is notable for the coexistence of congenital developmental abnormalities and acquired degenerative changes.[111,116–118] The most common CNS malformations are cerebral and cerebellar heterotopias, centrosylvian pachygyria and polymicrogyria, and olivary hypoplasia. Partial agenesis of the corpus callosum, hypoplasia of the cerebellar vermis, and septo-optic dysplasia are also common. Another unusual characteristic of patients with Zellweger syndrome is increased brain water and correspondingly increased brain weight. In addition to these abnormalities, most of which can be attributed to defective neuronal migration, myelin synthesis is qualitatively abnormal, and in longer-surviving individuals, a demyelinating process occurs.[116,118–120] When active demyelination is present (most commonly in the centrum semiovale, corpus callosum, occipital white matter, and cerebellum), macrophages with vacuolar lipid inclusions and "angulate" lysosomes are found. These storage macrophages are essentially the same as those found in the degenerating white matter of patients with X-linked ALD.[116,118,121] The recognition of an ALD-like white matter disease in Zellweger syndrome led to the discovery that patients with Zellweger syndrome, like those with X-linked ALD, have increased concentrations of VLCFAs, both in the CNS and systemically.[122–124]

Severe liver disease is almost universal in patients with Zellweger syndrome who survive the neonatal period.[106,107,125–128] Although the liver disease is often minimal during the first few months of life and may even appear to be absent, some combination of lobular disarray, focal hepatocytic necrosis, portal fibrosis or cirrhosis, intracellular and intracanalicular cholestasis, and increased iron storage can usually be found on biopsy. Foamy, lipid-filled hepatocytes, biliary dysgenesis, multinucleated giant cells, and focal areas of parenchymal collapse are also found, but less commonly (Figure 55.8-8). By electron microscopy and histochemistry (for the marker enzyme catalase), peroxisomes have been undetectable in the liver of almost all patients with classic Zellweger syndrome.[2,127] Abnormally shaped and dark-staining mitochondria with tubular cristae and paracrystalline inclusions, as well as scattered lipid-storage macrophages with angulate lysosomes, are also often found (Figure 55.8-9).[126] The chemical composition of the lamellar lipid material causing the distortion of lysosomes is not known but is suspected to consist of condensations of VLCFAs. Except for scattered cellular necrosis, the histology of individual hepatocytes is surprisingly normal, particularly in older infants, despite the progression of fibrosis and cirrhosis. By the age of 6 months, advanced cirrhosis and its many sequelae may dominate the clinical picture. Rapid progression from giant cell transformation without fibrosis to hepatocyte necrosis to cirrhosis in 3 to 4 months has been documented by serial biopsy in several patients. The cause of cirrhosis in Zellweger syndrome is not known, but increased levels of compounds such as hydrogen peroxide and unsaturated VLCFAs, which have aberrant metabolism in a peroxisome-deficient liver, have been proposed as hepatotoxins.

Another complication of liver disease in Zellweger syndrome and its related disorders is fat malabsorption and its multiple metabolic consequences, such as deficiencies of fat-soluble vitamins and nutritional failure to thrive. The cause of the malabsorption is often attributed to both the primary deficiency of bile acids and to the biliary abnormalities that follow the progressive cholestatic liver disease. These problems are not uncommon and, in milder forms of the disease, may even be the mode of presentation.[129,130]

Two biochemical abnormalities may have special importance in the evolution—and treatment—of the hepatic disease in Zellweger syndrome. First, the many abnormal species of bile acid that accumulate in the liver and other tissues of patients with Zellweger syndrome may cause injury to the liver. For this reason, there have

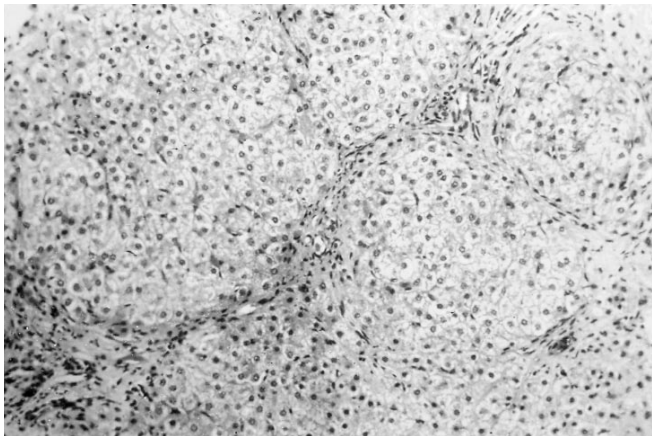

FIGURE 55.8-8 Liver histology in Zellweger syndrome showing lobular disorganization and early bridging fibrosis. Courtesy of H. Moser, MD.

been attempts to ameliorate possible bile acid toxicity by administration of bile acid supplements such as ursodeoxycholic acid, with some evidence of benefit.[131] Another more recently recognized abnormality is an almost universal severe deficiency of docosahexaenoic acid (DHA) and other related essential polyunsaturated fatty acids.[132,133] The cause of DHA deficiency in peroxisomal biogenesis disorders is reduced retroconversion of the precursor, $C_{24:6}$, to DHA via one cycle of peroxisomal β-oxidation.[134,135] Accordingly, many surviving patients with Zellweger syndrome and related disorders have been treated with supplements of DHA.[133] A third deficiency that may have a role in the degenerative pathology of Zellweger syndrome is the severely depressed level of plasmalogens, which, some have speculated, have important antioxidant properties in cell membranes.[136]

In addition to a variety of diagnostically useful anatomic and histologic abnormalities, several laboratory tests are now available for diagnosis of defective peroxisomal metabolism; these are listed in Table 55.8-5 and discussed in more detail in following sections. All of these peroxisomal abnormalities can usually be demonstrated in an infant with Zellweger syndrome, and their documentation in the proper clinical setting usually obviates the need to demonstrate absent peroxisomes by liver biopsy. Other less specific but relatively common biochemical abnormalities are also listed in Table 55.8-5. Once biochemical studies have established that a patient with a Zellweger phenotype has abnormalities in multiple peroxisomal biochemical pathways, complementation analysis and now mutational studies can be undertaken to identify the specific genetic lesion.

Infantile Refsum Disease. Infantile Refsum disease was first described in 1982 by Scotto and colleagues as a syndrome of developmental retardation, pigmentary retinopathy, sensorineural hearing loss, and mildly to moderately increased plasma levels of phytanic acid.[137] Although infantile Refsum disease differs clinically from Zellweger syndrome, early fibroblast complementation studies indicated

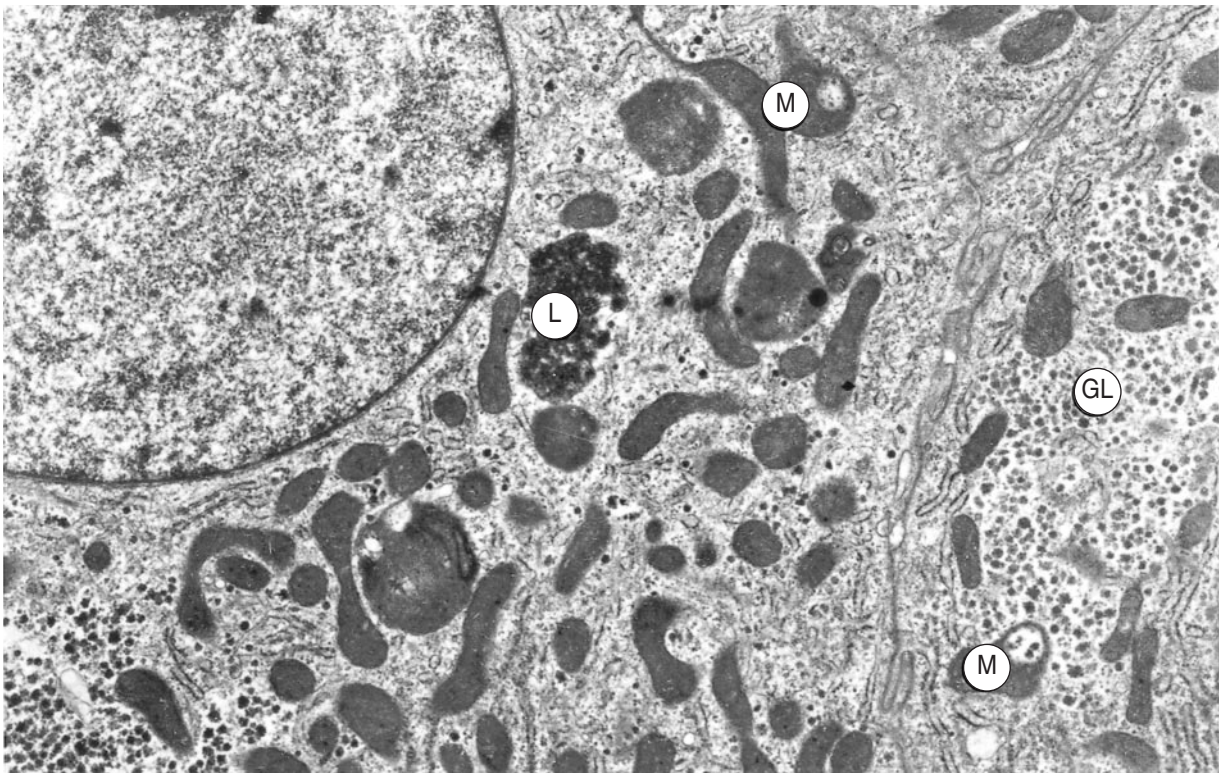

FIGURE 55.8-9 Liver ultrastructure in Zellweger syndrome. Mitochondria (M) with bizarre shapes and dense matrices are seen, together with normal lysosomes (L) and glycogen (GL). The mitochondrial abnormalities are most likely secondary phenomena because many patients with Zellweger syndrome have normal-appearing mitochondria. Courtesy of Sydney Goldfischer, MD.

TABLE 55.8-5 LABORATORY ABNORMALITES COMMON IN ZELLWEGER SYNDROME

ABNORMALITIES OF PEROXISOMAL METABOLISM
Increased levels of
Very-long-chain fatty acids (p, u, t)
Di- and trihydroxycholestanoic acids (p, u)
Pipecolic and hydroxypipecolic acids (p, u)
Phytanic and pristanic acids (p, t)
Dicarboxylic and epoxydicarboxylic acids (p, u)
Decreased levels of
Plasmalogens, platelet activating factor (p, t)
Phytanic acid β-oxidation (t)
Peroxisomal fatty acid β-oxidation (t)
Particulate catalase (t)
Normal bile acids (p, u)
Docosahexaenoic and related acids (p, t)
SECONDARY OR UNEXPLAINED BIOCHEMICAL ABNORMALITIES
Increased levels of
Serum transaminases, bilirubin
Serum iron and iron saturation (early months)
Cerebrospinal fluid protein (variable, late)
Threonine (p, u)
Urinary amino acids (generalized aminoaciduria)
4-Hydroxyphenyllactate (u)
Decreased levels of
Cholesterol (p)
Prothrombin, other coagulation factors (p)

p = plasma; t = tissues/fibroblasts; u = urine.

that infantile Refsum disease is actually a mild form of Zellweger syndrome,[102,103] as originally suggested by Poulos and colleagues[138] and now fully confirmed by mutational studies of the involved *PEX* genes.[101,139]

The first patients with infantile Refsum disease described in the literature lacked the characteristic facial appearance of Zellweger syndrome, except for mild craniofacial abnormalities such as epicanthal folds, anteverted nares, and midfacial hypoplasia. Unlike patients with classic Zellweger syndrome, who rarely achieve any psychomotor development, those with infantile Refsum disease have learned to walk and even have acquired some language.[139,140] Similarly, hypotonia is less severe in infantile Refsum disease, and a few patients have shown improving or normal muscle tone and brisk deep tendon reflexes beyond infancy. However, because pigmentary retinopathy, macular degeneration, and sensorineural hearing loss are progressive conditions, the majority of patients with infantile Refsum disease become blind and deaf because of their longer survival.

Liver disease is much less prominent in infantile Refsum disease than it is in Zellweger syndrome. Levels of serum transaminases and bilirubin are usually normal or only slightly increased, and hepatomegaly is less common. Nevertheless, major complications of hepatic disease, such as cerebral and gastrointestinal hemorrhages secondary to coagulopathy, have occurred.[140] Both intrinsic liver disease and malabsorption of vitamin K secondary to defective bile acid synthesis probably contribute to the coagulopathy. Associated vitamin A and vitamin E deficiencies are also common and may exacerbate visual and neurologic degeneration, as likely does the associated deficiency of DHA.[141] Histologically, the livers of patients with infantile Refsum disease do not have the lobular disorganization and biliary dysgenesis typical of Zellweger syndrome, but progressive fibrosis, apparently, is common. Morphologically recognizable peroxisomes are absent or at most represented by small numbers of catalase-positive microperoxisomes.[142] Hepatocytes and especially Kupffer cells often have inclusions of lipid vacuoles and leaflets similar to those of X-linked and neonatal ALD. In addition, unusual hepatocytic glycogen inclusions, only infrequently seen in Zellweger syndrome, appear to be relatively common in infantile Refsum disease. In one 12-year-old boy with infantile Refsum disease who came to autopsy, advanced micronodular cirrhosis was found.[143] Other important pathologic abnormalities in that patient included hypoplastic adrenal glands without degenerative changes, extensive infiltrates of lipid-storage macrophages in the lymph nodes, severe hypoplasia of the cerebellar granule layer, and severe degenerative changes in the retina and cochlea.

In addition to the full spectrum of peroxisomal biochemical abnormalities—increased levels of VLCFA, phytanic acid, pipecolic acid, and bile acid intermediates and depressed levels of DHA and erythrocyte plasmalogens—patients with infantile Refsum disease commonly have persistently low levels of serum cholesterol and both α- and β-lipoproteins.[140] Because plasmalogens and their precursors can be assimilated from the diet, erythrocyte plasmalogen levels, which are very low in infants with Zellweger syndrome, may increase and even normalize over a period of 6 to 12 months after birth. Nevertheless, when assayed in liver or fibroblasts, tissue levels and rates of synthesis of plasmalogens are consistently depressed.[140]

Neonatal Adrenoleukodystrophy. Between 1978 and 1982, several reports were published describing a total of 11 infants and young children of both sexes who suffered from a constellation of CNS, adrenal, and biochemical abnormalities almost identical to those of childhood (X-linked) ALD.[144,145] However, the apparent autosomal recessive inheritance of the disorder, its neonatal presentation, and a variety of associated systemic abnormalities uncharacteristic of X-linked ALD suggested that this new "neonatal" ALD was a genetically distinct disorder. The observation that some infants with neonatal ALD resembled patients with Zellweger syndrome then led to the discovery of multiple defects of peroxisomal metabolism and absent or severely diminished peroxisomes on liver biopsy in a number of these patients. Several review articles have described in detail the full spectrum of clinical and biochemical abnormalities in neonatal ALD.[145–147]

As in Zellweger syndrome, most infants with the neonatal ALD phenotype are severely hypotonic at birth and develop myoclonic seizures in the newborn period or the first few weeks of life. Dysmorphic features may be limited to midfacial hypoplasia, epicanthal folds, and simian creases or be absent altogether (Figure 55.8-10). Psychomotor development is globally retarded, and few patients achieve a mental age greater than 2 years. Growth is usually moderately retarded, although some patients have had normal linear growth. In addition, nystagmus, pigmentary retinopathy,

optic atrophy, limited vision, and deafness further handicap most of these children. After many months or years of slow psychomotor development, children with neonatal ALD may develop a leukodystrophy, a progressive demyelination process, and begin to lose skills and enter a phase lasting over several months or years, during which complete neurologic deterioration to a terminal vegetative state ensues. The onset of the destructive white matter loss cannot be predicted, and the course is extremely variable.

The diagnosis of neonatal ALD is sometimes unsuspected until autopsy, when the finding of demyelination and adrenal atrophy suggests the diagnosis of a form of ALD. However, in contrast to the postnatally acquired CNS defects of X-linked ALD, signs of prenatal CNS maldevelopment such as dysmyelination, polymicrogyria, and cerebral and cerebellar heterotopias, similar to those of Zellweger syndrome, are found at autopsy. In addition, infiltrates of macrophages filled with lamellar lipid inclusions are typically dispersed throughout the nervous system and the reticuloendothelial system. The principal difference between the CNS disease of neonatal ALD and that of patients classified as having Zellweger syndrome and infantile Refsum disease is the greater degree of demyelination in the children with neonatal ALD.

Unlike Zellweger syndrome, but more like infantile Refsum disease, most patients with neonatal ALD have hepatic disease that is clinically silent or very mild. Typically, only limited fibrosis or early cirrhosis is found by biopsy or at autopsy.[145,147] By electron microscopy, hepatic peroxisomes are severely reduced in both number and size but usually detectable, unlike in patients with Zellweger syndrome and most patients with infantile Refsum disease. Renal cysts and punctate cartilage calcification have been absent in neonatal ALD, an apparent distinction between neonatal ALD and Zellweger syndrome when the presence of demyelination and frank adrenal atrophy is used as a primary criterion for the diagnosis of neonatal ALD.[145,147]

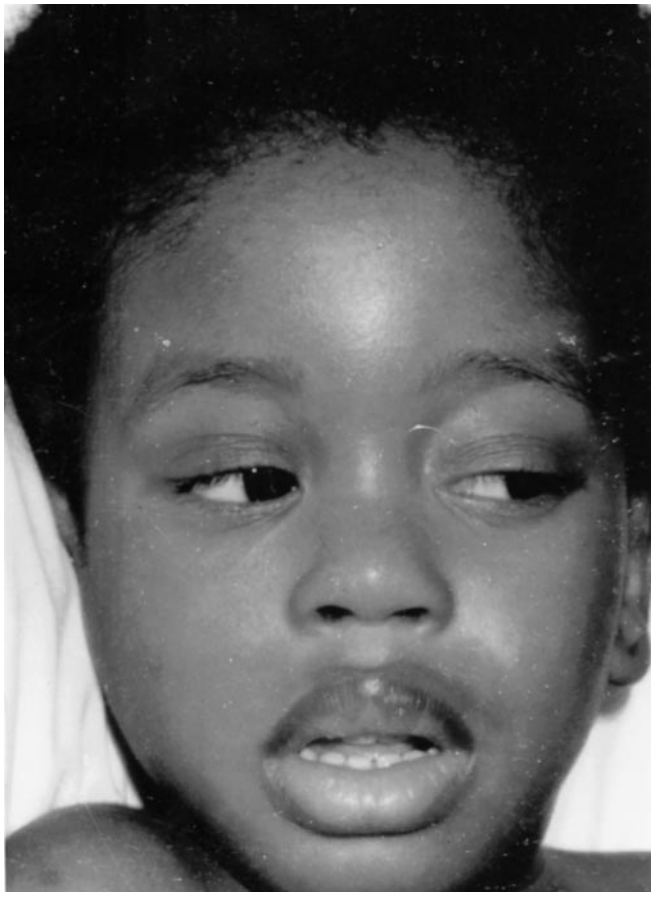

FIGURE 55.8-10 Facial appearance of a young child with neonatal adrenoleukodystrophy. Dysmorphic features are few but include a low nasal bridge and mild ptosis.

Most patients with neonatal ALD manifest all of the peroxisomal biochemical abnormalities characteristic of Zellweger syndrome, although the measured activity of some enzymes, such as phytanic acid oxidase and DHAP acyltransferase, may be somewhat higher than in Zellweger syndrome. Similarly, the plasma levels of VLCFA are often lower than in Zellweger syndrome and may be limited to increases of only saturated VLCFAs.[145,147]

Hyperpipecolic Acidemia. Three separate reports have described infants with progressive neurodegenerative disease and hyperpipecolic acidemia who, for a variety of reasons, were not considered to meet criteria for the diagnosis of Zellweger syndrome.[148–150] Although these patients are often grouped separately in reviews of peroxisomal disorders, studies of cultured fibroblasts or autopsy tissues from these patients later showed that all of them also had increased levels of VLCFA.[151] Accordingly, because of apparent deficiencies of at least two metabolically unrelated peroxisomal enzyme systems, these cases should be reclassified as examples of neonatal ALD or other disorders of peroxisomal biogenesis rather than cases of isolated hyperpipecolic acidemia. Although there are several as yet unreported patients who are suspected to have isolated hyperpipecolic acidemia (R. Kelley, unpublished observations, 1989) and who differ clinically from Zellweger syndrome, infantile Refsum disease, or neonatal ALD, none has yet been proven enzymatically to have a deficiency of either D-pipecolic acid oxidase or L-pipecolic acid oxidase, both of which appear to be peroxisomal enzymes in humans.[95] Isolated marked hyperpipecolic acidemia may be an unrelated, common autosomal recessive variant because it has been reported as an associated abnormality in isolated cases of autosomal recessive Joubert syndrome and Dyggve-Melchior-Clausen dwarfism.[152,153] Thus, it remains unknown whether isolated hyperpipecolic acidemia exists as a disease or only a biochemical curiosity of no clinical significance.

Diseases Caused by Deficiency of a Single Peroxisomal β-Oxidation Enzyme

X-Linked Adrenoleukodystrophy. The degradation of VLCFAs begins with their apparently specific transport into peroxisomes followed by activation to CoA thioesters by a specific very-long-chain fatty (VLCF)–acyl CoA synthetase (ligase). The VLCF–acyl CoA esters are then degraded by successive cycles of β-oxidative cleavage of 2-carbon acetyl CoA units mediated by three peroxisome-

specific enzymes: acyl CoA oxidase, MFE2, and 3-ketoacyl CoA thiolase (see Figure 55.8-2). The cause of reduced VLCFA oxidation in X-linked ALD was thought for many years to be caused by an isolated deficiency of peroxisomal VLCF–acyl CoA synthetase activity.[154,155] However, in 1993, the biochemical defect in X-linked ALD was shown to be caused by mutations in the *ABCD1* gene, which encodes ALDP.[40,156] ALDP is a peroxisomal membrane protein that is a member of the large family of ATP-binding cassette transmembrane transporter proteins, of which, for example, the cystic fibrosis protein (cystic fibrosis transmembrane receptor) is also a member.[157,158] This large class of membrane proteins transports substrates as diverse as chloride ions and entire proteins. Although ALDP has been studied intensely since its discovery, its exact role in the metabolism of VLCFAs remains unclear. Studies by Yamada and colleagues suggested that ALDP was important for the correct localization of the VLCF–acyl CoA synthetase within the peroxisome.[159] However, more recent work by Heinzer and colleagues has cast doubt on this hypothesis.[160]

X-linked ALD is the most common peroxisomal disorder and is unusual in its varying presentations from early childhood to late adult years.[161] When onset is between 5 and 10 years of age, X-linked ALD usually begins with a combination of behavioral, gait, and auditory disturbances and ends fatally after several years of devastating, global neurologic degeneration, with or without adrenal insufficiency. In adults, in whom a milder form of ALD is known as adrenomyeloneuropathy, peripheral nerve dysfunction and adrenal insufficiency predominate over relatively mild CNS disturbances. Occasionally, adults with isolated Addison disease or even clinically unaffected older adults with classic biochemical findings are discovered within pedigrees of cases of typical X-linked ALD or adrenomyeloneuropathy.

In contrast to the multiple congenital abnormalities characteristic of neonatal ALD, all of the neurologic and endocrinologic problems of X-linked ALD are acquired after birth. Nevertheless, diagnostic elevations of VLCFA in plasma and other tissues in X-linked ALD are present at birth.[161] Moreover, because of its mode of inheritance, X-linked ALD is also often manifest to a milder degree clinically and biochemically by carrier female patients.[162,163] Although X-linked ALD offers an excellent opportunity to understand the mechanism by which increased levels of VLCFA affect the CNS and steroid-secreting organs, very little is known at this time about the pathogenesis of VLCFA-associated CNS degeneration or endocrine dysfunction in either neonatal or X-linked ALD.

Individuals with X-ALD may come to the attention of the gastroenterologist when in childhood they present with cyclic vomiting, which is secondary to primary adrenal insufficiency. However, in the majority of individuals, there are no abnormalities of the liver or other gastrointestinal system abnormalities.

CADDS. Corzo and colleagues recently reported three boys with an apparently new syndrome characterized by neonatal hypotonia and cholestatic liver disease.[164] They were determined to have elevated VLCFA levels and were initially felt to have either Zellweger syndrome or one of the single enzyme defects in peroxisomal β-oxidation other than X-linked ALD. However, further study showed that they lacked immunoreactive ALDP. Mutation analysis identified deletions in the 5′ region of the *ABCD1* gene that extended through the promotor region and the neighboring gene, *DXS1357E*. The authors proposed the term CADDS for "contiguous *ABCD1 DXS1357E* deletion syndrome." The lack of ALDP in CADDS is noteworthy in that patients with childhood X-linked ALD typically present at 7 years of age but never < 3 years of age. In addition, liver disease is not found in patients with X-linked ALD. Liver biopsies of all three CADDS patients showed intracanalicular and ductal cholestasis. All three boys died in infancy (4–11 months), and causes of death included liver failure, gastrointestinal bleeding, and respiratory failure.

Acyl CoA Oxidase Deficiency (Pseudo–neonatal ALD). Two siblings who had severe hypotonia and myoclonic seizures in the first week of life but who lacked the dysmorphic appearance and other malformations characteristic of Zellweger syndrome were found by Poll-The and colleagues to have an apparently isolated deficiency of peroxisomal acyl CoA oxidase.[33] VLCFAs were markedly elevated in plasma and fibroblasts, but all other markers of peroxisomal dysfunction, including levels of bile acid intermediates, were normal. After a number of months of slow development, the children developed progressive sensorineural deafness, pigmentary retinopathy, and adrenal insufficiency and died in a vegetative state at age 4 years. There was no clinical or biochemical evidence of liver disease during life, and liver histology was notable only for somewhat increased peroxisomal size and lipoid deposits in hepatocytes. Cirrhosis or fibrosis, present in almost all patients with a disorder of PTS1-linked peroxisomal biogenesis, was not found. Isolated acyl CoA oxidase deficiency was documented by enzymatic assay of fibroblasts and immunologic methods[33] and, more recently, by DNA mutational analysis in the original and a second similar patient.[165] Additional patients with acyl CoA oxidase deficiency have been reported by Watkins and colleagues.[34]

Peroxisomal MFE2 Deficiency. More than 30 patients with a deficiency of peroxisomal MFE2 ("bifunctional enzyme") have now been described.[34,166,167] MFE2 is also known as D-BP. In contrast to the multisystem involvement of Zellweger syndrome, infants with MFE2 deficiency have a disorder dominated by their abnormal nervous system.[168] At birth, affected infants are severely hypotonic, macrocephalic, and neurologically depressed but usually lack hepatosplenomegaly, skeletal disease, or other important systemic abnormalities of Zellweger syndrome. However, neonatal seizures and severe psychomotor retardation are typical, and some of the infants have had dysmorphic facies reminiscent of Zellweger syndrome.[34] Although MFE2 deficiency is, in general, not a multisystem disorder like Zellweger syndrome, variable degrees of adrenal insufficiency, hepatic fibrosis, sensorineural hearing loss, pig-

mentary retinopathy, glomerulocystic kidney disease, and central white matter deterioration have been found. In contrast to Zellweger syndrome, however, hepatic peroxisomes are present. Despite the lesser systemic involvement, the severe CNS disease leads to the death of most MFE2-deficient patients in the first year.

Peroxisomal biochemical abnormalities in MFE2 deficiency are limited to increased tissue and plasma levels of VLCFAs and increased levels of bile acid intermediates, which MFE2 has a role in synthesizing. The absence of peroxisomal MFE2, but not peroxisomal oxidase or thiolase, was demonstrated by immunoblot analysis in the first patient identified.[166] Subsequently, more than 30 additional patients have been identified, mostly based on the clinical biochemical profile[166] and complementation analysis of cultured skin fibroblasts.[169] Of the three possible single enzyme defects of peroxisomal VLCFA β-oxidation, MFE2 deficiency is by far the most common.

Peroxisomal 3-Ketoacyl CoA Thiolase Deficiency (Pseudo–Zellweger Syndrome). Goldfischer and colleagues described a female infant who had Zellweger-like biochemical abnormalities but abundant hepatic peroxisomes and referred to this patient as having "pseudo–Zellweger syndrome."[170] As a result, a more detailed study of the enzymes of peroxisomal β-oxidation was undertaken by Schram and colleagues.[171] They reported that a single peroxisomal enzyme, 3-ketoacyl CoA thiolase, appeared to be deficient in liver tissue from this patient. Ferdinandusse and colleagues reinvestigated this case and determined that this patient with the only reported deficiency of peroxisomal 3-ketoacyl CoA thiolase actually had MFE2 deficiency.[172] The group of single peroxisomal β-oxidation enzyme deficiencies therefore appears to be limited to straight-chain acyl CoA oxidase, MFE2, and α-methylacyl CoA racemase (below) deficiency, and there is no longer evidence for the existence of peroxisomal 3-ketoacyl CoA thiolase deficiency as a distinct clinical entity.

2-Methylacyl CoA Racemase Deficiency. Ferdinandusse and colleagues reported three patients with elevations in pristanic acid and C_{27}-bile acid interediates, specifically DHCA and THCA, who were found to have a deficiency of 2-methylacyl CoA racemase.[173] This peroxisomal enzyme catalyzes the conversion of (2*R*)-methyl branched-chain fatty acyl CoAs to their (2*S*)-isomers. Only the *S*-conformers can undergo further β-oxidation. Two of the original patients had adult-onset neuropathy, but children with liver disease have also been reported. Setchell and colleagues described two sisters.[174] The proband was a 2-week-old girl with coagulopathy, vitamin D and E deficiencies, and mild cholestasis. A sibling who died at 5.5 months of age had had similar findings. Liver biopsy specimens showed neonatal hepatitis with giant cell transformation and hepatocyte necrosis, and peroxisomes were reduced in number. A high concentration of (25*R*)-THCA was found in the urine, bile, and serum and was similar to that seen in Zellweger syndrome. Serum phytanic acid was normal, whereas pristanic acid was markedly elevated. This patient was successfully treated with cholic acid therapy.

Other Peroxisomal Syndromes with Abnormal β-Oxidation. There are a number of other peroxisomal syndromes in which abnormal peroxisomal β-oxidation has been found, but not all are necessarily genetically distinct from the known peroxisomal disease complementation groups. Rather, they may differ only in the relative severity of the measured deficiencies. In our experience, there exist cases of peroxisomal disease featuring almost any combination of normal and abnormal levels of peroxisomal metabolites. Some of these, especially those with abnormal bile acid species, have been associated with progressive cholestatic liver disease. Similarly, several syndromes have been described in which phytanic acid oxidation and one or more peroxisomal functions are impaired.[175] Again, however, whether these represent genetically distinct syndromes or variants of other peroxisomal diseases is often unclear.

Pathophysiology of Zellweger Syndrome and Other Disorders with Abnormal Peroxisomal β-Oxidation. All of the primary defects of peroxisomal β-oxidation except X-linked ALD are associated with some degree of hypotonia, abnormal reflexes, seizures, and, in some instances, neuronal migration defects. This suggests that prenatal elevations of VLCFA-CoA esters, or possibly other acyl CoA substrates of the enzymes, may be involved in the congenital CNS abnormalities. However, there is not a clear understanding of how these compounds lead to abnormalities. It has been suggested that the increased levels of bile acid intermediates or the increased levels of unsaturated or 3-hydroxy-VLCF acyl CoA compounds may be contributing to maldevelopment. Such an association is also supported by the description of a patient with only impaired phytanic acid and bile acid metabolism who had the large fontanels and other craniofacial features of classic Zellweger syndrome.[47] Similarly, the abnormal CNS development of chondrodysplasia punctata may be dependent on deficient plasmalogen synthesis, which appears to be the biochemical common ground shared by Zellweger syndrome and various forms of RCDP, described below.

D-Specific Acyl CoA (Trihydroxycoprostanyl CoA) Oxidase Deficiency. The finding of increased levels of DHCA and THCA in peroxisomal MFE2 and thiolase deficiencies,[175] but not in peroxisomal acyl CoA oxidase deficiency, first suggested the existence of a specific oxidase for the initial hydroxylation step in the β-oxidative cleavage of the cholesterol side chain. This has now been confirmed enzymatically and by the description of patients who have normal VLCF acyl CoA oxidase activity but defective oxidation of trihydroxycholestanoyl CoA.[47,176] Interestingly, these infants resembled Zellweger syndrome physically and had progressive cholestatic liver disease. In addition, the patients had elevated levels of phytanic acid, presumably secondary to elevations of its metabolite, pristanoyl CoA, which, like VLCFA-CoA, is a substrate of the D-specific acyl CoA oxi-

dase. In contrast, in disorders of peroxisomal biogenesis and in adult Refsum disease, the elevation of phytanic acid occurs without bile acid abnormalities and is caused by a primary genetic (adult Refsum disease) or secondary (peroxisomal biogenesis disorder) deficiency of PAHX.[60]

Other Disorders of Peroxisomal Metabolism

Rhizomelic Chondrodysplasia Punctata. Although named for its severe rhizomelic dwarfism and diffuse epiphyseal and extraepiphyseal punctate calcification, RCDP is a complex, multiple congenital malformation syndrome with major nonskeletal abnormalities in the CNS (neuronal migration defect, seizures, deafness), eye (cataracts, blindness, corneal defects), and skin (ichthyosis).[177] Children with classic RCDP have severe growth retardation and profound mental deficiency, and most die before 1 year of age from respiratory insufficiency or complications of the CNS disease.

Recognizing that the punctate cartilage calcification of RCDP resembles that of Zellweger syndrome, Heymans and colleagues tested patients with RCDP for abnormalities of peroxisomal metabolism and discovered that both plasmalogen synthesis and phytanic acid oxidation were severely deficient.[178] By enzymatic assay, alkyl-DHAP synthase, the second enzyme of peroxisomal plasmalogen synthesis, and phytanic acid oxidase activity were depressed to less than 10% of normal activity. Plasma levels of phytanic acid in RCDP patients are usually higher than those of age-matched patients with Zellweger syndrome and may even reach the very high levels characteristic of adult Refsum disease in longer-surviving patients with RCDP.[179]

Because levels of intermediates of phytanic acid oxidation are not increased, the defective phytanic acid oxidation in RCDP is presumed to be limited to the initial α-hydroxylation step, as in adult Refsum disease. Plasma levels of pipecolic acid, VLCFAs, and bile acids are normal in RCDP, and liver disease and renal disease have also been absent.[179] Although one RCDP patient was reported to have reduced numbers of hepatic peroxisomes, others have had normally sized and abundant peroxisomes.[178] In addition to its multiple enzymatic deficiencies, the first clue that RCDP could be caused by a disorder of peroxisomal enzyme import distinct from that of the Zellweger syndrome group was the finding that fibroblasts of patients with RCDP contained a peroxisomal thiolase of a larger than normal molecular weight, indicating a failure of normal peroxisomal processing.[61,179] Molecular studies have now shown that, indeed, patients with RCDP have mutations in *PEX7*, a gene that codes for the PTS2 receptor.[61] Additional studies have shown that other PTS2-targeted enzymes, such as mevalonate kinase and phosphomevalonate kinase, are deficient in patients with RCDP.[180] Thus, RCDP is now classified with Zellweger and related peroxisomal syndromes as a disorder of peroxisomal biogenesis.

In recent years, several mild variants of RCDP have been found,[181,182] including children with normal stature who may have mild mental retardation and cataracts as only clinical problems. Others with the full RCDP biochemical phenotypes but normal stature have nevertheless had severe progressive neurologic disease.[181] Conversely, some patients with relatively typical adult Refsum disease and very high phytanic acid levels have been found to have milder but definite abnormalities in plasmalogen biosynthesis and, by complementation analysis, have been shown to have RCDP instead.[181] These milder forms of RCDP, wherein the major pathology may be caused by accumulation of phytanic acid, will likely be more treatable than classic RCDP using dietary restriction and direct elimination (plasmapheresis) of phytanic acid.[183] Although classic RCDP is clearly a disease affecting multiple peroxisomal enzymes, Barr and colleagues reported a patient with otherwise typical RCDP who had an isolated deficiency of DHAP acyltransferase.[184] This observation suggested that the clinical phenotype in classic RCDP is caused largely by defective plasmalogen biosynthesis. Other similar patients with isolated DHAP acyltransferase deficiency have been reported.[185,186] Rare cases of isolated DHAP synthase deficiency have also been reported.[187] Although abnormalities of plasmalogen synthesis and catalase distribution in a patient with the X-linked form of chondrodysplasia punctata, also known as Conradi-Hünermann syndrome, were reported by Emami,[188] these must be secondary abnormalities because the primary defect in Conradi-Hünermann syndrome was recently found to be a deficiency of 3β-hydroxysteroid-Δ8,Δ7-isomerase, a primary enzyme of cholesterol biosynthesis located in the microsomes.[189,190]

Heredopathia Atactica Polyneuritiformis: Adult Refsum Disease. In contrast to the early onset of infantile Refsum disease, adult Refsum disease (heredopathia atactica polyneuritiformis) is usually not evident clinically until the second or third decade. The major abnormalities in adult Refsum disease, all of which are acquired and progressive, include pigmentary retinopathy, sensorineural deafness, cerebellar ataxia, polyneuritis, ichthyosis, and cardiac conduction abnormalities.[191] Although clinical hepatic disease is absent, ultrastructural changes in the liver have been found to include excessive hepatocytic deposits of lipofuscin, vacuoles containing various types of lipoid accumulations, and an apparent deficiency of rough ER.[192] Vacuolization of renal tubular cells and structural abnormalities of their mitochondria have been reported and related to mild to moderate degrees of proximal renal tubular insufficiency in adult Refsum disease. Biochemically, Refsum disease is characterized by increased levels of free and esterified phytanic acid in the blood and tissues and a corresponding absence of phytanic acid β-oxidation activity as measured in fibroblasts and other solid tissues[193] and now known to be represented by a deficiency of phytanyl CoA α-hydroxylase.[60,194] All other peroxisomal functions appear to be normal. Because of the many years of accumulation of phytanic acid before diagnosis, levels of phytanic acid in plasma at the time of diagnosis of adult Refsum disease are often greater than 1,000 μg/mL compared with typical plasma levels of 10 to 200 μg/mL in Zellweger syndrome, infantile Refsum disease, or RCDP.[136,195] Refsum disease, which is one of the rarest inborn errors of metabolism, is inherited as an autosomal recessive genetic trait.

Stabilization and even partial reversal of the complications of adult Refsum disease can be achieved by restriction of dietary phytanic acid combined with direct elimination of accumulated phytanic acid by plasmapheresis, if necessary.[193] Although phytanic acid oxidase activity segregates with mitochondria in rats,[196] the localization is clearly peroxisomal in humans[60,194]; thus, adult Refsum disease is now securely classified as a peroxisomal disorder.

Primary Hyperoxaluria: Alanine:Glyoxylate Aminotransferase Deficiency. Primary (type I) hyperoxaluria is characterized by excessive oxalate synthesis, precipitation of calcium oxalate in the kidney, and progressive nephrocalcinosis.[197,198] Renal insufficiency usually develops during the first decade and may be followed by extrarenal calcification of the joints and, especially, myocardium. Except for some patients for whom pharmacologic doses of pyridoxine can substantially reduce the synthesis and excretion of oxalate, renal failure is inevitable. Although most of the oxalic acid in primary hyperoxaluria is produced by the liver, the liver is not subject to oxalate deposition or otherwise clinically diseased. Hepatic and peroxisomal ultrastructure is normal, apart from a mild to moderate increase in lipofuscin deposits.[199]

Danpure and colleagues have shown that type I hyperoxaluria is caused by deficient reclamation of glyoxylate, most of which is normally transaminated to glycine by alanine:glyoxylate aminotransferase (AGT).[200] A deficiency of this pyridoxine-dependent enzyme causes glyoxylate instead to be further oxidized to the metabolic end product oxalate. AGT has for many years been known to be located exclusively within the peroxisome,[201] and its deficiency appears to be the only peroxisomal defect in primary hyperoxaluria. In some cases of type I hyperoxaluria, an abnormality in the tripeptide peroxisomal targeting sequence causes AGT to relocate to the mitochondrial space.[202] Interestingly, hyperoxaluria does not occur in Zellweger syndrome, in which tissue levels, but not subcellular distribution, of AGT are normal.[203] Apparently, location of AGT in the cytoplasm does not impair its function as a transaminase. Because the AGT-deficient liver is the major source of oxalate and the kidney is the major target organ for the disease, combined kidney-liver transplant has become a standard of therapy for type I hyperoxaluria. As reviewed by Cochat and colleagues, patient survival after combined liver-kidney transplant at 5 and 10 years has been approximately 80% and 70%, respectively.[204] As anticipated, renal function in surviving transplant recipients has remained stable.

Acatalasemia. Catalase is present in all peroxisomes at high concentrations and serves the vital function of peroxidatic and catalatic disposal of hydrogen peroxide produced by the many peroxisomal oxidases.[5] Catalase is also present in the cytosol of erythrocytes, which lack recognizable peroxisomes. Acatalasemia is a rare, autosomal recessive disorder first identified in patients with progressive oral gangrene and characterized biochemically by a complete absence of enzymatically and, in some patients, immunologically detectable catalase in erythrocytes.[205] Because the only pathology associated with human acatalasemia is oral gangrene,[206] catalase in other tissues is presumed to be at least partially active. The pathogenesis of oral gangrene in this disorder is not fully understood, and only a minority of patients have any recognizable pathology. One theory holds that erythrocyte catalase detoxifies hydrogen peroxide produced by bacteria that invade superficial mucosal capillaries.[205] In the absence of catalase, tissue destruction by bacterial hydrogen peroxide proceeds unchecked and encourages further invasion of bacteria.

DIAGNOSIS OF PEROXISOMAL DISEASES

Clinical Problems Suggesting a Peroxisomal Disease

Even though children with Zellweger syndrome, infantile Refsum disease, or neonatal ALD are almost always considered abnormal at birth, the diagnosis of a peroxisomal disorder is often delayed for many months. For example, the not uncommon neonatal history of a difficult breech delivery, severe neonatal hypotonia, and abnormal neonatal reflexes characteristic of Zellweger syndrome is often misdiagnosed as perinatal asphyxia before the later development of liver disease, pigmentary retinopathy, or degenerative neurologic disease suggests a different diagnosis. The initial clinical impression of birth injury is often reinforced by the occurrence of myoclonic seizures during the newborn period. Alternatively, the finding of a combination of salt-and-pepper retinopathy and psychomotor retardation in some of the less severely affected children may lead to a mistaken diagnosis of congenital rubella or other prenatal infection. Cockayne syndrome, Leber congenital amaurosis, and Usher syndrome are other diagnoses commonly given to the more mildly affected patients with prominent pigmentary retinopathy and an extinguished electroretinogram. Similarly, Zellweger syndrome is occasionally misdiagnosed as Alagille syndrome (arteriohepatic dysplasia), another diagnosis that combines cholestatic liver disease with dysmorphic facial features. For such children, it is usually the appearance of an unexpected abnormality for the assigned diagnosis, such as pigmentary retinopathy or frank neurologic deterioration, that leads to the ultimate diagnosis of a peroxisomal disorder. Table 55.8-6 lists some of the diagnoses most commonly considered or given to patients with disorders of peroxisomal biogenesis. The clinician should consider the possibility of an underlying peroxisomal disorder when consulted about a patient with any of these diagnoses. Conversely, it is important to recognize that children with primary defects of bile acid biosynthesis (see Chapter 55.4, "Bile Acid Synthesis and Metabolism") may present as cholestatic liver disease and seizures and be initially considered for the diagnosis of Zellweger syndrome.

A number of clinical abnormalities that are especially important clues for the diagnosis of a peroxisomal disorder are summarized in Table 55.8-7.

Gastroenterologists are not uncommonly the first to suggest the diagnosis of a peroxisomal disease when consulted about a neurologically handicapped child who has

TABLE 55.8-6 DIFFERENTIAL DIAGNOSIS OF ZELLWEGER SYNDROME, INFANTILE REFSUM DISEASE, AND NEONATAL ADRENOLEUKODYSTROPHY

Down syndrome; other chromosomal disorders
Congenital hepatic fibrosis/polycystic kidneys
Congenital infection (TORCH) syndrome
Rhizomelic chondrodysplasia punctata
Smith-Lemli-Opitz syndrome
Lowe oculocerebrorenal syndrome
Usher syndrome
Leber congenital amaurosis
Cockayne syndrome
Septo-optic dysplasia (de Morsier syndrome)
Meckel syndrome (encephalosplanchnocystica)

TORCH = toxoplasmosis, other agents, rubella, cytomegalovirus, herpes simplex.

been found to have hepatomegaly, hepatic dysfunction, or simply persistently elevated serum transaminases. Gastrointestinal bleeding secondary to a coagulopathy, varices, or both is another common cause for involvement of the gastrointestinal specialist. However, more than once, evidence by liver biopsy of lipid inclusions in the Kupffer cells has been misdiagnosed as Niemann-Pick disease or another lysosomal lipidosis in a child with neonatal ALD or infantile Refsum disease who has marked VLCFA storage in macrophages. In these cases, careful electron microscopic examination of the storage material should differentiate the lipoid globules with associated birefringent lamellar lipid structures characteristic of the peroxisomal diseases from the lipid inclusions of the lysosomal sphingolipidoses. Other diverse routes have led to the diagnosis of a peroxisomal disease. The finding of either chondrodysplasia punctata (usually without rhizomelic shortening) or characteristic glomerular polycystic kidney disease in a neonate with typical neurologic signs is virtually diagnostic of Zellweger syndrome. In the older, more mildly affected child without clinically evident renal or skeletal lesions, neurosensory defects—optic atrophy, pigmentary retinopathy, abnormal electroretinogram, and deafness—are the most common problems that should lead to the consideration of a peroxisomal disease.

In general, a patient with any two of the major diagnostic criteria listed in Table 55.8-8 should lead the clinician to serious consideration of a disorder of peroxisomal biogenesis or one of the single enzyme defects of peroxisomal β-oxidation, which can closely mimic Zellweger syndrome, infantile Refsum disease, and neonatal ALD. The two newly described entities of CADDS and α-methylacyl CoA racemase deficiency may prove to be important causes of neonatal cholestatic jaundice, and appropriate investigations should be made.

Laboratory Evaluation of Patients with Peroxisomal Disorders

Although a definitive diagnosis of a generalized peroxisomal disorder at one time required demonstration of abnormal or absent peroxisomes by liver biopsy, now the diagnosis can virtually always be established with certainty by measurement of specific peroxisomal metabolites and enzymes in plasma, erythrocytes, fibroblasts, and other tissues, as outlined in Table 55.8-9. Nevertheless, whenever the diagnosis of a peroxisomal disease is entertained for a patient who is to have a liver biopsy, a portion of the biopsy specimen should be processed for study of peroxisomal ultrastructure and specific staining. Although for many of the peroxisomal disorders discussed in this chapter, the relevant genes have been cloned and causative mutations have been found, clinical findings and biochemical studies are still the mainstay of peroxisomal diagnosis and are likely to remain so for many years, especially for the peroxisomal biogenesis disorders, which have the most distinctive and usually unequivocally diagnostic biochemical abnormalities.[101,207]

TABLE 55.8–7 CLINICAL AND PATHOLOGIC CHARACTERISTICS OF THE PEROXISOMAL DISORDERS OF INFANCY AND EARLY CHILDHOOD

CHARACTERISTIC	ZELLWEGER SYNDROME	INFANTILE REFSUM DISEASE	NEONATAL ADRENOLEUKO-DYSTROPHY	ACYL COA OXIDASE DEFICIENCY	BIFUNCTIONAL ENZYME DEFICIENCY	3-KETOACYL COA THIOLASE DEFICIENCY	RHIZOMELIC CHONDRODYSPLASIA PUNCTATA
Abnormal facies	+++	+	+	–	±	+++	+++
Congenital hypotonia	+++	++	++	+++	+++	+++	–
Neonatal seizures	+++	+	++	+	+	++	+
Psychomotor retardation	+++	++	++	++	+++	+++	+++
Pigmentary retinopathy	++	+++	+++	++	++	+++	–
Sensorineural deafness	++	++	++	++	++	++	–
Absent or diminished hepatic peroxisomes	+++	+++	+	–	–	–	–
Hepatic fibrosis/cirrhosis	+++	+	+	–	±	±	–
Coagulopathy	+++	++	++	–	–	–	–
Adrenal lipid inclusions and/or atrophy	+	+	++	+++	+++	++	–
Polycystic kidneys	+++	±	±	–	±	+	–
Epiphyseal/apophyseal calcific stippling	++	–	–	–	–	+	+++
Growth retardation	+++	++	++	–	+	+	+++
Mean survival (yr)	0.6	> 5	3	4	1	0.9	1

– = absent; + = mild or occasional; ++ = moderate or common; +++ = severe or universal.

In general, the measurement of VLCFA levels in plasma, which is now available in several laboratories, is a good screening test for a disorder of peroxisomal biogenesis, X-linked ALD, or a single enzyme defect of peroxisomal β-oxidation.[101,207,208] The most important measurements in plasma are the absolute level of $C_{26:0}$ VLCFAs and the ratio of $C_{26:0}$ to $C_{22:0}$ VLCFAs, both of which are markedly elevated in Zellweger syndrome and related disorders. VLCFA abnormalities can even be detected in autopsy tissues preserved in formalin for many years. If RCDP or one of its variants is the diagnosis under consideration, then the measurement of plasmalogen levels in erythrocyte cell membranes, or, better, plasmalogen synthesis in cultured fibroblasts, and a plasma phytanic acid level are required. False-negative results are uncommon, and more detailed testing of plasma or fibroblasts is not usually required to diagnose Zellweger syndrome, neonatal ALD, infantile Refsum disease, RCDP, or X-linked ALD. There have been only a few cases of autopsy-confirmed neonatal ALD in which plasma levels of VLCFAs were only slightly increased, but subsequent studies in cultured fibroblasts were diagnostic of a multiple peroxisomal deficiency syndrome (R. Kelley and A. Moser, unpublished observations, 1987–1990). Also, it is important to recognize that tissue specimens obtained postmortem or from patients with severe hepatic disease or sepsis may have mild elevations of VLCFAs or pipecolic acid as secondary phenomena. As expected for a relatively newly delineated group of diseases, the clinical spectrum of peroxisomal diseases continues to widen, and new variants, particularly those with partial deficiencies, probably remain to be described.

When plasma levels of VLCFAs are increased in a patient suspected to have a peroxisomal biogenesis disorder, additional metabolite measurements are needed to help define the disorder. When all or most other basic peroxisomal metabolite measurements (pipecolic acid, plasmalogens, phytanic acid, bile acid intermediates) are abnormal, then Zellweger syndrome, infantile Refsum disease, or neonatal ALD is the diagnosis. On the other hand, if plasma levels of pipecolic acid and plasmalogen metabolism in fibroblasts are normal in a Zellweger-like patient or the nondysmorphic, severely hypotonic patient with elevated VLCFAs, then one of the isolated defects of peroxisomal β-oxidation is likely, that is, D-specific acyl CoA oxidase deficiency or MFE2 deficiency. The clinical distinction between X-linked ALD and one of the other isolated peroxisomal β-oxidation defects is usually not a question. However, the diagnosis of CADDS requires the demonstration of a lack of ALDP or a deletion in the appropriate region of the *ABCD1* gene. Despite the often severe adrenal atrophy present in patients with single β-oxidation enzyme defects, neonatal ALD, and infantile Refsum syndrome, adrenal insufficiency is usually not evident clinically but can sometimes be demonstrated by provocative tests of adrenal function.[145.147]

TABLE 55.8-8 MAJOR DIAGNOSTIC CRITERIA FOR A DISORDER OF PEROXISOMAL BIOGENESIS

Abnormal peroxisomal enzyme or metabolite level
Characteristic facial appearance
Evidence of cerebral dysgenesis
Hepatic fibrosis/cirrhosis, cholestasis, biliary dysgenesis
Polycystic (cortical) kidney disease
Abnormal electroretinogram, optic atrophy, pigmentary retinopathy
Sensorineural hearing loss
Punctate calcification of cartilage

TABLE 55.8-9 DIAGNOSTIC TESTS FOR PEROXISOMAL DISORDERS

Plasma	VLCFAs, phytanic acid, pipecolic acid, bile acid intermediates, essential fatty acids
Erythrocytes	Plasmalogens
Urine	Pipecolic acid; long-chain, odd-carbon, and epoxy dicarboxylic acids; bile acids
Fibroblasts, tissues	VLCFA levels, VLCFA β-oxidation, phytanic acid oxidation, plasmalogen levels and biosynthesis, DHAP acyl-transferase activity, alkyl-DHAP synthase activity, sedimentable catalase, peroxisomal size and abundance

DHAP = dihydroxyacetone phosphate; VLCFA = very-long-chain fatty acid.

Because renal tubular immaturity limits pipecolic acid reabsorption (via the iminoglycine transport system) in newborns, some infants with Zellweger syndrome may have normal or near-normal plasma pipecolic acid levels but diagnostically increased urinary pipecolic acid levels.[209] Conversely, after maturation of renal imino acid transport, previously diagnostic urinary levels of pipecolic acid may revert to normal at the same time that plasma levels become markedly increased. In addition, because red cell plasmalogens may with time normalize from dietary sources, specific assay of plasmalogen synthesis in cultured fibroblasts may be necessary in some cases.[101]

Except for X-linked ALD and the associated disorder CADDS, all of the other known peroxisomal disorders are inherited as autosomal recessive traits and therefore carry a 25% recurrence risk in future pregnancies. Prenatal diagnosis of peroxisomal diseases is possible both by traditional amniocyte culture and by chorionic villus biopsy technique. Measurement of VLCFAs, plasmalogen synthesis, and phytanic acid oxidase are commonly performed and reliable.[210–212] In addition, assay of VLCFA oxidation and measurement of particulate (ie, peroxisomal) catalase versus soluble catalase can be performed on amniocytes and cultured chorionic villus cells as backup tests.[210,211] Although there is less experience with prenatal diagnosis of the single enzyme defects of peroxisomal β-oxidation, they are detectable by measurement of peroxisomal β-oxidation rates in prenatally obtained specimens. The absence of a peroxisomal β-oxidation enzyme can also be detected in some cases by indirect immunofluorescence analysis or immunoblot analysis, whereas except for acyl CoA oxidase deficiency, direct enzymatic assay of tissues would be technically difficult because of the presence of much greater quantities of homologous mitochondrial enzyme. Of course, these analyses can be normal if a defect in a peroxisomal β-oxidation enzyme does not change the amount and electrophoretic character of the enzyme.

Finally, because prenatal diagnosis of most of the peroxisomal disorders involves the determination of metabolite and enzyme activity levels that are secondarily abnormal rather than the measurement of the primary genetic defect, it is important to measure more than one diagnostic metabolite or enzyme level and also to have evidence of the feasibility of a tissue biochemical diagnosis documented by prior studies of the proband's fibroblasts or other tissues. The recent finding of genetic mutations causing most of the more common peroxisomal disorders will eventually permit confirmation of a prenatal diagnosis by molecular methods in families with known mutation.

Treatment of Peroxisomal Disorders

General Measures: Zellweger Syndrome, Infantile Refsum Disease, and Neonatal ALD. Treatment of peroxisomal disorders is mostly supportive. For the more severe disorders of peroxisomal biogenesis, neurologic deficits appear to be dictated largely by primary brain malformations and, as such, are irreversible. Even if the postnatal demyelination and CNS degeneration that occur in the disorders of peroxisomal biogenesis could be prevented, it is unlikely that any of the classically affected patients would achieve even a marginal level of function. Supportive therapy for longer-surviving children with generalized peroxisomal deficiency syndromes should address at least five main areas: nutrition, seizures and other neurologic disabilities, progressive liver disease, adrenal insufficiency, and sensory or communication deficits.

Nutrition. Growth retardation in Zellweger syndrome, infantile Refsum disease, and neonatal ALD is common but not universal. Nutritional efforts to improve growth may be beneficial if significant malabsorption exists. Most often, however, even intensive nutritional therapy does little to ameliorate the growth retardation, which appears to be intrinsic in most cases and not caused by inadequate nutrition. Nevertheless, the absorption of fat and fat-soluble vitamins should be monitored. In the more severely affected children, swallowing dysfunction and gastroesophageal reflux are common problems that require medical attention, including nasogastric or gastrostomy feeding for many, if not most, patients.

Neurologic Problems. Seizures in this group of patients are typically myoclonic and may respond poorly to traditional one- or two-drug anticonvulsant therapy. However, there are no associated contraindications to medications, and any appropriate anticonvulsant may be used. Apnea, primary or secondary to seizures, is almost universal in Zellweger syndrome and is one of the more common causes of death.

Liver Disease. Liver disease is rapidly progressive in classic Zellweger syndrome but more variably progressive in patients with milder forms of the disorders. Early in the course of Zellweger syndrome, the only therapy needed for liver disease may be pharmacologic amounts of vitamin K to ameliorate (but not usually cure) a coagulopathy and special dietary measures to minimize the complications of fat malabsorption. Later, the expected complications of cirrhosis and end-stage liver disease—variceal bleeding, ascites, hemorrhoids, rarely hepatic encephalopathy, deteriorating seizure control, multiple hepatic synthetic deficiencies, and delayed drug metabolism—may emerge and require appropriate clinical management. Variceal bleeding in children with neonatal ALD and infantile Refsum disease has been treated by endoscopic sclerosing therapy.

Adrenal Insufficiency. In X-linked ALD, clinically significant adrenal insufficiency is common and requires appropriate adrenal steroid replacement therapy. Addison disease may be the only sign of X-linked ALD in the older child. It may appear at any age in individuals with the biochemical defect but is rarely seen in heterozygous women. It is rarely seen in Zellweger syndrome but may be seen in older individuals with peroxisome assembly disorders and does respond to appropriate replacement therapy.

Sensory Deficits. For patients with neonatal ALD or infantile Refsum disease phenotypes, who occasionally may achieve a developmental level of 2 or 3 years, visual and auditory deficiencies often become important management issues. The use of hearing aids may enable some patients to make surprising gains in communication skills and interactions with others. Even the use of sign language by severely hearing- and/or speech-impaired patients is known. Seemingly poor cognitive development in these children should not automatically be attributed to their congenital and acquired CNS defects if auditory and visual deficits remain unaided.

Other Peroxisomal Disorders. Because of its greater rarity, there is much less experience with the care of children with RCDP than the other disorders of peroxisomal biogenesis. Whereas liver disease is absent in RCDP, seizures, respiratory insufficiency, and recurrent pneumonia are common management problems. Despite excellent care, most RCDP patients die before 1 year of age from the complications of respiratory insufficiency. Growth retardation in long-term survivors is quite severe and, of course, not specifically treatable. Treatment of the single enzyme defects of peroxisomal β-oxidation varies. Patients with isolated defects of acyl CoA oxidase or MFE2 require therapy for the same range of neurologic and sensory deficits found in neonatal ALD, but significant liver disease has been absent in the few reported cases. However, as older, more mildly affected variants of these newer peroxisomal diseases are found, liver disease may yet emerge as a clinical problem.

Specific Metabolic Therapies. In part because of the often excellent response of patients with adult Refsum disease to dietary restriction of phytanic acid, a number of attempts have been made to treat patients with Zellweger syndrome or related peroxisomal diseases by correction of one or more of the characteristic biochemical abnormalities. Specifically, diets to limit the intake of pipecolic acid, phytanic acid, and VLCFAs have been given to several patients with Zellweger syndrome or infantile Refsum disease.[115,213,214] No therapy, however, has been clearly beneficial, despite sometimes substantial improvement in the

metabolite levels. In one patient with infantile Refsum disease treated with a low phytanic acid diet, phytanic acid levels normalized and VLCFA levels improved, but the abundance of hepatic lamellar lipid inclusions continued to increase.[213,214] Batyl alcohol, an octadecyl ether of glycerol that can be converted to plasmalogens in the microsomes, has also been given as a dietary supplement. Although red cell plasmalogen levels rose to normal on batyl alcohol supplements, there was no definite clinical improvement.[213,214] Other therapies without obvious benefit have included adrenal steroids and treatment with clofibrate, which in rats, but apparently not humans, increases peroxisomal numbers.[215] The deficient synthesis of DHA ($C_{22:6}$)[132] in peroxisomal biogenesis disorders can be ameliorated by dietary supplementation.[216,217] A more recent report of DHA treatment of children with peroxisomal biogenesis disorders showed remarkable improvement in clinical function and diverse biochemical abnormalities, including normalization of serum transaminases.[216] DHA supplementation was even associated with improved cerebral myelinization by magnetic resonance imaging.[218] Confirmation of efficacy of this as therapy awaits completion of an ongoing placebo-controlled study (G. V. Raymond and colleagues, unpublished data, 2003). Although the low levels of cholesterol and the antioxidant protection afforded by cholesterol may explain some of the evidence for hepatic fibrosis and cirrhosis, there is as yet no experience with prolonged cholesterol supplementation in children with Zellweger syndrome.

Another area of therapy for Zellweger syndrome and related disorders of peroxisomal biogenesis being explored has been the use of bile acid supplements. Anecdotally, liver function, seizure frequency, and growth all improved in a 6-month-old boy with Zellweger syndrome who was treated with a combination of cholic acid and chenodeoxycholic acid.[131] If, as speculated, the cholestatic liver disease characteristic of Zellweger syndrome is caused by the accumulation of abnormal bile acid species, then bile acid replacement therapy may indeed be beneficial. Despite the poor results of metabolic therapies for the peroxisomal disorders, there remains the possibility that extended trials of this nature may affect the course of sensory or other neurologic deterioration in some of the more mildly affected, longer-surviving patients if treatment is begun before degenerative neurologic changes have advanced.[219,220]

A mixture of triolein ($C_{18:1}$ triglyceride) and trierucin ($C_{22:1}$ triglyceride) ("Lorenzo's oil") has been used in X-linked ALD. There is very clear evidence of improvement of levels of VLCFAs in plasma. However, there is no clinical improvement in boys affected with cerebral disease or in men with adrenomyeloneuropathy.[221] Ongoing trials are presently evaluating this therapy as a preventive therapy.[222] A striking improvement in a case of X-linked ALD was achieved following bone marrow transplant.[223] Additional experience has allowed the development of guidelines for the appropriate selection of patients.[224] Whether bone marrow transplant will be a successful long-term therapy remains to be determined.

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9. Lysosomal Acid Lipase Deficiencies: Wolman Disease and Cholesteryl Ester Storage Disease

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HISTORICAL OVERVIEW

Abramov and colleagues described patients with severe malnutrition, hepatosplenomegaly, calcified adrenal glands, and death occurring within the first few months of life.[1] Additional patients in this family were described in 1961,[2] and the term Wolman disease was applied to such patients. These patients accumulated cholesteryl esters and triglycerides in many organs, most predominantly in the liver, spleen, adrenal glands, and lymph nodes. However, in 1946, Alexander described a patient with Niemann-Pick disease with a similar phenotype and calcification of the adrenal glands.[3] This is likely to be the earliest description of Wolman disease. During the period of 1963 to 1968, additional variants similar to but more mild than Wolman disease were described by Fredrickson, Schiff, Langeron, and Infante and their colleagues.[4–7] These individuals ranged in age from childhood to the fourth decade with hepatomegaly and increased levels of cholesteryl esters in the liver. The intestinal biopsy abnormalities in two such patients were described by Partin and Schubert; they coined the term cholesteryl ester storage disease (CESD).[8]

These two disorders represent a continuum of disease severity and are due to mutations in the locus for lysosomal acid lipase (LAL), an enzyme essential to the degradation of cholesteryl esters and triglycerides that arrive in the lysosome by receptor-mediated endocytosis. These allelic variants of LAL deficiency are termed Wolman disease and CESD. Because of the apparent rarity of the disorders, the full clinical spectra have yet to be fully described. These disorders represent a continuum of disease from the most severe variants, termed Wolman diseases, to progressively less severe involvement in CESD (Figure 55.9-1). The complete clinical spectrum, biochemistry, and pathophysiology have yet to be elucidated, although the availability of animal models should provide additional insight into the disease development and progression.[9,10] The work of Horton, Brown, and Goldstein and their colleagues has made clear the central role that LAL plays in intracellular cholesterol and lipid metabolism.[11–13] The disruption of intracellular lipid regulation leads to severe global metabolic derangements and lipodystrophy.[14]

CLINICAL/PATHOLOGIC PRESENTATION OF WOLMAN DISEASE AND CHOLESTERYL ESTER STORAGE DISEASE

Wolman Disease

The clinical course of Wolman disease has remarkable uniformity. Patients present within the first month of life with vomiting and diarrhea, hepatosplenomegaly, abdominal distention, and inanition with pyrexia and severe failure to thrive. This clinical course perpetuates itself in a downhill spiral to death by age 3 to 7 months.[15] An occasional patient survives beyond 1 year depending on the supportive medical interventions.

The first overt sign is protracted and persistent vomiting associated with abdominal distention. This occurs within

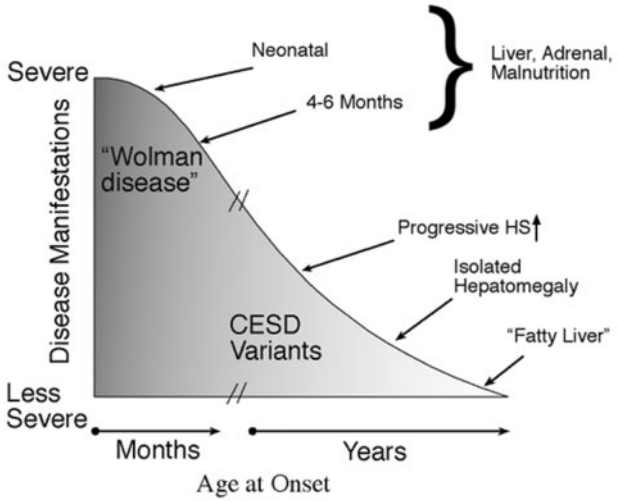

FIGURE 55.9-1 Schematic diagram of a continuum of lysosomal acid lipase deficiency states. Early-onset variants have manifestations in the first few days to weeks of postnatal life and by 4 to 6 months display characteristics of Wolman disease, including hepatosplenomegaly, adrenal enlargement and calcifications, and malnutrition. Variants with components of these signs occur from 1 to 12 years of age, and patients with cholesteryl ester storage disease (CESD), adrenal calcification, and pulmonary hypertension have been reported. At advanced ages, the only manifestation of poorly characterized "fatty liver" may represent late-onset CESD.

the first weeks of life and is accompanied by watery diarrhea, jaundice (occasionally), and low-grade fever. These are intractable to medical interventions or changes in diet and lead to severe weight loss, cachexia, and malnutrition.[1,16,17] Physical examination reveals hepatosplenomegaly with predominant liver enlargement. Enlargement of these organs can occur very soon after birth and has been noted as early as 4 days of age.[18] The first indication of Wolman disease is the abdominal radiograph showing massive enlargement and calcification of the adrenal glands.[16] The adrenal glands have punctate calcification throughout their parenchyma, are symmetrically and massively enlarged, and retain their normal shapes (Figure 55.9-2). Although not well delineated in published reports, these children also manifest adrenal cortical insufficiency (W. Krivit, personal communication, 2003).[18] Analyses of the abdomen by magnetic resonance imaging, computed tomography, or radiography also disclose massively enlarged liver and spleen with lymph node hypertrophy.[19] The latter can include the tonsils and adenoids that may be enlarged sufficiently to cause obstruction.

No specific signs or symptoms are referable to the central nervous system.[15] General deterioration is noted, with the progressive malnutrition and inanition that these children experience.[18,20] However, seizure, convulsions, and paralysis are reported occasionally in Wolman disease. These few central nervous system abnormalities are not primarily related to Wolman disease but rather are related to the malnutrition and specific nutritional deficiencies because of the severe malabsorption (see below).

No specific routine blood chemical abnormalities are present in Wolman disease patients. Anemia, abnormal liver function studies, or other chemical abnormalities referable to severe malnutrition can be present. Malabsorption of fats and other food stuffs has been documented. Specifically, triolein malabsorption is present.[20] This correlates with the infiltration of the jejunum with lipid-laden macrophages (see below). Indeed, intestinal damage may be so severe as to prevent enteral nutrition, and parenteral supplementation should be considered.[21] Because of the adrenal calcification, it is not surprising that patients have adrenocortical insufficiency and require supplementation.[16]

Wolman disease should be considered in the differential diagnosis of any child presenting with persistent vomiting or diarrhea, failure to thrive, hepatosplenomegaly, and malabsorption within the first month of life. The presence of enlarged calcified adrenal glands should be an immediate clue to the diagnosis of Wolman disease.

Cholesteryl Ester Storage Disease

In comparison with Wolman disease, CESD is a more variable and poorly delineated clinical syndrome. Although CESD is thought to be quite rare, this is an underestimate because of poor disease recognition. The clinical spectrum ranges from children diagnosed at age 3 to 4 years to adults in the fifth to sixth decade who present with variable manifestations but including isolated hepatomegaly.[22] The clinical course and progression of CESD in young children have been poorly documented. The relationship between apparent involvement early in life and the potential for significant progression to severe disease later in life remains to be elucidated. Importantly, the fulminant course is poorly documented and includes development of cirrhosis, pulmonary hypertension, onset of adrenal calcifications in adolescence, and failure to thrive.[23–25] In comparison, other patients may have a much more indolent course, with slow progression throughout their lifetimes. Except for mild hepatomegaly, these patients may go undiagnosed throughout their lives. The number of such patients and lack of follow-up information severely limit the ability of physicians to provide accurate information on disease progression to involved families.

Clearly, only the most severely involved patients are usually reported in the literature. This skews the perception of patients and physicians as to the degree of severity and progression of this disease. In particular, the presence of hepatic fibrosis may not be associated with progression to nodular cirrhosis, although both have been reported. Furthermore, the degree of variation within subpopulations

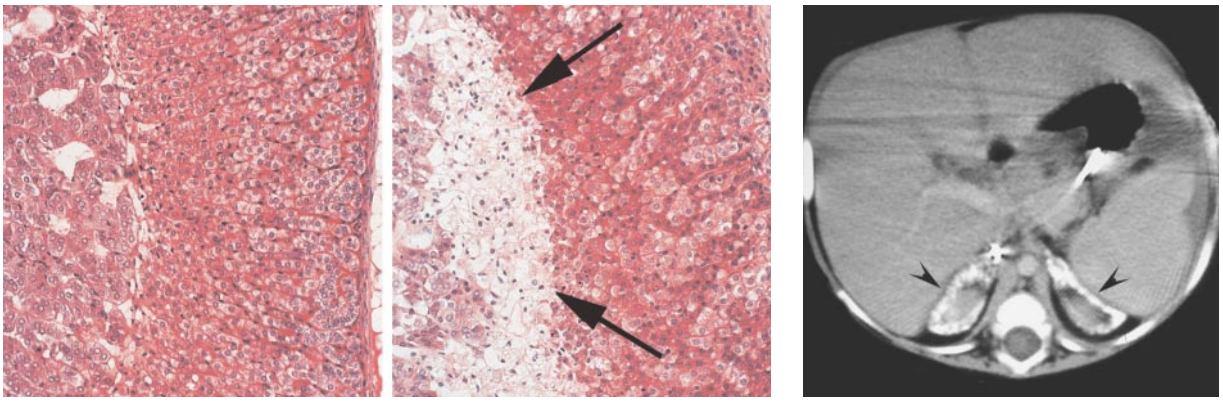

FIGURE 55.9-2 Histology and computed tomographic (CT) scan of abdomen. The zona reticularis of the lysosomal acid lipase (LAL)-deficient adrenal gland (*right*) becomes infiltrated with lipid-laden cells (*arrows*) containing birefringent material under polarized light. Normal adrenal gland is shown on the left. These are from the LAL-deficient mouse at age 4 months. The *arrows* show the calcific deposits in very enlarged adrenal glands in a human patient with Wolman's disease. The adrenal outlines are symmetric, as is the calcification. The CT scan is courtesy of William Krivit, MD, PhD.

may be significant and may or may not relate to the types of mutations that are present in these patients (see below).

Except for some hepatomegaly, there may be no specific abnormality leading the physician to the diagnosis. Specifically, malabsorption, malnutrition, and cachexia have not been reported as consistent components of CESD. Acute or chronic liver failure has occurred, as have progressive fibrosis and cirrhosis with varices.[23,24] Pulmonary hypertension has been described in one patient, but its relationship specifically to CESD is unknown.[24]

Hyperlipidemia may be the most consistent chemical abnormality in CESD patients. Some patients manifest a type IIb hyperlipoproteinemia profile, whereas others do not. Although CESD patients may have intestinal abnormalities on biopsy, abdominal pain and other indications of malabsorption, or cholelithiasis, have not been present in reported patients. Secondary abnormalities of the coagulation system may be noted and may depend on the degree of hepatic dysfunction.

Clearly, the literature is skewed toward reports of more severe CESD patients, and the disease may be much more common than such reports would suggest. The disease should be suspected in patients with uncharacterized vacuolization of the liver, hepatic steatosis involving Kupffer cells, and hepatomegaly, uncharacterized hyperlipidemia, or type II-b hyperlipidemia. Isolated hepatomegaly early in life or isolated hepatosplenomegaly may be the only finding in CESD, and the differential diagnosis of these findings should include CESD.

PATHOLOGY OF WOLMAN DISEASE AND CESD

Liver Pathology

The liver in Wolman disease is characteristically enlarged (> twofold) and progressively increases in size during the life span of the affected infant. Grossly, the liver is yellow in color and greasy in appearance. This is very similar to that in the LAL-deficient mouse.[9] Early in the disease course, the portal and lobular architecture is normal. Later, distortion of the portal spaces occurs with development of fibrosis and, occasionally, frank cirrhosis. Lymphoid infiltration without hepatitis also can be present. Progressive hepatic fibrosis is associated with marked accumulation of a mixture of triglyceride and cholesteryl esters in the lysosomes of hepatocytes, in Kupffer cells, and in portal area macrophages that develop in clusters. The lipid in macrophages tends to be more finely vacuolated than that in hepatocytes. Appropriate lipid stains performed on unfixed frozen sections demonstrate cholesterol and triglyceride in hepatocytes and an admixture of more complex, partly insoluble lipid in the macrophages. Ultrastructural study reveals lipid accumulation, mostly in lysosomes, and increased ceroid-lipofuchsin bodies. The latter are more evident in the macrophages than in hepatocytes.

In CESD, the liver has a gross appearance that is similar to that in Wolman disease (Figure 55.9-3), except that the liver may be more orange and less greasy. Biopsy specimens have a bright orange-yellow color and tend to float in formalin. The hepatocytes contain cytoplasmic vacuoles of variable size that can resemble nonalcoholic steatosis with septal fibrosis (Figure 55.9-4, A and B). The Kupffer cells and portal area macrophages have finely vacuolated cytoplasm and tend to occur in clusters, as in Wolman disease. Acid phosphatase activity is abnormally high in hepatocytes (Figure 55.9-4C). Lipid stains demonstrate a mixture of neutral lipid, cholesterol, cholesteryl esters, and ceroid (Figure 55.9-4D). The cholesteryl ester content of liver tissue is much higher in CESD than in Wolman disease. Because the lipid in tissues is extracted during processing into paraffin or plastic, cholesteryl ester crystals can be demonstrated only in unfixed frozen sections viewed in polarized light (Figure 55.9-4E). However, electron microscopy reveals a high content of cholesterol clefts (crystal-shaped voids) mixed with other lipids in membrane-bound vesicles (Figure 55.9-4F), presumably lysosomes where esterified lipid crystals had existed prior to processing. Septal fibrosis, periportal lymphocytic infiltration, and occasional plasma cells can develop. Portal fibrosis (see Figure 55.9-4B) and cirrhosis occur, but the fibrogenic mechanism is unknown, and progressive liver disease is not established as an inevitable outcome. Foamy macrophages, similar to the Kupffer cells, occur in the spleen, bone marrow, and lymph nodes.

Adrenal Glands

In Wolman disease, the adrenal glands are two to three times normal weight (~ 13–15 g) and have a yellow color. Characteristically, the zona reticularis has broad infiltration by large vacuolated cells that are scattered in haphazard clumps throughout this zone. Areas of necrosis and calcification occur. The adrenal glands have not been well studied in CESD. The adrenal gland in the LAL-deficient mice is very similar to that in Wolman disease, but the massive enlargement and necrosis with calcification are not present (see Figure 55.9-2).

Small Intestine

Intestinal malabsorption, a primary feature of Wolman disease but not of CESD, is associated with severe infiltration of

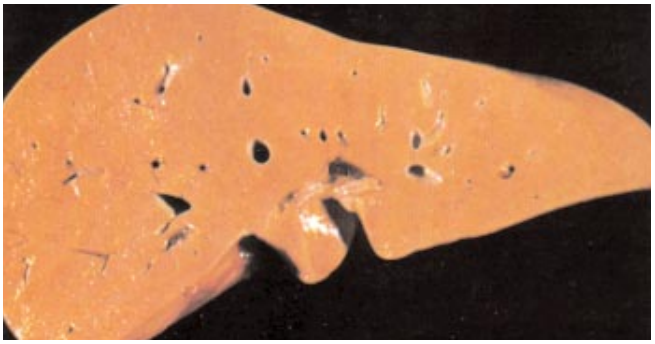

FIGURE 55.9-3 Cross-section of the liver in a patient with cholesteryl ester storage disease. The liver has an orange-yellow color that is immediately apparent on percutaneous or open liver biopsy. The color derives from the deposition of the major storage lipids and also carotene-like lipids (see CD-ROM for color image). Reproduced with permission from Dincsoy HP et al.[76]

the lamina propria by foamy lipid-laden macrophages, as well as accumulation of lipid in small intestinal epithelium. In contrast, Partin and Schubert reported that the proximal small intestinal epithelium of CESD is normal, the macrophages and the extracellular space of the lamina propria contain abundant lipids, including cholesteryl ester crystals, and macrophages at the tips of villi contain autofluorescent material, suggestive of ceroid-lipofuchsin.[8]

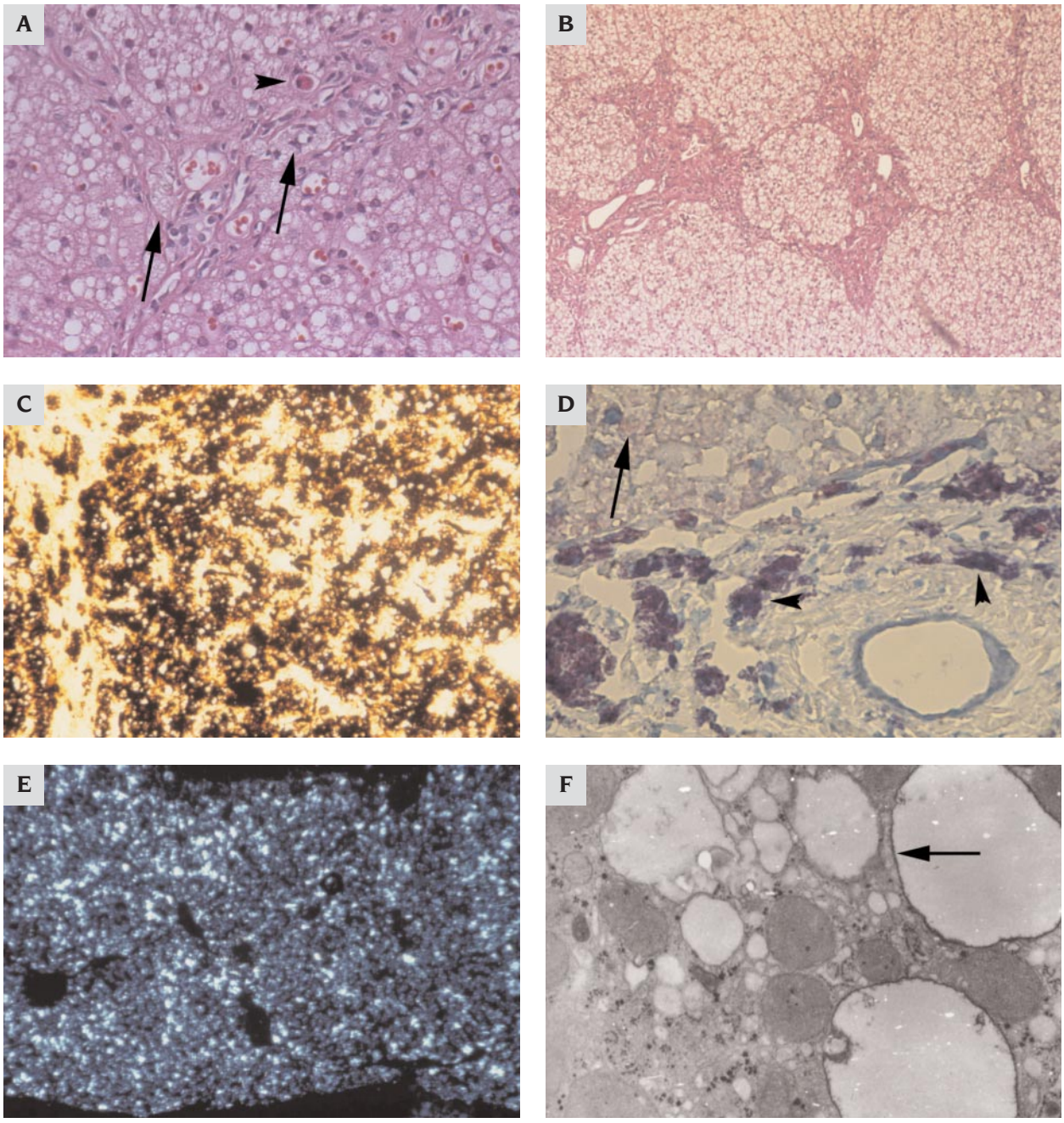

FIGURE 55.9-4 Histology and ultrastructure of the liver and small intestine in cholesteryl ester storage disease (CESD). *A*, Paraffin section of a liver biopsy in an adolescent with CESD. Hepatocyte cytoplasm is pale and vacuolated owing to lipid accumulation. Enlarged vacuolated cells in portal zone are macrophages (*arrows*). Occasional necrotic hepatocytes are present (*arrowhead*) (hematoxylin and eosin; x200 original magnification). *B*, Delicate septal fibrosis in liver from a child with CESD appears to originate in the portal zones without accompanying inflammation (hematoxylin and eosin; x200 original magnification). *C*, Acid phosphatase staining on unfixed cryostat section demonstrates massive increase of activity within hepatocytes in CESD (x200 original magnification). *D*, Nile blue sulfate lipid stain of unfixed cryostat section of liver in CESD demonstrates pale peach–colored neutral lipid in hepatocytes (*long arrow*) and dark blue-purple complex lipid mixture in portal area macrophages (*arrowheads*) (x200 original magnification) (see CD-ROM for color image). *E*, Unfixed unstained cryostat section of liver in CESD viewed in polarized light contains large numbers of anisotropic cholesteryl ester crystals. *F*, Liver cytoplasmic droplets in CESD have well-defined dense membranes (*arrow*), unlike usual hepatocyte lipid vesicles. This is consistent with the presumed lysosomal location of most of the lipid in CESD. Electron micrograph.

Liver, Adrenal Glands, Small Intestine, Spleen, and Other Tissues in Rodent Models of LAL Deficiency

Most published descriptions of Wolman disease are based on autopsy findings in infants, whereas most reports of CESD are based on clinical findings and study of biopsy tissue obtained during childhood and adulthood. The phenotypes are distinct in many ways, but overlapping phenotypes occur, and the concept of an incompletely defined spectrum is appealing (see Figure 55.9-1). The paucity of fully studied patients with Wolman disease or CESD limits the ability to define the morphology and natural history of these two closely related disorders. The two rodent models of LAL deficiency have provided insights into its pathogenesis and progression.[9,10] The most detailed descriptions are from the knockout mice with a complete deficiency of LAL in all tissues. These mice present with a phenotype that exhibits some of the manifestations of Wolman disease and CESD.[9,14] However, the calcification of the adrenal glands of Wolman disease and the hyperlipidemia of CESD are lacking. The mouse knockout model does not have progressive hepatic cirrhosis, although some fibrotic changes are present.

The mouse model provides insight into time-dependent changes in the hepatic disease. Specifically, the liver begins as a normal organ with relatively little change in hepatic weight or consistency in the first 2 to 4 weeks of life. During the second month, vacuolization and lipid storage of the hepatocytes become evident, as does the development of discrete areas of macrophages that are engorged with neutral lipids. Although the progression of hepatocellular storage may continue, the major pathologic abnormality from 2 to 8 months of life is the proliferation of Kupffer cells and portal macrophages that become engorged with cholesteryl esters and triglycerides. These cells can account for over 50% of the liver. The hepatomegaly is progressive and may reach 25% of body weight by 8 months of age, the life span of these mice. This progressive hepatomegaly is accompanied by macrophage storage cell infiltration in the spleen, small intestine, and lymph nodes throughout the body. Although the spleen has large numbers of storage cells, splenomegaly does not become as massive as that observed for the liver. Lymph nodes can be completely replaced by macrophage storage cells. Strikingly, macrophage infiltration of the villi of the jejunum is progressive (Figure 55.9-5). This extends progressively from the proximal to the distal small intestine so that the entire small intestine becomes infiltrated by these storage cells. The histopathology of the small intestine is nearly identical to that described by Partin and Schubert in the original human cases of CESD[8] and in the autopsy cases of Wolman disease.

Concomitant with the progression of hepatomegaly and storage cell infiltration throughout the body, LAL-deficient mice develop lipodystrophy leading to a complete loss of subcutaneous white fat and brown fat. By 6 months of age, essentially all white adipose tissue has disappeared: insulin resistance and low leptin levels develop simultaneously. The LAL-deficient mice are not hyperlipidemic, probably owing to malabsorption and massive redirection of lipids to liver Kupffer cells. This is reminiscent of the "triglyceride steal syndrome." The infiltration of the adrenal glands precisely mimics that in human Wolman disease, albeit without calcification. The zona reticularis is replaced progressively by storage cells (see Figure 55.9-5). The lungs have a few interstitial cells with vacuolization but no specific abnormalities. No gross behavioral or histologic abnormalities have been noted in the central nervous system.

This mouse model indicates that the complete absence of LAL leads to a macrophage and hepatocellular disease with macrophage proliferation and storage of cholesteryl esters and triglycerides as the primary pathologic abnormality. This leads to malabsorption and malnutrition, hepatic dysfunction, and infiltration of the adrenal cortex. The absence of significant fibrosis and/or cirrhosis in the mouse model suggests that either this is not a major or consistent component of the human disease or that mice simply do not survive long enough to develop reactions to the stored lipid in the macrophages.

In addition, mice do not develop the "premature arthrosclerosis" that has been described in human CESD. Thus, the mouse model more closely resembles the human Wolman disease model, with normal to subnormal plasma lipids. In the absence of hyperlipidemia, potentially caused by malabsorption, arteriosclerosis may not develop. If the LAL deficiency is placed against the background of an apolipoprotein E deficiency, a spontaneous atherogenic model in the mouse, doubly homozygous mice for apolipoprotein E and LAL deficiency have much more rapidly progressive lesions with apolipoprotein E deficiency alone.[26] Thus, the premature arteriosclerosis in the CESD may be accentuated by the presence of dietary or other genetic abnormalities in some patients, leading to hyperlipoproteinemia and progression of arteriosclerosis.

BIOCHEMICAL PATHOLOGY OF LAL DEFICIENCY

Lipoprotein and Lipid Abnormalities

Plasma triglyceride and cholesterol levels are usually normal in Wolman disease. Three cases showed elevated

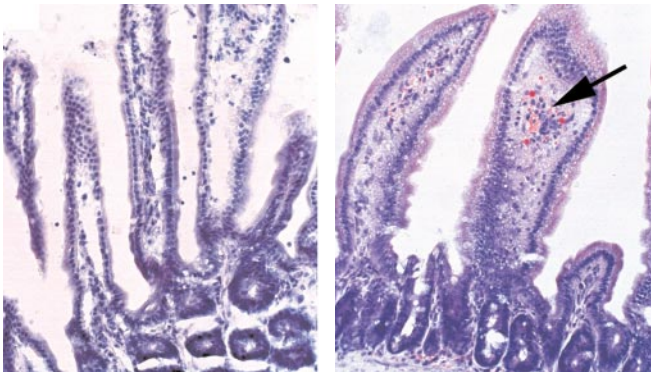

FIGURE 55.9-5 Small intestine in lysosomal acid lipase deficiency. The small intestinal villi become infiltrated with lipid-laden macrophages that contain cholesteryl esters and triglycerides. Normal and lysosomal acid lipase–deficient samples are on the left and right, respectively. The arrow shows oil red O–positive macrophages (see CD-ROM for color image).

triglycerides and very-low-density lipoprotein (LDL) cholesterol,[17,27] whereas plasma high-density lipoprotein (HDL) was decreased in others.[27–29] In comparison, CESD patients usually have hypercholesterolemia with increases of very LDL and LDL cholesterol levels. The plasma HDL in some CESD patients can be less than the 5th percentile (ie, < 20 mg/dL). In some other cases, the ratio of HDL_2-cholesterol to HDL_3 cholesterol was reversed (10:1) compared with the normal ratio (1:10).[30]

Patients with Wolman disease accumulate cholesteryl esters and triglycerides in the liver, spleen, small intestine, and adrenal glands. Compared with normal levels, the triglyceride concentrations can be 2- to 10-fold increased in the liver and 8- to 100-fold increased in the spleen. Quantitative analyses are not available for the patient's small intestine, but gross and histologic observations of the intestine from Wolman disease and CESD patients show accumulation of these lipids.[31] The mouse model of LAL deficiency showed total cholesterol increases of 43-fold in the liver, 27-fold in the spleen, and 2.5-fold in the small intestine by 8 months of age.[14]

All patients with Wolman disease have increased total cholesterol levels in the liver (~ 5- to 160-fold), spleen, small intestine, and adrenal glands (8-fold).[32] More than 98% of the accumulated total cholesterol are cholesteryl esters.[9] The fatty acid content of liver and spleen also can be increased, whereas the phospholipid and glycolipid contents are not, even at 8 months of age.[33,34]

In cases of CESD, triglyceride and cholesteryl esters were elevated in the liver. The cholesteryl ester storage in liver can be 120- to 350-fold higher than normal. Triglyceride levels have minor increases. Inconsistently elevated values were in spleen, small intestine, kidney, and lung. Analysis of acyl groups in the cholesteryl esters in the liver and small intestine of CESD patients revealed predominantly oleic and linoleic acids.[29,35–37]

INTRACELLULAR DERANGEMENTS IN LAL DEFICIENCY

LAL plays a central role in the modulation of cholesterol metabolism in all cells. The LDL receptor or other receptors on the plasma membranes of various cells can deliver LDL-bound cholesteryl esters and triglycerides to the lysosomes. Once delivered to the lysosome, the released cholesteryl esters and triglycerides are cleaved by LAL to free cholesterol and fatty acids. After LAL cleaves these lipids, they exit the lysosome and enter the cytosol. Normally, cholesterol will interact with the sterol response element binding protein system to modulate the intracellular production of cholesterol (Figure 55.9-6).[11] This system, elucidated by Goldstein and Brown over the past three decades, is a major component of modulation of neutral lipid metabolism in the body.[11] In LAL deficiency, cholesteryl esters and triglycerides cannot be cleaved; therefore, free cholesterol and fatty acids cannot leave the lysosome.[12,38] The cells detect an intracellular (cytosolic) cholesterol deficiency, and the cholesterol biosynthetic pathway is up-regulated to compensate. The increased production of cholesterol leads to enhanced esterification of cholesterol via the cytoplasmic enzyme acyl CoA:cholesterol acyltransferase and enhances the synthesis of very LDL to export the synthesized cholesterol. Similarly, the lack of fatty acid egress from the lysosome leads to up-regulation of a series of other biosynthetic fatty acid enzymes with enhanced fatty acid synthesis. The details of this fatty acid synthetic pathway are not fully elucidated, but the control of this pathway from lysosomally derived free fatty acids appears clear.[11]

The up-regulation of these systems in LAL deficiency has led to the use of statins for the treatment of hypercholesterolemia in CESD.[39] Apolipoprotein B synthesis and the plasma lipid abnormalities are partially corrected by statins, but this may be transient. Statins may be important for the therapy of CESD in the prevention of secondary disease but do not theoretically or practically have a major effect on the lipid accumulation in the lysosomes of various tissues, although re-esterification and the pathway should be diminished.

LAL PROPERTIES

LAL is a typical lysosomal hydrolase that is synthesized in the rough endoplasmic reticulum and is cotranslationally glycosylated as it emerges into the endoplasmic reticulum lumen.[38,40] Following clipping of the leader sequence, the enzyme is modified during transit through the Golgi apparatus but without further proteolytic modification. The oligosaccharides are remodeled to attach the mannose 6-phosphate targeting signal for lysosomal sorting. The newly synthesized LAL is delivered to the lysosome by the mannose 6-phosphate receptor system. LAL is not known to require cofactors for optimal hydrolysis, and it functions as a monomer. LAL has significant similarity to other acidic lipases, for example, hepatic or gastric lipases that cleave similar substrates in the hepatocyte cytosol or stomach, respectively. Because the isolation of LAL from natural tissues has been difficult, purified LAL has been characterized using recombinant expression systems.[41] The enzyme can be produced in large quantities from appropriately designed genetic sequences from humans using insect cells, Chinese hamster ovary cells, *Pichia pastoris* and *Pombe* yeasts, and other heterologous expression systems (G. A. Grabowski and H. Du, unpublished data, 2003).[42–44] As indicated above, LAL has significant homology to other acid lipases but is clearly distinct from hormone-sensitive lipase, pancreatic lysophospholipid lipase, lecithin cholesterol acyl transferase, lipoprotein lipase, hepatic lipase, and pancreatic lipase. A significant homology exists with a shared amino acid motif, –Gly-X-Ser-X-Gly, that is common to most lipases and is an essential pentapeptide in the active site.[43,45] This pentapeptide occurs twice in LAL, and serine 153 appears to be important to catalytic activity. Several other polymorphic variants of LAL have been described, and their physiologic significance and importance are under investigation.[46]

Recombinant heterologous expression and characterization of purified human LAL have proven that the triglyceridase and cholesteryl esterase activities are present in

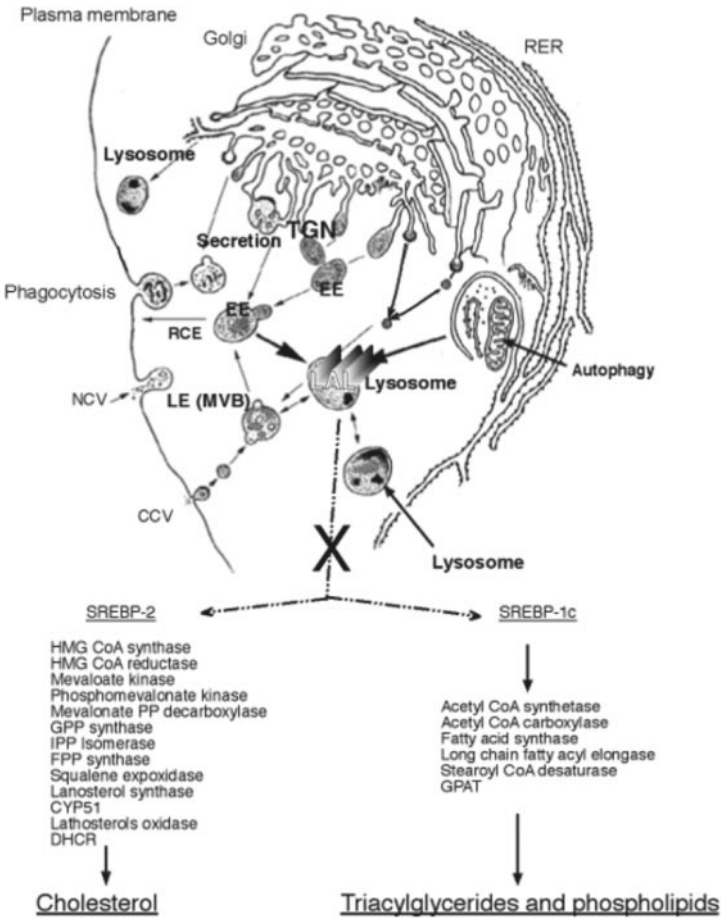

FIGURE 55.9-6 Schematic of the lysosomal acid lipase (LAL) system and its connection to cellular cholesterol and fatty acid metabolism. There are several types of lysosomes within cells that can contain different substrates and/or storage materials. Material can enter the lysosome from the surface, by autophagy of intracellular organelles, and after transit through the Golgi apparatus. LAL is synthesized in the rough endoplasmic reticulum (RER) and is post-translationally modified (oligosaccharides) and targeted to the lysosome following passage through the trans-Golgi network (TGN). CCV = clatherin CV; CV = coated vesicle; EE and LE = early and late endosomes, respectively; MVB = multivesicular body; NCV = nonclatherin CV; RCE = recycled coated endosome. The egress of cholesterol or fatty acids from the lysosome feeds back to the cytoplasmic, plasma membrane, ER-controlled SREBP systems for altering translation of specific enzymes leading to the synthesis of cholesterol and/or triacylglycerides and phospholipids.

the same polypeptide sequence.[44] This also is evident from the LAL knockout mouse. More importantly, serine 153 appears to be critical to the catalytic function for both substrates.[44] Thus, the deficiencies and abnormalities of LAL in Wolman disease and CESD must be explained by a single enzyme deficiency.

How can one explain the massive accumulation of cholesteryl esters and triglycerides in Wolman disease and just cholesteryl esters in the tissues from CESD patients? From mutagenesis data, the following appears clear: complete deficiency of LAL leads to Wolman disease with subsequent accumulation of cholesteryl esters and triglycerides. Diacylglycerides and monoacylglycerides also accumulate in smaller amounts because these are also LAL substrates. The catalytic activity of human LAL has a significant preference for tri-, di-, and monoacylglyceride substrates compared with cholesteryl esters.[44] The presence of apparently small amounts of normal LAL in tissues from CESD patients may provide an explanation for the variant phenotypes. A recurrent mutation in CESD leads to abnormal splicing and production of nearly normal amounts of a LAL messenger ribonucleic acid (RNA). But the mutation allows for only ~ 1 to 3% normal levels of LAL messenger RNA in the tissues of CESD patients.[30,47–49] This small amount of LAL RNA allows for the production of low levels of normal LAL that may be sufficient to nearly normalize the preferential acylglycerol substrate cleavage. However, this is insufficient to normalize metabolism of the less efficiently cleaved substrate, cholesteryl esters with their resultant accumulation in various cells. This indicates that large amounts of enzyme are not required for normalization and that low levels of LAL activity may be sufficient for prolonged survival with minor manifestations of disease.[50] LAL deficiency is very similar to other lysosomal hydrolase deficiency disorders in which small amounts of residual activity can normalize the flux of substrates through the lysosome. Higher thresholds of enzymatic activity are consistent with later-onset mild disease and, potentially, normalcy.

GENETICS AND MOLECULAR BIOLOGY

Wolman disease and CESD result from allelic mutations at the LAL locus on human chromosome 10q23.2–q23.3. The gene spans 45 kb, has 10 exons (Figure 55.9-7), and contains no unusual structures except for a large intron 3. The mutations found in Wolman disease and CESD are listed in Table 55.9-1 and represent patients from a variety of nationalities. A curious finding is the presence of mutations in intron 8 that result in abnormal splicing in either Wolman disease or CESD. This abnormal splice could eliminate intron 8 as owing to a splice junction abnormality. However, the location of the base change, either one or three bases following this donor slice, appears to affect the ability of the cell to produce normal LAL messenger RNA. The recurrent mutation in Wolman disease is a base substitution in exon 8 either one or three bases prior to the

intron 8 splice donor site. These appear to be incompatible with normal splicing and lead to a deletion of exon 8. The resultant truncated unstable protein in Wolman disease has no LAL activity and is rapidly degraded. In comparison, the substitution of a base at the +1 position downstream from the spliced donor site has been found in several CESD patients. This leads to ~ 5% of normal LAL messenger RNA splicing and the production of low levels of normal LAL protein from this mutant allele. Most (95%) of the mutant RNA produces a deletion of exon 8 and a truncated protein. The major difference, then, between Wolman disease and CESD is the absence or presence, respectively, of small amounts of normal LAL in cells and, as indicated above, the preferential cleavage of triglycerides compared with cholesteryl ester.

The most common mutation, a deletion of 254-277_1, Δ254-277_1 allele, was screened for in a randomly selected population in northwestern Germany.[15] In a cohort of 1,887 people aged 20 to 70 years, the frequency of Δ254-277_1 was 0.0019, giving an estimated homozygosity frequency of 1 in 300,000 or approximately 260 cases of CESD in Germany. If such calculations apply to the US population, there would be about 1,000 homozygotes for CESD. These calculations suggest that the frequency may be high enough to entertain screening of hyperlipidemic patients, particularly those with a type II-b profile, for CESD. The frequency of Wolman disease has been estimated at < 1 in 500,000 live births.[51]

Diagnosis

The diagnosis of Wolman disease should be considered in any infant with persistent vomiting and hepatomegaly in the first month of life, with progressive failure to thrive, and/or with hepatosplenomegaly and malabsorption. A radiograph of the abdomen may reveal adrenal calcifications (see Figure 55.9-2). The presence of adrenal calcifications can be observed in other conditions, but most occur later in life or are asymmetric. Thus, symmetric adrenal calcifications in the first few months of life may be pathognomonic of Wolman disease. Other lysosomal storage disease presenting this early in life with hepatosplenomegaly and severe failure to thrive includes the Niemann-Pick A variant with severe central nervous system involvement and inanition owing to sphingomyelinase deficiency. The severe variants of Gaucher disease type 2 have their onset in the similar time frame but with lesser degrees of hepatosplenomegaly, no malabsorption or diarrhea, and a predominant finding of rapidly developing neurologic signs with bulbar involvement. Suspect cases should have peripheral blood leukocyte LAL activities measured. The isolated deficiency of LAL is diagnostic. Contributing and supporting evidence for the diagnosis includes massive involvement of Kupffer cells on liver biopsy with or without some fibrosis and cholesterol crystals in the liver. Bone marrow biopsy may also show foam storage cells. Importantly, hypercholesterolemia and hypertriglyceridemia are not components of Wolman disease. Once the diagnosis is made by LAL enzyme assay, LAL mutations can be determined, and these may be used in further family studies or for prenatal diagnostic testing.

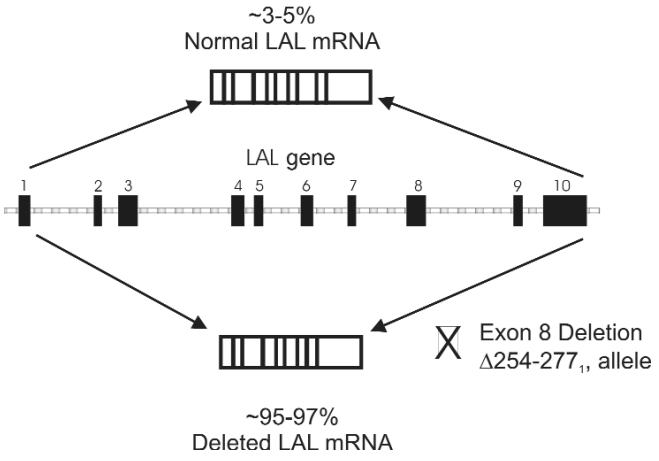

FIGURE 55.9-7 Schematic of the lysosomal acid lipase gene. The *LAL* is about 45 kb in size with 10 exons (*numbered rectangles*). Exons in the gene (*center*) are filled rectangles, and introns are represented by the thin horizontal rectangle. The spliced messenger ribonucleic acid (mRNA) is shown above and below to represent different mRNA products, resulting in a small amount of normal mRNA being formed. The majority of mutant mRNA leads to the splicing of exon 8 and a truncated translated protein.

CESD early in life must be considered in any child with hepatosplenomegaly and hyperlipidemia. CESD may be confused with glycogen storage disease (GSD) type IA or B because that disorder also presents with hepatomegaly and hypertriglyceridemia. Lactic acidosis can be a component of GSD IA or B but is not a component of CESD. Young children with CESD generally do not have failure to thrive but can have isolated hepatomegaly, prompting liver biopsy. The presence of engorged Kupffer cells with or without some fibrotic changes and cholesterol crystal deposition is highly suggestive. The CESD liver is distinguished from other lysosomal storage diseases, that is, Gaucher disease, Niemann-Pick A or B, and Pompe disease (GSD II), by its characteristic orange color and neutral lipid storage. GSD IA or B is not lysosomal, and glycogen storage predominates, not neutral fat. The diagnosis is made by isolated LAL deficiency in any nucleated cell. Peripheral white blood cells and cultured fibroblasts are the usual and most general sources for enzyme diagnosis. Thus, in the presence of hepatomegaly and hyperlipidemia, the diagnosis of CESD should be entertained in any child. On liver biopsy, the core may be orange in color, immediately suggesting the diagnosis.

In older children or adults, the findings are nonspecific. Mild hepatosplenomegaly or mild hepatomegaly with or without significant hyperlipidemia suggests the diagnosis of CESD. Characteristic liver biopsy findings and, occasionally, bone marrow biopsy findings should prompt LAL assays. The spectrum of the adult variants of CESD may be quite broad and include a large number of asymptomatic individuals. The diagnosis should be suspected in individuals with a type II-b hyperlipidemic pattern. LAL assays may obviate the need for liver biopsy for diagnosis. Mutation

TABLE 55.9-1 WOLMAN DISEASE AND CESD MUTATIONS

ALLELE	EXON	cDNA NUMBER*	BASE CHANGE	AA NUMBER†	AA CHANGE	WD	CESD	REFERENCE
E3Δ8bp	3	159–166	del 8 bp	19	Frame shift/stop	+		42
Y22X	3	169	C→A	22	Tyr-X	+		56
R44X	3	233	C→T	44	Arg-X	+		30, 48, 57
E4skip	4	270–468	?	56	Frame shift/stop	+		30
Q64R	4	294	A→G	64	Gln-Arg		+	58
G66V	4	300	G→T	66	Gly-Val		+	59, 60
W95X	4	387	G→A	95	Trp-X	+		30
H108P	4	426	A→C	108	His-Pro		+	59, 61, 62
H108R	4	426	A→G	108	His-Arg		+	63, 64
E4Δ2bp	4	435–436	del TC	111	Frame shift/stop	+		42
S112X	5	438	del C	112	Ser-X	+		60, 65
fs112	5	437–438	del TC	112	Frame shift/stop		+	30
fs177	6	634	T→TT	177	Frame shift/stop	+		30, 66
L179P	6	639	T→C	179	Leu-Pro	+	+	66, 67
P181L	6	645	C→T	181	Pro-Leu		+	59
E7SJM	6 (intron)		a→g	205-253	del 48 aa		+	59
fs219	7	722	del T	219	Frame shift/stop	+		30, 57
G245X	7	836	G→T	245	Gly-X		+	47
T267I	7	903	C→T	267	Thr-Ile		+	58
S268C	8	906	C→G	268	Ser-Cys		+	30
L273S	8	921	T→C	273	Leu-Ser		+	59
H274Y	8	923	C→T	274	His-Tyr		+	49
Q277X	8	932	C→T	277	Gln–X	+		68
E8SJM-1	8	863–934	G→A	254–277	del 24 aa		+	30, 62, 64, 67, 69–71
E8SJM+1	8 (intron)		g→a	254–277	del 24 aa	+		47
E8SJM-3	8	863–934	C→T	254–277	del 24 aa	+		68
E10ΔAG	10	1,007–1,008	del AG	302	Frame shift/stop		+	69
Y303X	10	1,012	T→A	303	Tyr-X	+		72
E10ΔC	10	1,020	del C	306	Frame shift/stop		+	30
T316A	10	1049	A→G	316	Thr-Ala			73
E10ΔG	10	1064	del G	321	Frame shift/stop		+	57
G321W	10	1064	G→T	321	Gly-Trp		+	42
L336P	10	1110	T→C	336	Leu-Pro		+	74

CESD = cholesteryl ester storage disease; LAL = lysosomal acid lipase.
*The complementary deoxyribonucleic acid (cDNA) number is according to human LAL cDNA clone that was published by Anderson and Sando in 1991.[75]
†The amino acid (AA) number is from mature N-terminus, which is started from 22 aa from the first Met.

analysis in affected individuals may be helpful for family screening and additional diagnostic and correlative studies.

TREATMENT

Currently, there is no specific treatment for Wolman disease or CESD that is generally available. One patient with CESD had a liver transplant for chronic liver failure and was apparently well 2 years after the procedure,[52] but additional data have not been forthcoming. For Wolman disease, bone marrow or stem cell transplant has been attempted in several cases.[53,54] Because of the severity of the illness and the adrenal insufficiency, the mortality rate has been high. In one series, four of five patients succumbed to transplant complications. In the surviving patient, the bone marrow transplant appeared to be successful, with disappearance of storage macrophages in the liver, spleen, and, presumably, other tissues. The adrenal glands appear to atrophy following bone marrow transplant (W. Krivit, personal communication, 2003), and lifelong corticosteroid replacement therapy is necessary. Enzyme replacement and gene therapy studies have been conducted in the mouse knockout model with resolution of the hepatocellular and macrophage storage.[41,55] These remain investigational and provide proof of principle for the treatment of this disease. Currently, no clinical trials are ongoing for these modes of therapy. The approaches of enzyme replacement or gene therapy for enzyme replacement should be efficacious in both Wolman disease and CESD. Prenatal diagnosis is available for families who wish to exercise this option.

Supportive therapies for Wolman disease have been inadequate, and long-term therapies with intravenous alimentation have not been reported. Because enteral nutrition may be impossible owing to the severity of involvement of the gastrointestinal tract in Wolman disease, supportive parenteral nutrition may be the only option. In CESD, adjunctive therapy with statins or other suppressors of 3-hydroxy-3-methylglutaryl CoA reductase function has been useful in suppressing very LDL and LDL with subsequent lowering of plasma lipoprotein levels. This may lead to a decreased risk of heart disease. Statins have not been shown to prevent the development of progressive hepatocellular disease or adrenal insufficiency. Because of the intestinal involvement in CESD, studies for malabsorption should be undertaken, and supplementation with vitamins A, D, E, and K may be appropriate. Other supplements should be undertaken if malabsorption is present.

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10. *Wilson Disease*

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Wilson disease is a rare autosomal recessive disorder of copper metabolism. S. A. Kinnear Wilson described the entity in 1912 and considered it to be a degenerative disorder of the central nervous system (CNS) associated with asymptomatic cirrhosis.[1] In 1921, Hall reported the hepatic symptoms and introduced the name "hepatolenticular degeneration."[2] It is generally accepted that the disorder is related to excessive accumulation of copper in the liver, CNS, kidneys, cornea, skeletal system, and other organs. The prevalence of the disorder is 1 in 30,000 persons worldwide, with a carrier frequency of 1 person in 90.[3] Wilson disease frequently presents in childhood, although the diagnosis may not be confirmed until adulthood. Early recognition of this disease and institution of appropriate therapy may be lifesaving.

PATHOPHYSIOLOGY

Wilson disease has been recognized as an entity for more than 80 years; the genetic defect has been identified, and the basic biochemical abnormalities are continuing to be elucidated. Wilson disease is a disorder of copper balance in which the biliary excretion of copper is inadequate, leading to excess accumulation in the other organs.[4] Copper is an essential trace element required in a number of enzyme systems. The main dietary sources of copper include liver, kidney, shellfish, chocolate, dried beans, peas, and unprocessed wheat. The average American diet includes 1.0 mg of copper per day.[5,6] Under normal circumstances, 50% of ingested copper is unabsorbed and lost in the feces[7] and 30% is lost through the skin.[8] A negligible amount normally is excreted in the urine. The remaining 20%, which is critically balanced for homeostasis, is normally excreted into the feces via bile.[4,5,9] Wilson disease is caused by the inability to excrete this remaining 0.2 mg of copper into bile; copper absorption from the gastrointestinal tract is normal. Studies measuring the peak copper concentration in blood after an oral dose of radiocopper have shown no difference between patients with Wilson disease and control patients.[7]

MOLECULAR GENETICS

Initial genetic linkage studies showed that the Wilson disease locus segregated with the red cell enzyme esterase D on chromosome 13.[10] Subsequent linkage analysis confined the disease locus proximally by the deoxyribonucleic acid (DNA) marker D13S31 and distally by the DNA marker D13S59.[11,12] Soon after it was confirmed that the Menkes disease gene (*MNK*) encoded a copper-binding P-type adenosine triphosphatase (ATPase) protein,[13–15] four independent groups identified the gene responsible for Wilson disease.[16–19] Three of these groups used the human Menkes disease gene as a probe,[16–18] whereas the fourth group used linkage disequilibrium and haplotype analysis.[19] The Wilson disease gene transcript encodes a transmembrane copper-transporting ATPase protein with a strong homology to the Menkes disease gene. The messenger ribonucleic acid for the Wilson disease gene is highly expressed in the liver, with limited expression in other tissues. The strong homology between the Wilson disease gene and the Menkes disease gene is interesting considering the different clinical manifestations of these two diseases. This can be better understood if both are considered to be disorders of ineffective intracellular transfer of copper.

The mechanism by which the Wilson protein, ATPase 7B, participates in copper metabolism is beginning to be understood (Figure 55.10-1). Dietary copper is absorbed in the upper intestine, where it binds to the proteins, albumin, copper histidine, and transcuprein.[20] Most of the copper is transported to the liver via the portal system and enters the hepatocyte, possibly by the cell surface transporter human copper transporter 1.[21] Once inside the hepatocyte, copper is transported by "copper chaperones," which traffic the metal within the cytoplasm. The identified copper chaperone in humans, known as human ATX homologue 1 (HAH1), functions as both a regulator of copper homeostasis and an antioxidant.[22,23] HAH1 transports copper to the Wilson ATPase 7B, which is located in the *trans*-Golgi network and, under basal conditions, also facilitates transfer of copper into ceruloplasmin.[24,25] However, an increase in cellular copper concentration has been shown to cause movement of the ATPase 7B protein to a cytoplasmic vesicle by a mechanism that appears to be reversible and independent of new protein synthesis.[24] This vesicle is hypothesized to be one of the mechanisms by which copper normally leaves the hepatocyte. Interestingly, ATPase 7B has also been found in the hepatocyte membrane and may represent a direct route to transport copper through the canalicular membrane.[25] Mutations in the ATPase 7B protein represent

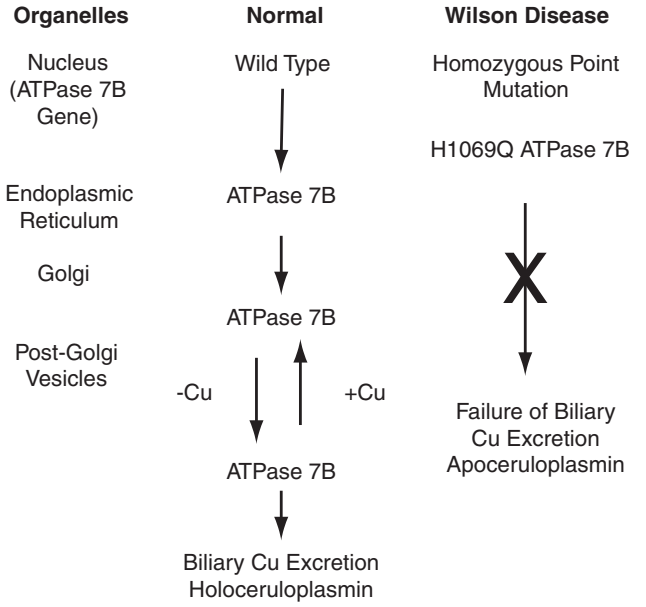

FIGURE 55.10-1 Molecular defect in Wilson disease. Under normal conditions, adenosine triphosphatase (ATPase) 7B is a transmembrane protein that traffics from the endoplasmic reticulum to the Golgi apparatus, where it is localized under basal conditions. ATPase 7B is responsible for the movement of copper into the Golgi, where ceruloplasmin probably acquires its copper. When copper is abundant, ATPase 7B traffics to a post-Golgi compartment, where it delivers copper to biliary excretory pathways that are thought to involve transfer to lysosomes and then to bile by a canalicular membrane transporter. In Wilson disease, the mutant protein fails to leave the endoplasmic reticulum and therefore is unable to transfer copper in the Golgi or facilitate copper excretion in bile. Reproduced with permission of Lippincott, Williams and Wilkins from Schilsky M. Inherited metabolic disease. Curr Opin Gastroenterol 1999;15:200–7.

the molecular basis of Wilson disease and are thought to cause a disruption in the transport of copper from the hepatocyte. Greater than 60 disease-specific mutations already have been identified[16,18,26] that make genetic screening of Wilson disease challenging. The most common mutation of the Wilson disease gene, His 1069 Glu (*H1069Q*), represents 30 to 60% of the Wilson disease alleles in North American, Austrian, Russian, and Swedish samples.[26,27] Two mutations not yet described in European samples have been found in Japanese children: 2874 del C in exon 13 and Arg 778 Leu in exon 8.[28]

The ability to study Wilson disease is enhanced with the recent identification of the Long-Evans cinnamon rat as an animal model.[29] This model has been used to demonstrate correction of the defect using complementation with a wild-type ATPase 7B complementary DNA incorporated into a recombinant adenovirus.[30] Under these conditions, ceruloplasmin synthesis was restored as well.

Much attention also has been given to the role that ceruloplasmin may play in the pathogenesis of disease. Ceruloplasmin is a blue-colored, α-globulin with a molecular mass of 132 kD. The gene for ceruloplasmin is on chromosome 3. It is produced exclusively in the liver, but its role in copper metabolism is unknown; it may function in iron transport.[20] Typically, patients with Wilson disease have low serum levels of ceruloplasmin. However, 5 to 25% of patients with Wilson disease have normal levels.[31] At times, this is secondary to an acute-phase response associated with active liver disease, but approximately 10% of patients have unexplainably normal values. Low values may be found in some heterozygotes who have no manifestations of the disease.[32]

The cause of the decreased and the occasional normal values of ceruloplasmin in Wilson disease is unknown, but available data do provide interesting clues. Both apoceruloplasmin and the number of atoms of copper per ceruloplasmin molecule are normal in Wilson disease, but the rate of holoceruloplasmin synthesis seems to be reduced.[3] Knowledge that Wilson disease results from a mutation of a copper transport protein is useful in explaining the variations observed in ceruloplasmin levels. Copper is incorporated during the biosynthesis of ceruloplasmin by transfer from the ATPase 7B located in the *trans*-Golgi network; ceruloplasmin is then secreted from the hepatocyte into the plasma.[33] Yet reduction of copper transport into excretory pathways still occurs, resulting in hepatic copper accumulation.[34] These mutations may also affect ATPase 7B interaction with copper chaperones or the excretion of copper across the canaliculus. In in vitro experiments, the *H1069Q* mutation appears to demonstrate an abnormal mechanism of protein folding that affects its ability to be expressed in the *trans*-Golgi network in the murine-mottled fibroblast cell line.[33]

The transfer of copper into the pathway for ceruloplasmin synthesis is altered by Wilson disease mutations, thereby causing decreased ceruloplasmin levels. Different mutations may alter copper transport to different degrees and at different cellular sites. Therefore, certain mutations may allow normal transport of copper for ceruloplasmin synthesis so that normal ceruloplasmin levels are maintained. Yet reduction of copper transport into excretory pathways still occurs, resulting in hepatic copper.

CLINICAL MANIFESTATIONS

The clinical manifestations of Wilson disease usually are related to hepatic or CNS involvement (Table 55.10-1). The presenting features are variable, and clinical disease is rarely present before patients reach 5 years of age. Most of the manifestations are related to deposition of copper in specific organs. In the series of Scheinberg and Sternlieb, the initial clinical manifestations were hepatic in 42% of patients, neurologic in 34%, psychiatric in 10%, hematologic or endocrinologic in 12%, and renal in 1% (Table 55.10-2). Approximately 25% of patients have more than one organ involved.[3] Of 50 cases reviewed by Walshe, 31 cases had hepatic and 17 had neurologic presentations.[35] In the pediatric age group, it is common for the hepatic manifestations to precede the neurologic manifestations by many years.

HEPATIC MANIFESTATIONS

Manifestations of liver disease are greatly varied. Wilson disease may present as acute self-limited hepatitis; full

TABLE 55.10-1 MANIFESTATIONS OF WILSON DISEASE

HEPATIC
Acute hepatitis
Chronic active hepatitis
Cirrhosis
Fulminant hepatic failure
CENTRAL NERVOUS SYSTEM
Neurologic
Psychiatric
OPHTHALMOLOGIC
Kayser-Fleischer ring
Sunflower cataracts
MISCELLANEOUS
Hemolytic anemia
Endocrinologic
Renal
Skeletal
Cardiac
Cholelithiasis

recovery may appear to occur, in which case, the patient is thought to have had a viral hepatitis. Many months or years may elapse before the patient again has evidence of liver disease. Patients, especially younger ones, may come to medical attention because of fulminant hepatic failure with jaundice, hypoalbuminemia, coagulation defects, ascites, hepatic encephalopathy, and, frequently, hemolysis.[36–38] Large amounts of copper are released by the liver, resulting in high serum copper levels associated with hemolytic anemia. Without a family history of hepatic or neurologic conditions, or Wilson disease itself, it is difficult to distinguish fulminant Wilson disease from fulminant hepatic failure of another cause. Patients with fulminant Wilson disease have a particularly poor outcome even if the diagnosis of Wilson disease is made. Interestingly, in one series, 16 of 21 patients with fulminant Wilson disease were female.[39] This observation of a female predominance for fulminant Wilson disease has been confirmed by others and is hypothesized to be due to the influence of sex hormones. Children and adolescents may also present with clinical features of chronic liver failure and cirrhosis with ascites, edema, hypoalbuminemia, and evidence of portal hypertension. These patients may be jaundiced. In contrast to other causes of cirrhosis, few reported cases exist of hepatocellular carcinoma evolving from Wilson disease.[40] Young patients also may come to medical attention with a clinical and histologic picture similar to that of chronic hepatitis. The presenting symptoms and signs range from elevations in liver-derived serum enzymes to symptoms resulting from complications of portal hypertension or liver failure. In such patients, neurologic dysfunction and Kayser-Fleischer (KF) rings may not be found[31,41] and the serum ceruloplasmin level may be normal,[31] adding to the difficulty in diagnosis.

CNS Involvement

When Wilson initially described hepatolenticular degeneration, he thought that the CNS damage was limited to the basal ganglia, especially the putamen.[1] CNS involvement is now known to be more extensive, and a wide spectrum of neurologic findings ensues. Neurologic manifestations have been reported to occur as early as 6 years of age,[3] but more typically they begin in the second to third decade of life and are usually associated with the presence of KF rings. Reports of Wilson disease without KF rings illustrate that this diagnosis should be considered in patients who exhibit the typical neurologic manifestations without other signs of disease.[42] The onset of neurologic symptoms is gradual, and severity progresses without treatment. CNS damage in Wilson disease is limited almost exclusively to the motor system, with the sensory system being spared. Common first neurologic symptoms are tremor, incoordination, dystonia, and difficulty with fine motor tasks such as dressing and writing. Later, other manifestations such as mask-like facies, drooling, dysarthria, rigidity, and gait disturbances may become apparent. The patient often becomes highly frustrated because the intellect is unchanged. Older patients are frequently misdiagnosed as having a pure psychiatric disorder or neurologic disease, such as multiple sclerosis or a disorder of the basal ganglia.[43] In pediatric patients, the first neuropsychiatric symptom of Wilson disease may be deteriorating school performance.

Computed tomographic (CT) scan of the head may be helpful in making the diagnosis (Table 55.10-3). CT findings are more likely to be abnormal in patients with neurologic involvement but also may be abnormal in patients who are asymptomatic or have only hepatic involvement. In one study of 60 patients with Wilson disease, 73% had ventricular dilatation, 63% had cortical atrophy, 55% had brainstem atrophy, 45% had basal ganglia hypodensity, and 10% had posterior fossa atrophy; in 18%, the findings were normal.[44] Other studies have shown changes in the internal capsule, thalamus, and white matter.[44,45] The CT

TABLE 55.10-2 PRESENTING SYMPTOMS OF WILSON DISEASE*

SYSTEM INVOLVED	STERNLIEB AND SCHEINBERG[3] (%)	WALSHE[35] (%)
Hepatic	42	62
Central nervous system		34
Neurologic	34	
Psychiatric	10	
Hematologic and endocrinologic	12	
Renal	1	

*More than one organ was involved in 25% of patients.

TABLE 55.10-3 FINDINGS FROM COMPUTED TOMOGRAPHY OF THE HEAD IN WILSON DISEASE

OBSERVATION	FREQUENCY (%)
Ventricular dilatation	73
Cortical atrophy	63
Brainstem atrophy	55
Basal ganglia hypodensity	45
Posterior fossa atrophy	10
Normal	18

Adapted from Williams FIB and Walshe JM.[44]

abnormalities do not represent actual copper deposition because this would be expected to show as hyperdense areas. Rather, the changes are likely to result from the damage caused by copper deposition. The hypodense areas, along with areas of generalized atrophy, are fairly characteristic of Wilson disease.[44] The severity of the CT abnormalities does not correlate with clinical symptoms[46] and is also of little prognostic value because patients with extensive involvement often do well in response to therapy.[44] Magnetic resonance imaging (MRI) supports the abnormalities seen on CT scan and may be more sensitive in identifying abnormal regions.[43–47] The hypodense areas seen on CT scan appear as regions of increased intensity on MRI, suggesting that edema may produce the abnormality seen on CT scan.[48] In one report, high signal intensity was seen in the basal ganglia, with involvement in nearly all areas of gray and white matter with generalized atrophy. MRI has also identified abnormalities in the lentiform and dentate nuclei, substantia nigra, and vermis cerebelli.[49] Both positron emission tomography and MRI spectroscopy are beginning to be used to correlate the clinical manifestations with the gross findings in Wilson disease.[50]

Psychiatric Manifestations

Psychiatric manifestations may be dramatic in patients with Wilson disease. These include poor school performance, anxiety, depression, compulsive behavior, phobias, aggressive outbursts, neurosis, and even psychosis.[51,52] Affected patients frequently are labeled with erroneous psychiatric diagnoses before the correct diagnosis of Wilson disease is made. It is sometimes difficult to distinguish the behavioral symptoms resulting from excessive copper deposition from those secondary to the individual's reaction to having a chronic disease. This is particularly an issue in adolescent patients, and psychological intervention is often helpful.

Ophthalmologic Manifestations

Ophthalmologic manifestations of Wilson disease have received considerable attention because their presence may help lead to the diagnosis before any laboratory result is available. Kayser first described the "ring" in a patient thought to have multiple sclerosis,[53] and several years later, Fleischer reported an association of the ring with Wilson disease.[54] The KF ring may have a variable color, depending in part on the color of the iris. It has been described as a golden brown, brownish green, greenish yellow, bronze, or tannish green discoloration in the zone of Descemet membrane in the limbic region of the cornea (Figure 55.10-2). It can sometimes be seen with the naked eye, but a slit-lamp examination is mandatory. The rings consist of copper granules; however, they represent only a small fraction of the total corneal copper content. The bulk of copper deposition is in the stromal layer, but no color change is seen in any of the corneal layers except in Descemet membrane. Copper is initially taken up by the aqueous humor and diffuses into the cornea. Movement of water-soluble substances such as copper is a function of the evaporation of tears from the surface of the cornea. Evaporation is less at the superior poles and somewhat less at the inferior poles. Because the solvent flow is less in these areas, copper deposition is first seen there. Therefore, the rings first form superiorly and then inferiorly and finally extend laterally to complete the ring. Likewise, with treatment, the rings fade in the reverse order in which they appear.[55]

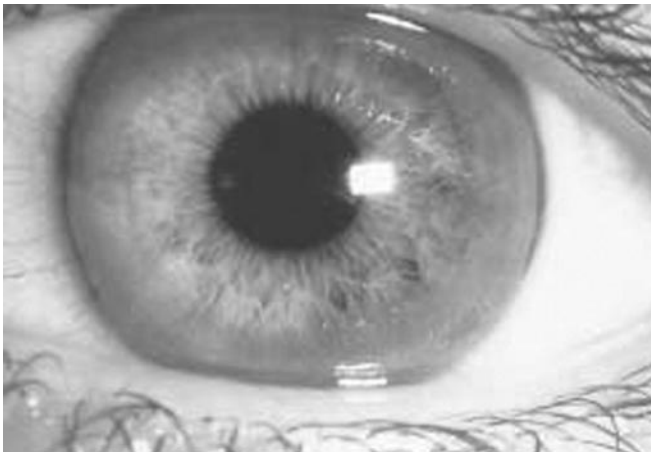

FIGURE 55.10-2 Appearance of the Kayser-Fleischer ring in a patient with Wilson disease. Note the deposition surrounding the limbus in this eye. With a darker iris, the ring may be observed only when using the slit lamp.

KF rings usually are present in patients with neurologic findings but frequently are present in those with only hepatic manifestations, as well as in some asymptomatic patients.[56] KF rings are not specific for Wilson disease. They have been seen in patients with chronic active hepatitis, primary biliary cirrhosis, and cryptogenic cirrhosis and in children with chronic intrahepatic cholestasis (Table 55.10-4).[55–61]

Sunflower cataracts are seen less frequently than KF rings and, when present, are accompanied by KF rings.[55] They can be seen with an ophthalmoscope as a greenish gray or golden disk in the anterior capsule of the lens, with spokes radiating toward the lens periphery.[61,62] Most of these cataracts resolve with therapy and will not affect vision.[55]

Cardiac Manifestations

Although Wilson disease is a multisystem disorder, few studies have evaluated the cardiac manifestations. One study of 53 patients showed electrocardiographic (ECG) abnormalities in 34%, including left ventricular hypertrophy, ST depression, T-wave inversion, premature ventricular contractions, sinoatrial block, and atrial fibrillation.[63] Thirteen percent of patients had arrhythmias, whereas 40 control patients of similar age all had normal ECG results. Of the patients with Wilson disease, 19% had mild asymp-

TABLE 55.10-4 CONDITIONS ASSOCIATED WITH KAYSER-FLEISCHER RINGS

Wilson disease
Chronic active hepatitis
Primary biliary cirrhosis
Cryptogenic cirrhosis
Intrahepatic cholestasis with cirrhosis

Adapted from references 38 to 45.

tomatic orthostatic hypotension. Response to a Valsalva maneuver (as a test for normal autonomic functioning) was abnormal in 6 of 18 patients with Wilson disease who were able to perform the maneuver.[63] Autopsy reports have shown cardiac hypertrophy, fibrosis, small vessel sclerosis, and myocardial inflammatory cell infiltrates, although gross abnormalities are not impressive. Pathologic findings did not correlate with myocardial copper content, which may be low or high. Several cases of sudden death are reported, presumably secondary to cardiac arrhythmia that may be related to Wilson disease.[64]

Renal Manifestations

Renal involvement is a widely recognized complication of Wilson disease. It is characterized by proximal tubular dysfunction as indicated by aminoaciduria, glycosuria, increased excretion of uric acid and calcium, and a decrease in filtration rate and effective renal blood flow.[65] There is an acidification defect that is likely a distal tubular dysfunction, in which patients are unable to acidify urine to a pH of less than 5.2 despite an acid load. Renal acidification defects can cause renal potassium wasting and recurrent hypokalemia.[66] Usually, however, patients are able to maintain normal or nearly normal plasma pH levels despite this renal tubular defect.[67–69] Renal stones are common and may predate the diagnosis of the disease. Hypercalciuria and inadequate acidification of urine may contribute to stone formation.[67] The histopathologic changes in renal biopsy specimens are not impressive. Membranoproliferative glomerulonephritis has been reported in association with Wilson disease but is more likely related to the presence of liver disease than Wilson disease itself.[70] Scheinberg and Sternlieb reported elevated copper concentrations in the kidney at autopsy in eight patients with untreated Wilson disease.[3] Rubeanic acid staining has demonstrated granules, presumed to be copper, within the tubular epithelium.[71] Renal function has been shown to improve with penicillamine therapy.[72]

Skeletal Manifestations

A variety of skeletal changes are observed in patients with Wilson disease. These include osteoporosis, rickets, osteomalacia, spontaneous fractures, osteochondritis dessicans, and osteoarthritis.[73] Bone demineralization is the most common abnormality seen. Renal defects causing hypercalciuria and hyperphosphaturia, with resultant hypocalcemia and hypophosphatemia, are the main cause of demineralization.[74,75] Other factors include dystonic contractures and immobilization. Chronic liver disease itself may cause skeletal abnormalities.[76] High levels of copper have been found in cartilage in some patients who underwent biopsy.[77] Pediatric patients rarely have significant skeletal changes on radiograph. Acute rhabdomyolysis has been reported as a presenting feature of Wilson disease.[78]

Other Manifestations

Hemolysis is a recognized complication of Wilson disease. It may precede other clinical manifestations of the disease and be short-lived or may progress to anemia and be the first recognized abnormality of the disease.[79] Hemolysis may occur secondary to an oxidative injury to red blood cell membranes from excess copper,[80] but the exact mechanism remains unknown.

As a consequence of hemolysis and cirrhosis, cholelithiasis may complicate Wilson disease. The stones are a mixed type, containing both cholesterol and pigment. Patients with Wilson disease should be examined for gallstones; likewise, in a child with gallstones, Wilson disease should be considered in the differential diagnosis.[81] Spontaneous splenic rupture has been reported as a presenting feature of Wilson disease.[82]

LIVER PATHOLOGY

The liver is the major organ for storage of copper. From more than 260 patients with Wilson disease analyzed by Scheinberg and Sternlieb, none of the liver specimens were normal; even a specimen from a 3.5-year-old boy was abnormal.[3] Cirrhosis has been seen in patients as young as 5 years of age.[83] Characteristic histologic findings are present but not pathognomonic. Fat deposition is one of the earliest changes seen in the liver biopsy specimen. Fine lipid droplets composed of triglycerides are dispersed throughout the cytoplasm.[3,84] As the disease progresses, these lipid droplets increase in size until hepatic steatosis is manifested. In early stages, electron microscopic study shows the mitochondria to be of varying shapes and sizes. The matrix density is increased, with vacuolated and crystalline inclusions. Inner and outer mitochondrial membranes, which are normally opposed, become separated, and the intercristal spaces expand. Peroxisomes, which are involved in cellular lipid metabolism, may become enlarged with a granular, flocculent matrix of varying density rather than with the homogeneous matrix seen in normal peroxisomes.[85] With progression of the hepatic lesion, there is collagen deposition and eventually development of fibrosis. Histologic features that are indistinguishable from those of autoimmune chronic hepatitis may develop, as well as hepatic necrosis (Figure 55.10-3).

If the diagnosis of Wilson disease is not made, and the patient survives, cirrhosis develops. Once cirrhosis is established, the fatty changes disappear, as do those changes seen in the mitochondria and peroxisomes. The electron microscopic findings are then relatively normal, except for excessive amorphous or globular copper-containing lipofuscin granules and lipid-containing lysosomes.[86]

A high copper content is found normally in the fetal and neonatal liver.[87] The cause is not known, but it is postulated that immaturity of bile excretion plays a role in this increased copper level.[88] Some of the copper binds to a sulfhydryl-rich protein, known as copper-associated or copper-binding protein, which is bound in hepatic lysosomes.[89] This lysosomal copper may be stained by orcein.[89] Between the third and sixth month postnatally, hepatic copper levels fall to within the normal adult range, and these orcein-positive granules are no longer seen in the normal liver. In children older than 6 months of age, orcein-positive granules indicative of elevated hepatic lysosomal copper are found only in abnormal conditions, including Wilson dis-

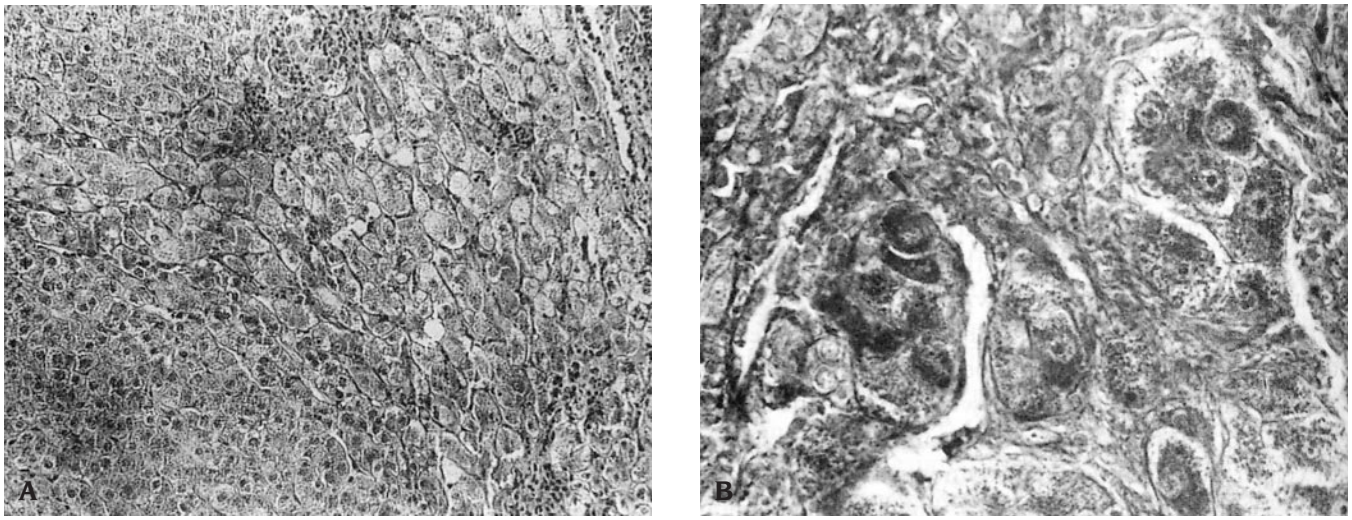

FIGURE 55.10-3 *A*, Wedge biopsy specimen from a child with Wilson disease showing a broad band of fibrous tissue at the right margin. An intense portal inflammatory response can be seen with lymphocytes spilling across the limiting plate into the lobule. Considerable hepatocellular necrosis exists with marked variations in cell size and some fat and pigment deposition. *B*, High-power view showing irregularities in cell size, hepatocellular necrosis, pigment deposition, and bile ductular proliferation. The limiting plate has been distorted, as shown by interdigitation of connective tissue and hepatocellular elements. Inflammatory cells are seen crossing the limiting plate into the lobule. Reproduced with permission from Grand RJ and Vawter GF.[122]

ease, biliary atresia, paucity of intrahepatic ducts, primary biliary cirrhosis, sclerosing or chronic cholangitis, cirrhosis, and primary hepatic tumors (Table 55.10-5). Orcein-positive granules are not seen in acute liver disease, except hepatitis B. In contrast to Wilson disease, the orcein-positive granules in other disease states are found mainly at the periphery of the liver lobules.[90] In Wilson disease, these granules are widespread in some lobules but may be completely absent in others.[90] Not all of the livers from patients with Wilson disease contain stainable copper-associated protein. In the early stages of the disease, when the liver copper concentration is highest, the copper is distributed diffusely in the cytoplasm and is absent from the lysosomes[91]; it is therefore not stainable. In the later stages of the disease, copper is redistributed to the lysosomes, and then copper may be stained by rubeanic acid and copper-associated protein by orcein.[86] However, histochemical techniques cannot confirm a diagnosis of Wilson disease, and they cannot be used to rule it out. Confirmation depends on quantitative measurement of hepatic copper content. Other disorders associated with elevated hepatic copper concentrations are listed in Table 55.10-5.

DIAGNOSIS

The diagnosis of Wilson disease may be made readily when the classic triad of hepatic disease, neurologic involvement, and KF rings is present. However, in the absence of this triad, the diagnosis begins with a high index of suspicion, especially in children. No single test can confirm the diagnosis with 100% accuracy (Table 55.10-6). Rather, the clinical and family history, physical examination, and certain key laboratory investigations collectively may establish the diagnosis. In ambiguous cases, genetic analysis may be required.

The first diagnostic test should be measurement of serum ceruloplasmin. Most children and adolescents with Wilson disease have decreased serum ceruloplasmin values, and at least 75% of those presenting with hepatic manifestations have low values.[92–94] Decreased values also may be seen in conditions associated with decreased hepatic synthetic function, such as malnutrition and severe hepatic insufficiency.[3,95] Ceruloplasmin also may be low in protein-losing enteropathy, nephrotic syndrome, and hereditary hypoceruloplasminemia.[92] Infants younger than 6 months of age normally have low serum ceruloplasmin levels.[93] Because ceruloplasmin is an acute-phase reactant, its value may be low-normal to normal in patients with Wilson disease during periods of active hepatic inflammation.[96] Its synthesis is stimulated by estrogens; hence, pregnancy (and estrogen therapy) is associated with near-normal to normal ceruloplasmin levels (Table 55.10-7).[96] Difficulty in establishing a diagnosis also occurs in 10% of heterozygotes who have low serum ceruloplasmin levels but no manifestations of Wilson disease.[96] Therefore, serum ceruloplasmin level should not be used as the sole determinant in diagnosing the disease.

TABLE 55.10-5 CONDITIONS ASSOCIATED WITH ELEVATED HEPATIC COPPER CONCENTRATION

Normal infant younger than 6 mo of age
Cholestasis syndromes
Biliary atresia
Paucity of intrahepatic ducts
Sclerosing cholangitis
Primary biliary cirrhosis
Indian childhood cirrhosis
Primary hepatic tumors
Wilson disease

TABLE 55.10-6 DIAGNOSIS OF WILSON DISEASE

Clinical information
Family history
Kayser-Fleischer rings
Laboratory tests
Hematologic
Liver function
Copper status
Serum copper: < 20 µg/dL
Urinary copper: > 100 µg/24 h
Hepatic copper: > 250 µg/g dry weight of liver
Low serum ceruloplasmin
Radiocopper excretion abnormal
Genetic analysis

Serum or plasma copper levels should be obtained at the time of diagnosis, although it should be noted that their concentration cannot be used as a diagnostic test for Wilson disease. In classic cases of Wilson disease, the serum or plasma copper levels are decreased, in conjunction with the low ceruloplasmin values. However, in certain stages of the disease, copper levels are elevated as a consequence of the flooding of the plasma with nonceruloplasmin-bound copper released from the liver. As will be discussed below, serum copper levels are also necessary for the assessment of the patient's adherence to therapy. Total serum copper levels are readily obtained in most laboratories.

In contrast to plasma copper determination, urinary copper excretion is a useful diagnostic test. It is normally less than 40 µg/24 h. The conventional level taken as diagnostic for Wilson disease is greater than 100 µg/24 h in symptomatic patients.[97] However, recent studies have shown that basal determination may be less than 100 µg/24 h in up to 25% of patients diagnosed with Wilson disease,[94,98,99] indicating that 24-hour urinary excretion of copper greater than 40 µg/24 h may be a better threshold for diagnosis of Wilson disease and requires further investigation. Abnormal urinary copper excretion is not specific for Wilson disease because it may be elevated in patients with primary biliary cirrhosis,[100] chronic active hepatitis,[31] fulminant hepatitis, and cholestasis (Table 55.10-8).[35] The urine collections must be obtained in copper-free containers.

TABLE 55.10-7 CONDITIONS ASSOCIATED WITH ALTERED CERULOPLASMIN CONCENTRATIONS

DECREASED
Malnutrition
Protein-losing enteropathy
Nephrotic syndrome
Hepatic insufficiency
Hereditary hypoceruloplasminemia
Neonates
Menkes syndrome
Wilson disease
Heterozygosity for Wilson disease
ELEVATED
Estrogen therapy
Infection/inflammation
Pregnancy

The use of the penicillamine-stimulated copper excretion test remains controversial. Many studies have found no difference in penicillamine-induced copper excretion between Wilson disease and chronic hepatitis.[101] However, one study exclusively in children suggested that this test might be valuable. After collecting one 24-hour urine sample in a copper-free container, patients received 500 mg penicillamine at the start of, and 12 hours into, the second 24-hour urine collection. Urinary copper excretion was measured in both specimens. Although there was overlap between copper excretion in patients with Wilson disease and those with chronic hepatitis, a penicillamine-induced cupruresis (> 25 µmol/24 h) was shown to be valuable in the diagnosis of Wilson disease.[102] There is some question as to the capacity of such brief exposure to penicillamine to sensitize the patient and render allergic reactions later more likely. This remains to be resolved. Whether this test is useful for diagnosis, 24-hour urinary copper excretion is a good measurement to follow during treatment of patients with Wilson disease because it allows quantification of the success of chelation therapy.

The clinician should look for KF rings by using slit-lamp examination, but the rings are not pathognomonic for Wilson disease (see Table 55.10-4).[57,58] However, in Wilson disease, they are present in approximately 50% of patients with a hepatic presentation and 95% of those with neurologic or psychiatric symptoms. They may also be helpful when considering the possibility of nonadherence to treatment, as in a patient whose KF rings have faded and then returned.

A liver biopsy should be performed whenever possible because the quantification of hepatic copper concentration will, in most cases, establish the diagnosis in the absence of known obstructive liver disease. Microscopic and ultrastructural analyses are valuable, whereas copper staining is not. Measurements of quantitative hepatic copper concentrations are mandatory. Normal hepatic copper concentration is less than 50 µg/g of dry weight of liver.[103] Patients with Wilson disease generally have values greater than 250 µg/g of dry liver, and values may be greater than 1,000 µg/g of dry liver. Presymptomatic homozygous patients, especially young children, may not always have levels greater than 250 µg/g. Heterozygotes may have values up to 150 to 200 µg/g. A normal hepatic copper concentration rules out the diagnosis of Wilson disease, whereas an elevated value confirms the diagnosis in the proper clinical setting. Elevated values may be seen in

TABLE 55.10-8 CONDITIONS ASSOCIATED WITH ELEVATED URINARY COPPER EXCRETION

Wilson disease
Primary biliary cirrhosis
Chronic active hepatitis
Fulminant hepatic failure
Cholestasis syndromes
Biliary atresia
Paucity of intrahepatic ducts
Sclerosing cholangitis

other conditions (see Table 55.10-5).[104–106] These usually can be distinguished by other techniques.

If the diagnosis is still uncertain, the rate of incorporation of radiocopper into ceruloplasmin may be determined or genetic testing obtained. The radiocopper test is performed after a fast of 8 hours; a dose of 2.0 mg of radiocopper is administered orally. The concentration of radiocopper is measured in the serum at intervals of 1, 2, 4, 24, and 48 hours later. The radiocopper rises in the 1-hour and 2-hour samples and then falls. In normal individuals, the serum concentration rises again to a higher level in the 24-hour or 48-hour sample, representing incorporation into ceruloplasmin. However, in patients with Wilson disease, even those with normal ceruloplasmin levels, the secondary rise is not achieved. One needs to be cautious in interpreting these results because considerable overlap occurs with heterozygotes, especially those with low serum ceruloplasmin concentrations.[107] If the index patient's serum ceruloplasmin is relatively high, other family members also may have near-normal values yet still have Wilson disease.

Currently, genetic analysis is mostly limited to identification of first-degree relatives of newly diagnosed patients. The pedigree analysis is performed using haplotypes based on polymorphisms surrounding the Wilson disease gene and requires the identification of a patient within the family (the proband). De novo diagnosis by molecular studies remains difficult at present owing mostly to the large numbers of disease-specific mutations of *ATP7B*; it is done only in the context of research protocols.[108]

Asymptomatic relatives, especially siblings of patients with Wilson disease, should be screened (Table 55.10-9). They should have a careful physical examination, ophthalmologic slit-lamp examination, measurement of serum ceruloplasmin and copper concentration, hepatic transaminase levels, and 24-hour urinary copper excretion. If all of these screening tests give absolutely normal results, the diagnosis is most likely excluded, although it may be prudent to repeat them at least once several months later. However, if even one test result is abnormal, a liver biopsy should be performed and samples sent for quantitative copper determination and histologic examination. In the young pediatric age group, if the 24-hour urinary copper result is normal, it should be repeated when the child is older, at which time enough copper would have accumulated to be reflected in an elevated urinary value. Subtle evidence of Wilson disease may be reflected in the presence of hemolysis, elevated reticulocyte values, depressed haptoglobin levels, and hypercalciuria. In equivocal cases, genetic analysis can be used to establish a diagnosis. In general, any liver disease in pediatric patients should be considered to be Wilson disease until proven otherwise.

NATURAL HISTORY

Deiss and others have devised a valuable staging system that explains many of the confusing findings in Wilson disease.[109] Revisions of the scheme also are available.[3] In stage I, a progressive accumulation of copper occurs in the cytosol of the hepatocytes. The process continues until all hepatic binding sites for copper are saturated. This stage is asymptomatic and usually occurs before age 5 years. In stage II, copper in the hepatocyte is redistributed from the cytosol to the lysosomes, and, at the same time, copper is released from the liver. If this release occurs gradually, the patient remains asymptomatic. If the redistribution is rapid, hepatic necrosis may occur, and the patient may become symptomatic from liver disease. In addition, rapid release of copper into the blood may result in hemolytic anemia. This stage is often accompanied by fulminant hepatic failure, leading to liver transplant. However, if patients pass through stage II without clinical illness, they remain asymptomatic. In stage III, copper continues to be stored in the lysosomes, and varying degrees of fibrosis or cirrhosis develop. In this stage, accumulation of copper also occurs in other tissues, such as brain, cornea, kidney, or skeleton. Patients may remain asymptomatic for years if the brain deposition of copper progresses slowly. Stage IV is characterized by symptomatic CNS disease. If copper accumulation occurs rapidly, then liver disease, CNS disease, or both become apparent in a short time. Stage V occurs when treatment is begun before the patient dies from hepatic failure or irreversible brain damage. In stage V, cupruresis leads to reductions in copper accumulation, repair of tissue injury, and improvement in the clinical status of the patient.[110]

Few series of patients with Wilson disease have partially characterized the long-term prognosis of patients with this disease.[111–113] These series have suggested that the long-term outcome of these patients is mainly dependent on adherence to lifelong treatment with excellent prognosis in those patients who are compliant with effective medical therapy, even if cirrhosis is present at the time of diagnosis. However, it is important to note that none of these studies have compared the long-term survival of patients with Wilson disease with that of an age- and gender-matched population. Furthermore, there is no study assessing the quality of life of patients on long-term treatment for this condition.

TREATMENT

At the time of the discovery of Wilson disease, and for many years subsequently, early diagnosis had little clinical

TABLE 55.10-9 SCREENING OF ASYMPTOMATIC RELATIVES OF PATIENTS WITH WILSON DISEASE

MANDATORY
History and physical examination
Ophthalmologic slit-lamp examination
Serum ceruloplasmin and copper concentrations
Hepatic transaminase levels
24-Hour urinary copper excretion
ADDITIONAL
Blood smear for hemolysis
Reticulocyte count and haptoglobin
Urinary calcium level
Genetic analysis

If any of the above is abnormal, liver biopsy becomes mandatory with examination of histology and measurement of quantitative liver copper content.

significance. In 1951, Denny-Brown and Porter and Cumings[115] introduced dimercaprol (BAL) as an effective treatment for Wilson disease.[114,115] However, the daily painful intramuscular injections made BAL impractical. In 1956, Walshe drastically changed the outcome of patients with Wilson disease by showing that D-penicillamine is an effective treatment.[95] Wilson disease is fatal if untreated, but successful outcome is achieved with effective pharmacologic therapy. The US Food and Drug Administration approved D-penicillamine as effective and safe for Wilson disease in 1963. In 1968, in a landmark paper, Sternlieb and Scheinberg showed its effectiveness in presymptomatic homozygous patients.[116]

Penicillamine

Penicillamine remains the chelating agent with which there has been the greatest experience. Initial therapy with trientine is emerging as an acceptable alternative. Penicillamine is a sulfur-containing amino acid that is a metabolite of penicillin; it chelates copper and then is excreted in the urine. When initiating therapy, a small dose should be used; the dose should then be gradually increased and administered orally on an empty stomach in four divided doses, 30 to 45 minutes before meals and at bedtime, or 2 or more hours after eating. In children and adolescents, the dose is 20 mg/kg of body weight; the customary adult dose is 1.0 g/d.[3] Penicillamine is better absorbed in the absence of food.[117] Patients should also receive 25 mg of pyridoxine three times a week because of the potential antipyridoxine effects of penicillamine.[118] As a consequence of treatment, urinary copper excretion may be more than 5,000 µg in a 24-hour period. However, this decreases with time; after months to years of therapy, it can be as low as 600 µg in a 24-hour period.[109] Usually, there is a dramatic improvement in symptoms within weeks of beginning therapy. If no improvement occurs, the daily dose of penicillamine may be raised to 1.5 to 2 g/d or its equivalent according to body weight, although one must consider faulty patient compliance as a possible cause of poor response. The higher dose of penicillamine is associated with an increased risk of side effects. Most patients become clinically asymptomatic, or nearly so, within months of beginning treatment, but some may not show significant functional improvement before 1 year. One concern when deciding on the correct drug for treatment is that neurologic symptoms may worsen with the initiation of penicillamine treatment. It is postulated that with treatment, large amounts of hepatic copper are mobilized and then deposited in the brain, worsening neurologic symptoms. There is also concern that treatment with penicillamine may initiate neurologic symptoms in previously asymptomatic patients with Wilson disease.[119] Such patients may not return to pretreatment baseline.[120] Trientine appears to be associated with a lower risk of this phenomenon and may be preferred as first-line therapy for neurologic Wilson disease. Perhaps gradual introduction of penicillamine prevents this occurrence. In general, neurologic manifestations improve, although dysarthria associated with mask-like facies may not disappear.[121] KF rings may disappear or fade partially. As a consequence of therapy, liver function test results improve and hepatic concentration of copper decreases.[122]

Studies have shown improvement in liver biopsy findings with decreased portal fibrosis, inflammation, and necrosis.[110,120,123] There is a report of a 10-year-old child who had advanced liver cirrhosis whose repeated liver biopsy showed practically normal results 27 months after starting penicillamine therapy. The biopsy samples were obtained by laparotomy, with multiple samples taken to decrease sampling error.[123] This report is unusual in showing virtually complete reversal of liver disease.

The patient's adherence to therapy is best assessed using sequential determinations of 24-hour urinary copper excretion. In addition, Scheinberg and Sternlieb recommend the assessment of free serum copper.[3] This is accomplished by spot determinations of total serum copper (µg/dL) and ceruloplasmin (mg/dL) concentrations. The unit designations are then ignored. A factor of 3 is multiplied by the ceruloplasmin value, and that value is subtracted from the total serum copper level. A resulting figure not greater than 20 indicates compliance.[124]

Undesirable side effects of penicillamine therapy may occur within the first 3 weeks of treatment in 20% of patients. These include fever, skin rash, lymphadenopathy, granulocytopenia, and thrombocytopenia.[125] Other reactions that may occur later include nephrotoxicity with proteinuria or even nephrotic syndrome,[126] lupus-like syndrome,[127] Goodpasture syndrome (which was fatal in three patients),[128] elastosis perforans serpiginosa,[129] and pemphigoid lesions of the mouth, vagina, and skin.[130,131] A penicillamine dermatopathy may occur in patients receiving more than 2 g of penicillamine for several months. Penicillamine interferes with crosslinking of collagen and elastin, which leads to a weakening of the subcutaneous tissue so that bleeding into the subcutaneous tissue may occur with even slight trauma.[129] If a reaction occurs, penicillamine should be stopped. The clinician may then pretreat with 20 to 30 mg of prednisone (0.5 mg/kg of body weight) daily for 2 to 3 days before reinstituting therapy. Penicillamine should be introduced in a much lower dose and gradually increased. Once penicillamine is tolerated, the prednisone may be withdrawn.[129] If the reaction was severe, the clinician may not wish to attempt this but to institute other decuprinizing agents (Table 55.10-10). The success of treatment with oral D-penicillamine may be limited by the presence of renal failure. Adding penicillamine to peritoneal dialysis solution is not beneficial.[132,133] Postdilution hemofiltration and continuous arteriovenous hemofiltration with oral penicillamine have been effective.[134] Although there are incidental reports of connective tissue defects and cleft palate in the offspring of pregnant women taking penicillamine[135,136] and other therapeutic agents available (see below), Scheinberg and Sternlieb point out that cessation of treatment is dangerous, and such complications are extremely infrequent.[3] Population-based, case-controlled studies of birth defects in the children of women taking penicillamine for Wilson disease and other disorders, compared with in the random population, are not available.

Death has occurred as early as 8 months after discontinuation of D-penicillamine in a patient who had become asymptomatic with treatment and then was noncompliant. There are several reports of death within 1 year of stopping therapy in noncompliant patients. This raises the question of the exact mechanisms of D-penicillamine action. A patient who has been decuprinized with therapy should not die after just 8 months of copper reaccumulation (because initial copper accumulation takes more than 5 years in stage I). Sternlieb and colleagues suggest that penicillamine may form a nontoxic complex with copper.[130] When penicillamine treatment is suddenly stopped, there may be a sudden dissociation of this complex, and massive amounts of copper may be released, accounting for the rapid hepatic decompensation that occurs in suddenly noncompliant patients. The first sign of relapse after stopping penicillamine is a silent rise in serum transaminase levels.[130] The rise may be low compared with the amount of ongoing hepatic injury. Bilirubin becomes elevated later, and there is a decrease in serum albumin concentration, an elevation in free serum copper levels,[3] and an elevation in 24-hour urinary excretion of copper. The urinary copper excretion may be greater than 2,000 µg in a patient who has been noncompliant and then begins taking penicillamine again before urine collection.[130] It is rarely greater than 1,000 µg per 24 hours in patients taking penicillamine regularly.

TRIENTINE

In 1969, Walshe introduced triethylene tetramine dihydrochloride (trientine) as an altemative chelating agent to penicillamine for a patient who had developed an immune complex nephritis after 6 years of penicillamine treatment.[137] Cupruresis, as great as or greater than that achieved with penicillamine, may be achieved with trientine.[138] Most patients have complete reversal of the side effects seen with penicillamine, although at least one patient with elastosis perforans serpiginosa did not improve with trientine.[139] Two other patients with penicillamine-induced lupus did not improve on discontinuation of penicillamine and introduction of trientine. Iron deficiency anemia may develop in patients treated with trientine, especially women. This resolves with daily iron supplements.[139] Trientine is safe during pregnancy.[140] Trientine is given orally in divided doses of 1 to 1.5 g daily, 1 hour before or 2 hours after meals. In children younger than 10 years of age, 0.5 g (approximately 20 mg/kg) daily is recommended.[139] Renal complications from trientine can occur.

ZINC

Zinc, a known antagonist of copper absorption, has been introduced as a possible alternative maintenance treatment for Wilson disease in patients previously successfully treated with penicillamine. In 1946, Smith and Larson reported on the antagonistic effects of zinc on copper balance in rats.[141] A decrease in liver copper content secondary to zinc supplementation in sheep was reported in 1954.[142] Patients with sickle cell anemia treated with zinc had been observed to develop copper deficiency.[143] In 1961, the role of zinc in producing negative copper balance in Wilson disease was first described.[144] Patients have subsequently demonstrated clinical improvement when treated with zinc alone.[145,146]

TABLE 55.10-10 TREATMENT OF WILSON DISEASE

Dietary restriction of copper
D-Penicillamine (with pyridoxine)
Triethylene tetramine (trientine)
Ammonium tetrathiomolybdate
Zinc
Liver transplant

Copper is absorbed mainly in the proximal small intestine.[147] Its absorption is increased in the presence of chelating agents, a high-protein diet, anions, and L-amino acids. Fiber, bile, ascorbic acid, and zinc inhibit its absorption. Once copper crosses the intestinal brush border, it binds to metallothioneine in the cytosol of the enterocytes. Zinc, copper, cadmium, glucagon, glucocorticoids, and bacterial infections induce the synthesis of intestinal metallothioneine.[148] Metallothioneine has a higher affinity for copper than for zinc.[149] The copper that is metallothioneine bound cannot pass the serosa but is sloughed with the intestinal cells into the lumen and then excreted in the stool. Therefore, copper levels in stool are increased in patients treated with zinc.[150]

Experience is growing in the use of zinc in Wilson disease, and thus far, no treatment failures have occurred.[150,151] The adult recommended dose is 50 mg of elemental zinc taken three times a day, spacing each dose from food or liquids by at least 1 hour. Children and pregnant women should receive 25 mg per dose three times a day.[150] Treatment can be monitored by measuring 24-hour urinary copper. Because decoppering occurs in the gastrointestinal tract, urinary copper reflects body copper burden. A value greater than 125 µg per 24 hours suggests patient noncompliance. Twenty-four-hour urinary zinc levels average 3.5 mg and should be at least 2 mg when a therapeutic dose is taken.[150,152]

Because it takes 1 to 2 weeks to induce metallothioneine levels in the intestine, in addition to its slower rate of decoppering, zinc is not practical for initial treatment in symptomatic patients. As maintenance therapy, it has less toxicity than penicillamine, and there is more experience with zinc than with trientine.

Although zinc seems to be a basically safe medication, long-term effects are not known. Lymphocyte response to phytohemagglutinin, neutrophil chemotaxis, and bacterial phagocytosis were reduced in normal male subjects taking 150 mg of zinc twice a day for 6 weeks.[153] This observation has been challenged in a subsequent study because of the possibility that inducing copper deficiency in normal subjects may have been responsible for the decrease in lymphocyte function. Examining mitogenic response and levels of natural killer cell activity in patients treated with zinc for 15 years showed no evidence of decrease in lymphocyte function.[152] Zinc has been reported to reduce high-density lipoprotein cholesterol in normal male subjects.[154] Finally,

an elevation in serum amylase and lipase has been reported during zinc therapy; however, it is believed to be caused by higher levels of these proteins induced by zinc rather than by pancreatic damage.[150]

Tetrathiomolybdate

A new potential therapeutic option is ammonium tetrathiomolybdate (TM). It has two anticopper mechanisms. It complexes ingested copper, thereby preventing absorption. Second, TM forms complexes with copper and albumin in blood, making the copper unavailable for cellular uptake. Most recently, it has been found that TM has antifibrotic and anti-inflammatory effects mainly through inhibition of profibrotic and proinflammatory cytokines.[155] In a trial of 33 patients with neurologic manifestations of Wilson disease treated with TM, only 1 patient had deterioration of neurologic function, with good to excellent recovery in a 1- to 6-year follow-up period.[156] This appears to be a promising alternative to penicillamine because it is associated with a low prevalence of complications. However, this medication remains experimental in the United States and is not commercially available.

Antioxidants

Antioxidants, mainly vitamin E, may have a role as adjunctive therapy. Experimental studies have suggested that an increase in oxidative stress may play a central role in the liver injury seen in patients with Wilson disease.[157] Moreover, serum and hepatic vitamin E levels have been found to be low in patients with this condition.[158,159] Although symptomatic improvement when vitamin E was added to the treatment has been occasionally reported, further studies are still needed to better determine the possible role of this vitamin and other antioxidants in the treatment of patients with Wilson disease.

With the potential of genetic testing to establish the diagnosis of Wilson disease in presymptomatic homozygous relatives, treatment may begin at an early age. A recent retrospective review showed that 32 asymptomatic homozygous children were treated safely with penicillamine prophylaxis as early as 1.5 years of age.[125] However, the necessity for such early treatment must be balanced against the risks of increasing the total duration of exposure to penicillamine.

Liver transplant has been performed in a number of patients with Wilson disease[39] (Table 50.10-11). At least four groups of patients can be defined who should be considered for liver transplant[39]: (1) patients presenting with a clinical picture of fulminant hepatic failure, often adolescent or young patients; (2) patients with findings of severe hepatic decompensation who have not improved after several months of adequate chelation therapy; (3) patients who have been effectively treated but have developed severe progressive hepatic insufficiency acutely after stopping penicillamine; and (4) patients with progressive and/or irreversible neurologic dysfunction. Reports of Wilson disease patients surviving liver transplants have demonstrated extremely favorable outcomes, with a recent study showing a quality of life comparable to that of age- and sex-matched controls from the general population.[160] Tests of copper status, including serum ceruloplasmin, serum copper, and 24-hour urinary copper excretion, normalize within 1 to 2 months.[39,161] Several reports have shown improvement of neurologic symptoms after transplant. Polson and colleagues described two patients: one preoperatively had continued worsening of neurologic manifestations despite penicillamine treatment and the other had continued worsening of hepatic and neurologic symptoms. In both patients, recovery of neurologic function occurred but was slow.[162] The findings that clinical and laboratory abnormalities normalize after liver transplant confirm the accepted theory that the metabolic defect of Wilson disease is localized within the liver.

TABLE 55.10–11 INDICATIONS FOR ORTHOTOPIC LIVER TRANSPLANT IN WILSON DLSEASE

Fulminant hepatic failure
Cirrhosis with decompensation
Progression of hepatic dysfunction despite treatment
Exacerbation after discontinuation of therapy
Progressive and irreversible neurologic disease

Plasma exchange has been used to reduce serum copper levels and treat the hemolytic anemia associated with fulminant Wilson disease prior to liver transplant.[163] Experimental treatment for Wilson disease may involve the use of hepatocyte transplant. Using the Long-Evans cinnamon rat, hepatic disease has been prevented with infusion of normal rat hepatocytes.[164]

SUMMARY

Wilson disease should be considered in every pediatric patient with liver disease of unknown origin. Early diagnosis and institution of therapy prevent progression of disease and permit normal life expectancy. A variety of therapeutic strategies are available, but penicillamine remains the initial therapy of choice. Lifelong copper chelation or zinc therapy is mandatory.

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CHAPTER 56

PARENTERAL NUTRITION–ASSOCIATED LIVER DISEASE

Julie E. Bines, MD, FRACP

Liver dysfunction is reported to occur in 7.4 to 84% of patients receiving parenteral nutrition (Table 56-1).[1–4] In most patients, this presents as a transient abnormality in serum liver enzyme levels that return to normal with cessation of parenteral nutrition. Cholestasis is the most common manifestation in infants and children, whereas steatosis is more common in adults.[3,4] The incidence of parenteral nutrition–associated liver disease (PNALD) in neonates receiving parenteral nutrition for more than 2 weeks has decreased from 31 to 25% over the past 10 to 15 years, although mortality related to PNALD has not significantly altered.[5] Children with short bowel syndrome requiring long-term parenteral nutrition are at increased risk for the development of complicated PNALD, and liver failure is reported in 3 to 19% of these patients.[6–8]

The etiology of PNALD is unknown. Initially, it was presumed that parenteral nutrition solutions contained a toxic substance or lacked an essential component required for normal hepatic function.[9,10] However, in animal studies, the administration of parenteral nutrition solutions results in hepatic injury but not liver failure.[11,12] The role of sepsis in the early development of PNALD has been the focus of a number of studies.[6,13–15] Current evidence suggests that the developing liver is particularly sensitive to injury resulting from a range of individual factors, such as infection and intestinal stasis, and that PNALD has a multifactorial etiology that reflects the underlying clinical indication for administration of parenteral nutrition (Table 56-2).[6,9,16]

TABLE 56-1 SPECTRUM OF HEPATOBILIARY DYSFUNCTION IN PATIENTS RECEIVING PARENTERAL NUTRITION

Abnormal liver function tests
Cholestasis
Cirrhosis
Liver failure
Hepatocellular carcinoma
Acalculous cholecystitis
Biliary sludge
Cholelithiasis, cholecystitis

RISK FACTORS FOR THE DEVELOPMENT OF PNALD

Patient-Related Factors

Birth Weight and Gestational Age. Low birth weight is a key predisposing factor for the development of PNALD.[9,17] PNALD was reported in 50% of infants with a birth weight < 1,000 g compared with 7% of infants weighing > 1,500 g at birth (Figure 56-1).[17] Gestational age has been identified as an independent factor for the development of PNALD in some but not all studies.[9,17–19] There is a direct relationship between the duration of parenteral nutrition administration and the prevalence of PNALD in premature infants.[17] Almost two-thirds of all infants weighing less than 2,000 g at birth develop cholestasis after 2 weeks of parenteral nutrition therapy.[17]

The increased incidence of PNALD observed in premature infants is thought to reflect the immaturity of hepatic function and the enterohepatic circulation of bile acids.[17,20–22] Normal enterohepatic circulation requires that bile acids are delivered to the intestinal lumen, reabsorbed, and then returned to the hepatocyte for recirculation. However, neonates have reduced hepatic uptake, bile acid synthesis, and the volume of the total bile salt pool.[20–23] Intraluminal concentration and intestinal reabsorption of bile acids are also reduced in premature infants.[24,25] Glutathione is required for normal bile secretion. Glutathione levels are depleted in weanling rats during administration of parenteral nutrition.[26]

Ninety percent of the normal bile acid pool consists of cholic acid, deoxycholic acid, and chenodeoxycholic acid.[27] Bacterial overgrowth of the small intestine induces intraluminal deconjugation of bile salts and increases production of potentially toxic bile acids such as lithocholic acid.[27,28] Patients with PNALD have increased serum and bile concentration of lithocholic acid.[29,30] Lithocholic acid impairs bile flow and, in animals, is associated with hepatic injury similar to the histologic changes observed in patients with PNALD.[30,31] Lithocholic acid is solubilized in the liver by sulfation. Infants have reduced hepatic sulfation and, as a result, may be at increased risk of hepatic injury owing to toxic bile acids.[32]

TABLE 56-2 FACTORS ASSOCIATED WITH DEVELOPMENT OF PARENTERAL NUTRITION–ASSOCIATED LIVER DISEASE

PATIENT FACTORS
Low birth weight
Prematurity
Male sex
Preexisting liver disease
DISEASE FACTORS
Gastrointestinal disease status
Primary disease etiology
Length of residual small intestine
Dysmotility in remaining segment of small intestinal
Residual intestinal disease (ie, Crohn disease)
Length of time with diverting ileostomy or colostomy
Small intestinal bacterial overgrowth
Sepsis
Type of microorganism
Source of infection
NUTRITIONAL FACTORS
Enteral nutrition
Lack of enteral feeding
Undigested nutrients and bacterial overgrowth
Substrate related
Proportion of enteral calories
Formula composition
Parenteral nutrition
Duration of parenteral nutrition administration
Overfeeding
Substrate related
Glucose excess
Specific amino acid excess/deficiency
Lipid excess
Accumulation of phytosterols
Specific micronutrient excess/deficiency

Gender. An association between male sex and the development of PNALD was recently reported.[13] The investigators postulate that a genetic and/or hormonal effect on immune function predisposes males to infection and PNALD.

Preexisting Illness. Preexisting liver disease or a parenteral nutrition–independent risk factor for liver disease is associated with an increased incidence of PNALD.[33]

DISEASE-RELATED FACTORS

Gastrointestinal Disease. Massive small bowel resection is an important risk factor for the development of PNALD in children and adults.[6,33] Parenteral nutrition–associated cholestasis is reported in 30 to 60% of children with short bowel syndrome.[7,8] Residual small intestinal length was identified as the only independent predictor of peak serum bilirubin level in infants and children with parenteral nutrition–associated cholestasis.[34] Seventy percent of infants with < 50 cm of residual small intestine and all adults with entire small intestinal resection developed parenteral nutrition–associated cholestasis.[33,35,36] Chronic cholestasis is the precursor of complicated PNALD and liver failure.[33,37,38]

A link between gastroschisis and PNALD has been reported.[6] Infants with gastroschisis frequently develop intestinal obstruction in utero, and, following surgery, there is a high rate of dilatation and dysmotility of the proximal intestine.

The approach to surgical management of short bowel syndrome in the neonatal period may influence the development of progression of PNALD. Although emphasis is placed on retaining the maximal length of intestine, there is a risk of enhancing exposure of the liver to intestinally derived endotoxin from retained ischemic bowel.[16] Absence of disease in the remaining small intestine is associated with improved survival in patients receiving home parenteral nutrition for intestinal failure.[33] The number of operations and the length of time with a diverting ileostomy or colostomy have been identified as risk factors for the development of PNALD.[34,37] Conversely, the absence of the ileocecal valve did not predispose patients to PNALD.[34]

As part of the adaptive process following resection, the residual small intestine becomes dilated and intestinal transit is slowed in an attempt to compensate for loss of bowel length. Intestinal stasis predisposes the patient to the proliferation of strict anaerobic and facultative anaerobic bacteria within the small intestine. Incomplete absorption of enteral feeds provides a rich source of nutrients for luminal bacteria. Sixty-four percent of infants receiving parenteral nutrition who have bacterial overgrowth later develop sepsis with the same microorganism (such as *Klebsiella*, *Escherichia coli*, enterococci, *Candida*).[38] Bacterial overgrowth is also associated with the deconjugation of bile acids and the production of potentially hepatotoxic bile acids.

Sepsis. Sepsis is associated with cholestasis in infants receiving parenteral nutrition and in infants who have never received parenteral nutrition.[3,14,17,37,39,40] Cholestasis occurs more commonly after gram-negative bacterial infections (in particular *E. coli*).[41] In patients with a normal gastrointestinal tract, cholestasis during an episode of sepsis is usually transient.[16] However, in patients with short bowel syndrome, sepsis has been closely linked to the development of parenteral nutrition–associated cholestasis and progressive PNALD.[6,16,33]

Lipopolysaccharide and peptidoglycan-polysaccharide endotoxins produced by gram-negative and gram-positive

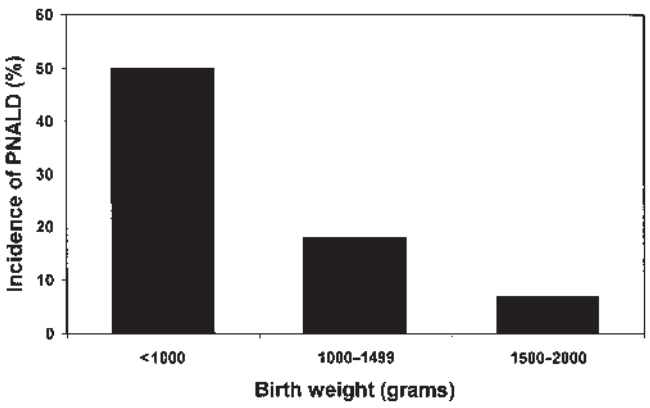

FIGURE 56-1 Relationship between the incidence of parenteral nutrition–associated liver disease (PNALD) and low birth weight. Adapted from Beale EF et al.[17]

bacteria promote the release of tumor necrosis factor (TNF)-α and interleukin-2 from Kupffer cells in rats, resulting in hepatic inflammation and fibrosis.[42] The potential role of TNF in the pathogenesis of PNALD has been a recent focus of interest.[43,44] The administration of antibodies to TNF to rats fed parenteral nutrition results in an improvement in PNALD.[44] Polymyxin B is effective against gram-negative bacteria. In rats with PNALD, polymyxin B blocked endotoxin activity and TNF production and resulted in an improvement in steatosis.[45]

Cholestasis occurred in 26% of 152 neonates who developed an infection while receiving short-term parenteral nutrition.[14] In contrast, cholestasis was not observed in any patient who did not develop an infection.[14] A close association between invasive bacterial or fungal infection and the onset of jaundice in neonates with short bowel syndrome has been observed.[6] Infection occurred before the onset of jaundice in 90% of infants and occurred earlier in cholestatic patients compared with noncholestatic patients (Figure 56-2).[6] Cholestatic patients had more episodes of infection in the first 6 months of life, although the total number of episodes was the same in both groups. Importantly, once established, cholestasis did not resolve with antibiotic therapy and continued to rise, either progressing to hepatic failure or gradually resolving.[6] These data suggest that early exposure to infection is important in the development of PNALD in infants with short bowel syndrome, in whom the liver is immature and susceptible to cholestatic injury and potentially sensitized or stressed by parenteral nutrition administration.[6]

Nutrition-Related Factors

Enteral Nutrition. ***Lack of Enteral Feeding.*** A lack of enteral feeding has been reported as an important risk factor for the development of PNALD in a number of studies.[15,37,46] Enteral feeding induces hormonal stimulation of bile flow, gallbladder emptying, and hepatobiliary development.[47,48] Serum cholecystokinin, glucagon, enteroglucagon, gastrin, motilin, gastric inhibitory polypeptide, and secretin levels differ markedly between infants who are enterally fed and parenterally fed infants.[47,48] In animals with PNALD, treatment with cholecystokinin-octapeptide resulted in decreased periportal inflammation and fibrosis but no improvement in bile flow, bile acid secretion, or hepatocellular injury.[49] Human studies suggest that cholecystokinin (or cholecystokinin-octapeptide) may improve the conjugated hyperbilirubinemia associated with PNALD provided that liver failure is not established.[50,51]

Luminal nutrients aid in the maintenance of gut mucosal barrier function. In the absence of enteral nutrition, the intestine undergoes atrophy, which may increase the risk of bacterial translocation and portal sepsis.[52] In the absence of enteral feeding, intestinal motility and the enterohepatic circulation of bile acids are decreased.[53] These factors may contribute to hepatocyte stress and injury.

Enteral Nutrition Substrates. In neonates with short bowel syndrome, there is a significant relationship between the proportion of calories received enterally at 6 and 12 weeks and subsequent weaning from parenteral nutrition therapy.[6,34] The composition of the enteral feed may also contribute to the development of PNALD. Breast milk or amino acid formulas are associated with greater success at weaning from parenteral nutrition in children with short bowel syndrome.[34,54] Formula composition or excess macronutrients may increase the risk of bacterial overgrowth. Increased bacterial overgrowth and bacterial translocation were observed in mice fed a commercial liquid enteral formula compared with control animals fed chow.[55]

Parenteral Nutrition. ***Duration of Parenteral Nutrition Administration.*** There is a direct relationship between the duration of parenteral nutrition administration and the prevalence of PNALD in premature infants.[17] In surgical neonates, the incidence of cholestasis is reported to increase from 35% after 2 weeks of parenteral nutrition therapy to 75% after 90 days and 100% after 180 days of therapy.[41] The incidence of cholestasis is higher in infants commencing parenteral nutrition at an earlier age and in infants who have had a delay in the introduction of enteral feeds.[37] Chronic cholestasis developed in 65% of adults receiving parenteral nutrition for a median of 6 months.[33] Complicated PNALD developed in 50% of adults after receiving parenteral nutrition for 6 years.[33]

Overfeeding. Total caloric and carbohydrate overfeeding is associated with metabolic changes and alteration in bile flow and liver function.[56] In normal healthy subjects and in clinically stable patients, excessive glucose intake increases insulin concentration, resulting in a decrease in ketogenesis, an increase in glucose oxidation and lipogenesis, and a decrease in fatty acid oxidation.[57] The insulin-to-glucagon ratio is increased in the portal vein. These metabolic changes have been associated with increased levels of serum hepatic enzymes and hepatic steatosis.[57] Clinically stable adult patients fed a glucose-based parenteral nutrition solution to an average of 177% of predicted energy expenditure developed fatty infiltration and intrahepatic cholestasis on liver biopsy within 5 days of commencing parenteral nutrition.[56] Abnormal serum tests of liver function were detected by 14 days in 83% of

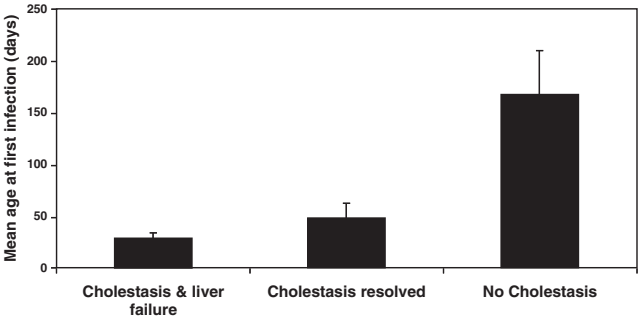

FIGURE 56-2 The effect of age at first infection on the development and severity of parenteral nutrition–associated cholestasis in neonates with intestinal resection. Adapted from Sondheimer JM, Asturias E, Cadnapaphornchai M. Infection and cholestasis in neonates with intestinal resection and long-term parenteral nutrition. J Pediatr Gastroenterol Nutr 1998;27:131–7.

patients, with abnormalities occurring in proportion to the increase in carbohydrate load.[56] Repeat liver biopsy at day 21 showed bile duct proliferation, canalicular bile plugs, centrilobular cholestasis with accumulation of bile pigment within hepatocytes, and periportal inflammation.[56] Similar abnormalities have been observed in stable infants receiving glucose-based parenteral nutrition.[58]

Acute metabolic stress may exacerbate the impact of carbohydrate overfeeding on hepatic morphology and function. With acute stress, lipolysis and fatty acid oxidation increase relative to glucose oxidation owing to the action of counterregulatory hormones, resulting in insulin resistance.[59] In the presence of excessive glucose administration, serum glucose and insulin concentrations are elevated.[59] These factors may increase the risk for hepatic injury. Pyruvate dehydrogenase is a rate-limiting step in glucose oxidation; however, during sepsis, the activity of this enzyme may be inhibited.[60] Increased glucose load during a period of inhibition of this enzyme may further add to the insult to the hepatocyte during stress.[60] Overfeeding during a stress-free period is reported to diminish the hepatic response to subsequent injury, in particular sepsis.[61] In addition, bacterial translocation from the gut is increased during periods of nonprotein overfeeding in animals.[62] Overfeeding during critical illness may further contribute to the hypermetabolic response and hepatocyte injury.

Overfeeding with lipid emulsions containing long-chain triglycerides has been associated with decreased clearance of bacteria from the reticuloendothelial cells. Although this may be clinically significant in terms of posing an increased risk of bacterial sequestration into the lung, these changes have not been associated with characteristic features of hepatocyte injury or disturbances in bile flow.[63]

Substrate-Related Mechanisms. The chemical composition of parenteral nutrition solutions has been implicated in the development of PNALD.[9,28,56] These solutions may include specific nutrient deficiency or excess/toxicity. Contaminants infused with the parenteral nutrition solution may also contribute to hepatic injury.

Glucose infusion has been associated with hepatic steatosis.[9,28] Bile flow is reduced during glucose infusion in animal studies.[64,65] Preterm infants require approximately 4 to 8 mg/kg/min of glucose to suppress hepatic glucose oxidation; however, infusions should not exceed 12.6 mg/kg/min because excess exogenous glucose that is not oxidized may be converted to glycogen or fat in the liver.[66]

Amino acid infusions have been associated with cholestatic liver disease in both human and animal studies.[19,56] A more rapid rise and higher bilirubin concentration were reported in premature infants receiving 3.6 g amino acids/kg/d compared with infants receiving 2.5 g amino acids per kilogram body weight per day.[19]

Individual amino acids have been implicated in the development of parenteral nutrition–associated cholestasis.[9,28] This may reflect immaturity of amino acid metabolism, resulting in an excess of precursor amino acids or a defect in the synthesis of amino acids or proteins. Serum methionine levels are increased in some infants receiving parenteral nutrition owing to a block in the transsulfuration pathway, remethylation of homocystine, or impaired oxidation of sulfur-containing amino acids.[67] Hepatocellular injury observed in neonatal rats receiving infusions of methionine was prevented by supplementation with arginine and glycine.[68,69] Increased cystine has been related to cholestasis and morphologic alterations, including bile duct proliferation, periportal necrosis, portal fibrosis, and inflammation of portal triads.[70] Increased homocystine causes hepatocellular injury and iron deposition in animal studies.[71] Photo-oxidation of amino acid solutions may result in production of hepatotoxic metabolites.[72] The extent of cholestasis was dependent on the dose of tryptophan and degree of light protection in newborn rats receiving an intraperitoneal amino acid solution.[72]

Owing to the immaturity of amino aid metabolic pathways, some amino acids become conditionally essential in premature infants. Evidence supporting the role of a lack of taurine in the development of PNALD remains controversial.[73] Taurine is important in the conjugation of bile acids in neonates, and a lack of taurine results in bile acids becoming predominantly glycine conjugated. These bile acids are potentially hepatotoxic in the infant.[73] The benefit of taurine-supplemented parenteral nutrition to reduce the incidence of PNALD has not been confirmed.[74,75]

Carnitine is absent from most commercially available amino acid solutions. Carnitine is synthesized from methionine and lysine; however, in infants, this conversion may be limited owing to immature metabolic pathways.[76] Carnitine is required for fatty acid oxidation in the mitochondria, and carnitine deficiency is associated with hepatic steatosis. Low serum carnitine levels have been observed in infants and adults receiving parenteral nutrition; however, intravenous carnitine supplementation has not consistently improved serum and hepatic carnitine levels or features of PNALD.[77–79]

High-dose infusion of lipid emulsion is associated with impaired bilirubin excretion in adults.[79] Early lipid emulsions containing cottonseed oil were associated with the development of cholestasis and liver damage.[80] These solutions are no longer in use. Long-chain triglyceride lipid emulsions are a rich source of linoleic acid that promote the synthesis of leukotriene B_4, a proinflammatory cytokine, and may contribute to an increased inflammatory response to cytokines.[81] Commercially available long-chain triglyceride lipid emulsions were not thought to cause PNALD if administered in doses of 1 to 2 g per kilogram body weight per day.[19,56,80] However, in a recent study in intestinal failure patients receiving home parenteral nutrition, cholestasis and complicated PNALD were associated with a dose of lipid emulsion ≥ 1 g/kg/d.[33]

Clayton and colleagues have reported that the long-term use of a long-chain triglyceride lipid emulsion (Intralipid) results in the progressive accumulation of phytosterols.[82] In some patients, plasma concentrations of phytosterols were up to 25% of total plasma sterols and were even higher than in the Intralipid preparation.[82] Phytosterols are present in small amounts as contaminants of the lipid emulsion. Because they are insufficiently metabolized by the liver, it has been suggested that phytosterols

accumulate in the macrophages of hepatic sinusoids and disrupt normal macrophage function.[83] Intravenous phytosterol infusion caused reduced bile flow but not liver injury in newborn piglets.[82]

Choline is absent from commericially available parenteral nutrition solutions, and low serum choline levels have been reported in adults on parenteral nutrition therapy.[84] Phosphatidylcholine is required for the synthesis of lipoproteins. Choline deficiency in rats is associated with hepatic steatosis; however, it is not known if this relationship also exists in man.[85]

Selenium and molybdenum deficiency has been reported in association with PNALD; however, the role they may play in the pathogenesis is uncertain.[86] Serum manganese levels are increased in 79% of long-term parenteral nutrition patients.[87] Manganese can be toxic to the brain and the liver. There is a significant correlation between serum manganese levels and asparate transaminnase levels; however, it is unclear whether this is a primary or a secondary effect owing to delayed biliary excretion.[88]

CLINICAL MANIFESTATIONS

Hepatic Steatosis

Fatty infiltration of the liver is the most common manifestation of PNALD in adults.[4] It usually occurs secondary to excessive parenteral carbohydrate intake and resolves with a reduction in parenteral carbohydrate intake. Hepatic steatosis clinically presents as hepatomegaly associated with a mild to moderate increase in serum aminotransferase levels.[4]

Cholestasis

Chronic cholestasis is the primary manifestation of PNALD in infants and children and in adults with intestinal failure.[4,6,33] Persistent elevation of serum conjugated bilirubin level is the most consistent biochemical predictive marker of progressive PNALD.[16,89] Cholestasis is usually defined as a serum conjugated bilirubin level of > 1.5 mg/dL or 40% total bilirubin concentration. In infants, serum bilirubin levels may begin to rise as early as 1 to 2 weeks after initiation of parenteral nutrition therapy. Elevation of serum bile acids, either total bile acids or specific cholic acid conjugates, including lithocholate, has been reported to detect early evidence of PNALD prior to elevation of serum conjugated bilirubin level.[90,91] Total serum bile acids and conjugated bilirubin levels correlate with histologic changes in the liver and duration of exposure to parenteral nutrition in animals receiving parenteral nutrition.[92]

Cirrhosis and Complicated PNALD

With progression of disease, the serum alkaline phosphatase gradually increases. However, this may be difficult to interpret in infants and children because the bone isoenzyme may be elevated owing to bone disease or decreased owing to zinc deficiency. γ-Glutamyl transpeptidase or 5′-nucleotidase may also be elevated but does not add significant diagnostic benefit over serum bilirubin concentration alone in infants.[16,89] Levels of serum transaminases and alkaline phosphatase did not correlate with the severity of liver histology in PNALD.[92]

Reduced serum albumin levels are associated with increased mortality owing to PNALD.[4] However, in patients with excessive protein loss or severe protein-energy malnutrition, hypoalbuminemia and hypoproteinemia may reflect nutritional deficit and not liver dysfunction. Similarly, nutritional vitamin K deficiency may also result in a prolonged prothrombin time.

Biliary Tract Abnormalities

Biliary sludge and/or cholelithiasis occur in 12 to 40% of children receiving long-term parenteral nutrition.[93,94] These may occur as a result of gallbladder hypomotility, changes in the composition of bile, or altered enterohepatic circulation of bile acids. The risk of cholelithiasis is increased in children with ileal resection, ileal disease, or hemolytic anemia or in those receiving furosemide therapy. Biliary stones usually consist of both cholesterol and pigment. Acalculous cholecystitis is associated with high morbidity and mortality.[95]

HISTOPATHOLOGY

Characteristic histologic features are observed in liver biopsies of patients receiving parenteral nutrition; however, these changes are not specific or diagnostic.[9,96,97] The histologic changes progress with duration on parenteral nutrition. The initial lesion, steatosis, may occur within the first 2 weeks of parenteral nutrition administration. The degree of steatosis correlates with the amount of energy infused.[98] Cholestasis occurs predominantly in the centrilobular region and involves hepatocytes, canaliculi, and Kupffer cells (Figure 56-3). Serum conjugated bilirubin concentration may not accurately reflect the extent of cholestasis observed on liver

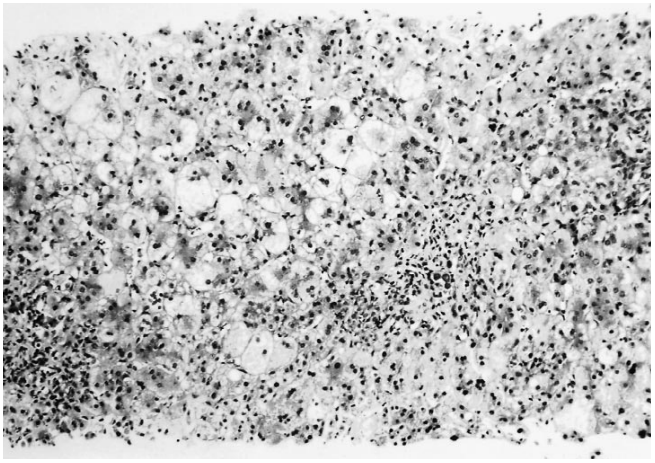

FIGURE 56-3 Light microscopy of a liver biopsy of a patient with parenteral nutrition–associated liver disease showing nonspecific periportal inflammation and mild lobular disarray, with ballooning of hepatocytes, microvesicular steatosis, and focal pseudoacinar arrangement of hepatocytes. Cholestasis is present within canaliculi, hepatocytes, Kupffer cells, and occasional bile ducts. Courtesy of Antonio Perez, MD, Department of Pathology, Children's Hospital, Boston.

biopsy. Mild to moderate periportal inflammation is observed. This is usually a lymphocytic infiltrate, although neutrophils and eosinophils may also be present. With disease progression, hepatocytes become ballooned with lipofuscin granules identified in the periportal region. Kupffer cells are hyperplastic and may also have lipofuscin granules. The lobular architecture becomes disordered with periportal fibrosis observed in most patients. Bile duct proliferation and, less commonly, bridging fibrosis may occur. In infants, extramedullary hematopoiesis is common. If parenteral nutrition administration continues, steatosis and extramedullary hematopoiesis tend to improve, but cholestasis, fibrosis, and progression to cirrhosis can occur.[97] If parenteral nutrition treatment is ceased before the development of cirrhosis, many of the histologic features of liver disease improve.[97] The estimated time from onset of long-term parenteral nutrition to the development of moderate fibrosis (< 50% portal spaces involved) in children with short bowel syndrome is reported to be about 40 months.[96] After the development of fibrosis, there tends to be a more rapid progression to cirrhosis (mean duration 14 months). Hepatocellular carcinoma was reported in a 6-month-old infant dependent on long-term parenteral nutrition.[99]

MANAGEMENT

Cessation of parenteral nutrition is the most effective therapy for the management of PNALD.[4,97,99,100] However, this may not be possible in some patients, and treatment is aimed at minimizing the impact of parenteral nutrition on the liver. This includes the identification of individual risk factors and the development of a multidirected strategy to reduce the adverse impact on the liver. Meehan and Georgeson reported the prevention of liver failure in long-term parenteral nutrition patients using taurine, prevention and aggressive treatment of sepsis, strict catheter care, early parenteral nutrition cycling, "appropriate" enteral feeding, and inhibition of bacterial translocation.[101] The timing of the progression of liver disease is critical in planning which patients may require small bowel or combined liver–small bowel transplant.[4,16,89] In the absence of liver transplant, PNALD can be fatal.[6]

Enteral Feeding

For patients who are unable to discontinue parenteral nutrition, small volumes of enteral nutrition have been shown to reduce the progression of PNALD.[9,102] Minimal or "trophic" enteral feeding is associated with gallbladder contraction, increased bile flow, increased gastrin and glucagon secretion, a reduction in intestinal stasis, and bacterial overgrowth.[4,102] Supplementation of enteral feeds with fish oil rich in Ω-3 long-chain polyunsaturated fatty acids has been advocated in an attempt to limit exposure to Ω-6 polyunsaturated long-chain fatty acids.[103] Excess Ω-6 polyunsaturated long-chain fatty acids have been linked to hepatic inflammation. Supplementation of feeds with Ω-3 long-chain polyunsaturated fatty acids and/or antioxidants (vitamins C and E, *N*-acetylcysteine) has been proposed for the prevention of PNALD.[104]

Prevention of Infection

Meticulous catheter care and prompt and aggressive treatment of infection are important in the prevention of PNALD.[4,6,101] The supervision of central line care and nutrition therapy by a multidisciplinary nutrition support team is associated with a significant benefit in terms of the incidence and age at development of liver disease owing to parenteral nutrition.[105] Routine throat swabs have been used to predict bacteremia in infants receiving parenteral nutrition who have abnormal small intestinal flora.[106] Prompt surgical intervention with possible resection is encouraged in patients with suspected intestinal ischemia in an attempt to minimize hepatic exposure to endotoxin in the neonatal period.[16]

Bacterial Overgrowth

Bacterial overgrowth is a frequent complication of functional and mechanical disorders of the small intestine. The clinical hallmarks of bacterial overgrowth include abdominal distention, vomiting, halitosis, and loose, offensive stool. The diagnosis is established by the quantitative culture of small intestine fluid. A high fasting breath hydrogen level may also be suggestive. Treatment usually involves the use of antibiotics. Metronidazole and the oral nonabsorbable antibiotics gentamicin, kanamycin, neomycin, and polymyxin B have been studied in animals and in limited human trials.[28] Metronidazole prevents PNALD in rats; however, its role in the prevention of human disease is uncertain.[107] In infants receiving parenteral nutrition, intravenous metronidazole was associated with lower serum hepatic transaminase levels but had no effect on the incidence of hyperbilirubinemia.[108] Suppression of bacterial overgrowth may be assisted by avoiding acid-suppressing agents and carbohydrate malabsorption.[16] The use of probiotic microorganisms such as lactobacillus GG and bifidobacteria to suppress the growth of potentially harmful bacteria has been proposed, although the efficacy of probiotics for the prevention of PNALD is yet to be determined.[16,109] Patients with permanent dilatation of a loop of small intestine may benefit from surgical intervention, including resection of a stricture or stenosis or an infolding or tapering procedure.[101]

Modification to Parenteral Nutrition Solution and Administration

Modification of the macronutrient concentrations and balancing their composition in the parenteral nutrition solution may be required in patients with PNALD. Energy should be provided to meet individual needs for growth and activity. Infants receiving energy at 110 kcal/kg/d or greater had a higher incidence of cholestasis compared with infants receiving a lower energy intake.[3] Glucose infusions should be limited to less than or equal to 15 g/kg/d or 12.6 mg/kg/min in premature infants.[66]

Recommendations for intravenous amino acid intakes for premature infants are generally aimed at providing between 2 and 3 g/kg/d.[66] Specialized pediatric amino acid solutions have been developed with the goal of limiting the complications of amino acid imbalance owing to immature

metabolism in the premature and young infant. However, the efficacy of these solutions in preventing or minimizing PNALD has not yet been established.[110,111] Supplementation of parenteral nutrition solutions with taurine has not been shown to reduce serum hepatic enzyme, bilirubin, or bile acid concentrations in premature infants receiving short-term parenteral nutrition.[73–75] Intravenous glutamine supplementation has been shown to improve gut barrier integrity, although its role in the prevention and treatment of PNALD in infants and children requires further study.[112]

Current evidence suggests that in infants and children, lipid emulsions should be limited to < 3 g/kg/d and less than 60% of total daily energy.[19,113] Lipid doses of > 1 g/kg/d were associated with cholestasis and complicated liver disease in adults with intestinal failure receiving home parenteral nutrition. However, this has not yet been confirmed in children.[33] In vitro studies suggest that exposure of lipid emulsion to ultraviolet light results in the production of potentially toxic hydroperoxidases.[114] Therefore, lipid emulsions should be protected from ultraviolet light, particularly during phototherapy. The addition of fat-soluble vitamins to the lipid emulsion may provide some protection from this effect.[115]

In the presence of progressive cholestasis and cirrhosis, modification of the micronutrient composition of the parenteral nutrition solution may include the removal of copper and manganese.[37] This underscores the importance of routine monitoring of micronutrient status in patients receiving long-term parenteral nutrition. Cycling of parenteral nutrition for up to 12 hours a day may help in limiting the progression of PNALD in patients requiring long-term parenteral nutrition.[116,117]

Pharmacologic Management

A number of pharmacologic agents have been proposed for the prevention and treatment of PNALD. Nonsteroidal anti-inflammatory drugs, including acetylsalicylic acid, have been shown to be beneficial in the prevention of PNALD in animal studies.[118,119]

Cholecystokinin-octapetide and the cholecystokinin analog ceruletide stimulate gallbladder contraction and prevent the development of biliary sludge and cholelithiasis in patients receiving parenteral nutrition.[49,50,120,121] The use of these agents in the prevention and treatment of PNALD is encouraging but warrants further clinical investigation.

Ursodeoxycholic acid improves bile flow and reduces serum and liver bilirubin concentrations in piglets with PNALD.[122] In humans, treatment with ursodeoxycholic acid has been associated with a reduction in markers of liver dysfunction, including serum γ-glutamyltransferase, aspartate transaminase, alanine transaminase, alkaline phosphatase, and/or serum bilirubin concentration.[123] A rebound increase in serum γ-glutamyltransferase, alkaline phosphatase, and alanine transaminase concentration was observed in children in whom ursodeoxycholic acid was discontinued in the presence of ongoing parenteral nutrition administration.[123] These patients responded to reintroduction of ursodeoxycholic acid therapy. This raises the question of whether ursodeoxycholic acid reduces serum markers of liver dysfunction in PNALD but does not influence the progression of liver disease. Enteral administration of tauroursodeoxycholic acid was not found to be effective in the prevention of PNALD in neonates.[124]

Other pharmacologic agents, including antibiotics, cholestyramine, rifampin, and phenobarbital, have been studied in the context of management of PNALD.[28] Surgical approaches aimed at improving bile flow, including biliary irrigation, have also been associated with improvement in PNALD in some patients.[125,126]

Liver–Small Bowel Transplant

Most children requiring long-term parenteral nutrition have underlying intestinal disease, either short bowel syndrome or severe intestinal dysfunction. If transition to full enteral nutrition cannot be achieved and parenteral nutrition cannot be discontinued, over time, PNALD may progress to overt liver failure.[4] Small bowel transplant prior to progression to severe liver disease and combined liver–small bowel transplant are therapeutic options for the management of both primary gut disease and the complication of parenteral nutrition–associated liver failure. Because the morbidity and mortality associated with small bowel transplant are probably more favorable compared with combined liver–small bowel transplant, it is important that referral to a transplant center is made before the liver disease becomes irreversible.[16,89,127,128] Elevation of the total serum bilirubin level of > 3 mg/dL for over 3 months in a parenteral nutrition–dependent infant and/or early clinical features of progressive liver disease, including mild splenomegaly, dilatation of abdominal wall veins, and a deteriorating platelet count, are indications for referral.[16,89] Serum hepatic or biliary enzyme levels provide no additional predictive benefit in patients with PNALD.[16] Hypoalbuminemia, coagulopathy, and hypoglycemia during cycling of parenteral nutrition are markers of hepatic synthetic dysfunction. These are considered late features of liver disease and are associated with a poor prognosis.[16] In the setting of progressive PNALD and portal hypertension, severe bleeding may occur from stomas in patients with short bowel syndrome.

CONCLUSION

Infants and children receiving parenteral nutrition are at risk of developing PNALD. No single factor has been shown to be responsible for the development of this potentially fatal complication. Current evidence suggests that the developing liver is particularly sensitive to injury resulting from a range of individual factors, such as infection and intestinal stasis, and that PNALD has a multifactorial etiology that, in part, is determined by the underlying clinical indication for parenteral nutrition administration. Discontinuation of parenteral nutrition is the most effective treatment, but this cannot be achieved in some patients. Treatment strategies that include the identification of risk factors and the development of a multidirected approach to the prevention and treatment of PNALD are required. Although new pharmacologic and

surgical approaches have been reported, the risks and benefits of these therapies still need to be assessed in randomized prospective controlled trials. Small bowel or combined liver–small bowel transplant provides a therapeutic option for long-term parenteral nutrition–dependent patients with PNALD in the presence of short bowel syndrome or severe intestinal dysfunction.

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CHAPTER 57

SYSTEMIC CONDITIONS AFFECTING THE LIVER

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An understanding of the effects of systemic diseases on hepatobiliary function in infancy and childhood requires an understanding of liver physiology and, in particular, how that physiology changes with increasing maturity. This is described in detail in Chapter 5, "Liver Function and Dysfunction." It is particularly important to recognize that many of the cells that comprise the liver and biliary tract respond to potentially toxic insults differently at different ages and that the process of cell repair and wound healing varies with the insult to the liver and the age of the patient. The liver may be injured as a passive bystander or may actively exacerbate the systemic conditions described below.[1–19] The following discussion is organized by organ system or major categories of systemic disease and is meant to provide a clinical overview of hepatobiliary function during system illnesses.

ACUTE CARDIAC DISEASE

The pediatric hepatologist may be called on to evaluate the infant and child with cardiac disease in several situations. These include (1) the patient with acute liver injury from cardiogenic circulatory compromise, (2) the patient with chronic congestive heart failure or outflow obstruction, and (3) the patient with complex congenital anomalies in which the liver and heart are affected by associated malformations.[20–22]

Acute circulatory failure results in a typical pattern of liver injury that is a result of the unique dual blood supply to the liver and of the heterogeneity of the liver acinus. Two-thirds of the blood supply to the liver is from the portal circulation, and the remainder is from the hepatic artery. The oxygen-rich blood perfuses the liver acinus, creating a gradient of oxygenation, with the maximal concentration of oxygen delivered to the periportal zone 1 of Rappaport and the lowest concentration of oxygen to the pericentral zone 3. Blood flow to the liver is regulated locally by the concentration of adenosine, a vasodilator that is produced by endothelial cells. With poor perfusion, the local concentration of adenosine increases, and vasodilation occurs. This results in increased portal flow that compensates partially for the reduced arterial flow. Outflow resistance may also affect oxygenation of the liver, as in acute cardiogenic shock. Thus, cells in the pericentral zone of the liver are most susceptible to perfusion-related injury.

Clinical Presentation

The infant or child with ischemic hepatic injury is usually critically ill. Cardiac symptoms may predominate, but, occasionally, the cardiac origin may not be recognized, especially when hepatic symptoms predominate and initial attention focuses on liver dysfunction.[19] This is particularly true in cases of noncyanotic heart disease. The liver is uniformly enlarged, and the edge is round and smooth, reflecting generalized engorgement of the organ.[23,24] Jaundice, which is usually a later manifestation of liver disease, may not be apparent in the first few days after the acute event.

Concentrations of hepatic transaminases are increased up to 200 times the upper limit of the reference range. Lactic dehydrogenase is also increased and, if fractionated, is mostly of hepatic origin (Table 57-1). This increase in lactic dehydrogenase, seldom seen in viral hepatitis, may be used to distinguish ischemic hepatic injury from acute viral hepatitis.[25] The dramatic increase in aspartate aminotransferase and alanine aminotransferase reflects the predominant injury to the central zone of the liver.[26–28] Not uncommon is an association with renal hypoperfusion and increases in serum urea nitrogen and serum creatinine. Acute renal tubular necrosis may also occur. The peak concentration of the transaminases occurs in the first 3 days after the insult. The concentrations return to normal in 5 to 7 days except in patients with ongoing liver ischemia. Coagulation is significantly altered, and, typically, the prolongation of the prothrombin time, as indicated by the international normalized ratio, is not corrected by the

TABLE 57-1 THE LIVER IN HEART DISEASE

Hepatomegaly with a smooth rounded edge of the liver
Jaundice and hyperbilirubinemia
Splenomegaly with chronic cardiac insufficiency
Differential elevation of liver-derived enzymes AST > ALT
Coagulopathy unresponsive to vitamin K

ALT = alanine aminotransferase; AST = aspartate aminotransferase.

administration of vitamin K. Jaundice and the increase in bilirubin occur later, usually by the third to fifth day after the original insult. Typically, the degree of hyperbilirubinemia is mild compared with the increase in transaminases and is by no means universal.[29] Cholestasis occurs with a decrease in transaminases. Persistence of cholestasis and coagulopathy with normalizing concentrations of enzymes indicates a poor prognosis and an exhaustion of the hepatocyte mass owing to the acute ischemic event.

A pig model of cardiogenic shock showed that immediately following decreased liver perfusion, there is an increase in messenger ribonucleic acid for acute-phase proteins.[30] Expression of certain heat shock proteins was also demonstrated, with the potential for preservation of cellular integrity. The significance of this expression and its relationship to the degree of liver injury and potential for recovery are unknown.[31]

Histologically, the pericentral area shows variable degrees of parenchymal necrosis and hepatocyte loss directly related to the duration of shock. There is little inflammatory activity, and the necrotic areas are clearly defined. At this stage, cholestasis is not a prominent feature.[32] The differential diagnosis of ischemic hepatic injury is usually not a problem because cardiac signs and symptoms predominate. Patients with other conditions associated with shock and ischemic events, including septic and hypovolemic shock, may present similarly. Protracted seizures and drug-induced hepatitis must be considered as well with this constellation of biochemical and histopathologic features. Patients with severe rhabdomyolysis may present with a marked increase in aspartate aminotransferase that is out of proportion to the concentration of alanine aminotransferase. The presence of muscle pain, easy fatigability, and, occasionally, bulky muscle mass should be sought. The presence of myoglobinuria and an increase in creatinine kinase help in the differential diagnosis.

Therapy for ischemic hepatic injury is directed toward the original insult. Supportive measures include the administration of vitamin K and, if bleeding is a problem, fresh frozen plasma. The injury to zone 3 hepatocytes, the site of xenobiotic and drug metabolism, may increase the susceptibility of the liver to potentially toxic metabolites of medications administered to these patients.

Prognosis

Correction of the original insult results in resolution of the hepatopathy. An ischemic event with a duration of more than 24 hours usually results in severe liver injury and may progress to liver failure. The lack of resolution of the coagulopathy together with a decrease in transaminases and deepening jaundice is ominous. In several adult and pediatric series, the most important prognostic factors for survival were the degree of shock and, most importantly, the duration of the ischemic event. A duration of circulatory disturbance of more than 24 hours is associated with significant morbidity and mortality.[33,34] The cardiac lesions that most commonly result in significant liver involvement are hypoplastic left heart syndrome and coarctation of the aorta.[24]

CHRONIC CARDIAC DISEASE

The liver is vulnerable to a chronic increase in pressure on the right side of the heart. Chronic passive congestion results in sinusoidal dilatation and engorgement, fibrosis, and, eventually, cirrhosis.[34,35]

Clinical Manifestations

Clinically, the liver is enlarged and hard; the edge is usually smooth, but nodularity is apparent in the advanced stages. The increase in liver-derived enzymes is minimal or nonexistent at first. With time, a slow, mild increase in transaminase values, as well as increases in the biliary tract–derived enzymes, is seen. Presumably, the degree of fibrosis is related to the development of microthrombi in the sinusoids and in the hepatic veins. The distribution of fibrosis can be uneven, with the right and left lobes affected to different degrees.

In a series of 150 pediatric patients, the severity and duration of hypoxia correlated with the amount of connective tissue present at autopsy.[35,36] Fibrosis had asymmetrically affected the lobes of the liver. Inhomogeneous liver parenchyma was noted on computed tomography in 24 of 25 patients with congestive heart failure.[36] Other patterns noted included hepatomegaly, enlargement of the inferior vena cava, and early reflux of contrast material into the inferior vena cava. The patient may or may not be jaundiced. The degree of hyperbilirubinemia fluctuates and occasionally results in overt jaundice. In late stages, jaundice in cardiac cirrhosis indicates a poor prognosis.

Histologic Features

Bands of fibrosis and, occasionally, cirrhosis are seen. Sinusoidal engorgement and dilatation are common (Figure 57-1). With chronic congestion, zone 3 hepatocytes atrophy, and the liver grossly resembles a nutmeg because of the association of areas of engorgement and atrophy. In cases of fetal hydrops, the liver congestion is accompanied by hemosiderosis in zone 1 hepatocytes.[37]

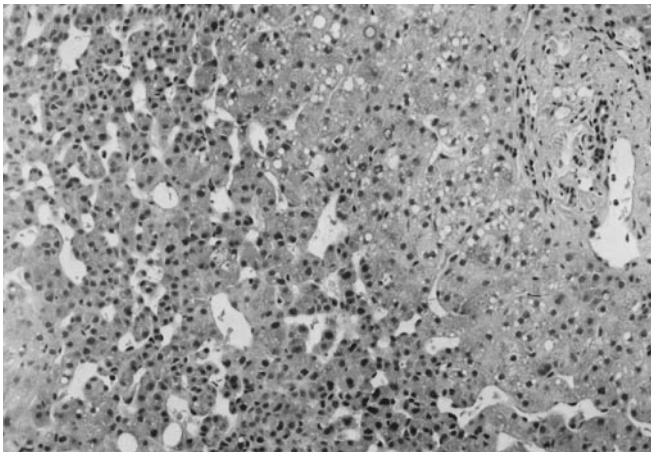

FIGURE 57-1 Venous outflow obstruction of cardiac origin. Note sinusoidal dilatation (hematoxylin and eosin; ×40 original magnification). Courtesy of Dr. L. Burgart, Mayo Clinic and Foundation.

TREATMENT

For children who have a chronic increase in right-sided ventricular pressure from surgical correction of congenital heart disease, definitive correction is not always possible. With advances in medical and surgical care, more of these children are surviving into young adulthood, and cardiac cirrhosis may become the most important factor for their survival. The chronically congested liver is more susceptible to other insults. Animal studies have shown that endotoxemia with congestive heart failure can lead to fulminant hepatic failure.[35]

PROGNOSIS

Prognosis is variable because the distribution of pressure is not uniform throughout the liver, and there may be significant potential for compensated cirrhosis. Correction of the underlying cause is therapeutic in most cases.

OPEN HEART SURGERY

It is not uncommon for patients to have both congenital heart disease and congenital liver disease. The infant with a paucity of bile ducts or biliary atresia may have hemodynamically significant congenital heart disease, and the cardiac surgeon often inquires about the possibility of liver damage during open heart surgery and extracorporeal oxygenation. The infant or child with a paucity of bile ducts is usually jaundiced. The serum concentrations of biliary tract–derived enzymes are markedly increased, but the increases in transaminases are only moderate. In our experience, the prothrombin time is the best indicator of the ability of the patient to avoid fulminant hepatic failure as a result of the operation. Often the cardiac lesion is more serious than the liver lesion in patients with syndromic paucity of intrahepatic bile ducts. The same is not entirely true for the association of biliary atresia, polysplenia, dextrocardia, and congenital heart and vascular lesions. In terms of the liver, the earlier a portoenterostomy is performed, the better the hepatic outcome. If biliary drainage is accomplished and the infant is thriving, the cardiac lesion can be corrected at a later, optimal time. If biliary drainage is not accomplished, the cardiac lesion will be corrected in conjunction with liver transplant to optimize the outcomes of both operations. Once again, the prothrombin time and the degree of cholestasis and growth failure are used to determine the timing of the intervention and the degree of support required during and after the intervention.

Liver abnormalities are not uncommon after open heart surgery.[38,39] The increase in liver-derived enzymes is similar to that in ischemic hepatitis, although much smaller. Hyperbilirubinemia occurs later and is related to the duration of hypothermia and extracorporeal circulation. The infant or child with persistently low cardiac output is most susceptible to the development of liver injury. Blood transfusions during the operation add to the hyperbilirubinemia. The duration of hypoperfusion and the response to inotropic agents are important factors in the degree of liver involvement. Liver failure with encephalopathy has been reported for both adult and pediatric patients.[40–43]

CARDIAC TRANSPLANT

The liver may show evidence of being affected immediately within the first 2 weeks after cardiac transplant or not until the development of chronic liver disease. In the setting of established pulmonary hypertension, after heart transplant, the immediate insult to the liver is due to right-sided heart failure if the transplanted organ fails. This is similar to heart failure from other causes; however, the clinical case may be further complicated by acute rejection and infections. Chronic liver disease after heart transplant may be due to a lymphoproliferative disorder involving the liver or to chronic viral hepatitis acquired after transplant. The infection is due to hepatitis B and C viruses and to non-A–E hepatitis viruses, as well as to de novo infections with cytomegalovirus. Most cases of chronic hepatitis B and C are mild, and fulminant cases are rare except in cases of precore mutants of hepatitis B virus. Immunosuppression may lead to faster progression of liver fibrosis.[44]

SEPSIS

Infants and children with systemic or localized infections may have significant liver involvement.[45–48] One example is the classic description of neonatal cholestasis in the context of a urinary tract infection. Underestimation of liver dysfunction is likely because usually only jaundiced patients are evaluated. Jaundice from septicemia occurs infrequently. In an adult series of 1,150 bacteremic patients, only 7 had jaundice.[46]

The organisms implicated in the development of hepatic dysfunction include gram-negative enteric pathogens, streptococci, and staphylococci. The site of infection varies. For instance, jaundice with septicemia is one such manifestation, and hepatic abscess is known to complicate appendiceal infections. Another example is soft tissue infections leading to hepatopathy.

The onset of jaundice occurs 2 to 5 days after the infection, and the degree of direct hyperbilirubinemia varies from 5 mg/dL to more than 60 mg/dL. Hepatomegaly occurs in about 50% of the cases, and, in this instance, exclusion of hepatic abscess or cholangitis is important in the differential diagnosis.[47,48] The hepatomegaly is due to the activation of the reticuloendothelial system.[46–50]

The increases in the liver- and biliary tract–derived enzymes are only moderate compared with the degree of hyperbilirubinemia, and this dissociation is characteristic of septicemia. However, the increase in alkaline phosphatase may be significant at the onset of jaundice and during the resolution of jaundice.[46,50–52]

The recognition of the polarity of the hepatocyte has shed new light on the transport mechanisms of organic acids and bile salts. Evidence has shown that the cholestasis of septicemia is associated with abnormal regulation of the localization of the multidrug resistance protein 2. This transporter is responsible for the secretion of conjugated bilirubin at the canalicular membrane. The effect of endotoxin on this transporter results in its translocation to the inner cytoplasmic membrane. Thus, the degree of hyper-

bilirubinemia is out of proportion to the degree of hepatocyte damage.[53,54]

Histologically, the liver may show suppurative cholangiolitis without large duct cholangitis, in association with intrahepatic cholestasis (Figure 57-2). The lesion in the context of the clinical findings is characteristic.[48] Cholestasis alone is also common (Figure 57-3).

One important consideration is that septic patients have other confounding factors that may contribute to cholestasis. For example, some patients with multiorgan failure may have received blood products or are receiving total parenteral nutrition (TPN) and various medications. In particular, cephalosporins and biliary sludge may contribute to cholestasis. Evaluation of the patient centers on the exclusion of an hepatic abscess or ascending cholangitis. Treatment of the underlying cause is essential. There are no data on the efficacy of supportive treatment, including bile acid supplementation and early feeding, but their use is common. The prognosis is excellent after the infection is controlled.[47,50]

Characteristic features of certain infections are worth mentioning. For example, the liver may be involved in pneumococcal pneumonia.[46] The right lobe is most commonly affected, and the male-to-female ratio is 10:1.[46,47] There is swelling of hepatocytes and focal necrosis. The increase in serum transaminases may be moderate. Usually, the increase in aspartate aminotransferase is greater than the increase in alanine aminotransferase. Streptococcal infections may be associated with jaundice, and an association with scarlet fever has been described in which the liver may be tender and enlarged. Hepatomegaly and hepatitis with jaundice are not uncommon in ehrlichiosis, a tickborne infection. A history of exposure in endemic areas is helpful, and the typical rash on the palms and soles aids in the diagnosis. Hepatic involvement resolves with antibiotic therapy.[54–56]

A mild hepatitis that is often subclinical is not infrequent with varicella. The increases in serum transaminases are about three times the upper limit of the reference range. The increase is transient, and the hepatitis usually follows a benign course.[46]

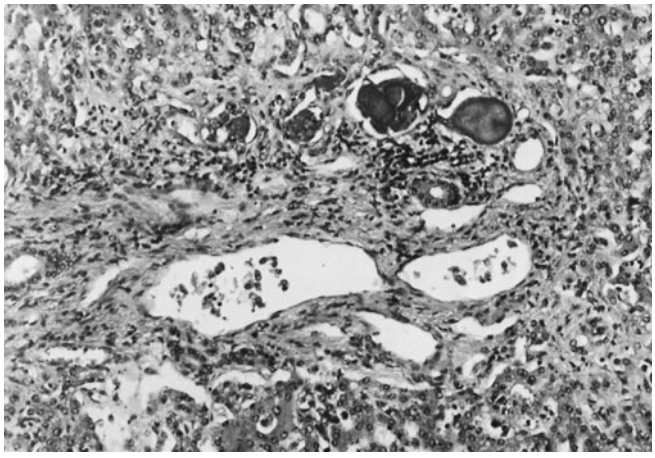

FIGURE 57-2 Cholangitis lente in the context of prolonged septicemia. Note severe cholestasis (hematoxylin and eosin; ×40 original magnification). Courtesy of Dr. L. Burgart, Mayo Clinic and Foundation.

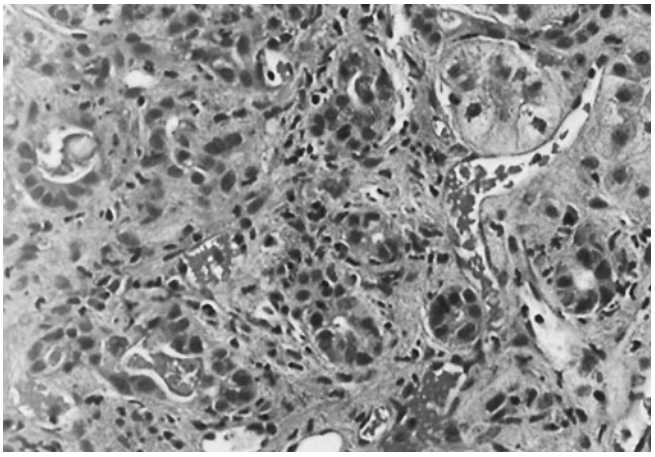

FIGURE 57-3 Cholangiolar cholestasis in the context of septicemia (hematoxylin and eosin; ×40 original magnification). Courtesy of Dr. L. Burgart, Mayo Clinic and Foundation.

CONNECTIVE TISSUE DISEASE

Liver involvement is often considered in the child with connective tissue disease for a number of reasons: (1) the child has a new-onset disease and autoimmune hepatitis is a consideration; (2) the child has connective tissue disease and involvement of the hepatobiliary system, usually evident as increases in transaminases and alkaline phosphatase and hepatosplenomegaly; and (3) the child is receiving medications that may impair liver function and questions arise as to the potential hepatoxicity of these medications.

Juvenile Rheumatoid Arthritis

Hepatosplenomegaly is not uncommon in juvenile rheumatoid arthritis (JRA). Approximately 10 to 15% of the children may have hepatomegaly sometime during the course of the disease. Splenomegaly is usually more prominent and occurs more frequently than hepatomegaly. The occurrence of splenomegaly with neutropenia and active systemic JRA characterizes Felty syndrome.[57] Hepatomegaly and increases in transaminases may occur with systemic JRA.[58,59] The histologic findings are nonspecific, showing Kupffer cell hyperplasia and focal hepatitis.[57–59] The occurrence of a hepatitis exacerbation is sometimes associated with improvement of the arthritis, but the mechanism is unknown.

Long-standing JRA, usually of more than 8 years, can be associated with amyloidosis.[60] The incidence is about 4% in adult series. Hepatosplenomegaly with proteinuria should increase awareness of amyloid deposition in multiple organs, but this complication is rare in pediatric patients.

Another feature is the asymptomatic increase in serum alkaline phosphatase and transaminases. The increase in alkaline phosphatase reported in the adult population is not as prevalent as in the pediatric population.[58] Some of this increase is from multiple sources, and some is from the inflamed synovium. The number of joints involved seems to

correlate with the increase in the alkaline phosphatase. More common, however, is the increase in transaminases, which has a dose-dependent association with aspirin therapy.[61] The patient with JRA who is receiving aspirin therapy is more susceptible to the development of Reye syndrome.[61–64] One may speculate about the existence of subtle mitochondrial fatty acid oxidation genetic defects that are triggered by particular environmental factors in this population. With the discovery of more mitochondrial errors of metabolism, it is possible that concomitant administration of toxic medications may unmask these conditions.

The use of aspirin has been largely replaced by the use of nonsteroidal anti-inflammatory drugs that are less toxic to the liver. However, the potential for an idiosyncratic reaction in the form of fulminant hepatic failure is possible.[65,66] Gold toxicity manifests as cholestasis; histologically, the liver shows cholestasis and deposits of gold particles in Kupffer cells. The liver may have a brown-black pigmentation. Fulminant hepatic failure has been reported with the use of gold compounds, but the use of this treatment is uncommon in the pediatric age group.

Methotrexate is increasingly used for JRA. Two patterns of liver involvement occur. One is a mild increase in liver enzymes, about three to four times the upper limit of the reference range, that occurs in 15% of patients.[67,68] The other is progressive fibrosis that may be clinically silent. The progression to fibrosis seems to be related to a cumulative methotrexate dose of more than 1.5 g. In pediatrics, our experience and the experience of others with small series is that fibrosis is not common, even when methotrexate doses exceed 1.5 g. In adults, the toxicity of methotrexate is significantly increased with obesity and alcohol consumption.[67–71]

Systemic Lupus Erythematosus

The liver is involved in up to 40% of patients with systemic lupus erythematosus (SLE). Hepatomegaly occurs in about one-third of the patients sometime during the illness. Increases in liver-derived enzymes occur in two contexts: with use of hepatotoxic medications and with use of aspirin, the most important culprit.[72–81] In addition, the use of corticosteroids may be involved with steatosis and the use of azathioprine with cholestasis.

The association of SLE and autoimmune hepatitis is well described in detail elsewhere.[73,74] The presence of autoimmune markers common to both diseases is not uncommon, and the liver lesion of SLE is indistinguishable from classic autoimmune hepatitis. This spectrum of liver disease fits into the overlap syndrome seen in adult patients who have features of autoimmune hepatitis and primary sclerosing cholangitis. The liver enzyme concentrations and the histologic changes improve rapidly with immunosuppression therapy.

The presence of a hypercoagulable state may lead to vaso-occlusive disease (VOD) of the liver or to Budd-Chiari syndrome. The association with lupus anticoagulant and anticardiolipin antibodies is well described.[78–82]

The liver may be involved in the transient abnormalities of neonatal SLE.[82,83] This occurs because of the transplacental transfer of antibodies from the mother to the fetus and is associated with anti-Ro and anti-La antibodies. The characteristic pattern is congenital heart block, dermatitis, and hematologic abnormalities. Cholestasis that resolves by 6 months of age has been described and is associated with portal fibrosis, bile duct obstruction, and inflammation. Any infant with a combination of cardiac arrhythmias and cholestasis should be evaluated for SLE, especially because the maternal disease may be asymptomatic and unrecognized.

Juvenile Dermatomyositis

The liver is seldom involved in juvenile dermatomyositis. However, the increase in the aspartate aminotransferase from muscular origin can masquerade as hepatitis early in the disease. Hepatosplenomegaly is not uncommon in the severe form of the disease. The use of methotrexate may be associated with increases in liver-derived enzymes, and the long-term consequences are often of concern.[66,72–74] The use of nonsteroidal anti-inflammatory drugs can add to the concern for hepatotoxicity.

Miscellaneous Connective Tissue Disease

The liver and spleen may be involved in mixed connective tissue disease, an overlap syndrome with features of SLE, scleroderma, and polymyositis.[82] The involvement is from generalized vasculitis, and usually there is hepatosplenomegaly. The vasculitis involves medium-size vessels, with intimal thickening and inflammation in the periportal areas.[84]

Hydrops of the gallbladder and increases in the liver enzymes occur in Kawasaki disease.[83] Hepatomegaly and increases in the liver enzymes occur in about one-third of the patients. The use of high doses of aspirin causes dose-dependent toxicity in the liver, which has occasionally led to severe hepatitis. Histologically, Kupffer cell hyperplasia, bile duct inflammation, and gallbladder wall thickening have been reported. Interestingly, hydrops of the gallbladder may respond to therapy with low doses of nonsteroidal anti-inflammatory drugs.

HEMATOLOGIC DISORDERS

Sickle Cell Disease

Depending on the definition and the diagnostic methods used, the incidence of liver disease in sickle cell (SC) disease varies. If one uses abnormal concentrations of liver-derived enzymes as an indicator, liver disease may be present in up to 65% of the patients. If histologic criteria are used at autopsy, the liver is universally involved in SC disease.[84,85]

Several issues are often considered in patients with SC disease: (1) the distinction of acute crises from acute gallbladder disease, (2) the contribution of cardiac dysfunction to acute SC disease crises, (3) the chronic overload of iron and bilirubin in the liver and in other organs, and (4) the association of chronic hepatitis from contaminated blood products in patients with SC disease.

Any of the following factors lead to liver injury: (1) the increased hemolysis adds to the development of unconju-

gated hyperbilirubinemia[85]; (2) the increased concentrations of bilirubin and turnover lead to increased risk of pigment gallstones and acute and chronic gallbladder disease; (3) the additional transfusions increase the risk of bloodborne infections and contribute to the iron overload; iron overload with or without anemia may lead to cardiac dysfunction and secondary liver congestion; and (4) the liver may have repeated episodes of ischemic crises; the reticuloendothelial system is the first to be affected by the iron overload, and the hepatocytes are affected later. In severe cases, brown pigmentation of the hepatocytes is seen with hematoxylin and eosin stain.[84,86]

Acute Hepatic Crisis

The liver is involved in an acute hepatic crisis in about 10 to 15% of patients with SC disease.[87,88] Pain in the right upper quadrant of the abdomen, tender hepatomegaly, increases in serum transaminases, and conjugated hyperbilirubinemia develop. Fever is common. The results of coagulation studies are normal. The distinction of SC hepatopathy from acute cholecystitis may be difficult. The biliary tract–derived enzymes and serum transaminases may be equally increased. Conjugated hyperbilirubinemia is common, and concentrations may vary from 15 to 50 mg/dL. The transaminases are increased up to 10 times the upper limit of the reference range. An increase in lactic dehydrogenase is common and reflects ongoing hemolysis. Imaging of the intrahepatic and extrahepatic bile ducts is important and may help in the differential diagnosis. The difficulty arises when there is a hepatic crisis with incidental cholelithiasis. Careful observation and supportive therapy may lead to a rapid resolution of liver abnormalities, and a lack of dilated bile ducts and normal concentrations of pancreatic enzymes help in determining the course of the disease. Occasionally, a liver biopsy is indicated. The classic sinusoidal congestion, sickling, Kupffer cell hyperplasia, and erythrophagocytosis are characteristic of SC hepatopathy.

Acute and Chronic Cholecystitis

Acute cholecystitis and choledocholithiasis in SC patients are similar to those conditions in other patients.[87,89–95] The triad of abdominal pain and tenderness in the right upper quadrant, fever, and increased serum biochemical biliary tract inflammatory markers helps in the diagnosis. Imaging studies show dilatation of the biliary tree in some patients. Others with hepatic crisis improve rapidly.

The most difficult diagnostic decisions involve conditions without a clear cause. Intrahepatic or endoscopic imaging of the biliary tree is then essential. Endoscopic imaging can be coupled with therapeutic maneuvers and may obviate the need for operative intervention. Endoscopic treatment of complications should be considered in centers with expertise in therapeutic endoscopic retrograde cholangiopancreatography.

Chronic pigment gallstones occur in 70 to 80% of patients with SC disease.[87,89–95] The incidence increases with age and is related to the increased concentrations of bilirubin from hemolysis. The consultant is often asked to consider whether an incidental cholelithiasis needs to be addressed. If the child is well and there are no significant symptoms or increases in liver enzymes, careful observation and awareness of the finding are important if pancreatitis or choledocholithiasis occurs.

CHRONIC IRON OVERLOAD

Repeated transfusions result in chronic iron overload (Table 57-2). Iron overload is most common in thalassemia.[85,96] The identification of genetic markers for hemochromatosis is challenging because the development of iron toxicity in this population is variable and occurs at a later stage in life. When thalassemia and hemochromatosis occur in the same patient, it can be difficult to determine the best method of following iron overload. There are no known reports of associated diseases (Figures 57-4 and 57-5). However, hepatitis correlated with the accumulation of more than 300 μg of iron per gram of liver tissue, presumably as a result of oxidative injury.[97] Therefore, in patients with multiple transfusions, increases in the liver enzymes may be a manifestation of iron overload, requiring measurement of ferritin and histologic confirmation with iron stains of the liver. This would also exclude other infections and vascular hepatic insults.

Chronic Viral Hepatitis Complicating Iron Overload States

Chronic viral hepatitis occurs in about 25 to 30% of patients with SC disease.[94,98] Screening for hepatitis C virus has reduced the risk of transfusion-associated infection. In a series of 99 patients, 23% had evidence of hepatitis C virus infection.[94] The risk of infection is related to the frequency of blood transfusions, and multiple transfusions may result in repeated infections with different genotypes. The frequency of transfusions, combined with chronic liver disease, may complicate the response to treatment. There are no reports that clarify the optimal antiviral therapy and the response to therapy for this population.

Thalassemia

The transfusion requirements of patients with thalassemia lead to iron overload and eventually to liver fibrosis and cirrhosis. The risk of viral infection is high in this patient population, and the patients who received transfusions before the availability of screening for hepatitis C virus have a high incidence of infection. Histologic findings in the liver are nonspecific and may show the characteristic features of hepatitis C infection.[94,98]

TABLE 57-2 CHRONIC IRON OVERLOAD

Arrhythmia
Congestive heart failure
Bronzed skin
Hepatomegaly
Splenomegaly
Diabetes
Increased susceptibility to *Yersinia* infection

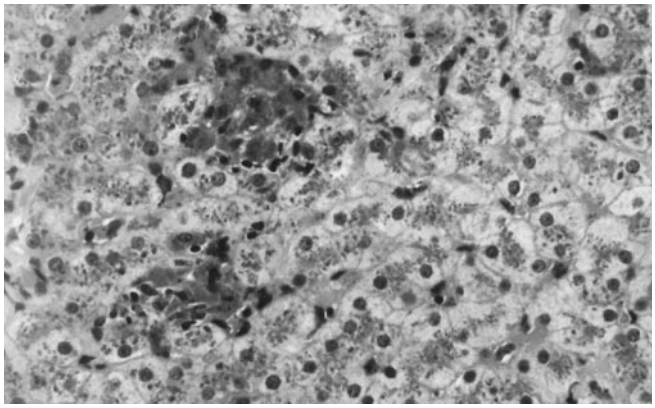

FIGURE 57-4 Severe iron overload with sickle cell disease iron content 23,000 µg/g of tissue (hematoxylin and eosin; ×40 original magnification). Courtesy of Dr. L. Burgart, Mayo Clinic and Foundation.

Infection with hepatitis C virus constitutes an important cause of morbidity in patients with β-thalassemia.[99] The patient may be infected more than once and with more than one strain of the virus. The combination of iron overload and viral burden may accelerate the progression to fibrosis.

Coagulation Disorders

The use of contaminated blood products, especially before 1990, has resulted in a high incidence of hepatitis C infection in patients with hemophilia.[94,98] In this population, the liver is commonly involved, and increases in the liver-derived enzymes are not unusual. Because the patients have had multiple transfusions, infection may have occurred through more than one genotype. The results of therapeutic trials in this population are pending at this time.

Budd-Chiari Syndrome

Budd-Chiari syndrome can be a manifestation of either coagulation disorders or vascular diseases affecting the liver (Table 57-3). Because coagulation disorders are associated with this syndrome, it is another example of how the liver is involved in hematologic diseases. Yet, worldwide, membranous obstruction of the hepatic veins is the most frequent cause of this syndrome.[100]

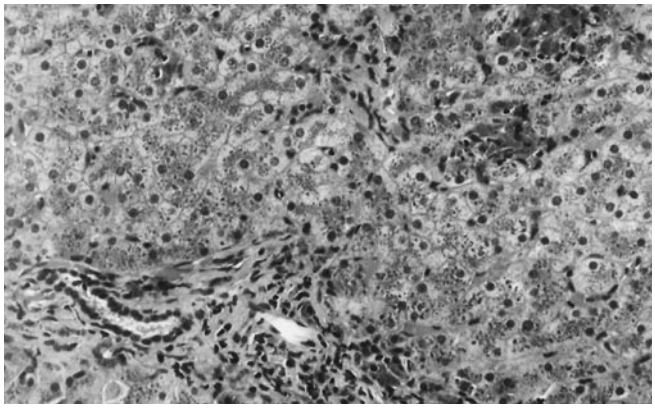

FIGURE 57-5 Severe hemosiderosis with sickle cell disease (hematoxylin and eosin; ×40 original magnification). Courtesy of Dr. L. Burgart, Mayo Clinic and Foundation.

The acute form is characterized by the sudden onset of abdominal pain in the right upper quadrant, tender hepatomegaly, and ascites. The acute event leads to mild increases in the liver enzymes, and in a similar fashion to cardiac congestion, the increases in enzymes reflect zone 3 involvement. Prolongation of the prothrombin time is common, but this finding may be confusing if a hypercoagulable state is present.[101,102]

Acute events may result from thrombosis of the hepatic veins as a result of protein C deficiency, antithrombin III deficiency, mutations in the genes for factor V Leiden and thrombin, polycythemia vera, primary lymphoproliferative disorders, inflammatory bowel disease, paroxysmal nocturnal hemoglobinuria, Behçet syndrome, and collagen vascular diseases, including those associated with anticardiolipin antibodies and lupus anticoagulant.

Acute obstructive lesions are usually due to tumor invasion from adrenal and renal cancers. Occasionally, patients who have hepatic tumors that have spread beyond the liver may present with acute Budd-Chiari syndrome.

Chronic Budd-Chiari syndrome is usually due to membranous obstruction of the hepatic veins, a condition common in developing countries. The lesion may be congenital or, perhaps more commonly, due to thrombosis. Thrombosis is often found histologically, but it is difficult to know whether it is a consequence of congenital occlusion. The increases in the liver-derived enzymes are mild, and coagulation is normal. Progression of the disease leads to manifestations of portal hypertension and liver failure, including ascites, hepatic encephalopathy, and variceal bleeding. Treatment of membranous occlusion is by invasive radiologic techniques. Advanced liver disease can be corrected with transplant, although recurrence of disease in the transplanted liver is possible, especially when polycythemia or primary thrombotic conditions are present.[100–120]

The diagnosis of Budd-Chiari syndrome is made with the use of several types of imaging techniques, from Doppler ultrasonography to magnetic resonance imaging. Hypertrophy of the caudate lobe is a peculiar feature found on technetium uptake because of the differential drainage of this lobe. The caudate lobe drains directly into the inferior vena cava and is enlarged because of hypertrophy from portal hypertension in Budd-Chiari syndrome.[104,118,121–124]

A dual approach is used to manage Budd-Chiari syndrome: identify the original insult and treat the present

TABLE 57-3 BUDD-CHIARI SYNDROME

Membranous obstruction of the hepatic veins
Idiopathic hypercoagulable states
Contraception
Pregnancy
Inflammatory bowel disease
Myeloproliferative disorders
Paroxysmal nocturnal hemoglobinuria
Chronic cardiac congestion
Renal and suprarenal tumor invasion
Behçet syndrome

condition. Diagnosis of hypercoagulable states should be sought and the evaluation for polycythemia pursued vigorously to prevent recurrence of vascular compromise. If the liver shows only congestion and the response to diuretic therapy is good, anticoagulation may be attempted. If a subsequent liver biopsy shows persistent congestion, only then can medical therapy be continued. If severe necrosis is present, surgical shunting is offered. If fibrosis is present, medical therapy and evaluation for liver transplant are initiated.[112–115,121]

Vascular Diseases

The dual supply of blood to the liver is a fundamental difference between it and other organs.[116,117,125] Development of a liver infarct requires that occlusion to both the arterial and the venous supplies occurs simultaneously. Occasionally, occlusion of the hepatic and portal veins occurs, leading initially to loss of hepatocytes and subsequently to bridging fibrosis. After atrophy of the hepatocyte mass has occurred, compensatory hypertrophy occurs in the remaining liver tissue.[126] Depending on the location and the extent of vascular compromise and the reparative adaptation of the liver, several clinicopathologic entities may occur. In noncirrhotic portal hypertension, reactive nodular hyperplasia and idiopathic portal hypertension accompany the liver cell atrophy. The liver function is normal,[126–135] and the liver is characteristically normal in size.

Nodular hyperplasia of the liver occurs with systemic diseases (Table 57-4). The hyperplasia occurs in different forms, depending on the extent of vascular compromise. In large nodular hyperplasia, extensive areas of the liver are affected, and several large nodules are present. In regenerative nodular hyperplasia, the liver has many small nodules affecting multiple areas of the liver. Large nodules are not accompanied by portal hypertension unless they become numerous. These large lesions occur with congenital anomalies of the portal vein, such as absence of the portal vein, patent ductus venosus, or large arteriovenous shunts. A recent report of focal nodular hyperplasia mentions its association with multiple clonal chromosomal aberrations.[136] The patient presents with silent hepatomegaly owing to focal nodular hyperplasia, and the diagnosis is established by imaging studies and analysis for clonal abnormalities in the mesenchymal origin of the vascular stroma.

When the vascular supply is severely impaired, leading to significant and mainly venous injury, cirrhosis may be the end result. This leads to the loss of hepatocyte function, marginal blood supply, capillarization of sinusoids, and various degrees of cholestasis.

TABLE 57-4 NODULAR REGENERATIVE HYPERPLASIA

Juvernile rhumatoid arthritis
Systemic lupus erythematosus
Polyarteritis nodosa
Glomerulonephritis
Cryoglobulinemia
Antiphospholipid syndrome
Chronic cardiac congestion
Pulmonary hypertention
Portal vein thromobosis
Persistence of ductus venosus
Contraception

The involvement of hepatic arteries in systemic conditions is not uncommon. The dual supply of the liver is protective for the most part; however, arterial compromise affects the large intrahepatic bile ducts, and stricture formation secondary to biliary necrosis is a well-recognized complication of hepatic artery injury, whether surgical or thrombotic.

One report describes ischemic necrosis of bile ducts complicating Henoch-Schönlein purpura owing to hepatic vasculitis. The vasculitis affected the integrity of the biliary system and resulted in biliary cirrhosis, requiring transplant.[137]

Research into the biology of the endothelial cell has led to several conceptual advances: (1) the identification of endothelin, a potent vasoactive peptide; (2) the identification of overproduction of endothelin in the injured liver; and (3) the elucidation of the synthesis and function of nitric oxide.[13–18] The recognition that endothelin and nitric oxide are produced by endothelial cells and that they have dramatic effects on the surrounding stellate cells and on the microvasculature is shedding new light on the function of these cells in the development of portal hypertension. The recognition of the importance of the endothelial cell during ischemic liver injury and its targeting by host lymphocytes during organ rejection has become critical to our understanding of the cellular and molecular events in liver transplant and disease.[10–17]

MALIGNANCIES

Leukemia

Hepatosplenomegaly is common in leukemia. About one-third of patients with leukemia have clinical and biochemical abnormalities sometime during their illness. One rare presentation of neonatal leukemia is liver failure, and biopsy results are usually needed for the diagnosis. The response to chemotherapy is dramatic. The histologic finding is leukemic infiltration of the liver by malignant cells (Figure 57-6).[138]

Increases in liver-derived enzymes occur often, especially during therapy, but the increase is transient. In our experience, several patients continued to have abnormal serum concentrations of liver enzymes while they received low-dose maintenance therapy with azathioprine. The increases in liver enzymes correlated with the metabolic activity of thiopurine methyltransferase.

Neutropenia predisposes the patient to opportunistic infections, and severe hepatitis from adenoviral infection has been reported in this population. More ominous is fungal infection, for which the only clues may be hepatomegaly, increases in liver enzymes, and fever.[139,140]

Hodgkin Lymphoma

The extent of liver involvement in Hodgkin disease varies with the timing of the evaluation.[141–146] Whereas the liver is clinically involved in about 5% of the patients at pre-

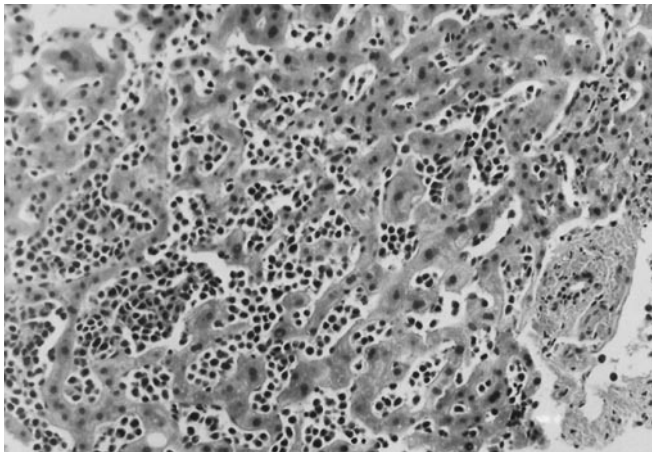

FIGURE 57-6 Liver involvement by leukemia (hematoxylin and eosin; ×40 original magnification). Courtesy of Dr. L. Burgart, Mayo Clinic and Foundation.

sentation, evidence of liver involvement is found in 50% at autopsy.

Histologic findings show either direct involvement of the liver or nonspecific inflammation and infiltration and noncaseating granulomas. Reed-Sternberg cells are found in about 25% of the cases. More commonly, nonspecific liver inflammation is found in about 50% of the cases. The detection rate of Reed-Sternberg cells is higher with laparoscopic biopsy than with percutaneous biopsy. Noncaseating granulomas, found in about 25% of the cases, are not considered to be part of the direct involvement of the liver in Hodgkin disease.

The most commonly increased biliary tract enzyme in the adult population is alkaline phosphatase, showing a correlation with disease stage. The fact that this enzyme is often elevated owing to normal bone growth in healthy children makes it a less useful indicator in pediatric patients than in adults.

Jaundice occurs with direct liver involvement. Obstructive lesions of the biliary tree are much less common. There are reports of vanishing bile duct syndrome occurring in patients with Hodgkin lymphoma. Whether this syndrome represents a variant of Hodgkin presentation or coincidental occurrence of primary sclerosing cholangitis is not clear.

Langerhans Cell Histiocytosis

The liver is involved in two large groups of disorders of histiocytes. Langerhans cell histiocytosis (LCH) refers to the group that includes eosinophilic granuloma, Letterer-Siwe disease, Hand-Schüller-Christian syndrome, Hashimoto-Pritzker syndrome, and histiocytosis X.[147,148]

This group of disorders is characterized by abnormal infiltration of various tissues with phagocytic mononuclear histiocytes with particular markers. These markers indicate the dendritic lineage of the Langerhans cells that infiltrate various organs. The definitive diagnosis is determined by the presence of Birbeck granules on electron microscopy. The demonstration of T-6 antigens on the surface of cells with tissue involvement is also diagnostic. In addition, the finding of CD1a antigen on the surface of histiocytes is a relatively easy way to demonstrate the cell lineage in paraffin-embedded tissue specimens. Although the presence of the CD1a marker is not 100% specific for LCH, the procedure is rapid and easy.[149]

The other group of disorders that involve histiocytes but not of the Langerhans cell type includes the familial hemophagocytic lymphohistiocytosis and the infection-associated hemophagocytic syndromes.[149–152]

Depending on the degree of organ involvement, LCH is categorized as involving either one site or multiple sites in a single system or as being multisystemic. In multisystemic LCH, patients are classified as having either organ involvement alone or organ involvement with dysfunction.[149]

Liver involvement in LCH is mostly a manifestation of multisystemic disease. The patient may have the characteristic skin lesion with diaper dermatitis, drainage from the external auditory canal, or hepatosplenomegaly. Other presentations include gastrointestinal tract bleeding and protein-losing enteropathy. The liver is almost universally involved with gut disease and is involved in up to 30% of patients with LCH. The spectrum of liver disease includes acute hepatitis, severe biliary cholestatic liver disease, and silent hepatomegaly with incidental increases in serum liver enzymes. The histologic findings vary from liver infiltration with histiocytes to destructive sclerosing cholangitis, in which the biliary enzymes are often increased, but jaundice is not prominent. The progression of the liver lesion does not parallel the response of the skin and other organs to chemotherapy. Cirrhosis may also be the presenting manifestation, especially when the disease progresses silently.[147–153] The progression to biliary cirrhosis is rapid compared with other causes of biliary cirrhosis, such as primary sclerosing cholangitis. Liver disease in LCH is progressive despite successful treatment of skin and bone lesions. After cirrhosis develops, the progression appears to be relentless.

The important point to remember is that patients with LCH require careful monitoring of liver involvement. A false sense of security should be avoided when the extrahepatic manifestations of the disease are controlled.

In a review at a single center, 75% of the neonatal patients with LCH presented with multisystemic involvement.[148] The characteristic skin lesion and the extent of the skin lesions did not correlate with systemic disease, and mortality was higher in this age group than in older children. Overall, the liver is involved in 30% of patients with LCH, but it is involved in 50% of patients with multisystemic disease. In patients with liver disease, only 25% have jaundice.[154] The others have hepatomegaly with or without increases in the liver enzymes. In a few patients, the onset of liver disease occurs well after successful treatment of skin and extrahepatic disease.

As expected, the outcome of LCH is dependent on the extent of disease at presentation. However, the progression of histiocytic infiltration of organs and the response to therapy in individual cases cannot always be predicted. A report has shown a clear association between the level of soluble interleukin-2 receptor and the extent of LCH. This association may be useful for monitoring a patient's

progress and possibly monitoring the response to chemotherapy. Interestingly, there seems to be activation of the tumor necrosis factor pathway in tissues with histiocytic infiltration, and one report showed a good response to the use of tumor necrosis factor receptor:Fc-fusion protein with regression of multisystemic invasion.[149] If confirmed in more cases, this report may pave the way for treatment of disease before pathologic changes in the liver become irreversible and the only option is liver transplant.

A key feature of the management of LCH is close cooperation between hematologists and hepatologists. The patient should have frequent and focused examinations for hepatomegaly and for screening laboratory values of hepatic and biliary enzymes. The use of magnetic resonance imaging of the liver and the biliary tree seems to be promising for identifying liver disease and biliary destruction. However, changes that are detectable by this technique may be indicative of late infiltration rather than early infiltration. Therefore, histologic examination of tissue remains an important tool for confirming and analyzing liver involvement.

Liver transplant has been successful for treatment of this disease. However, a review of the transplant experience in LCH patients has shown some that there is an increased risk of lymphoproliferative disease developing post-transplant,[155] perhaps owing to an altered immune status. This speculation is based on the demonstration of increased levels of interleukin-1, tumor necrosis factor, granulocyte-macrophage colony-stimulating factor, and soluble interleukin-2 receptor. Recurrence of LCH in the transplanted organ and extrahepatic sites is reported to occur in up to 33% of the patients. Only recently has it been shown that the graft is susceptible to LCH invasion.[155] Despite chemotherapy, the disease seems to be progressive in transplants, as well as in native livers. Finally, acute rejection is nearly universal among patients who receive transplants for LCH. Patients with certain liver conditions characterized by altered immune status, such as LCH and primary sclerosing cholangitis, who undergo liver transplant may require a different immunosuppressive regimen or may develop significant morbidity later, such as post-transplant lymphoproliferative disease or exacerbation of concomitant inflammatory bowel disease.[156,157]

Hemophagocytic Syndrome

Hemophagocytic syndrome (also called hemophagocytic lymphohistiocytosis), is a rare disease affecting infants and children. The hallmark of the disease is the accumulation in the reticuloendothelial system of lymphohistiocytes with features of hemophagocytosis. The diagnosis is based on a set of clinical and laboratory criteria (Table 57-5).

The liver is often involved in both infection-induced and familial hemophagocytic syndromes.[147–150] The sporadic form is a reactive proliferation of the lymphohistiocytic lineage, affecting multiple organs in reaction to a viral, bacterial, or fungal infection. The activated lymphocytes are benign, and there is evidence of hemophagocytosis. The most frequently associated infection is related to Epstein-Barr virus. The immune systems in children with hemophagocytic lymphohistiocytosis seem to lack the ability to control infections; they react with an uncontrolled inflammatory response with sustained activation of macrophages and T lymphocytes. Evidence of proliferation is present in the peripheral blood and numerous organs. Fever and hepatomegaly and other organomegaly are present. Concentrations of serum liver enzymes are abnormal in 80% of the patients. The lesion resolves with prompt resolution of the original infection in sporadic cases. The familial form is almost universally fatal without stem cell transplant.

The inheritance of the familial form is autosomal recessive. The condition occurs in the first 3 months of life and has a poor prognosis. The infant has hepatosplenomegaly, fever, and weight loss. Anemia, thrombocytopenia, hyperlipidemia, and low concentrations of fibrinogen are features of this disease as well. Neurologic involvement may also be present, manifesting as irritability, convulsions, meningitis, and altered consciousness.[158]

The liver and other organs are infiltrated with malignant histiocytes. The best area for the documentation of the diagnosis is the bone marrow because the liver lesion may show a nondiagnostic lymphocytic hepatitis without phagocytosis. Hyperlipidemia is an important feature of this condition and may help in the diagnosis. Acute fulminant hepatic failure mimicking neonatal hemochromatosis has been reported in two cases. In patients with hemophagocytic syndrome, the ferritin level may be increased, so the disease may be confused with neonatal iron storage disease. Ultrasonographic findings show hepatomegaly, thickening of the gallbladder wall, and increased periportal echogenicity. There is a report of one case successfully treated with intravenous administration of cyclosporine.[159] In one report, a deficiency in perforin, an important mediator of lymphocyte cytotoxicity, was shown to be present in familial hemophagocytic syndrome.[157]

Whereas the sporadic form occurs as a result of immunologic overactivity, as in Epstein-Barr virus–related hemophagocytic lymphohistiocytosis, the familial form is due to malignant transformation. Therefore, in the sporadic form, immunosuppression is the mainstay of therapy, whereas in the familial form, immunomodulation and immunosuppression of T-lymphocyte activation are performed in preparation for stem cell transplant as the definitive treatment.[160]

The gene encoding for perforin has been mapped to 10q22 and is mutated in familial LCH.[155] Perforin is a protein expressed in lymphocytes, macrophages, and bone marrow precursors. Its main role is the formation of pores

TABLE 57-5 CLINICAL AND LABORATORY CRITERIA FOR THE DIAGNOSIS OF HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS

Fever and hepatosplenomegaly
Cytopenia in 2 of 3 lineages
Hypertriglyceridemia or hyperfibrinogenemia or both
Hemophagocytosis in bone marrow, spleen, or lymph nodes
Absence of malignancy

in target cells. After this occurs, the disrupted membrane of the target cell allows the entry of granzymes that trigger apoptosis. Perforin-deficient mice cannot lyse target cells and have an impaired defense against cancer and intracellular pathogens.[161] In combination with the defective aspect of antigen-presenting cells lacking perforin, this leads to several disrupted immune pathways that combine to cause the clinical manifestations of the disease.

The familial form is autosomal recessive, with an incidence estimated at 0.12 per 100,000 children.[154] Despite the classic description of sporadic cases occurring with an infection, 50 of 122 children reported to the registry for hemophagocytic lymphohistiocytosis had evidence of infection; of those, 25 had a positive family history and characteristics that were similar to those of the other 25 sporadic and nonfamilial cases.

Finally, Griscelli syndrome, a rare condition characterized by partial albinism and immunodeficiency, is also associated with perforin mutation; therefore, patients may present with hemophagocytosis with liver involvement.[162]

Bone Marrow Transplant

The liver is involved in several ways in patients undergoing bone marrow transplant. Chronologically, liver involvement occurs during the conditioning regimen; during the period of marrow ablation and pancytopenia, immediately after engraftment; and 6 months after transplant.

When malignancy recurs, the liver may be affected by the original condition that led to the transplant. The liver may become one of the sites of opportunistic infections, or it may be injured by various medications. Prolonged fasting and the use of TPN may contribute significantly to the pathophysiology of liver disease in bone marrow transplant patients.

The hepatopathy of bone marrow transplant varies from fulminant failure owing to viral infections, such as herpes simplex and adenovirus, to chronic cholestasis from graft-versus-host disease (GVHD) and prolonged nutritional support (see Table 57-4).

Veno-occlusive Disease

The triad of hepatomegaly, increased liver enzymes, and ascites describes VOD owing to obstruction of the terminal hepatic venules. Originally, VOD was described in patients exposed to pyrrolizine alkaloids (Table 57-6). VOD of the liver occurs in about 20 to 25% of the patients undergoing bone marrow transplant.[152,153,157] The condition is more likely to occur with allogeneic transplants than with autologous transplants. It is more common in patients who undergo transplant for malignancies, such as leukemia, than for aplastic anemia. Preexisting liver disease, total-body irradiation, and the intensity of the conditioning regimen contribute to the endothelial injury and the development of VOD. Low levels of pseudocholinesterase, a marker of hepatic synthetic function, correlate positively with the development of liver disease, and levels of proteins C, S, and antithrombin III correlate inversely with the development of VOD.[155,163,164]

Diagnostic criteria for VOD include onset within 20 days after transplant, weight gain of 2 to 10%, hepatomegaly, ascites, and a serum concentration of bilirubin greater than 2 mg/dL. Using these criteria, the frequency of VOD after bone marrow transplant ranges from 1 to 50%, with a mortality rate of 3 to 50%. Poor outcome is correlated with the severity of hyperbilirubinemia and the presence of multiorgan failure.[165–167]

The pathophysiologic process of VOD is presumed to be direct injury to the endothelium of the central hepatic veins. The injury is often associated with zone 3 necrosis from irradiation. The propensity of zone 3 hepatocytes to injury is multifactorial and is related to the relatively low oxygen tension in this area and the concentration of drug metabolism pathways. Glutathione depletion in this area leads to increased injury, a process that may be prevented by administration of glutamine. The combination of radiation injury and tissue hypoxia seems to involve the endothelial cells of the terminal hepatic venules and to initiate local thrombosis that potentiates congestion and hypoxia. Eventually, significant fibrosis and collagen deposition around occluded central veins lead to the clinical triad of weight gain, jaundice, and hepatomegaly that manifests between the second and sixth week after transplant. There is considerable evidence to implicate intravascular thrombosis in the early events leading to VOD.[168] The hypothesis is that reactive compounds are produced by zone 3 hepatocytes that are directly toxic to the endothelial cell. The endothelial cell injury leads to intravascular thrombosis, thereby potentiating the initial insult and leading to activation of the stellate cell. Persistent activation of the stellate cell, the principal fibrogenic cell in the liver responsible for the accumulation of collagen, is also evident from studies of autopsy cases.

VOD is characterized by fibrous intimal thickening or occlusion of hepatic venules less than 0.3 mm in diameter.[169–171] Occasionally, lesions and significant congestion are seen in larger veins (Figures 57-7 and 57-8).

Treatment for moderate and severe VOD consists of infusion of tissue plasminogen activator and transjugular intrahepatic portosystemic shunting, which may be beneficial in the early stages of the disease. In uncontrolled studies, heparin showed some efficacy.[172] Early detection is crucial. Abdominal girth measurement and twice-daily measurement of body weight are essential for the initiation of therapy. Jaundice and frank ascites are ominous signs. In about 50% of the patients, the disease resolves slowly. Fatal

TABLE 57-6 VENO-OCCLUSIVE DISEASE OF THE LIVER

Pyrrolidine alkaloids
Aflatoxins
Cyclophosphamide
Azathioprine and 6-thioguanine
Vincristine
Busulfan
Hypervitaminosis A
Cysteamine
Immune deficiency
Estrogens
Pregnancy

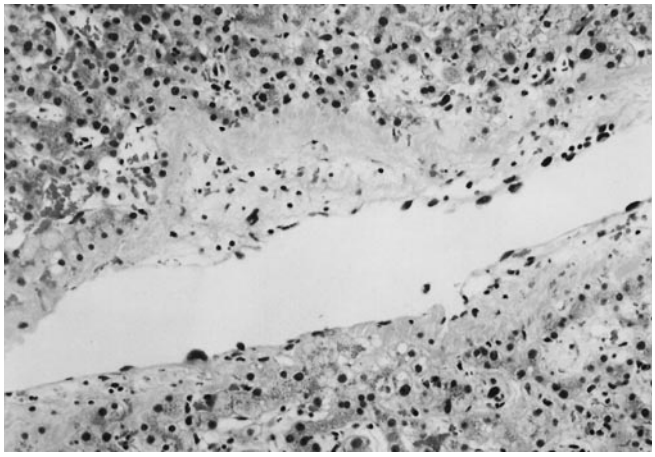

FIGURE 57-7 Veno-occlusive of the liver. Note wall thickness of hepatic venules (hematoxylin and eosin; ×40 original magnification). Courtesy of Dr. L. Burgart, Mayo Clinic and Foundation.

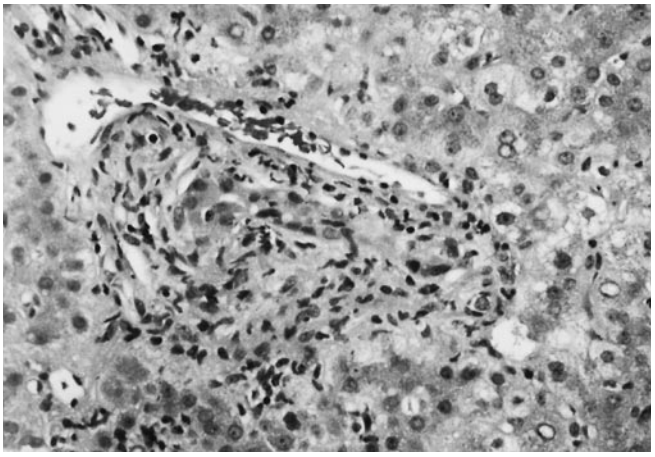

FIGURE 57-9 Graft-versus-host disease after bone marrow transplant (hematoxylin and eosin; ×40 original magnification). Courtesy of Dr. L. Burgart, Mayo Clinic and Foundation.

outcome is not uncommon, and liver transplant for VOD has been attempted.[171]

Graft-versus-Host Disease

Chronic GVHD occurs in up to 70% of the patients after bone marrow transplant. The disease most commonly affects the skin, liver, and gastrointestinal tract, but other organs and organ systems are affected as well. Isolated involvement of one organ system, such as the liver, is not uncommon. Chronic GVHD, by definition, occurs 100 days after transplant.[173,174] The disease may occur earlier, and in about one-third of the patients, it occurs after acute GVHD. The predisposing factors to GVHD include acute GVHD and the degree of human leukocyte antigen (HLA) mismatch.[173–182] The portal tracts are enlarged, and there is variable lymphocytic infiltration. Long-standing GVHD results in the vanishing bile duct syndrome and eventually may lead to cirrhosis. Treatment consists of administration of immunosuppressive medication, ursodeoxycholic acid, and thalidomide (Figures 57-9 and 57-10).

Acute GVHD may occur when an immunocompromised host receives immunocompetent T cells.[183] The condition has been described in patients after solid organ or stem cell transplant, in immunocompromised patients receiving unirradiated blood, and in newborns with hemolytic disease after exchange transfusion.[184,185] Acute GVHD, which occurs 3 to 6 weeks after the transplant, is characterized by anorexia, nausea, vomiting, and, occasionally, profuse diarrhea. A maculopapular rash is also common. Hepatocyte injury and cholestasis are common; however, severe insufficiency is rare. Histologically, the liver shows segmental destruction of small bile ducts in association with mononuclear infiltration. Rectal and skin biopsy findings may help in the diagnosis and the exclusion of hepatotoxicity and viral infections. Immunosuppression is the treatment of choice.

NUTRITIONAL DISORDERS

Malnutrition

The liver is affected in all forms of malnutrition.[186–189] Chronic starvation results in depletion of glycogen stores,

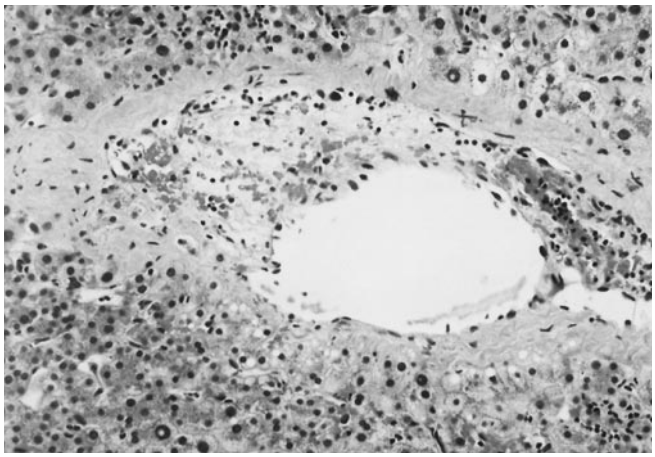

FIGURE 57-8 Idiopathic veno-occlusive disease of the liver (hematoxylin and eosin; ×40 original magnification). Courtesy of Dr. L. Burgart, Mayo Clinic and Foundation.

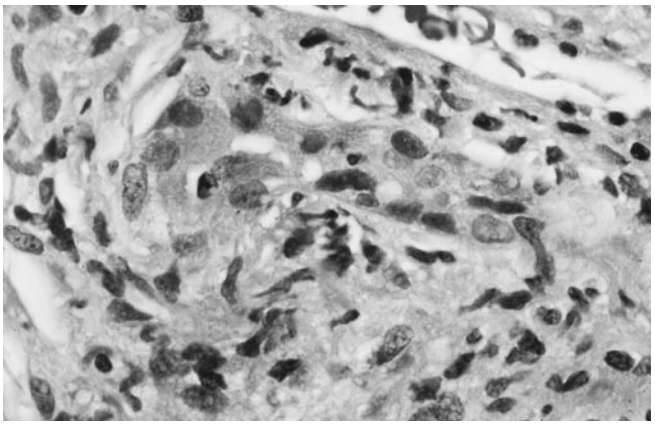

FIGURE 57-10 Hepatic graft-versus-host disease, higher magnification. Note inflammatory infiltrate (hematoxylin and eosin; ×160 original magnification). Courtesy of Dr. L. Burgart, Mayo Clinic and Foundation.

reduced neoglycogenesis owing to reduced protein stores, and production of ketones as fuel for cardiac muscle and the central nervous system.

Chronic malnutrition leads to the mobilization of free fatty acids even when the intake of carbohydrates is small. The free fatty acids are mobilized from the periphery and are not oxidized effectively in the liver; eventually, fat accumulates in the hepatocytes with resultant macrovesicular steatosis. Kwashiorkor, in particular, is associated with severe steatosis, whereas marasmus is associated less often. The livers of patients in prolonged starvation have decreased numbers of peroxisomes, and their carnitine stores are depleted, which are two factors that contribute to lipid metabolic abnormalities.[188–190]

Deficiencies of trace metals, such as zinc and selenium, may contribute to liver injury. Zinc is a cofactor in several enzymatic reactions and is considered to be an antioxidant. Selenium may function as an antioxidant as well.

Among children who have undergone starvation, chronic infections and bacterial overgrowth are often present and are presumed to contribute to liver disease in some way. In developing countries, the association of parasitic infestations, such as ascariasis, that may invade the biliary tree is well known.

Fatty Liver, Obesity, and Nonalcoholic Steatohepatitis

Fatty liver occurs with obesity, diabetes mellitus (especially type 2), jejunoileal bypass, hypertriglyceridemia, and certain metabolic diseases, such as galactosemia, tyrosinemia, and hereditary fructose intolerance (Table 57-7). Mitochondrial fatty acid oxidation defects and peroxisomal disorders also cause fatty liver. Rare causes of fatty liver include cholesterol ester storage disease and neutral lipid storage disease. Fatty liver can also be a feature of drug toxicity because the drugs undergo phase I metabolism through the cytochrome P-450 enzyme system. In cases of drug toxicity, mitochondrial function is presumably affected, resulting in microvesicular steatosis. Except for the known metabolic causes of fatty liver, nonalcoholic steatohepatitis (NASH) is considered to be the most severe form of nonalcoholic fatty liver.[190–210]

The normal liver is 5% fat by weight, in the form of triglycerides, cholesterol, cholesterol esters, and fatty acids. In steatosis, the liver may be 40% fat. The metabolism of fat depends on various factors, including the presence of fed or fasting states and the hormonal conditions, such as insulin and glucagon balance, adrenergic tone, and concentrations of thyroid hormones. During the fed state, the combination of triglycerides from chylomicrons and insulin and the activation of the parasympathetic system contribute to the accumulation of lipids in the adipose tissue and liver. During the fasting state, glucagon is released in response to the sympathetic nervous system, resulting in the increased formation of free fatty acids, which are released into the circulation as fuel for muscle and the brain.

The liver is crucial not only in energy metabolism but also in hormonal homeostasis. During excessive food intake, the liver stores fats, and steatosis may develop. During chronic starvation, fats are mobilized from the periphery, but oxidation in the liver is lacking, and steatosis is a hallmark of kwashiorkor, as previously mentioned. The same processes that occur during chronic starvation occur with prolonged use of TPN, especially if the administration of nutrients is continuous. In diabetic patients who have newly diagnosed diabetes, who are noncompliant, or whose diabetes is poorly controlled, accumulation of fat in the liver is common. The use of corticosteroids likewise affects the metabolism of fats, and steatosis is a common occurrence during prolonged administration.

Many metabolic disorders are associated with steatosis.[201–205,208,209] Classic Reye syndrome and severe steatosis occur with mitochondrial fatty acid oxidation defects. The presence of steatosis is also common in hereditary fructose intolerance, galactosemia, and tyrosinemia. Cholesterol ester storage disease produces a peculiar orange-colored liver and is also associated with steatosis. Certain medications, such as tetracycline, colchicine, and asparaginase, cause steatosis.[199]

Chronic intake of vitamin A leads to steatosis and to stellate cell hyperplasia. Long-term intake may lead to perisinusoidal fibrosis, presumably from activation of the stellate cell by accumulation of retinyl esters.[199–203]

The number of children with a body mass index (BMI) greater than the 95th percentile for age and sex has doubled since 1976.[211] About 15% of obese children have NASH, characterized by increases in serum liver enzymes that are associated with inflammatory changes and with occasional bridging fibrosis on liver biopsy. Pure macrovesicular steatosis without increases in liver enzymes is very common in obesity and reflects the accumulation of fat in the liver and in other organs.

The reason why increases in transaminases (usually four times the upper limit of the reference range) occur in certain children is unknown. Often the only clue to liver disease is an incidental finding of increased serum liver-derived enzymes on routine testing. The biliary tract–derived enzymes may be mildly increased as well. Occasionally, ultrasonography shows increased echogenic-

TABLE 57-7 CAUSES OF STEATOSIS

Malnutrition
Essential fatty acid deficiency
Celiac disease
Diabetes mellitus
Galactosemia
Hereditary fructose intolerance
Glycogen storage disease
Tyrosinemia
Homocystinuria
Mitochondrial oxidation and respiratory chain defects
Carnitine deficiency
Cholesterol ester storage disease
Abetalipoproteinemia
Cystic fibrosis
Drugs
Total parenteral nutrition
Obesity
Reye syndrome

ity of the liver parenchyma as an indicator of steatosis. However, many patients with NASH have minimal changes on ultrasonography. A more accurate, quantifiable measure of liver steatosis can be determined with magnetic resonance imaging of the liver.

All of the cases of NASH identified at the Armed Forces Institute of Pathology during a 5-year period were reviewed; 71% of the patients were obese.[199] Therefore, the association of obesity and NASH is strong in the adult population. Nevertheless, one-third of the patients were not obese. The relationship between a nonobese BMI and the development of NASH is not known, and the prevalence of NASH in the obese population is not known. Normalization of the increases in liver enzymes may occur with a decrease in the BMI. Whether this is accompanied by a decrease in the frequency of liver fibrosis in patients with NASH is unknown. Ultrasonographic echogenicity and the absence of other causes help in the determination of the diagnosis. Reports of fatty liver in adult patients followed up for 18 years showed that the group at highest risk of progression to cirrhosis is the one with fibrosis, Mallory hyaline, and significant histologic inflammation on liver biopsy.[203,204] There are no comparable data for children.

The generally benign nature of this condition and the slow progression to cirrhosis outweigh the risks involved in a biopsy. The steatosis in this condition is usually macrovesicular and occasionally microvesicular (Figures 57-11 and 57-12). There are anecdotal reports of familial NASH, although diabetes and the illicit use of alcohol are confounding variables. The familial forms seem to be associated with mild obesity. Whether there are subgroups of patients with a metabolic basis for their microvesicular steatosis is unknown.[212,213] Proposed explanations include abnormalities of lipid oxidation, chronic endotoxemia, iron overload, and concurrent infection with hepatitis C virus.

The association of type 2 diabetes and NASH is well known in adults. Whether similar changes occur in juvenile diabetes is not entirely clear.[202–208] It is well known that about 5% of patients with type 1 diabetes have associated celiac disease. Left undiagnosed, this may lead to significant malnutrition and subsequent steatosis.

Short-Bowel Syndrome

The liver is affected in short-bowel syndrome in several ways: malnutrition and vitamin and trace element deficiencies may be present,[214–216] TPN may contribute to liver injury, intra-abdominal infections may result in liver abscesses, and bacterial overgrowth may affect the development of liver inflammation and injury. The early institution of enteral feeding and the cycling of nutritional supplementation have decreased the incidence of liver injury. The recognition of the contribution of bacterial overgrowth to the malabsorption of nutrients has led to the aggressive use of antibiotics, which has a beneficial effect on the liver (see Chapter 39, "Gastrointestinal Manifestations of Immunodeficiency").

Total Parenteral Nutrition

TPN is the best characterized cause of nutrition-related liver disease. Cholestatic liver disease predominates in premature and young infants and steatohepatitis in older children and adolescents.[217–229] Clinical cholestasis develops in about 25% of premature infants receiving TPN.[220] The severity of the cholestasis depends on the duration of nutritional support, and cholestasis usually resolves after the cessation of parenteral support. Associated sepsis, severe lung disease, congenital heart disease, and short-bowel syndrome add to the liver insult. Amino acid and glucose concentrations may be the primary culprits in the development of liver disease, although this remains controversial. The effect of fat emulsions is not entirely clear.[220–225]

The composition of the current amino acid formula results in a serum amino acid profile that is similar to that of breastfed babies. This formulation has resulted in a decreased incidence of cholestatic liver disease. The decreased concentration of carbohydrates in the formula seems to be beneficial as well. The early institution of oral

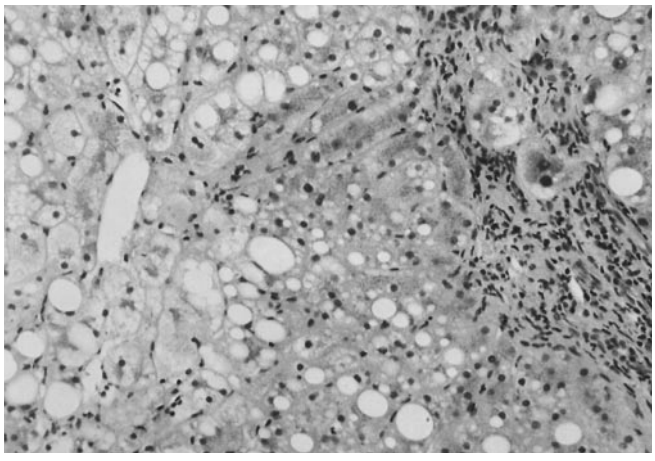

FIGURE 57-11 Nonalcoholic steatohepatitis (hematoxylin and eosin; ×40 original magnification). Courtesy of Dr. L. Burgart, Mayo Clinic and Foundation.

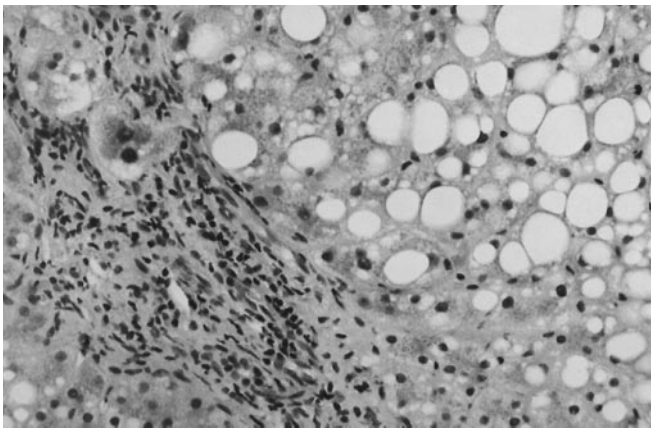

FIGURE 57-12 Nonalcoholic steatohepatitis. Note inflammatory infiltrate and macrovesicular steatosis (hematoxylin and eosin; ×100 original magnification). Courtesy of Dr. L. Burgart, Mayo Clinic and Foundation.

feeding and the cycling of TPN to nighttime feeding alone has also helped restore the natural process of gallbladder emptying and has protected the liver from injury.

The earliest biochemical abnormality is an increase in serum bile acids.[228] Hyperbilirubinemia and increases in serum alkaline phosphatase and γ-glutamyltransferase occur later. Usually, there is associated hepatomegaly. Ultrasonographic findings may show evidence of steatosis.[230] More commonly, biliary sludge and cholelithiasis can be seen, especially in premature infants who have received diuretic therapy. The development of sludge (calcium bilirubinate) occurs in 100% of infants receiving TPN for more than 6 weeks. However, gallstones develop in only a small proportion of patients. Among those patients, incidental ultrasonographic findings may be all that are documented; other patients have clear evidence of cholelithiasis and choledocholithiasis, with cholecystitis, pancreatitis, or both.

Early pathologic features consist of lobular cholestasis.[222–226] Portal inflammation and bile duct proliferation suggestive of obstruction may be present. Canalicular and cytoplasmic bile stasis, a common feature, occurs later. Giant cell transformation, indicative of a regenerative response, may occur. The lesion progresses to fibrosis and micronodular cirrhosis in a minority of patients (Figure 57-13).

The exact mechanism of TPN-related liver disease is unknown. The most important aspect of this cholestasis seems to be an exacerbation of the physiologically poor enterohepatic bile circulation already present in infants and children.

The physiologic immaturity of the enterohepatic circulation of bile is accompanied by an immaturity of the bile acid metabolism. For example, the gradient of bile acid synthesis that exists in the liver of the adult does not exist in the liver of the infant.[228] The physiologic concentration of serum bile acid in infants is clearly in the pathologic range for the adult. The result is a decreased bile acid pool, decreased circulation, increased concentration of serum bile acids, and decreased concentration of canalicular bile acid.

In animal studies, intravenous infusion of amino acids results in decreased output of bile. Infusion of large quantities of protein results in earlier and more severe cholestasis.[229] The combination of enteral starvation and parenteral nutrition results in the decrease or cessation of several hormonal stimulatory activities that are essential for proper enterohepatic circulation of bile. The lack of cholecystokinin, glucagon, insulin, and secretion affects not only cholestasis but also the formation of extrahepatic biliary sludge.

The short-bowel syndrome is associated with loss of surface area and interruption of the bile acid enterohepatic circulation. Moreover, loss of the ileocecal valve results in bacterial overgrowth, which is accompanied by increased translocation of bacteria into the bloodstream and increased incidence of septic episodes. Sepsis, especially with gram-negative enteric bacteria, can lead to dysregulation of the multidrug resistance protein 2 transport protein. The dependency of certain children on TPN for survival has led to the development of intestinal transplant as a lifesaving procedure. Occasionally, a combined liver–small bowel transplant is needed simply because of the development of end-stage liver disease from a combination of factors, including TPN.

The antioxidant role of vitamin E, selenium, and zinc in protecting the parenchyma and preventing chronic inflammation is not known.

Steatosis occurs with increased caloric intake in the form of glucose. There is a direct correlation between the amount of glucose given and the development of steatosis. The condition is usually asymptomatic, but mild increases in serum transaminases are not unusual (Figure 57-14).

Herbal Therapy and the Liver

The use of herbal medicines has increased during the past few years. Clinicians should ask patients whether they use herbal supplements or traditional remedies because herbal therapy may be associated with liver abnormalities.

A definition of *herb* is "a plant or plant part valued for its medicinal, savory, or aromatic qualities."[231] This broad definition includes plants and trees and their products or parts as possible sources of herbal products. Herbal products are also considered to be nutritional supplements

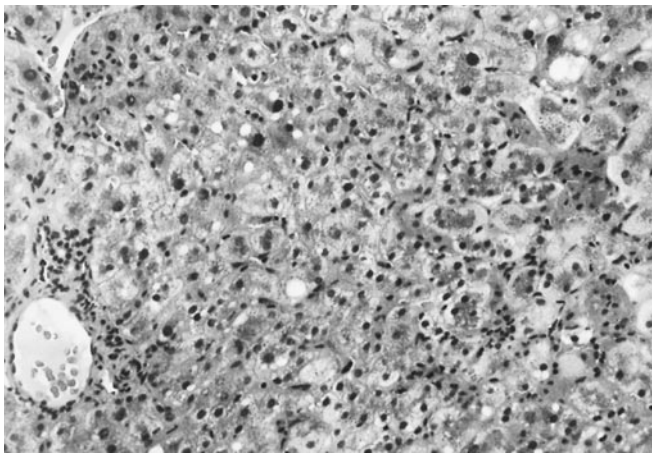

FIGURE 57-13 Total parenteral nutrition cholestasis (hematoxylin and eosin; ×40 original magnification). Courtesy of Dr. L. Burgart, Mayo Clinic and Foundation.

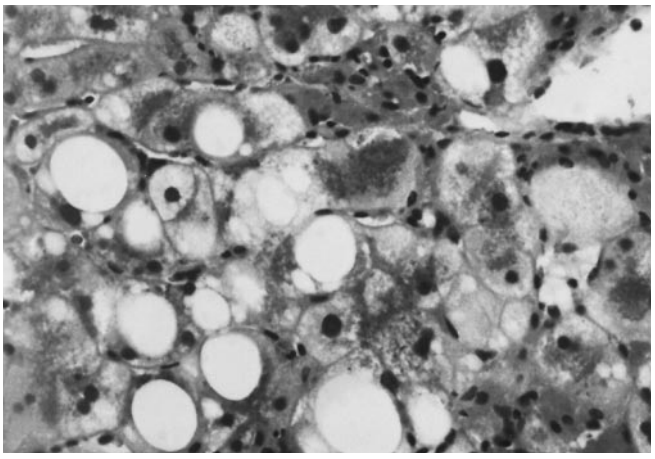

FIGURE 57-14 Total parenteral nutrition–associated steatosis (hematoxylin and eosin; ×100 original magnification). Courtesy of Dr. L. Burgart, Mayo Clinic and Foundation.

because they may contain a vitamin, an amino acid, or a trace element.

The classification of herbs and nutritional supplements as dietary supplements by the Dietary Supplement Health and Education Act of 1994 is outside the jurisdiction of many of the safety and regulatory rules that cover these categories. The purity and composition of products, as well as the consistency of dosages on labels, have varied. Some products were found to contain unlabeled compounds, and others were found to have significant heavy metal (lead) content. Table 57-8 shows the documented cases of hepatotoxicity associated with the use of herbal medicines.

Celiac Disease

The liver is involved in celiac disease in several forms.[232–236] Chronic malnutrition may lead to hepatic steatosis and fatty liver that is indistinguishable from the condition in kwashiorkor. Similar mechanisms of fatty acid mobilization occur in celiac disease and kwashiorkor (see Chapter 44.1, "Celiac Disease")

A few patients with celiac disease present with increases in liver enzymes. Concentrations of serum transaminases are usually increased two to five times the upper limit of the reference range. The patient may have subtle signs of malabsorption, such as iron or folate deficiency, or only growth failure. Overt symptoms, such as diarrhea, muscle wasting, and irritability, may also be present. The increases in enzymes resolve with the elimination of gluten from the diet. The exact pathogenesis of the liver involvement in celiac disease is not known but is presumed to be autoimmune.

Histologic evaluation shows nonspecific hepatitis, chronic active hepatitis, and, rarely, cryptogenic cirrhosis. A few reports of an association with primary sclerosing cholangitis show that the liver lesion does not improve with a gluten-free diet.

Celiac disease occurs with other autoimmune diseases, such as diabetes mellitus and Hashimoto thyroiditis, and in patients with Down syndrome. The association of diabetes and celiac disease may result in both steatosis from poorly controlled diabetes and chronic malnutrition. Awareness of these associations is important in the consideration of associated liver disease.

Celiac disease has also been associated with sarcoidosis. Therefore, the liver may be secondarily affected by granulomatous changes as well.

ENDOCRINE DISEASE

Hypopituitarism

The constellation of hypoglycemia, nystagmus, and cholestasis should prompt evaluation for hypopituitarism.[237,238] Hypopituitarism occurs in association with the absence of the septum pellucidum and septo-optic dysplasia. The lack of thyroid hormones and cortisol affects the bile acid–independent fraction of bile flow in the liver, with resultant cholestasis. In male infants, a micropenis may be evident. The cholestasis is similar to the cholestasis from other neonatal causes and can be associated with acholic stools. Acholic stools occur in obstructive lesions and hepatocellular disorders such as idiopathic neonatal hepatitis and α_1-antitrypsin deficiency. Hepatomegaly may be present.

Histologic cholestasis and neonatal hepatitis have been associated with hypopituitarism. The findings are nonspecific. The jaundice is initially due to an indirect hyperbilirubinemia, and it eventually develops into a mixed hyperbilirubinemia. Treatment is aimed at the underlying disorder, and rapid correction of the cholestasis occurs with initiation of thyroid and adrenal hormone replacement therapy.

Hypothyroidism

The association of neonatal hypothyroidism and jaundice is well described.[239–242] About 20% of infants with hypothyroidism have jaundice, which is due to indirect hyperbilirubinemia and presumably to the lack of maturation of the conjugation enzymes. Abnormal bile flow may also be present.

In older children and adolescents, liver and thyroid disease are associated with autoimmune disease. The association of Hashimoto thyroiditis with hypergammaglobulinemia occurs in about 70% of the patients. The association of autoimmune chronic active hepatitis and autoimmune thyroid disease is well known. Thyroid disease may precede or follow the development of liver disease.[243]

Diabetes Mellitus

In patients with diabetes mellitus, the liver may be involved in several ways.[243–247] The aforementioned association between celiac disease, chronic active hepatitis, and diabetes may result in chronic liver inflammation, steatosis, or even steatohepatitis, also seen in children with diabetes alone who have poor glycemic control. Poor glycemic control results in the accumulation of glycogen in the liver and hepatomegaly. The degree of hepatomegaly correlates with the amount of glycogen deposited and hence with the duration and severity of the diabetes.

There is also an association between diabetes and the development of cholelithiasis.

TABLE 57-8 HERBAL MEDICINES ASSOCIATED WITH DOCUMENTED CASES OF HEPATOTOXICITY

Soy phytoestrogens
Green tea leaf
Pyrrolizidine alkaloids and Jamaican tea preparations
Anthronoids
Protoberberine alkaloids
Germander (*Teucrium* spp)
Herbs rich in coumarin
Herbs rich in podophyllotoxin
Impila (*Callilepis laureola*) root
Kava (*Piper methysticum*) rhizome
Kombucha
Ma huang (*Ephedra* spp)
Skullcap (*Scutellaria* spp)

Adapted from Bauer BA. Herbal therapy: what a clinician needs to know to counsel patients effectively. Mayo Clin Proc 2000;75:835–41.

Autoimmune Polyglandular Syndrome Type 1

The association of autoimmune disease and its effect on the parathyroid glands, adrenal glands, and ovaries constitutes autoimmune polyglandular syndrome type 1,[248] which includes an association of mucocutaneous candidiasis and autoimmune hepatitis. The autoimmune hepatitis (type 2) is liver, kidney, and microsomal antibody positive. Liver disease occurs after the earlier manifestations in other organs. Hypocalcemia is common and is often exacerbated by the administration of corticosteroids for control of the liver lesion. Screening for the development of autoimmune hepatitis, with at least yearly serum liver enzyme determinations, is essential (see Chapter 48, "Systemic Endocrinopathies").

RENAL DISEASE

The association of liver and renal disease is well recognized.[249–256] Renal and hepatic disease occur concomittantly in both hepatitis C and B and in patients on dialysis. Liver abnormalities can occur in cases of glomerulonephritis and in hemolytic uremic syndrome as well. Some anomalies, such as autosomal recessive polycystic kidney disease and congenital hepatic fibrosis, affect both organs, but the degree of disease in the liver and kidney can be variable. In the infantile form of polycystic kidney disease, early renal failure predominates, whereas in Caroli disease, the liver lesion is predominant. Both entities are covered in detail elsewhere in this textbook. When hepatopathy is present in the dialysis or renal transplant patient, the liver involvement is usually attributed to viral infections, immunosuppressive medications, opportunistic infections, or iron overload.

GENETIC SYNDROMES WITH LIVER INVOLVEMENT

Liver disease is a component of many genetic syndromes (Table 57-9). Three recently reported syndromes with concomitant liver involvement are described below.

Hereditary Fetal Growth Retardation with Aminoaciduria, Cholestasis, Iron Overload, and Lactic Acidosis

This new genetic disease was recently described in 17 newborns from Finland. The characteristic findings are growth retardation with Fanconi-like aminoaciduria, cholestasis, and iron overload. The patients have increased serum ferritin concentrations and hypotransferrinemia. The acronym GRACILE is used to summarize the findings. Treatment in two cases with apotransferrin and exchange transfusion resulted in prolonged survival and growth. The disease locus was assigned to 2q33-37.[257]

Familial Progressive Tubulointerstitial Nephropathy and Cholestatic Liver Disease

This recently described entity is characterized by tubular nephropathy and sclerosed glomeruli with intrahepatic biliary ductular irregularities akin to primary sclerosing cholangitis. Histologically, the liver shows fibrosis with enlarged portal areas and biliary ductular proliferation. Serum concentrations of liver-derived enzymes are moderately increased.[258]

Renal Hepatic Pancreatic Dysplasia with Multiple Congenital Anomalies

Syndromes of renal hepatic pancreatic dysplasias may be grouped together as dysplastic syndromes of several organs. The association of renal, hepatic, and pancreatic abnormalities is a constant feature.[259]

MISCELLANEOUS DISORDERS

Amyloidosis

Amyloid is an amorphous protein, and amyloidosis is the resultant end-organ damage from deposition of amyloid protein.[260–264] The two major amyloid proteins are designated "AL" for primary amyloid and "AA" for secondary amyloid. Primary amyloid consists of the light chain of immunoglobulin. Secondary amyloid or serum amyloid is associated with chronic inflammation. It appears to be secreted by the liver as an acute-phase reactant. Amyloid is deposited in the gastrointestinal tract, liver, and renal tissue as the result of chronic inflammation. The condition is rare in children younger than 15 years. Cystic fibrosis is the most common association, with reports of up to one-third of the patients having amyloidosis at autopsy. The association has been reported in familial Mediterranean fever, JRA, and tuberculosis as well. The association of hepatomegaly and proteinuria with a chronic inflammatory condition should prompt the search for amyloid in rectal and renal tissue. Also, a liver biopsy may be the first diagnostic test performed when a patient has silent hepatomegaly (Figures 57-15 and 57-16). There is no correlation between the degree of amyloidosis and the increases in liver enzymes.[265,266]

Sarcoidosis

Sarcoidosis is a multisystemic chronic granulomatous disease of unknown cause.[267–270] It is associated with Crohn disease, celiac disease, lymphoma, Addison disease, thyroiditis, and the use of various drugs. The lung is the organ involved most often. Hepatomegaly is a late phenomenon, occurring in about one-third of the patients. The differential diagnosis centers on the association of other granulomatous diseases, such as chronic drug therapy, cat-scratch disease, and immunodeficiency (Table 57-10). Long-standing sarcoidosis leads to portal hypertension, with minimal increases in liver enzymes, and eventually to fibrosis and micronodular cirrhosis. Treatment with corticosteroids and, eventually, methotrexate is advocated in reports of adult patients.

Behçet Syndrome

Behçet syndrome is a multisystemic disorder characterized by ocular, mucocutaneous, articular, vascular, gastrointestinal tract, and neurologic abnormalities.[60,271–274] The clinical syndrome consists of recurrent oral, genital, and gastrointestinal tract ulcers. The association with uveitis, arthritis, and renal amyloidosis is well known. The association of

TABLE 57-9 CHARACTERISTIC FEATURES OF GENETIC DISEASES WITH LIVER INVOLVEMENT

DIAGNOSIS	AGE AT ONSET*	FEATURES
Galactosemia, tyrosinemia, neonatal iron storage disease	Neonate; infant	Jaundice, ascites, edema, coagulopathy, lethargy, sepsis, hypogylcemia, hyperammonemia, hepatitis, growth retardation, lactic acidosis, cholestasis, hypotransferrinemia
Fructosemia	Infant, with introduction of fructose	Jaundice, ascites, coagulopathy, lethargy, hypoglycemia, acidosis, hyperammonemia, phosphorus abnormalities, hepatitis
Neonatal iron storage disease	48 h	Cholestasis, coagulopathy, and liver failure (prominent); hepatitis (mild)
Inborn error of bile acid synthesis	48 h	Similar to neonatal iron storage disease; normal GGT
Ataxia telangiectasia	Infant; child	Features of ataxia telangiectasia, immunodeficiency, autoimmune markers, mild hepatitis, and steatohepatitis
SYNDROMES WITH CHOLESTASIS AND HEPATOMEGALY		
Inborn errors of bile acid metabolism	Neonatal	Mild hepatitis, normal or low GGT
Familial cholestasis	Infant; child	PFIC 1: family history features; PFIC 1 and 2: low GGT, paucity of bile ducts; PFIC 3: proliferation of bile ducts; family history of gallstones; cholestasis during pregnancy
α_1-Antitrypsin deficiency	Neonate; infant	Acholic stools, hepatomegaly
Galactosemia	As above	As above
Tyrosinemia	As above	As above
Fructosemia	As above	As above
Niemann-Pick type C	Neonate; infant	Jaundice, neurologic signs
Peroxisomal disorders	Neonate; infant	Hypotonia; low or normal GGT; varied presentations: neurologic, renal, hepatic
Adrenoleukodystrophy, Zellweger syndrome, pipecolic acidemia	Infant	As in peroxisomal disorders; hypotonia with poor sucking and retinal pigmentation, fibrosis and cirrhosis, abnormal very-long-chain fatty acids, normal GGT
Tubular nephropathy and cholestasis	Child	PSC-like with tubular nephropathy, familial pattern
Cystic fibrosis	Infant	Meconium ileus equivalent
SYNDROMES WITH HEPATOMEGALY		
Glycogenosis, types I and III; Wolman disease; familial adenomatous polyposis	Infant; 1 mo to first years	Hypoglycemia; variable clinical and metabolic features; liver with normal consistency; splenomegaly (unlike glycogenosis, type I); Wolman disease: diarrhea, vomiting, organomegaly, foam cells in the marrow, lysosomal acid lipase deficiency with storage of triglycerides and cholesterol esters, adrenal calcifications (a hallmark); familial adenomatous polyposis associated with hepatoblastoma
Glutaricaciduria, type II	Neonate	Respiratory and cardiac failure at birth with hepatomegaly
Mucopolysaccharidosis, types I and II; α-mannosidosis; fucosidosis	Neonate; infant	Progressive neurologic deterioration, splenomegaly
α_1-Antitrypsin deficiency; congenital hepatic fibrosis; glycogenosis, type IV; carbohydrate-deficient glycoprotein; fibrosis	0–6 yr	With or without splenomegaly, hard liver, portal hypertension, carbohydrate-deficient glycoprotein syndromes with intractable diarrhea and developmental delay
Cystic fibrosis, congenital hepatic fibrosis, α_1-antitrypsin deficiency, Wilson disease	Variable: 3–6 yr or later	Portal hypertension, varices, cirrhosis, typical features of biliary ectasia with fibrosis
Sialidosis, type II; mucolipidosis; Gaucher disease; mucolipidosis, type II	Early infant	Coarse features, ocular symptoms, bone changes, vacuolated lymphocytes

GGT = γ-glutamyltransferase; PFIC = progressive familial intrahepatic cholestasis; PSC = primary sclerosing cholangitis.
*Age categories: neonate, 0–4 weeks; infant, 0–12 months; child, 1 year to puberty.

hepatic vein thrombosis and Behçet syndrome was described in a report of four cases, with a review of 17 previously published cases. There was a male preponderance; the inferior vena cava was thrombosed in 90% of the patients, and the thrombosis was acute in one-third of the patients.[273]

CONCLUSION

The liver is a frequent target in systemic diseases because of its complexity and its central role in homeostasis, xenobiotic metabolism, defense against endogenous and exogenous insults, and regulation of the vascular space.

In this chapter, cellular and molecular events were linked to pathophysiologic and clinical correlates of disease. However, to cover every aspect of liver involvement in these disorders is beyond the scope of this chapter, and many subjects are covered in detail elsewhere in this textbook.

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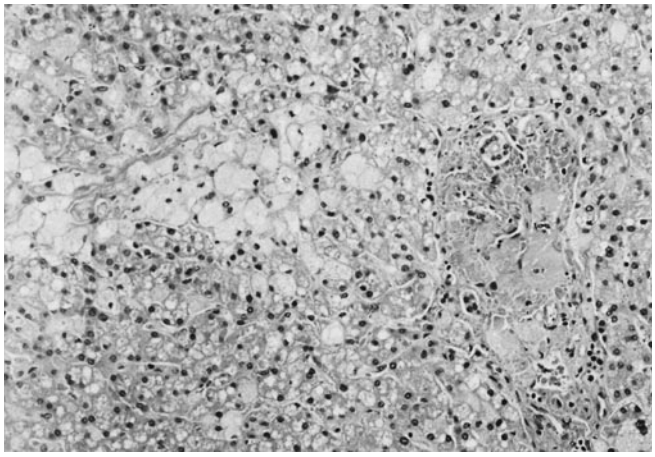

FIGURE 57-15 Amyloidosis, low power (hematoxylin and eosin). Courtesy of Dr. L. Burgart, Mayo Clinic and Foundation.

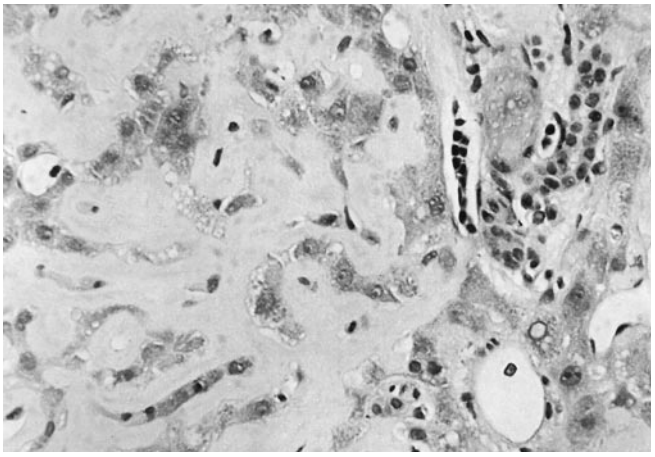

FIGURE 57-16 Amyloidosis (hematoxylin and eosin; ×100 original magnification). Courtesy of Dr. L. Burgart, Mayo Clinic and Foundation.

TABLE 57-10 GRANULOMATOUS HEPATITIS

Tuberculosis
Mycobacterial infections
Brucellosis
Typhoid fever
Listeriosis
Cat-scratch disease
Histoplasmosis
Aspergillosis
Candidiasis
Cryptococcus
Actinomycosis
Syphilis
Visceral larva migrans
Schistosomiasis
Q fever
Ehrlichiosis
Cytomegalovirus infection
Epstein-Barr infection
Erythema nodosum
Drug reaction
Systemic lupus erythematosus
Juvenile rheumatoid arthritis
Histiocytosis
Lymphoma
Sarcoidosis

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CHAPTER 58

ACUTE LIVER FAILURE

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Acute liver failure (ALF), a clinically heterogeneous and complex multisystem disorder, is a rare but devastating sequel of an insult to the liver. The liver damage, within a few days to weeks, can cause encephalopathy and multiorgan failure. Different underlying etiologies, the age of the patient, and the duration of time over which the disease evolves contribute to the heterogeneity of this disorder. The mortality is high without liver transplant. ALF is the indication for liver transplant in about 10 to 20% of pediatric recipients in major transplant centers, with a 1-year survival rate of 60 to 75%. Auxiliary liver transplant offers the opportunity for the native liver to regenerate with the possibility of withdrawal of immunosuppression. Liver assist devices (cleansing systems and biologic systems) are being evaluated as newer treatment options. Management requires early appreciation of the severity of the illness, treatment, and prevention of complications, and if the spontaneous recovery is doubtful, emergency liver transplant before the irreversible brain damage occurs. This involves an integrated, multidisciplinary approach involving hepatologists, intensivists, and transplant surgeons; therefore, this condition should ideally be managed in a specialist center.

DEFINITION

Although the occurrence of fatal hepatitis as a consequence of epidemic hepatitis was first reported in 1946,[1] the first attempt at a formal definition was made only in 1970 by Trey and Davidson.[2] Fulminant hepatic failure (FHF) was described as "a potentially reversible condition, the consequence of severe liver injury, in which the onset of hepatic encephalopathy was within eight weeks of the first symptoms of illness, and in the absence of pre-existing liver disease."[2] In 1989, the term "late-onset hepatic failure" was used on the basis of development of hepatic encephalopathy (HE) between 8 and 24 weeks from the onset of jaundice.[3] Bernuau and Benhamou described FHF as the onset of encephalopathy within 2 weeks of the onset of jaundice (rather than other symptoms); it was subfulminant hepatic failure if encephalopathy developed between 2 and 12 weeks from the first appearance of jaundice.[4] Subsequently, O'Grady and colleagues proposed a new classification based on a retrospective study of 539 adult patients. They suggested use of the terms hyperacute, acute, and subacute failure, depending on the interval between jaundice and onset of encephalopathy, less than 1 week, 1 to 4 weeks, and 5 to 12 weeks, respectively.[5] They also suggested that the lack of preexisting liver disease is not mandatory if it remained asymptomatic, for example, Wilson disease, autoimmune hepatitis, delta virus superinfection, and hepatitis B reactivation.

These definitions do not satisfactorily encompass the complexity of the condition in the pediatric population. The early stages of encephalopathy are very difficult to detect in infants and small children. Also, in children, particularly during infancy, encephalopathy may appear very late, if ever, and ALF may be the first manifestation of an underlying metabolic disease associated with a variable degree of chronic liver damage. The first pediatric definition was suggested by Bhaduri and Mieli-Vergani as "a rare multisystem disorder in which severe impairment of liver function, with or without encephalopathy, occurs in association with hepatocellular necrosis in a patient with no recognised underlying chronic liver disease."[6] This definition has addressed the issues relevant to the pediatric population.

ETIOLOGY

ALF is the final common pathway of a variety of insults to the liver. There is considerable variation in the etiologies around the world, with acute viral hepatitis and drugs accounting for the majority of cases. The frequency of ALF in all age groups in the United States is 17 cases per 100,000 population per year, but the frequency in the pediatric age group only is unknown.[7] The overall incidence of ALF complicating acute hepatitis in the United States is 0.9%, and it causes about 2,000 deaths annually, with non-A–E hepatitis being the most common cause.[8] In children, acute viral hepatitis is the most common identified cause in most of the series, but there is a lot of geographic variation, with hepatitis A being the most common cause in Asia, whereas in Europe and North America, it is seronegative hepatitis. The etiology of ALF as seen in a tertiary pediatric liver center is shown in Table 58-1.

INFECTIVE

Viruses. Infection with the hepatotropic viruses is probably the most identifiable cause of ALF. Patients usually present with icterus and markedly raised serum transaminase

TABLE 58-1 ETIOLOGIES OF ACUTE LIVER FAILURE IN NEONATES AND CHILDREN (KING'S COLLEGE HOSPITAL, 1991–2000)

ETIOLOGY	*n*
NEONATES (*n* = 31)	
Neonatal hemochromatosis	15
Hemophagocytic lymphohistiocytosis	4
Disseminated herpes simplex virus infection	5
Metabolic	4
Transplacental acetaminophen toxicity	1
Endocrine (isolated cortisol deficiency)	1
Sepsis/shock	1
CHILDREN (*n* = 100)	
Non-A–E hepatitis	45
Hepatitis A/B	7
Other viral infection	3
Metabolic	18
Acetaminophen toxicity	8
Other drug/toxin	5
Sepsis/hypoxia	3
Miscellaneous	11

levels. The magnitude of transaminase elevation and the rate of decline do not predict prognosis. In patients who spontaneously recover, serum bilirubin, international normalized ratio (INR), and serum transaminases gradually decline, whereas a continued rise in bilirubin levels and INR, despite declining serum transaminase levels, indicates massive hepatocyte necrosis and a poor prognosis.

The risk of developing liver failure in acute hepatitis A virus (HAV) infection is 0.1 to 0.4%.[9–12] A higher incidence of ALF is suggested when HAV infection occurs in patients with underlying chronic liver disease. Very high mortality is reported in an Italian study in which 7 of 17 children with chronic hepatitis C infection with superadded HAV infection developed ALF, and only 1 child survived.[13] The diagnosis of acute hepatitis A is made by the detection of the anti-HAV immunoglobulin (Ig)M antibody in serum. In 95% of cases, anti-HAV IgM antibody is present at the time of presentation, and the remaining 5% become positive on repeat testing.

The incidence of ALF owing to hepatitis B virus (HBV) is 1 to 4%.[8] The liver failure can present at (1) the time of acute infection, (2) reactivation of chronic HBV infection in immunocompromised patients, (3) superinfection or coinfection with hepatitis D virus, or (4) seroconversion from a hepatitis B e antigen–positive to a hepatitis B e antibody (HBeAb)-positive state. Infants born to HBeAb-positive mothers are a special group that can present with ALF around 3 weeks to 3 months of age.[14,15]

There is a theoretic risk of developing ALF after hepatitis C virus (HCV) infection, but in a large follow-up study of children with post-transfusion HCV infection, ALF was not observed.[16]

Hepatitis E virus (HEV) infection, a waterborne infection like hepatitis A and a well-recognized cause of ALF, is common in the Indian subcontinent and Africa. The risk of developing ALF in adult males is 0.6 to 2.8%, but the risk increases significantly in pregnant women, especially in the third trimester, with the case-fatality ratio of around 25%.[17] HEV infection may be responsible for up to 8% of cases, which would have been attributed to seronegative hepatitis. A history of travel to an endemic area is not always present. A study from northern India reported that 7 of 44 children with ALF had isolated HEV infection, whereas another 16 of 44 had mixed HEV and HAV infection. HEV infection is diagnosed by the presence of anti-HEV antibody in the serum.[18] Despite identification of hepatitis G (a flavivirus) virus in ALF of unknown etiology, it does not appear to cause ALF.[19] Similarly, TT virus (transfusion-transmitted virus), a single-stranded deoxyribonucleic acid (DNA) virus, was discovered in 1997 from the sera of non-A, non-B (NANB) hepatitis patients but has also not been shown conclusively to cause ALF.[20]

Non-A–E hepatitis (seronegative hepatitis) is the most common cause of ALF in the Western world. In our series, of 100 cases of ALF, 45 were due to non-A–E hepatitis.[21] Similar experience was reported from Chicago, with 26 of 42 children with ALF who were diagnosed with non-A–E hepatitis.[22] The diagnosis is one of exclusion in which other causes of ALF are eliminated with appropriate laboratory investigations and clinical examination. Non-A–E hepatitis is characterized by its propensity to cause severe hepatitis, a high fatality rate (low spontaneous remission) without liver transplant, and its association with bone marrow failure in up to 10% of patients.[23] Bone marrow failure can develop even a few weeks after the onset of symptoms of ALF.

Other Hepatotropic Viruses. Herpes simplex virus, cytomegalovirus, Epstein-Barr virus, and varicella-zoster virus, members of the herpesvirus family, can cause severe hepatic necrosis, particularly in immunocompromised patients and neonates. Herpes simplex infection–induced ALF in the neonatal period has a very high case-fatality ratio. The diagnosis is suspected in an unwell neonate with or without a vesicular lesion along with remarkably raised serum transaminases and coagulopathy. Maternal history suggestive of herpes simplex infection and/or positive serology is helpful in establishing the diagnosis. Viral studies (immunofluorescence or polymerase chain reaction) of vesicular fluid are diagnostic. Association of cytomegalovirus with ALF is not reported, although Epstein-Barr virus has been, rarely, associated with ALF.[24]

Parvovirus B19 infection can cause severe hepatitis, ALF, and, rarely, bone marrow failure in children. A retrospective study of 6 patients with ALF with peritransplant aplastic anemia owing to presumed NANB hepatitis showed the presence of parvovirus B19 DNA in 4 of 6 explanted livers, but all 6 patients had IgG antibodies against parvovirus.[25] In our experience, parvovirus infection as the cause of ALF was recognized in 2 of 8 children who had bone marrow aplasia with ALF.[23]

Echovirus (type 20) and coxsackieviruses have been reported to cause ALF, especially in neonates.[26,27] In an adult study, Toga virus–like particles were isolated in explanted livers in 7 of 18 patients who had liver transplant for ALF owing to NANB hepatitis but none in 26 explanted livers available for study after liver transplant owing to other causes. ALF, characterized by severe hemorrhagic

necrosis on histology, developed 7 days after transplant in 5 patients, all in the NANB group with Toga virus–like particles in native liver. Further analysis revealed no identifiable epidemiologic factors between these and the other NANB hepatitis–induced ALF patients.[28]

Nonviral Infections. Nonviral infective agents have been implicated in the pathogenesis of ALF, although rarely. Acute liver dysfunction is well recognized in severe sepsis. Bacterial infections, especially gram-negative infections (*Salmonella*, *Shigella*, *Escherichia coli*, and *Pseudomonas*), miliary tuberculosis, brucellosis, and Q fever (owing to *Coxiella burnetii*), have been reported to cause ALF. Spirochetal infections, particularly leptospirosis, can present with severe hepatitis or ALF. The clinical picture is predominated by high fever and renal failure. In endemic areas, there have been reports of ALF following plasmodium falciparum infection.[29]

Hepatic dysfunction in sepsis is the result of decreased hepatic perfusion, hypoxia, and lactic acidosis. Bacterial cell wall products (endotoxin and lipoteichoic acid) and cytokines (tumor necrosis factor, interleukin-1β, and interferons) induce the production of nitric oxide. The elevated levels of inducible nitric oxide are toxic to liver cells.[30]

Drugs and Toxins

Drugs and toxins are well known to cause liver failure in children. In general, the risk factors for drug-induced hepatotoxicity are age (very young or adolescents), abnormal renal function, concurrent use of other hepatotoxic agents, drug interactions, and preexisting liver diseases. Drug-induced hepatoxicity can be a dose-dependent response, an idiosyncratic reaction, or a synergistic reaction (Table 58-2).

Estimates of the risk of developing ALF as a result of an idiosyncratic reaction range from 0.001% for nonsteroidal anti-inflammatory drugs to 1% for the isoniazid-rifampicin combination. Drug toxicity produces a distinctive pattern of liver injury. The most common liver injury pattern owing to drugs is hepatitic (hepatocellular necrosis), accounting for about 90% of cases. Others could be cholestatic (biliary damage), mixed (both hepatitic and cholestatic), or steatosis.[31] Sodium valproate is known to unmask underlying mitochondrial cytopathies; hence, detailed investigations to exclude mitochondrial hepatopathies should be undertaken before injury is ascribed to sodium valproate. Use of antiretroviral drugs has also been associated with ALF. Ecstasy (3,4-methylenedioxymethamphetamine), a synthetic amphetamine, has been associated with a range of clinical syndromes ranging

TABLE 58-2 CAUSES OF ACUTE LIVER FAILURE

- Infective
  - Viral
    - Viral hepatitis
      - A, B, B + D, E
      - Non-A–E hepatitis (seronegative hepatitis)
    - Adenovirus, Epstein-Barr virus, cytomegalovirus
    - Echovirus
    - Varicella, measles
    - Yellow fever
    - Rarely, Lassa, Ebola, Marburg virus, dengue, Toga virus
  - Bacterial
    - Salmonellosis
    - Tuberculosis
    - Septicemia
  - Others
    - Malaria
    - Bartonella
    - Leptospirosis
- Drugs
  - Dose dependent
    - Acetaminophen
    - Halothane
  - Idiosyncratic reaction
    - Isoniazid
    - Nonsteroidal anti-inflammatory drugs
    - Phenytoin
    - Sodium valproate
    - Carbamazepine
    - Ecstasy
    - Troglitazone
    - Antibiotics (penicillin, erythromycin, tetracyclines, sulfonamides, quinolones)
    - Allopurinol
    - Propylthiouracil
    - Amiodarone
    - Ketoconazole
    - Antiretroviral drugs
  - Synergistic drug interactions
    - Isoniazid + rifampicin
    - Trimethoprim + sulfamethoxazole
    - Barbiturates + acetaminophen
    - Amoxycillin + clavulinic acid
- Toxins
  - *Amanita phalloides* (mushroom poisoning)
  - Herbal medicines
  - Carbon tetrachloride
  - Yellow phosphorus
  - Industrial solvents
  - Chlorobenzenes
- Metabolic
  - Galactosemia
  - Tyrosinemia
  - Hereditary fructose intolerance
  - Neonatal hemochromatosis
  - Niemann-Pick disease type C
  - Wilson disease
  - Mitochondrial cytopathies
  - Congenital disorders of glycosylation
  - Acute fatty liver of pregnancy
- Autoimmune
  - Type 1 autoimmune hepatitis
  - Type 2 autoimmune hepatitis
  - Giant cell hepatitis with Coombs-positive hemolytic anemia
- Vascular/ischemic
  - Budd-Chiari syndrome
  - Acute circulatory failure
  - Heat stroke
  - Acute cardiac failure
  - Cardiomyopathies
- Infiltrative
  - Leukemia
  - Lymphoma
  - Hemophagocytic lymphohistiocytosis

from asymptomatic hepatic liver function tests or subacute liver failure to rapidly progressive ALF.[32]

Acetaminophen, the most common drug associated with ALF, is safe when used in therapeutic doses in healthy individuals. It is normally a dose-dependent hepatotoxic agent. The hepatocyte necrosis is caused by accumulation of the *N*-acetylparabenzoquine amide, which is a toxic intermediate compound.[33] When cytochrome P-450 enzymes are induced either owing to drugs such as antiepilepsy drugs or chronic alcohol consumption, hepatic glutathione stores are depleted. Consequently, even therapeutic doses of acetaminophen can lead to accumulation of *N*-acetylparabenzoquine amide, causing ALF. Inadvertent administration of higher doses of acetaminophen can lead to ALF in children. A detailed history of exposure to acetaminophen is helpful. In 1995, a series of young children were reported with ALF without any identifiable cause but with minimal jaundice. All of these children had a history of exposure to acetaminophen, although in therapeutic doses. Fifty percent of these patients survived, and the histopathology was characterized by a varying degree of centrilobular necrosis, which is a characteristic lesion of acetaminophen toxicity, suggesting the possible role of toxicity owing to therapeutic doses of acetaminophen in the development of ALF.[34] Serum acetaminophen levels after 4 hours of ingestion are useful in identifying high-risk patients but are not informative in patients in whom toxicity is secondary to chronic administration.

Mushroom (*Amanita phalloides*) poisoning leading to ALF is mainly reported from Europe (France and eastern Europe), the west coast of the United States, and South Africa. Cases of poisoning peak in autumn, when mushrooms are plentiful. α-Amantin is a heat-stable toxin that is not destroyed by cooking. The usual presentation is severe diarrhea with or without vomiting commencing about 5 or more hours after ingestion. Liver failure is usually followed 3 to 4 days later. Other hepatotoxins include carbon tetrachloride, herbal medicines, and aflatoxins.

Autoimmune Hepatitis

Autoimmune hepatitis can present as ALF, most of these patients being liver-kidney microsomal antibody positive. The diagnosis may be difficult because some cases may not show an antibody response at presentation. Some reports have suggested a good response to steroids and azathioprine or cyclosporine.[35,36] In our experience, children with autoimmune hepatitis presenting with ALF along with encephalopathy do not respond to any form of immunosuppression and need urgent liver transplant.

Metabolic Diseases

Inherited disorders of metabolism merit special attention as a differential diagnosis while investigating ALF in pediatric patients, in particular newborn babies. Although all of these patients have variable degrees of liver damage before clinical presentation as ALF, overt signs and stigmata of chronic liver disease are usually absent. A high index of suspicion is important because urgent intervention such as dietary manipulation or disease-specific treatment may be lifesaving. Conditions that are common to the neonatal age group are listed in Table 58-3.

Galactosemia is usually associated with hypoglycemia and gram-negative septicemia. Immediate exclusion of galactose (from the diet and the medications) usually provides a quick recovery, but some cases do progress to liver failure. Tyrosinemia presents with severe coagulopathy, mild jaundice, and rickets. Hereditary fructose intolerance is rare, but a history of administration of fructose, as in fruits, sugar, or honey, may coincide with clinical symptoms.

Neonatal hemochromatosis (NH) is a disorder of iron handling of antenatal onset with excess iron deposition in the nonreticuloendothelial system. Liver failure usually presents in the first few days of life, but liver disease is generally present at birth. Maternal viral infection in the antenatal period or metabolic disease in the fetus is suggested as an underlying cause. Although an underlying genetic basis for NH has been suspected, no test is available for predictive analysis in at-risk pregnancies. In a systematic study of the mode of transmission of this disorder in a total of 40 infants born to 27 families, four pedigrees showed clear evidence of maternal infection associated with NH. One pedigree showed transmission of maternal antinuclear factor and ribonucleoprotein antibodies to the affected infants, and two families with possible matrilineal inheritance of disease in maternal half-siblings, but the large subgroup of the affected pedigrees points to the inheritance of an autosomal recessive trait. This included 14 pedigrees with affected and unaffected infants and a single pedigree in which all four affected infants were the sole offspring of consanguineous but otherwise healthy parents.[37] It can occur sporadically or recurrently, without an overt cause, in siblings. The diagnosis should be considered in every case of neonatal liver failure. Elevation of ferritin as a diagnostic test is sensitive but not specific because elevation of ferritin is commonly observed in sick babies. Hypersaturation of transferrin with relative hypotransferrinemia may be a valuable finding. Magnetic resonance imaging of the liver or pancreas to demonstrate iron is not usually rewarding, but a punch biopsy specimen of buccal mucosa is a useful diagnostic tool. Documentation of iron in salivary glands in buccal mucosa is diagnostic of NH. To ensure the presence of salivary glands in the buccal mucosal biopsy specimen, a frozen section examination is advisable. Most of the time, the biopsy can be performed safely after correction of coagulopathy with blood products. Iron chelation and antioxidant cocktail therapy (Table 58-4) for NH

TABLE 58-3 CAUSES OF NEONATAL LIVER FAILURE

Perinatal herpes simplex virus infection
Neonatal hemochromatosis
Galactosemia
Tyrosinemia
Hemophagocytic lymphohistiocytosis
Septicemia
Mitochondrial cytopathies
Congenital disorders of glycosylation
Severe birth asphyxia

has shown variable results.[38,39] Recently, successful fetal outcome following antenatal intravenous immunoglobulin therapy of pregnant mothers considered to have a high risk of carrying an affected fetus has been reported. In six pregnancies in five mothers, intravenous immunoglobulin was administered weekly at a dose of 1 g/kg body weight from the eighteenth week until the end of gestation. Four pregnancies progressed to term; one had spontaneous delivery at 36 weeks, and one had an induced birth at 32 weeks. All six were live births and were medically stable, with evidence of liver involvement. Four of six required an antioxidant or a chelation cocktail.[40]

Wilson disease, an autosomal recessive disorder, may present as ALF in an older child. The acute hepatic presentation is characterized by the presence of liver failure, Coombs-negative hemolytic anemia, and low alkaline phosphatase. Demonstration of Kayser-Fleischer rings is diagnostic of Wilson disease in a patient who presents with ALF. Serum ceruloplasmin is usually but not invariably low, and serum free copper concentration can be increased or normal. A serum alkaline phosphatase to total bilirubin ratio of < 2.0 has also been suggested as a diagnostic tool to discriminate Wilson disease from other causes of ALF.

Mitochondrial Disorders

In recent years, mitochondrial respiratory chain disorders have been implicated in the etiology of ALF in children.[41–43] This group of disorders encompasses a wide variety of diseases, including Pearson syndrome, mitochondrial DNA depletion syndrome, nuclear DNA defect, Alpers disease, and intestinal pseudo-obstruction with liver disease. Presenting symptoms could be hypoglycemia, vomiting, coagulopathy, acidosis, and increased lactate with or without neurologic symptoms. The presence of high serum lactate in the mother and a history of sibling deaths are suggestive of this condition. Diagnosis involves quantitative assessment of the respiratory chain enzyme complexes in the affected tissues (muscle, liver, skin fibroblast culture). Isolated hepatic involvement with successful liver transplant has been reported; however, the follow-up of these patients is not long enough to rule out future neurologic deterioration. Rarely, fatty acid oxidation defects and inborn errors of bile acid synthesis, especially Δ^4-3-oxosteroid 5β-reductase enzyme deficiency, can present as ALF.[44,45]

TABLE 58-4 DISEASE-SPECIFIC THERAPIES

Acetaminophen toxicity: *N*-acetylcysteine (100 mg/kg/d) until INR is < 1.5
Hereditary tyrosinemia: NTBC[93]
Neonatal hemochromatosis: iron chelation and antioxidant cocktail
N-Acetylcysteine (100 mg/kg/d IV infusion)
Selenium (3 µg/kg/d IV)
Desferrioxamine (30 mg/kg/d IV)
Prostaglandin E_1 (0.4–0.6 µg/kg/h IV)
Vitamin E (25 U/kg/d IV/PO)
Mushroom poisoning: benzylpenicillin (1,000,000 U/kg/d) or thiotic acid (300 mg/kg/d)

INR = international normalized ratio; IV = intravenously; NTBC = 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione; PO = orally.

Vascular/Ischemic Causes

Any condition causing obstruction of hepatic venous outflow (eg, Budd Chiari syndrome, veno-occlusive disease, cardiomyopathies, and acute heart failure) can present with ALF. A detailed cardiovascular examination, including an echocardiogram, is essential to exclude cardiac causes. Diagnostic clues include soft hepatomegaly and ascites.

Malignancies

Hemophagocytic lymphohistiocytosis is a spectrum of inherited and acquired conditions with disturbed immunoregulation and encompasses two main conditions that have common clinical and pathobiologic characteristics: familial (primary) hemophagocytic lymphohistiocytosis and secondary hemophagocytic lymphohistiocytosis. Familial hemophagocytic lymphohistiocytosis is an invariably fatal inherited disease seen mostly in infancy and early childhood,[46] but secondary hemophagocytic lymphohistiocytosis can affect people at any age and may subside spontaneously. The annual childhood incidence of familial hemophagocytic lymphohistiocytosis has been estimated (in Sweden) at 1.2 cases per 1,000,000, corresponding to 1 in 50,000 births.[47] Clinical presentations include fever, hepatosplenomegaly, and pancytopenia. There is reduced cytotoxic T- and natural killer cell activity, as well as a widespread accumulation of T lymphocytes and macrophages, some of which may engage in hemophagocytosis.[46–48] Biochemically, it is characterized by high serum triglycerides and low fibrinogen. Usually, patients with hemophagocytic lymphohistiocytosis bleed disproportionately from venepuncture sites because of the coagulation abnormalities present.

A varied form of hematologic malignancies (eg, leukemia[49] or lymphoma) can present with ALF. Diagnostic clues include high fever, hepatosplenomegaly, high alkaline phosphatase, high lactate dehydrogenase, and abnormalities on peripheral blood film. Bone marrow examination is diagnostic.

PATHOPHYSIOLOGY OF THE CLINICAL SYNDROME

ALF is a syndrome of multiorgan involvement. Most patients are jaundiced at presentation, except those with hyperacute liver failure, in whom jaundice follows encephalopathy. Peripheral stigmata of liver cell failure may be seen occasionally. A rapid decrease in liver span is usually seen, but patients with hematologic malignancies or cardiac failure have hepatomegaly.

Encephalopathy

The pathogenesis of encephalopathy is not clearly understood and probably is multifactorial. The most widely studied factor is ammonia (Figure 58-1). Ammonia is produced from the breakdown of proteins, amino acids, purines, and pyrimidines. Half of the ammonia in the intestine is produced by bacteria; the remainder is from the breakdown of dietary protein and glutamine. Ammonia is cleared in the liver by urea cycle enzymes. In brain, urea cycle enzymes

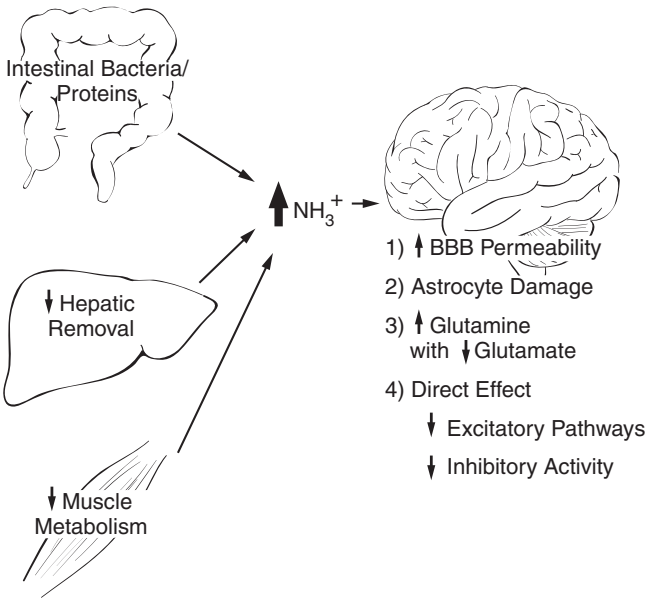

FIGURE 58-1 Summary of the role of ammonia in hepatic encephalopathy.

are absent; hence, ammonia is cleared by the formation of glutamine by enzyme glutamate synthetase. High concentration of glutamine in brain has been demonstrated by magnetic resonance spectroscopy.[50] Ammonia has a direct effect on the neural membranes and also causes postsynaptic inhibition.[50] Although the role of hyperammonemia in HE appears logical, 10% of patients with HE have normal serum ammonia levels.

γ-Aminobutyric acid (GABA), a principal inhibitory neurotransmitter in the brain, is increased in ALF.[51] GABA can act directly or in synergy with the benzodiazepine receptors. In addition to elevated blood levels, increased GABA receptor density and increased sensitivity have been shown in animal studies.

Intestinal decarboxylation of amino acids in the colon lead to the formation of β-phenylethanolamine, tyramine, and octopamine. These products inhibit dopamine- and catecholamine-mediated cerebral transmission by acting as false neurotransmitters.[52]There is also an imbalance in the ratio of plasma and intracerebral branched-chain and aromatic amino acids. Other toxins, such as mercaptans, phenols, fatty acids, and middle-molecular-weight substances, have all been implicated as causative agents. These toxins suppress neural energy metabolism and alter blood-brain barrier permeability.

Encephalopathy of variable degree is often present in patients with ALF, but early encephalopathy is difficult to recognize in children. Traditionally, the encephalopathy is believed to progress over four stages in adults. The symptomatology of stages 3 and 4 is similar in adults and children, but the following modifications could be applied to pediatric patients:

- Stage 1: Mild confusion/anxiety, disturbed or reversal of sleep rhythm, shortened attention span, slowing of ability to perform mental tasks (simple addition or subtraction). In young children, irritability, altered sleep pattern, unexplained bursts of excessive crying.
- Stage 2: Drowsiness, confusion, mood swings with personality changes, inappropriate behavior, intermittent disorientation of time and place, gross deficit in ability to perform mental tasks. In young children, excessive sleepiness, inability to interact with or recognize parents, lack of interest in favorite toys or activities.
- Stage 3: Pronounced confusion, delirious but arousable, persistent disorientation of time and place, hyperreflexia with a positive Babinski sign.
- Stage 4: Comatose with or without decerebrate or decorticate posturing, response to pain present (IVa) or no response to pain (IVb).

Electroencephalographic changes present in HE, although not very specific, may be useful in diagnosis and in assessment of treatment. These changes occur very early, even before the onset of psychological or biochemical disturbances. Initially, there is slowing of the alpha rhythm down to the delta range. Altering stimuli such as opening eyes may fail to reduce the background rhythmic activity. Usually, changes start in the frontal or central region and then progress posteriorly. In deeper coma, there is generalized slowing, and synchronous low-amplitude waves are recorded. It is still unclear whether routine electroencephalographic monitoring provides any advantage over clinical assessment alone except in patients who are ventilated and paralyzed, in whom it may reveal seizure activity.

Intracranial Hypertension and Cerebral Edema

Intracranial hypertension is uncommon in stages 1 and 2 but is invariably present in grade 4 encephalopathy. Raised intracerebral pressure can lead to brainstem herniation, which is the most common cause of death observed on autopsy in 80% of fatal cases.[53] Increased intracranial hypertension more commonly occurs in hyperacute liver failure (70%) rather than ALF (55%). The pathophysiology and clinical events related to it could be divided into three phases:

- Phase 1: Episodic increase in intracranial pressure (ICP) either spontaneously or in response to stimuli involved in the routine care of the patient. An intact Cushing reflex at this stage can maintain cerebral perfusion by increasing mean arterial pressure. Although the cerebral oxygenation is preserved at this stage but life-threatening, brainstem herniation can occur.
- Phase 2: At this stage, mean arterial pressure does not increase with further surges in ICP, leading to neuronal hypoxic injury.
- Phase 3: This phase is dominated by poor cerebral perfusion either owing to very high ICP or low mean arterial pressure, leading to hypoxic brain injury.

Factors responsible for encephalopathy cause cerebral edema, leading to an increase in ICP. This increase in ICP can lead to a cycle of poor cerebral perfusion, anoxic injury, and further worsening of cerebral edema. The clinical features vary depending on the severity of intracranial

hypertension. The factors and mechanisms that may lead to increased ICP are shown in Figure 58-2.

The mechanism leading to increased ICP could be broadly considered under two headings: the direct toxicity of neuronal cells and vasogenic. The neuronal toxicity leads to osmotic imbalance and an increase in intracellular water, whereas disruption of the blood-brain barrier leading to plasma seepage into the cerebrospinal fluid is of vasogenic origin. Circulating endotoxin from superimposed infections,[54] fluid overload, hypoglycemia,[55] and gastrointestinal bleeding all add to further worsening of intracranial hypertension (Table 58-5).

TABLE 58-5 FACTORS WORSENING INTRACRANIAL PRESSURE

Hypotension
Hypoxia
Hypoglycemia
Sepsis
Electrolyte disturbances (hyperkalemia)
Gastrointestinal bleeding

Renal Failure

Renal failure occurs in about 55% of all ALF patients referred to specialist centers.[56] A variable degree of renal dysfunction is invariably present in patients with acetaminophen-related ALF. In the pediatric population, the incidence of renal failure is lower (10–15%) than in the adult population.[57] Functional renal failure (hepatorenal syndrome) usually progresses to tubular damage as the encephalopathy advances. Avid sodium retention (urinary sodium < 20 mmol/L) and normal urine sediment may help to differentiate between functional renal failure and tubular damage. The hepatorenal syndrome recovers rapidly after liver transplant, whereas established tubular damage requires prolonged renal replacement therapy.

Renal failure could be due either to the direct toxic effect on kidneys, as in acetaminophen overdose, or to a complex mechanism such as hepatorenal syndrome or acute tubular necrosis secondary to complications of ALF (sepsis, bleeding, and/or hypotension). In hepatorenal syndrome, there is a hyperdynamic circulation with a decrease in renal perfusion pressure, leading to activation of the sympathetic nervous system and rendering the kidneys more susceptible to decreases in the renal perfusion pressure and increased synthesis of several vasoactive mediators such as renin, angiotensin, adrenaline, eicosanoids, and endothelins.[58] These vasoactive mediators not only cause vasoconstriction leading to a rise in renal vascular resistance but also a decrease in glomerular capillary ultrafiltration coefficient leading to a fall in the glomerular filtration rate over and above that caused by the vasoconstrictors alone.

Blood urea estimation is unreliable as a marker of renal dysfunction because gastrointestinal hemorrhage may increase urea disproportionately. Serum creatinine is a better indicator of kidney function.

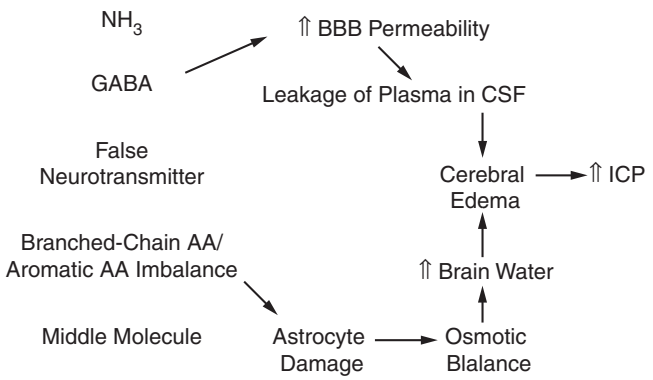

FIGURE 58-2 Schematic diagram of the pathophysiology of cerebral edema. AA = amino acid; BBB = blood-brain barrier; CSF = cerebrospinal fluid; GABA = γ-aminobutyric acid; ICP = intracranial pressure; NH_3 = ammonia.

Metabolic Derangements

Hypoglycemia is present in 40% of patients with ALF. This is due to increased plasma insulin levels owing to reduced hepatic uptake and reduced gluconeogenesis. The classic signs and symptoms of hypoglycemia are often masked, and regular blood glucose monitoring is mandatory because hypoglycemia can worsen HE and cause rapid neurologic deterioration.

Acid-base imbalance is common. Metabolic acidosis is present in about 30% of patients with acetaminophen-induced ALF and is a bad prognostic marker, with greater than 90% mortality if the arterial pH is less than 7.3 on or after the second day of overdose in adequately hydrated patients. This acidosis is independent of renal function and usually precedes the onset of encephalopathy, whereas in ALF owing to other etiologies, metabolic acidosis is present in only 5% of cases, occurs late in the disease process, and is associated with a poor outcome. Lactic acidosis develops in about 50% of patients, reaching grade 3 or 4 encephalopathy, and is related to inadequate tissue perfusion owing to hypotension or hypoxemia resulting from the impaired oxygen extraction owing to microvascular shunting of blood from actively respiring tissue. Sometimes respiratory alkalosis can be present owing to hyperventilation, probably related to direct stimulation of respiratory center by unknown toxic agents. Respiratory acidosis can be caused by pulmonary complications or by respiratory depression in association with increased ICP.

Hypokalemia is common and is due to excessive urinary potassium loss with inadequate replacement. Hyponatremia may be dilutional owing to excessive antidiuretic hormone secretion, or it may represent a true sodium-depleted state in patients who are vomiting. Hypophosphatemia is most commonly associated with acetaminophen-induced ALF when renal function is preserved. Other electrolyte disturbances include hypocalcemia and hypomagnesemia.

Hemodynamic Abnormalities

The early hemodynamic changes in ALF patients reflect a state of hyperdynamic circulation with decreased systemic peripheral vascular resistance and increased cardiac out-

put. These changes are similar to the one seen in systemic inflammatory response syndrome.[59] Profound vasodilatation, probably mediated by increased prostaglandin or nitric oxide, causes hypovolemia. Invasive monitoring is required to determine the adequacy of intravascular volume and appropriate fluid regimens. As the disease progresses, it can lead to circulatory failure either due to falling cardiac output or the inability to maintain an adequate mean arterial pressure despite inotropic support or depression of brainstem function as a result of cerebral edema. Cardiac arrhythmias of most types may occur in the later stages and are usually caused by electrolyte disturbances (eg, hypo- or hyperkalemia, acidosis, hypoxia, or cardiac irritation by a central venous catheter).

Pulmonary Complications

Pulmonary complications are common and are usually present in about 50% of patients. Aspiration of gastric contents is a significant early complication, particularly in encephalopathic patients who are vomiting; hence, early elective ventilation to protect the airway is critical in these patients. Other complications include atelectasis, infection, intrapulmonary hemorrhage, respiratory depression, or pulmonary edema. In a series of 100 patients with FHF, 52 had radiologic changes and 37 of 52 had pulmonary edema.[60]

Coagulopathy

The liver synthesizes not only the coagulation factors (except factor VIII) but also inhibitors of coagulation and factors involved in the fibrinolytic system. ALF is characterized by decreased synthesis of clotting factors (factors II, V, VII, IX, and X), accelerated fibrinolysis, and impaired hepatic clearance of activated clotting factors and fibrin degradation products. The prothrombin time expressed as an INR is markedly elevated and is used as an indicator of the severity of the liver damage. Factors V and VII have the shortest half-lives of all of the coagulation factors and are theoretically more sensitive markers than INR of hepatic synthetic function. Significant disseminated intravascular coagulation is unusual in ALF. Thrombocytopenia may develop rapidly, and a platelet count of less than 100×10^9/L has been reported in about two-thirds of the patients. Causes include increased immune-mediated platelet destruction and decreased hepatic synthesis of factors necessary for platelet maturation and release into the peripheral blood. Also, abnormalities of platelet morphology and function have been reported.

Clinically, significant bleeding tends to be less severe compared with the degree of INR prolongation, although the risk of hemorrhage correlates with thrombocytopenia. Common sites of internal hemorrhage include the gastrointestinal tract, nasopharynx, lungs, and retroperitoneum. Intracranial hemorrhage is uncommon. The incidence of gastrointestinal hemorrhage has declined since the use of histamine$_2$ blockers in the management of ALF. A mild degree of hemophagocytosis (hemophagocytic lymphohistiocytosis) is commonly observed and contributes to coagulation abnormalities. The presence of significant disseminated intravascular coagulation usually indicates sepsis or secondary hemophagocytic lymphohistiocytosis.

Infections

Patients with ALF are at increased risk of bacterial infections because of poor host defenses. There is impaired Kupffer cell and polymorphonuclear function and reduced levels of factors such as fibronectin, opsonins, and chemoattractants, including components of the complement system. The additional predisposing factors are poor respiratory effort and cough reflex and the presence of an endotracheal tube, urinary catheters, and central venous and arterial lines.

Infection can lead to development and progression of multiorgan failure. An active uncontrolled infection also can render potential candidates disqualified for emergency liver transplant. It is very difficult to detect infection in the setting of ALF because there is a poor correlation between infection and normal indicators of infection, such as leukocyte count and fever. The risk factors for infections include coexisting renal failure, cholestasis, treatment with thiopental, and liver transplant. The presence of encephalopathy (grade 2 or above) has been shown to be associated with bacterial infection in about 80% of cases and fungal infections in about 32% of cases.[61] The sources of positive cultures include blood, urine, sputum, and catheter tips. More than two-thirds of bacterial infections are due to gram-positive bacteria, usually *Staphylococcus aureus*, but streptococci or gram-negative organisms such as coliforms are also isolated. *Candida* spp are the most common fungal infections. These are often unrecognized and ominous. Deterioration of HE after initial improvement, a markedly raised leukocyte count, pyrexia unresponsive to antibiotics, and established renal failure are strong indicators of fungal infection.

Acute Pancreatitis

Mild elevation of serum amylase is not uncommon, but clinically significant pancreatitis is unusual. It should be suspected in patients who complain of abdominal pain or have hypocalcemia. Etiologic factors include causative virus, hemorrhage into or around the pancreas, sodium valproate–related ALF, or shock.

Adrenal Hyporesponsiveness

In a recent adult study, 62% of patients with ALF had a poor response to a short synacthen test and showed significant improvement of hemodynamic parameters after administration of replacement therapy with hydrocortisone.[62] No pediatric data are available, but corticosteroid replacement could be considered in patients with intractable hypotension unresponsive to conventional therapy.

PROGNOSIS

The prognosis of ALF varies greatly with the underlying etiology. In an adult series from King's College Hospital, London, 50% of patients survived following acetaminophen overdose, whereas the survival rate was only 12.5% following halothane-induced ALF—66% for hepatitis A and 39% for hepatitis B.[63]

Prothrombin time is the best indicator of survival.[64] Bhaduri and Mieli-Vergani have shown that the maximum INR reached during the course of illness was the most sen-

sitive predictor of the outcome, with 73% of children with an INR less than 4 surviving compared with only 4 of 24 (16.6%) with an INR greater than 4.[6]

Factor V concentration has been used as a prognostic marker, especially in association with encephalopathy (Clichy criteria). In children, a factor V concentration of less than 25% of normal suggests a poor outcome, and in many French centers, this criterion is used for listing for liver transplant.[65]

Liver biopsy is rarely helpful in ALF and is usually contraindicated because of the presence of coagulopathy. However, it can be done using a transjugular approach. Hepatic parenchymal necrosis of more than 50% is associated with a reduced survival,[66] but the potential for sampling error is considerable. A biopsy taken from an area of complete collapse will show very few viable hepatocytes, indicating a poor prognosis. On the other hand, a biopsy specimen taken from a regenerative nodule may falsely give a good prognosis (Figure 58-3). These limitations and difficulty in performing a liver biopsy in patients with ALF have limited its value as a prognostic test.

A small liver or, more particularly, a rapidly shrinking liver is an indictor of a poor prognosis. Computed tomographic volumetry of the liver has been used to assess both the size of the liver and its functional reserve.[66]

Fulminant Wilson disease is invariably fatal, and emergency liver transplant is the only effective treatment.[67,68] Predicting the outcome of decompensated Wilson disease presenting as ALF is usually difficult. In our experience, a prognostic score has been useful in identifying the patients who carry a high risk of mortality without liver transplant (Table 58-6). It incorporates bilirubin, INR, aspartate transaminase, white blood cell count, and albumin at presentation. A score of 11 or more indicates high mortality, with 93% sensitivity and 96% specificity.

Survival depends on the ability of the liver to recover from the ensuing insult, but it is very difficult to predict the potential of recovery. There are no single criteria that can predict the outcome with absolute certainty and be universally applicable for all patients with ALF with different etiologies. However, prediction of a low level of survival (chance of < 20%) is clinically useful to decide to list the patient for orthotopic liver transplant (OLT), which has a 1-year survival rate of 75%.[69]

MANAGEMENT

An initial contact with a specialist center at diagnosis should be made to establish a management plan. Depending on the local facilities and expertise, the timing of referral to hepatology and liver transplant centers may vary, but an early transfer is always recommended. Liver transplant has undoubtedly improved the survival of this group of patients, but better intensive care monitoring and early and better management of complications can sometimes avoid transplant or death. Also, monitoring of these patients is a continuous process of assessing the clinical status so that a child who is not considered for liver transplant initially may change his/her status. Likewise, a child who has been listed for liver transplant may show signs of unexpected improvement, hence not requiring transplant operation, or may develop complications that contraindicate it.

Initial Assessment

A careful and detailed history should include the mode of onset of illness, family history of liver disease, consanguinity, and exposure to drugs and toxins. Clinical examination

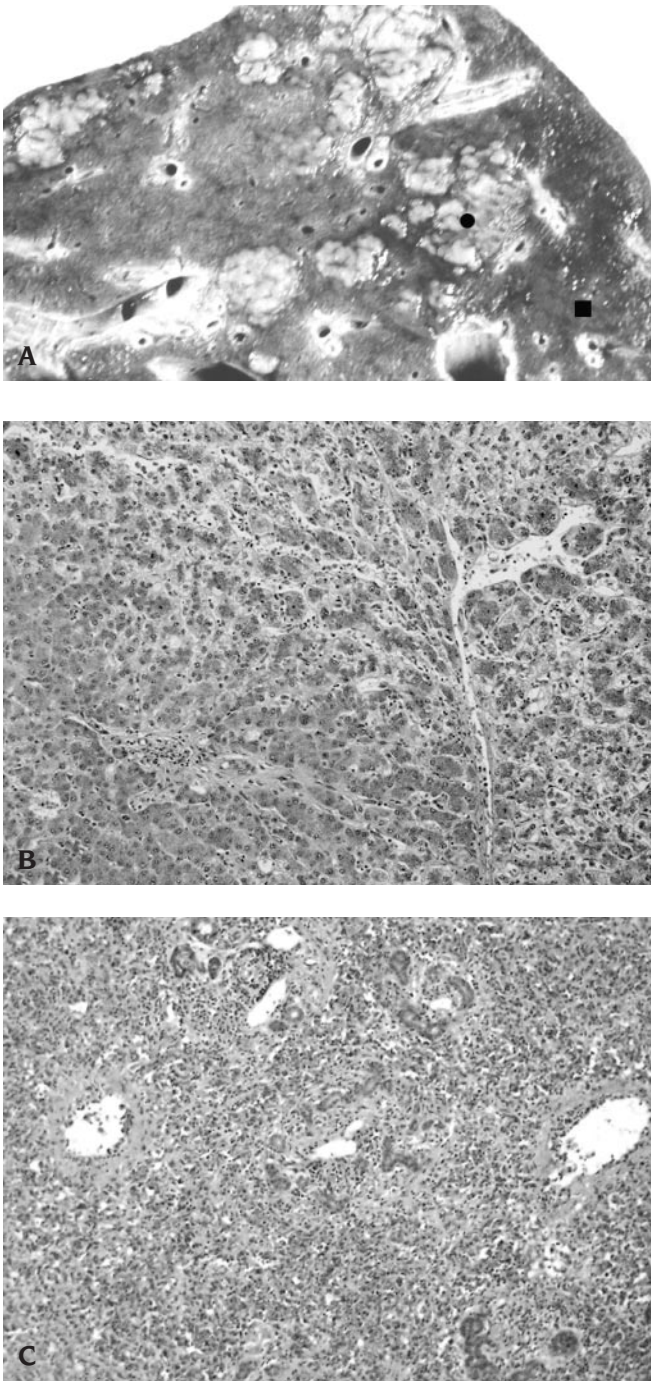

FIGURE 58-3 *A*, Macroscopic appearance of areas of collapse (■) and regeneration (●) in an explanted liver. Microscopic appearances from these areas show regenerating hepatic parenchyma (*B*) or collapse (*C*) (hematoxylin and eosin; ×100 original magnification). Courtesy of Professor Bernard Portmann, King's College Hospital.

TABLE 58-6 WILSON DISEASE INDEX

SCORE	BILIRUBIN (μMOL/L)	INR	AST (IU/L)	WBC (10^9/L)	ALBUMIN (G/L)
0	0–100	0–1.2	0–100	0–6.7	> 45
1	101–150	1.3–1.6	101–150	6.8–8.3	34–44
2	151–200	1.7–1.9	151–200	8.4–10.3	25–33
3	201–300	2.0–2.4	201–300	10.4–15.3	21–24
4	> 300	> 2.5	> 300	> 15.4	0–20

AST = aspartate transaminase; INR = international normalized ratio; WBC = white blood cell count.

could give diagnostic clues such as the presence of any herpetic vesicles, signs of underlying chronic liver disease, and the presence of Kayser-Fleischer rings on slit lamp examination. All children with ALF require elaborate investigations to establish the underlying cause (Tables 58-7 and 58-8).

General Measures

All children with ALF should be nursed in a quiet environment with as little stimulation as possible to minimize acute increase in the ICP. Children with encephalopathy or an INR greater than 4 (without encephalopathy) should be admitted to an intensive care unit for continuous monitoring. Sedation is contraindicated unless the patient is to be mechanically ventilated because of the possibility of aggravating the encephalopathy or precipitating respiratory failure.

Monitoring of nonventilated ALF patients should include the following:

- Continuous oxygen saturation monitoring
- 6-hourly urine output
- 6-hourly vital signs including blood pressure, neurologic observations, and blood glucose estimation
- 12-hourly electrolyte and coagulation studies (INR)
- Daily full blood count along with surveillance blood and urine cultures

In addition, patients on assisted ventilation have an arterial line for invasive blood pressure monitoring and frequent blood sampling. Blood gas analysis is performed every 4 hours, and electrolytes and prothrombin time are measured every 8 hours. Sedation in these ventilated patients is maintained by a morphine and midazolam infusion in our unit. The use of benzodiazepines for sedation in ALF is only recommended once a decision has been taken to ventilate the patient (management of specific complications is discussed later). Controlled trials in adults have failed to substantiate any beneficial effect of corticosteroids, interferon, insulin and glucose, prostaglandin E_1, bowel decontamination, and charcoal hemoperfusion in patients with ALF. The use of enemas and cathartics in patients who are not constipated is usually counterproductive, not only by increasing their nursing need but also by triggering surges of ICP. Hypoglycemia should be avoided by use of intravenous glucose infusion or by ensuring adequate enteral intake. Total fluid intake is restricted to two-thirds maintenance if there is no evidence of dehydration, with the idea of decreasing the possibility of development of cerebral edema. The idea of protein restriction to limit the possibility of HE has now been disregarded, and adequate calories should be provided. Oral or nasogastric feeding is usually well tolerated. Prophylactic broad-spectrum antibi-

TABLE 58-7 DIAGNOSTIC TESTS OF THE CAUSES OF ACUTE LIVER FAILURE

CAUSE	TEST
Hepatitis A infection	Anti-HAV antibody (IgM)
Hepatitis B infection	
Acute infection/seroconversion	Anti–core antibody (IgM)/HBV profile
Increased replication	Full HBV profile
Hepatitis D infection	Anti-HDV antibody (IgM)
Parvovirus, adenovirus, EBV	Viral serology/antigen tests, PCR
Seronegative hepatitis	Diagnosis of exclusion (all tests)
Acetaminophen	History, drug level in blood
Mushroom poisoning	History, diarrhea
Autoimmune hepatitis	Autoantibodies, immunoglobulins
Wilson disease	Urinary copper, Kayser-Fleischer rings, Coombs-negative hemolytic anemia
Galactosemia	Galactose-1-phosphate uridyl transferase level in blood
Tyrosinemia	Urinary succinylacetone
Neonatal hemochromatosis	Buccal mucosal biopsy, raised ferritin, high transferrin saturation
Hemophagocytic lymphohistiocytosis	Bone marrow aspiration (typical cells)
Mitochondrial hepatopathies	Muscle and liver biopsies for quantitative assay of respiratory chain enzyme
Veno-occlusive disease	Doppler ultrasonography/venography
Malignancies	Imaging (CT/MRI) and histology
Idiosyncratic drug reactions	History, eosinophil count

CT = computed tomography; EBV = Epstein-Barr virus; HAV = hepatitis A virus; HBV = hepatitis B virus; HDV = hepatitis D virus; IgM = immunoglobulin M; MRI = magnetic resonance imaging; PCR = polymerase chain reaction.

TABLE 58-8 INVESTIGATIONS IN INFANTS AND CHILDREN WITH ACUTE LIVER FAILURE

- Biochemical tests
  - Liver function tests (total and direct bilirubin, AST, ALT, GGT, ALP, albumin)
  - Blood sugar
  - Serum electrolytes
  - Serum calcium, phosphorus
  - Serum magnesium
  - Uric acid
  - Cholesterol
  - Triglyceride
  - Amylase
  - α_1-Antitrypsin phenotype
  - Galactose-1-phosphate uridyl transferase (in infants and neonates)
  - Serum copper and ceruloplasmin (in children > 3 yr old)
  - Serum amino acids
  - Plasma acylcarnitines
  - Blood gas analysis
- Hematologic tests
  - Full blood count
  - Reticulocyte count
  - Prothrombin time or INR
  - Blood for grouping and crossmatching
  - Direct Coombs test
  - Bone marrow examination (in seronegative hepatitis or if hematologic malignancy or HLH is suspected)
- Ultrasound scan of abdomen, especially liver, portal and hepatic veins, inferior vena cava, biliary system, and spleen
- Microbiologic tests
  - Bacterial cultures: blood, urine, stool, throat swab, sputum, skin lesion if present, ascitic fluid if present
  - Viral culture of urine and skin lesion if present
- Serologic tests
  - Viral hepatitis: anti-HAV IgM antibody, HBsAg, HBcAg, hepatitis D antigen and antibody, anti–hepatitis C antibody, anti–hepatitis E antibody
  - Cytomegalovirus
  - Epstein-Barr virus
  - Human immunodeficiency virus (HIV)
  - Measles
  - Varicella
  - Herpes simplex virus
  - Adenovirus
  - Echovirus
  - Others: toxoplasmosis, leptospirosis, and listeriosis
- Immunologic tests
  - Immunoglobulins(IgG, IgA, and IgM)
  - Tissue antibodies (anti-SMA, GPC, mitochondrial, liver-kidney microsomal and antinuclear antibodies)
  - Complement C3 and C4
- Ascitic fluid or cerebrospinal fluid cytospin for evidence of hemophagocytosis
- Urine
  - Toxicology
  - Chemical analysis, osmolality, and electrolytes
  - Organic acids
  - Succinyl acetone
  - 24-h urinary copper prepenicillamine and postpenicillamine (2 doses of 500 mg 12 h apart)
  - (5 mL of serum and aliquot of initial urine for possible subsequent investigations)
- Tissue studies
  - Buccal mucosal biopsy
  - Muscle biopsy
  - Skin fibroblast culture
  - Transjugular liver biopsy

ALP = alkaline phosphatase; ALT = alanine transaminase; AST = aspartate transaminase; GGT = γ-glutamyl transferase; GPC = gastric parietal cell; HAV = hepatitis A virus; HBcAg = hepatitis B core antigen; HBsAg = hepatitis B surface antigen; HLH = hemophagocytic lymphohistiocytosis; INR = international normalized ratio; SMA = smooth muscle antibody.

otics[70] and antifungals[71] significantly reduce the incidence of infective episodes. In neonatal liver failure, intravenous acyclovir should be commenced.

MANAGEMENT OF SPECIFIC COMPLICATIONS

Neurologic Complications

The most important neurologic complications are HE and cerebral edema. Management options for the treatment of HE are limited. Ammonia-lowering measures such as dietary protein restriction, bowel decontamination, or lactulose are of limited or no value in rapidly advancing encephalopathy. The use of branched-chain amino acids, flumazenil, and extracorporeal circuits has only shown transient improvement in encephalopathy, without any survival benefit in larger studies.

Cerebral edema has been documented at the time of postmortem in about 30 to 40% of all patients with fatal ALF. Typical features of raised ICP include systemic hypertension, hypertonia, hyperreflexia, decerebration, hyperventilation, dysconjugate eye movements, or squint. In infants, the anterior fontanel may be tense. If uncontrolled, the clinical features progress to loss of pupillary reflexes and, ultimately, impairment of brainstem reflexes. Despite severely increased ICP, papilledema is a rare sign. Systemic hypertension is a good surrogate marker for increased ICP in the initial stages but is absent in later stages.

To date, the opinion is divided toward the risk-to-benefit ratio of using ICP monitoring devices for early detection of increased ICP. Proponents of the use of ICP monitors argue that these devices allow early and accurate detection of changes in ICP, especially in ventilated and sedated patients, in whom the clinical signs are usually masked. It also helps in accurate monitoring of ICP during interventions such as central line insertions, tracheal suctioning, and hemodialysis or hemodiafiltration. Keays and colleagues have shown that ICP monitoring identified rises in ICP unaccompanied by clinical signs, and, as a consequence, treatment was given to the monitored patients more often than the nonmonitored group. The duration of survival from the onset of grade 4 encephalopathy was significantly greater in the ICP-monitored group, although the overall survival rate was unchanged. Monitoring also provided important prognostic information because the peak ICP was higher in nonsurvivors than in survivors.[72] The group that was not enthusiastic about invasive ICP monitoring argued that the procedure is associated with a high incidence of intracranial bleeding and is never proven

to improve patient survival. Epidural transducers are associated with a lower complication rate (3.8%) compared with subdural devices that carry a 22% risk of intracranial complications.[8,73] High ICP at the time of insertion of the device has been shown to be a major risk factor for the development of intracranial hemorrhage. Our practice is to insert a subdural bolt in children older than 2 years who have clinical signs of increased ICP and are awaiting liver transplant.

The aim of ICP monitoring is to maintain cerebral perfusion pressure (mean arterial blood pressure – ICP) at more than 50 mm Hg. If the cerebral perfusion pressure falls below 50 mm Hg, the adequacy of sedation and paralysis should be checked, along with $PaCO_2$ levels (in ventilated patients, $PaCO_2$ should be kept between 4 and 4.5 kPa). If the $PaCO_2$ is more than 4.5 kPa, then hyperventilation may be helpful. Excessive hyperventilation should be avoided because it may paradoxically compromise the cerebral perfusion pressure. A care pathway for the management of raised intracranial pressure is shown in Figure 58-4.

Mannitol remains the mainstay of treatment for increased ICP because of its property as an osmotic diuretic. It has also been suggested that a therapeutic response is due to the increase in cerebral blood flow, and the rapidity of action of mannitol is more consistent with this function. A rapid bolus of 0.5 g/kg as a 20% solution over a 15-minute period is recommended, and the dose can be repeated if the serum osmolarity is less than 320 mOsm/L. In anuric patients, a diuresis is simulated by ultrafitrating three times the administered volume over the next half hour.

Uncontrolled studies have shown sodium thiopental to be an effective agent in controlling mannitol-resistant cerebral edema.[74] A bolus dose of 2 to 4 mg/kg over 15 minutes is followed by a slow intravenous infusion of between 1 and 2 mg/kg/h. There has been no controlled trial of the use of sodium thiopental in lowering ICP. Major concerns are hemodynamic instability and increased incidence of infective complications following its administration. Hypothermia (core body temperature of 32°C) has been shown to be effective in the management of severe intracranial hypertension with lowering of ICP and improvement of cerebral perfusion pressure in adults.[75] Subclinical seizure activity also has been suggested as a contributing factor for the development of cerebral edema. In a study of 42 adult patients, Ellis and colleagues demonstrated a significant reduction in the seizure activity in the group treated with phenytoin infusion. Incidence of cerebral edema was also significantly less in the phenytoin-treated group compared with the control group.[76]

In severe unresponsive cerebral edema, the emphasis of management shifts toward preservation of cerebral perfusion pressure, increased oxygen delivery to the brain, and manipulation of the neuronal microcirculation to promote cerebral oxygen extraction. In this situation, inotropic agents can be used to increase the mean arterial pressure, consequently improving cerebral perfusion pressure. Normally, at this stage, spontaneous recovery is unlikely without liver transplant.

Hepatectomy with a portacaval shunt has been shown to stabilize the patients hemodynamically with reduction of ICP up to 48 hours followed by successful liver transplant. *N*-Acetylcysteine has been shown to increase the cerebral blood flow and cerebral metabolic rate, thereby improving the microcirculatory stability.

Infections

Bacterial and fungal infections have been documented in about 82 and 34% of patients with ALF, respectively.[61] About 60% of deaths in ALF have been attributed to sepsis.[77] Prophylactic intravenous antibiotics have been shown to reduce the incidence of culture-positive bacterial infection from 61.3 to 32.1%. The respiratory tract is the most common site (47%), followed by the urinary tract (23%).[77] A high index of suspicion, along with early and frequent bacteriologic investigations, is necessary for early diagnosis. Gram-positive bacteria are the most common organism isolated in about 70% of cases, 35% of these isolates being *S. aureus*.[77]

Topical antifungal prophylaxis has been used in combination with intravenous antibiotics. The efficacy of systemic antifungals as prophylaxis has not been studied systematically. Systemic fungal infection is very difficult to diagnose, but renal failure, severe cholestasis, previous or concomitant thiopental therapy, concomitant immunosuppressive therapy, and worsening coagulopathy are the high-risk factors. The choice of systemic antifungal agents is determined by the local experience. In our unit, fluconazole is the preferred agent because *Candida* spp. account for most of the fungal infections.

Hemodynamic Instability

Circulatory failure is a common mode of death in patients with ALF, often complicating sepsis or multiorgan failure. Invasive hemodynamic monitoring may provide early evidence of circulatory failure. Despite the presence of edema, frequently these patients have intravascular volume depletion and need an appropriate combination of colloids, crystalloids, or blood products. In the presence of persistent hypotension despite normal filling, pressure vasopressors such as noradrenaline and adrenaline are inotropic agents of choice. *N*-Acetylcysteine has been shown to improve the

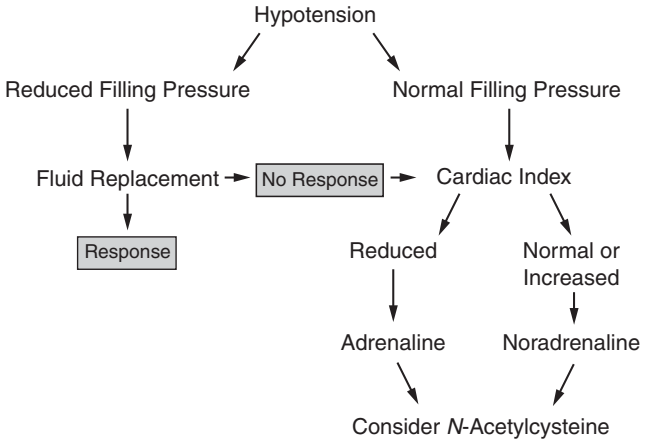

FIGURE 58-4 Care pathway for the management of hypotension.

parameters of oxygen metabolism. A combination of prostacycline and *N*-acetylcysteine has been found to be more beneficial for oxygen metabolism than either drug alone. In our experience, newer monitoring devices such as pulse contour cardiac output (PiCCO) and lithium dilutional cardiac output (LiDCO) monitoring, which can measure various body water compartments, are good devices to rationalize fluid management and the choice of vasopressors. The PiCCO method is a combination of transpulmonary thermodilution technique and arterial pulse contour analysis. It requires a central venous line and an arterial thermodilution catheter placed in either a femoral or an axillary artery. A bolus of cold saline is injected in the central venous line, and the thermodilution is detected from the special arterial catheter. It measures cardiac ouput (by thermodilution), continuous cardiac output using the pulse contour method, intrathoracic blood volume, and extravascular lung water.[78] LiDCO monitoring involves a bolus indicator dilutional technique for measuring cardiac output using isotonic lithium chloride as an injectate.[79] A care pathway for the management of hypotension is shown in Figure 58-5.

Renal Failure

Renal failure with severe oliguria often develops in ALF, especially in later stages. Although the mechanism of renal failure is not clear, it is essential to correct intravascular hypovolemia. Recent studies have shown that low-dose dopamine is not only ineffective[80] but can have deleterious effects,[81] especially in the setting of profound vasodilatation, which is seen typically in ALF. Extracorporeal renal support was required in 75% of cases with acetaminophen-induced ALF and in 30% of patients with other etiologies of ALF. Hemodiafiltration and hemodialysis should be instituted when the urine output is less than 1 mL/kg/h. Continuous filtration or dialysis systems are associated with less hemodynamic instability and consequently less risk of aggravating latent or established encephalopathy than intermittent hemodialysis. In spite of the presence of coagulopathy, heparin requirements have been shown to be increased. Recently, prostacycline infusion at a rate of 5 ng/kg/min has been found to be superior to heparin anticoagulation with respect to functional duration of the filters and the hemorrhagic complications.[82]

Coagulopathy

Bleeding diathesis, although always present in ALF, differs in severity from patient to patient. Normally, disseminated intravascular coagulation is not a feature of ALF but is usually an indicator of sepsis. The possible advantage of reduced bleeding by repletion of coagulation factors with fresh frozen plasma has not been established by clinical studies. Because coagulopathy is a very good tool for assessment of prognosis and monitoring of disease progression, correction of coagulopathy is indicated only if the patient is already listed for transplant or prior to an invasive procedure such as insertion of a central line or ICP monitors. This also carries a further disadvantage of volume overload and hyperviscosity. There has been a poor correlation between the severity of prolongation of prothrombin time and bleeding tendencies, but associated thrombocytopenia is an important risk factor for hemorrhage; hence, the platelet count should be maintained above 50×10^9/dL.

The most common site of bleeding is the gastrointestinal tract. Prophylactic ranitidine (histamine$_2$ blocker) or proton pump inhibitors have been shown to decrease the incidence of gastric bleeding.[83] A study comparing antacid with histamine$_2$ blockers showed equal efficacy if the gastric pH was kept above 3.5 by frequent administration of antacid. There is increased risk of gastric colonization with use of histamine$_2$ blockers or proton pump inhibitors. Sucralfate has the potential advantage of reducing gastric colonization and pulmonary infection by maintaining gastric acidity, but its efficacy in ALF has not been assessed.

Ventilatory Management

Ventilatory support in the form of mechanical ventilation is instituted when grade 3 encephalopathy develops or when patients in grade 1 or 2 encephalopathy require sedation. Inducing agents such as suxamethonium and fentanyl are generally safe. Sedation could be maintained with a combination of an opiate such as morphine or fentanyl and a hypnotic such as midazolam. There are no special ventilatory requirements in patients with ALF; however, a peak end-expiratory pressure above 8 cm of water should be avoided because it may increase ICP.

Initially, adult respiratory distress syndrome (ARDS) is unusual, and ventilation is easy. The most severe ventilatory problems arise when liver function is improving or the patient had liver transplant when there is a chance of developing ARDS. In patients with unresponsive ARDS, nitric oxide inhalation or intravenous prostacycline may be tried, although their role has not been properly assessed in ALF.

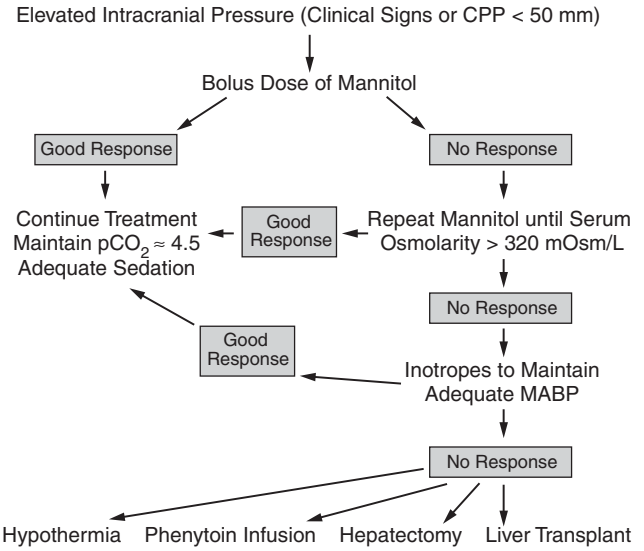

FIGURE 58-5 Care pathway for the management of raised intracranial pressure. CPP = cerebral perfusion pressure; MABP = mean arterial blood pressure; pCO_2 = carbon dioxide partial pressure.

Liver Assist Devices

In ALF, pathophysiologic changes are due to impairment of synthetic, detoxifying, and biotransformatory activity resulting from the loss of functioning hepatocytes and Kupffer cells. The severity and duration of these changes also depend on the rate and extent of liver regeneration. There has been increasing interest in the possibility of providing an extracorporeal liver support system either as a bridge to liver transplant or, ideally, to obviate the need for it by supporting liver function while the native liver regenerates. Liver support devices could be either cleansing devices or a bioartificial liver support system (Table 58-9). Cleansing devices perform only the detoxifying function of the liver, whereas bioartificial liver support systems have a theoretic advantage of providing the synthetic and detoxifying properties.

Charcoal hemoperfusion was extensively assessed in an initial study of 76 adults and suggested an increase in survival in patients with grade 3 HE,[84] but a subsequent controlled trial did not show any significant difference in outcome.[63]

Recently developed cleansing devices such as Biologic-DT and the molecular adsorbent recirculating system (MARS) attempt to remove protein-bound toxins by perfusion over resins or albumin. The MARS uses an albumin-impregnated dialysis membrane and dialysate containing 5% human albumin solution. This dialysate is perfused to remove water-soluble toxins, including ammonia. Preliminary studies in adults showed a survival rate of 69%, with hemodynamic stabilization, improvement in mental status, and HE.[85] More information is required before this device can be recommended universally.

The bioartificial liver support system uses bioreactors containing hepatocytes in columns. Of three bioartificial liver support devices, the bioartificial liver and the Berlin Extracorporeal Liver Support System use primary porcine hepatocytes, whereas the extracorporeal liver assist device uses the hepatoblastoma cell line. Anticoagulated whole blood or plasma is passed through a device, allowing metabolic transfer between perfusate and hepatocytes. Although preliminary results were encouraging, a controlled pilot study of the ELAD did not show any significant benefit.[86] The bioartificial liver system has shown statistical improvement in the level of consciousness, an increase in ICP, and an increase in cerebral perfusion pressure.[87] These devices appear to be promising, but pediatric experience is limited and anecdotal.

TABLE 58-9 TYPES OF LIVER ASSIST DEVICES

CLEANSING DEVICES	BIOARTIFICIAL LIVER SUPPORT SYSTEMS
Charcoal hemoperfusion	Bioartificial liver
Biologic-DT	Extracorporeal liver assist device
Plasmapheresis	Berlin Extracorporeal Liver Support System
Molecular adsorbent recirculating system	

Liver Transplant

Liver transplant is the only proven treatment that has improved the outcome of ALF. Taking into account all age groups, ALF accounts for 5 to 7% of all liver transplants in the United States[88] and about 11% in Europe.[89] Reports from Europe and the United States have shown that about 45 to 50% of cases with ALF undergo liver transplant, whereas in 13 to 27% of cases, it was contraindicated at the time of admission. About 6 to 18% of cases were removed from the waiting list because of development of a contraindication or the improvement in the clinical and prognostic status or died before a donor liver became available.[8]

Because of the scarcity of donor organs, optimal use of donor livers is essential. This entails proper selection of the patients as well as timing of the operation. In adults, the King's College Hospital criteria have been extensively used for listing for liver transplant (Tables 58-10 and 58-11).[64]

Contraindications for liver transplant are fixed and dilated pupils, uncontrolled sepsis, and severe respiratory failure (ARDS). Relative contraindications are accelerating inotropic requirements, infection under treatment, cerebral perfusion pressure of less than 40 mm Hg for more than 2 hours, and a history of progressive or severe neurologic problems in which the ultimate neurologic outcome may not be acceptable.

After successful transplant, cerebral edema can persist for 12 hours, and cerebral autoregulation is restored within 48 hours. In contrast, the hemodynamic and the neurologic parameters improve during the anhepatic phase of the operation. Extending this advantage, there have been reports of using a two-stage procedure in very unstable patients with hepatectomy followed by liver transplant as soon as the donor liver is available. The longest time of the anhepatic phase preceding OLT has been 48 hours in our experience. Ringe and Pichlmayr reported the largest series of this two-stage procedure, but only 59% of patients received liver transplant[90]

Auxiliary Liver Transplant

The rationale behind this technique is that the allograft provides liver function while the native liver regenerates.

TABLE 58-10 INDICATORS OF A POOR PROGNOSIS IN ACETAMINOPHEN-INDUCED ACUTE LIVER FAILURE

PARAMETER	SENSITIVITY (%)	SPECIFICITY (%)	POSITIVE PREDICTIVE VALUE (%)
Arterial pH < 7.3	49	99	81
All 3 of the following:	45	94	67
Prothrombin time > 100 s or INR > 6.5			
Creatinine > 300 µmol/L (2.3 mg/dL)			
Grade 3–4 encephalopathy			

INR = international normalized ratio.

TABLE 58-11 INDICATORS OF A POOR PROGNOSIS IN NONACETAMINOPHEN ETIOLOGIES OF ACUTE LIVER FAILURE

PARAMETER	SENSITIVITY (%)	SPECIFICITY (%)	POSITIVE PREDICTIVE VALUE (%)
Prothrombin time > 100 s or INR > 6.7	34	100	46
Any 3 of the following:	93	90	92
Unfavorable etiology (seronegative hepatitis or drug reaction)			
Age < 10 yr or > 40 yr			
Acute or subacute categories			
Serum bilirubin > 300 μmol/L (2.3 mg/dL)			
Prothrombin time > 50 s or INR > 3.5			

INR = international normalized ratio.

Once the native liver shows signs of recovery, immunosuppression can be weaned and eventually stopped. Auxiliary liver transplant could be heterotopic (the donor graft is placed alongside the native liver in the right upper quadrant) or orthotopic (part of the native liver is resected and replaced with a reduced-size graft), replacing the right lobe, left lobe, or left lateral segment. Auxiliary partial OLT is preferred over heterotopic. In our center, all patients who undergo auxiliary liver transplant undergo elective liver biopsies at day 7, 6 months, and 1 year, along with computed tomographic volumetry (to assess the volume of native liver and graft) and radionucleotide scan (diisopropyl iminodiacetic acid to assess the relative function of the native liver and the graft). Once the native liver has returned to normal morphology and function, immunosuppression is gradually weaned off. The donor liver is supposed to atrophy with time. In our experience, 12 of 14 children who had auxiliary liver transplant survived, and 7 of them have been able to stop immunosuppression after a median post-transplant interval of 9 months (M. Rela, unpublished data, 2003).

Because auxiliary partial OLT is technically demanding, the choice of this operation is ideally left to the local surgical team.There have been no universally accepted indications for auxiliary liver transplant in the setting of ALF.

Hepatocyte Transplant

Taking the success of auxiliary liver transplant further, hepatocyte transplant has been tried in experimental animals with improved survival. In a small number of clinical studies, variable improvement in encephalopathy, coagulopathy, and hyperammonemia has been reported. The procedure has shown some encouraging results as a bridge to transplant, and in one child, liver transplant was avoided; however, the technique remains experimental.[91,92]

CONCLUSION

ALF is a multisystem disorder with a very high mortality rate. In children, seronegative hepatitis is the most common cause of ALF in Western countries. Despite improvement in intensive care support, liver transplant is the only effective treatment. Liver assist devices and hepatocyte transplant hold a great potential of providing a bridge to transplant or avoiding it while the native liver regenerates. The condition should ideally be managed in a liver center with facilities for liver transplant.

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CHAPTER 59

TREATMENT OF END-STAGE LIVER DISEASE

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The challenges of treating children with end-stage liver disease are considerable. As liver function deteriorates, a cascade of complications ensues, involving every major organ system. The pivotal role of the liver as the body's biochemical "brain" becomes even more evident as liver function is lost. As well, the liver is positioned at the interface of the splanchnic and systemic circulations and has a profound influence on hemodynamics.

The many diverse causes of chronic liver disease in children are discussed elsewhere, but all share a similar final common pathway as chronic liver failure evolves. Although the spectrum of complications resulting from chronic liver disease is similar in children and adults, children are more vulnerable to the profound effects of the failing liver on growth, development, and nutrition. As well, the management of portal hypertension and bleeding varices is made more technically difficult in small children because therapeutic interventions are often limited by the diminutive size of the patient and their vasculature.

The success of liver transplant over the last two decades has given the pediatric hepatologist a new perspective on managing end-stage liver disease. Because many of these children are transplant candidates, medical care has changed dramatically from the pre-1980s philosophy of supportive, palliative treatment preceding inevitable death to anticipatory management that will maintain the child in the best possible condition until transplant. One of the most effective means that the pediatric hepatologist has to ensure survival after liver transplant is to prevent the child from becoming critically ill before transplant. Keeping this in mind, this chapter reviews the management of the major complications of end-stage liver disease in children.

NUTRITIONAL SUPPORT

Cholestatic liver diseases are the most important etiology of end-stage liver disease in children. Biliary atresia is the most common, followed by the intrahepatic cholestasis syndromes. Irrespective of the cause of cholestasis, all share an increased risk of malnutrition. Because most of these children are candidates for liver transplant and malnutrition is a known factor that adversely affects morbidity and mortality after transplant (Figure 59-1),[1–3] early recognition and timely intervention are essential.

The cause of malnutrition is multifactorial (Table 59-1).[4,5] Fat malabsorption is inevitable because of the dependence of long-chain fatty acids, the most important component of dietary fat, on the micellar action of bile acids in the intestinal lumen for absorption.[6] Compounding the risk, most children afflicted with cholestatic liver disease are infants, with high caloric requirements for growth and development. As well, increased intra-abdominal pressure, either secondary to ascites or organomegaly, is a frequent complication causing early satiety, diminished oral intake,

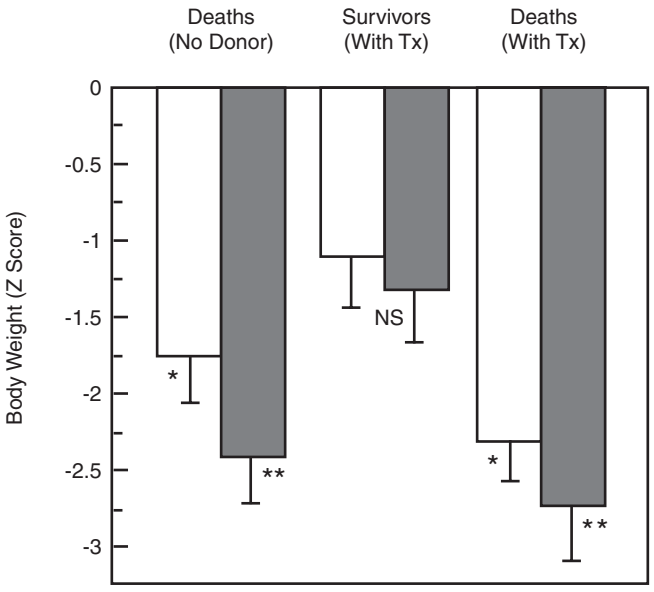

FIGURE 59-1 Z-scores for weight at the time of referral and at the time of transplant or death without a donor in three outcome groups of children with end-stage liver disease accepted for liver transplant. Open bars are scores at referral; black bars are scores at transplant or death. Deaths (no donor), $n = 8$; survivors, $n = 23$. Deaths (with transplant [Tx]), $n = 6$; $p < .05$ for deaths. *$p < .02$ versus survivors; **$p < .02$ versus survivors. NS = not significant. Reproduced with permission from Shepherd RW et al.[1]

TABLE 59-1 FACTORS CONTRIBUTING TO MALNUTRITION IN END-STAGE LIVER DISEASE

Fat malabsorption 2° to cholestasis
Diminished oral intake 2° to ↑ abdominal girth
Ascites
Organomegaly
↑ Abdominal pressure → ↑ emesis
Peripheral resistance to anabolic effects of growth hormone
Anorexia 2° to chronic disease
↑ Catabolic rate
Intercurrent infection
Complications

and an increased risk of postprandial emesis. These infants are often debilitated by the anorexia of chronic disease, which is further exacerbated when the complications of end-stage liver disease supervene. Oral intake further decreases at a time when catabolic stresses are increased, resulting in a widening discrepancy between caloric requirement and intake. As the cycle of cholestasis-induced malabsorption and inadequate calorie and protein intake intensifies, these small children rapidly decompensate to clinically evident protein-energy malnutrition. First, subcutaneous tissue stores are depleted (evidenced by decreasing triceps skinfold thickness), followed by loss of muscle mass (measured by decreases in midarm circumference). Eventually, if left untreated, these severely cholestatic infants will present emaciated, hypotonic, dehydrated, and in need of emergency medical care.

Important metabolic and endocrine derangements are associated with chronic liver disease, further intensifying protein-energy malnutrition. Peripheral insulin resistance causing increased gluconeogenesis depletes muscle protein stores. The ratio of aromatic amino acids to branched-chain amino acids (BCAAs) (leucine, isoleucine, valine) in the peripheral blood increases, secondary to increased use of BCAAs by peripheral muscle.[7] BCAAs have important anticatabolic effects and a role in optimizing nitrogen use. As well, caloric requirements are increased above normal estimates for age, as demonstrated by studies in which children failed to show improvement in nutritional status, unless they received in excess of 1.5 to 2 times the estimated caloric requirements for age.[4,8]

Of particular importance in children are the now well-described abnormalities in the growth hormone (GH) and insulin growth factor (IGF) axis in cholestatic liver disease.[9,10] GH mediates most of its anabolic effects through IGF-1. The IGF-binding proteins (IGFBPs), especially IGFBP-3, regulate the transport of IGF to the tissues. Although IGF-1 and IGFBP-3 are produced in a variety of tissues, the most important site of synthesis is the hepatocyte for IGF-1 and the Kupffer cell for IGFBP-3. In children without liver disease but with protein-energy malnutrition, IGF-1 levels fall, suggesting an important link between nutritional status and growth.[11] In children with cholestatic liver disease, including both biliary atresia and Alagille syndrome, several authors have shown that not only are GH levels increased above normal, but IGF-1 levels are decreased, suggesting peripheral resistance to GH. In a study of children with chronic liver disease, short stature, and increased GH levels, Bucuvalas showed that the expected effects of high GH levels, lypolysis, and decreased insulin sensitivity did not occur.[12] As well, several authors have shown that despite adequate protein calorie intake, GH supplementation of children with biliary atresia or Alagille syndrome had no beneficial effect on growth, anthropometric measurements, or body composition.[10,13] In addition, circulating IGF-1 and IGFBP-3 levels were unaffected.[14] This resistance to exogenous GH in the face of low levels of IGF-1 raises the possibility that these growth-retarded children may benefit from IGF-1 treatment.[10,14]

The assessment of nutritional status in children with chronic liver disease is complicated by the effects of fluid retention, ascites, and often massive hepatosplenomegaly, making changes in weight, or weight-for-height ratios, unreliable. Although height z-scores are more useful, a falloff in linear growth is a relatively late consequence of malnutrition. To accurately assess the earlier stages of protein-energy malnutrition and to follow the effects of nutritional interventions, anthropometric measurements are essential. Midarm circumference correlates with lean body mass (protein stores) and triceps or subscapular skinfold thickness with body adipose stores. More sophisticated estimates of body cell mass measure total-body potassium content. Because more than 90% of body potassium is intracellular, its measurement is not affected by fluid shifts between the intra- and extracellular compartments.[15] These principles are well demonstrated by a study that evaluated 56 children with chronic liver disease. Mean height z-scores were decreased, whereas mean weight and mean weight-for-height z-scores were close to normal.[16] Moreover, triceps skinfold z-scores were the most depressed, compared with either height or weight z-scores. Midarm circumference was also depressed but not as markedly as triceps skinfold thickness, suggesting that lean body mass was not as severely depleted as body adipose tissue reserves. This study demonstrates the important principle that children with chronic liver disease have evidence of both chronic malnutrition, as evidenced by stunted growth (decreased height z-scores), as well as acute malnutrition, characterized by depressed adipose reserves and lean body mass.

Improving nutritional status in children with chronic liver disease is challenging (Table 59-2).[17,18] Anorexia, increased abdominal girth, and intervening complications often severely limit the child's voluntary enteral intake. As well, the sodium and fluid restrictions required to manage ascites may sabotage efforts to increase caloric intake. Specialized formulas high in medium-chain triglycerides, which are not dependent on bile for absorption, are useful adjuvants. Caloric density may be further increased by the addition of medium-chain triglyceride and glucose.

However, these measures are often insufficient, necessitating supplemental nasogastic or jejunal feeds[19] or, failing this, parenteral nutritional support. Nocturnal drip feedings appear to be the best tolerated and can allow high caloric intakes without the rise in serum ammonia that might be anticipated with the increased protein intake.[8,20]

TABLE 59-2 ESCALATING LEVELS OF NUTRITIONAL SUPPORT

Change to specialized formula
Low Na
Enriched MCT content
Supplement vitamins A, D, K, and E
↑ Caloric density of voluntary enteral formula intake
24.27 cal/oz
Addition of MCT oil or polycose
Supplement voluntary enteral intake
Continuous nocturnal NG or NJ feeds
Parenteral nutritional support
If fails to grow on maximum enteral feed or unable to tolerate ↑ enteral feeds
Intralipid supplementation only
Intralipid + glucose/amino acid solutions + parenteral vitamins

MCT = medium-chain triglyceride; NG = nasogastric; NJ = nasojejunal.

The usefulness of supplementing enteral feeds with BCAA remains somewhat controversial. In one randomized study, infants receiving BCAA supplementation showed an increase in total-body potassium and improved anthropometric measurements compared with control infants fed an isocaloric formula.[15]

Supplemental enteral feeds fail in many infants because of the inability to tolerate the volumes required. Emesis and increased stool output often negate the hoped for benefits, and parenteral nutrition is the only remaining option. However, parenteral nutrition should not be viewed as a last resort but rather instituted at the first signs of failure of enteral support. Calories provided can be increased easily and weight-for-height z-scores improved[21] by using concentrated glucose solutions supplemented with intravenous lipids.[22] In addition, free water and sodium can be carefully controlled and trace element and vitamin deficiencies more effectively treated.[17,23] Although central venous catheter sepsis is always a concern, careful training of caregivers can greatly decrease this risk.[24]

Fat-Soluble Vitamin Supplementation (Vitamins A, D, E, and K)

Supplementation of the fat-soluble vitamins A, D, E, and K is especially important in children with cholestatic liver disease.[25] The decrease in intraluminal bile acids leading to malabsorption of fat, and therefore the fat-soluble vitamins, is the mechanism of these deficiencies.[26] Vitamin K deficiency, manifested by coagulopathy, is treated with oral vitamin K_1 (Mephyton, Merck & Co, Inc, West Point, PA) at a dose of 5 to 10 mg/d. Vitamin K_1 is virtually nontoxic, compared with vitamin K_3, which, although water soluble, is associated with hemolysis in large doses. The vitamin K–dependent coagulation factors are II, VII, IX, and X. Vitamin K deficiency decreases factor VII levels first so that monitoring prothrombin time is the most useful measure to assess the response to vitamin K therapy. However, as end-stage liver disease becomes more severe, decreased production of all of the coagulation factors produced in the liver occurs, even when vitamin K supplementation is adequate, with consequent prolongation of both prothrombin time and partial thromboplastin time. Occasionally, severely cholestatic children will be unable to adequately absorb oral vitamin K supplements, but the prothrombin time often normalizes after intramuscular vitamin K.

Vitamin E deficiency is a particular concern in children because of vitamin E's role in central nervous system development. Infants who develop cholestasis in the first weeks of life will have low vitamin E levels by about 4 months of age.[27] The first signs of vitamin E deficiency are a symmetric decrease in peripheral stretch reflexes. Left untreated, vitamin E deficiency will progress to cerebellar ataxia, posterior column dysfunction, and peripheral neuropathy.[28] Early recognition is therefore essential but is made more difficult by the unreliability of monitoring only serum vitamin E levels. Normal serum vitamin E levels range between 5 and 15 mg/dL. However, the most accurate evaluation of vitamin E status is the ratio of serum vitamin E to total serum lipids. In children < 12 years of age, the vitamin E–to–total serum lipid (sum of fasting cholesterol, triglycerides, and phospholipids) ratio should be > 0.6 mg/g.[29] No fully water-soluble vitamin E supplement is currently available, making effective vitamin E supplementation often difficult. The free form of vitamin E, available as an over-the-counter preparation, is most often used, but doses as high as 400 to 800 IU/d may be needed. The response to therapy is best evaluated by sequential neurologic evaluations and monitoring of serum vitamin E–to–total lipid ratios.

In those children who do not respond to supplementation of vitamin E by traditional methods, oral administration of a water-soluble form of vitamin E–α-tocopheryl polyethylene glycol–1,000 succinate (α-TPGS) has been found to correct biochemical vitamin E deficiency in doses of 15 to 25 IU/kg/d. In truly refractory cases, an admixture of all fat-soluble vitamins with TPGS may be more beneficial than administration of the supplement alone.

The familiar consequences of vitamin D deficiency—rickets and osteoporosis—can be avoided by early vitamin D supplementation. Although the liver is the site of 25-hydroxylation of vitamin D—a key step in the initiation of activation of vitamin D—malabsorption is the most important cause of vitamin D deficiency in cholestatic children.[30] 25-Hydroxyvitamin D is the major circulating form of vitamin D and, in normal children, has a serum concentration of between 25 and 30 ng/mL.[31] Orally administered 25-hydroxyvitamin D (Caderol, Orggnon Inc, West Orange, NJ) is superior to vitamin D_2 (Drisdol, Sanofi Pharmaceuticals, New York, NY). A dose of 25 to 50 µg/d or 5 to 7 µg/kg/d is adequate. Serum 25-hydroxy levels should be measured and maintained within the normal range. The 1,25-hydroxy form of vitamin D (Rocaltrol, Roche, Nutley, NJ) may be used in a dose of 0.1 to 0.2 µg/kg/d but is not superior to the 25-hydroxy form because kidney conversion of 25-hydroxy to 1,25-hydroxyvitamin D remains intact.

Vitamin A deficiency, characterized by conjunctival and corneal dryness (xerosis) and night blindness, occurs when serum levels fall below 100 to 200 mg/L. Vitamin A can be supplemented with a water-miscible form, such as Aquasol A, at a dose of 5,000 to 15,000 IU/d. Vitamin A

serum concentrations should be monitored both before and during therapy to avoid vitamin A toxicity.

PORTAL HYPERTENSION

Variceal bleeding as a consequence of portal hypertension is one of the most dramatic and life-threatening complications seen in pediatric liver disease. Aggressive management begins with cardiovascular resuscitation and requires knowledge of the full range of medical, pharmacologic, and surgical therapies available (Figure 59-2).

The pathophysiology of portal hypertension is described in detail in Chapter 5.2, "Fibrogenesis and Cirrhosis." In brief, the level of portal vein obstruction can be presinusoidal, sinusodial, or postsinusoidal.[32] Presinusoidal portal hypertension can be extrahepatic or intrahepatic. Extrahepatic portal vein obstruction used to be the most frequently recognized cause of portal hypertension in children and was most often secondary to instrumentation of the umbilical vein in neonates, omphalitis, congenital malformations, blunt trauma, or intra-abdominal infections. Intrahepatic presinusoidal portal hypertension is associated with congenital hepatic fibrosis and schistosomiasis and is relatively less common than extrahepatic or other intrahepatic postsinusoidal causes of portal hypertension in children. Cirrhosis, now the most common cause of portal hypertension in children, results in complex derangements of portal flow within the liver at all three levels but particularly within the sinusoids (reflected by an increased wedge hepatic pressure) and in the postsinusoidal space. Budd-Chiari syndrome, webs in the suprahepatic vena cava, veno-occlusive disease, and cardiac disease are some of the causes of postsinusoidal portal hypertension. The critical difference affecting management of the three levels of portal vein obstruction is that liver functional reserve is almost always normal with extrahepatic and presinusoidal obstruction but may be profoundly impaired in children with sinusoidal and postsinusoidal obstruction secondary to cirrhosis. In such children, coexistent ascites and coagulopathy make the management of variceal bleeding even more challenging.

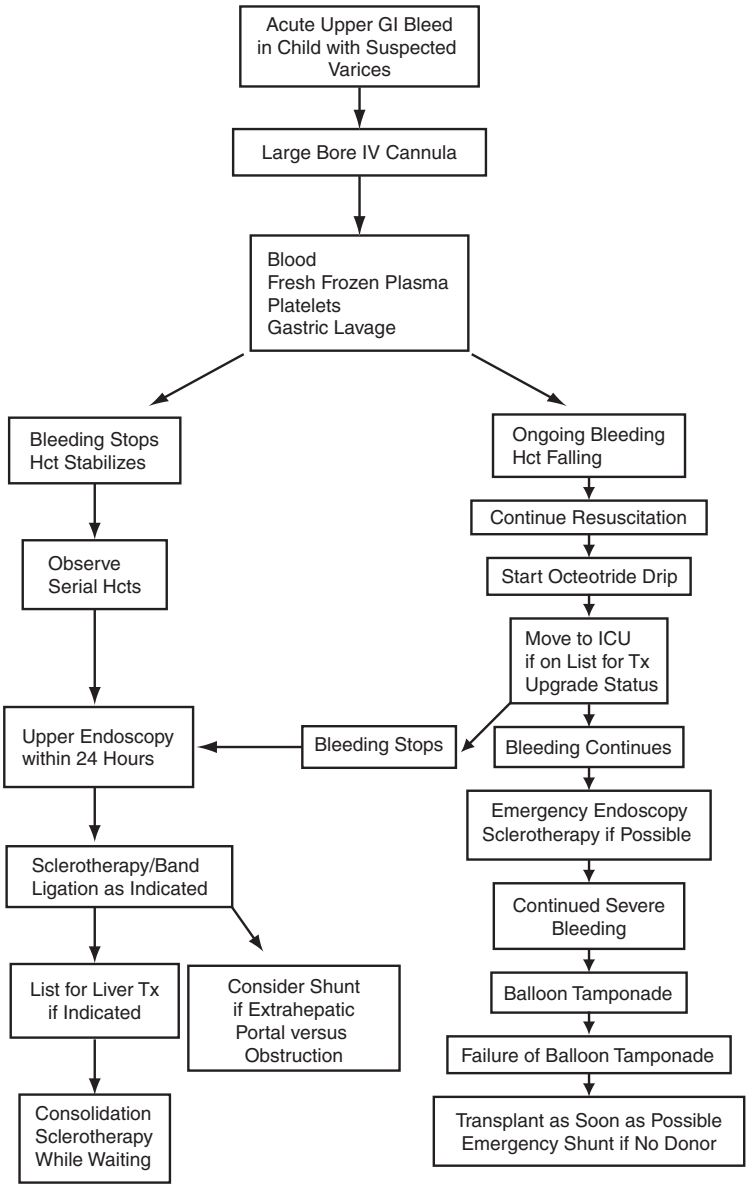

FIGURE 59-2 A management algorithm for the child with suspected bleeding. GI = gastrointestinal; Hcts = hematocrits; ICU = intensive care unit; IV = intravenous; Tx = transplant.

Definition, Diagnosis, and Prediction of Variceal Bleeding

Portal hypertension is defined as portal vein pressure > 5 mm Hg or a portal vein to hepatic vein gradient of > 10 mm Hg.[33]

Portal hypertension can frequently be diagnosed by physical examination. The spleen is usually palpable and sometimes massively enlarged, although it must be remembered that in biliary atresia, one of the most common causes of portal hypertension in children, there may be associated asplenia, polysplenia, or situs inversus, making this physical sign not always reliable. Ascites is generally only present when portal hypertension is at the sinusoidal level. The four main portal to systemic vein collateral systems that become prominent in portal hypertension can all be readily examined. Increased pressure in the paraumbilical venous network is manifested by engorged superficial abdominal wall veins radiating from the umbilicus—the caput medusa. With overfilling of the perirectal collateral venous system, hemorrhoids appear. When abdominal organs become adherent to the abdominal wall, the collaterals formed result in visible varices seen within the stomas[34] sometimes used in biliary drainage procedures or seen in old laparotomy scars.

Esophageal varices are best examined by endoscopy. Newer, less invasive radiologic techniques are being increasingly used. In a recent study, magnetic resonance angiography had a sensitivity of 100% and a specificity of 93% compared with endoscopy for detecting collateral vessels in children with biliary atresia after portoenterostomy.[35] In a study of 50 children, intravenous computed tomography (CT) using a helical CT scanner identified changes in the esophageal and gastric vasculature earlier than endoscopy.[36]

The size of the liver is generally not especially helpful. In older children with cirrhosis, the liver is frequently small and shrunken, whereas in younger children with biliary atresia, the liver may be moderately enlarged, hard, and with an irregular edge. In children with Budd-Chiari syndrome or congenital hepatic fibrosis, the liver may be massively enlarged and firm.

Ultrasonography, particularly when enhanced by duplex sonography, is very useful in the evaluation of portal hypertension.[37] In extrahepatic causes of portal vein obstruction, the characteristic absence of the portal vein signal is replaced by the findings of multiple dilated collateral veins (cavernous transformation). The echogenicity of the liver itself is normal. Not only can the presence or absence of the portal vein be determined, but the direction of flow can also be assessed. Hepatofugal flow (ie, away from the liver) is associated with severe portal hypertension. The ultrasonographer can also provide details of increased flow in the portal collateral circulation and may be able to demonstrate findings such as enlarged coronary veins or paraesophageal veins. Evaluation of the splenic vein is also important, particularly to look for splenic aneurysms, which are found in 8 to 14.7% of patients with portal hypertension.[38,39] Once portal hypertension is suspected on clinical or ultrasound findings, elective upper endoscopy can give useful information. The size of the varices and the presence or absence of portal hypertensive gastropathy help determine the medical management.

As variceal bleeding is the most serious complication of portal hypertension, with a 30 to 50% mortality and a high risk of rebleeding,[40] several studies have addressed the issue of whether the risk of bleeding can be predicted (Table 59-3). Although most of this information is obtained from adult patients, several observations also relevant to children can be made. First, variceal bleeding rarely occurs if the portal vein to hepatic vein gradient is < 12 mm Hg.[41,42] However, in patients with a portal vein to hepatic vein gradient > 12 mm Hg, only about one-third will bleed. Second, the risk of bleeding is related to the cause of portal vein obstruction. In the Bicêtre experience of 389 children with portal hypertension, 80% of children with extrahepatic portal vein obstruction bled compared with 46% of children with congenital fibrosis and 32% of children with cirrhosis. Moreover, 41% of children with portal vein obstruction bled before the age of 3 years. Children with Budd-Chiari syndrome had a very low incidence of variceal bleeding (Figure 59-3).[43] Third, the endoscopic appearance of the varices provides some estimate of the risk of bleeding.[44,45] Large, tense varices that do not flatten with insufflation of air are more likely to bleed than small, decompressed varices. Although large varices bleed more easily, the size of the varices is not related to the measured portal hypertension.[46] Varices with congestion of the overlying or surrounding mucosa, particularly those with characteristic red spots and red wale markings ("whip-like" discolorations from dilated venules), and gastric varices are also associated with increased risk of bleeding.[43]

In children, the risk of bleeding may change over time. In those with extrahepatic portal vein obstruction, the development of a decompressing collateral circulation may actually decrease the risk as children get older. However, in the Bicêtre study, almost half of the children studied serially showed a progression in the severity of varices over time, and more than one-third of these had a bleeding episode.[43] Finally, the severity of underlying cirrhosis increases not only the risk of bleeding[45] but also the mortality after variceal hemorrhage. In a study of 134 children with biliary atresia, children after a first esophageal variceal bleed, the relative risk of death or transplant was 12.0 if the total bilirubin was more than 10 mg/dL compared with 7.2 if the bilirubin was 4 to 10 mg/dL and 0.6 if the bilirubin was less than 4 mg/dL compared with a same-aged child without a variceal bleed.[47]

Management of Acute Variceal Bleeding

Hematemesis or melena as a result of variceal bleeding is often massive, and the child may present to an emergency

TABLE 59-3 FACTORS PREDICTING VARICEAL BLEEDING

Portal vein–hepatic vein gradient > 12 mm Hg
Large, tense varices
Red wale marks, red spots on varices
Severity of underlying liver disease

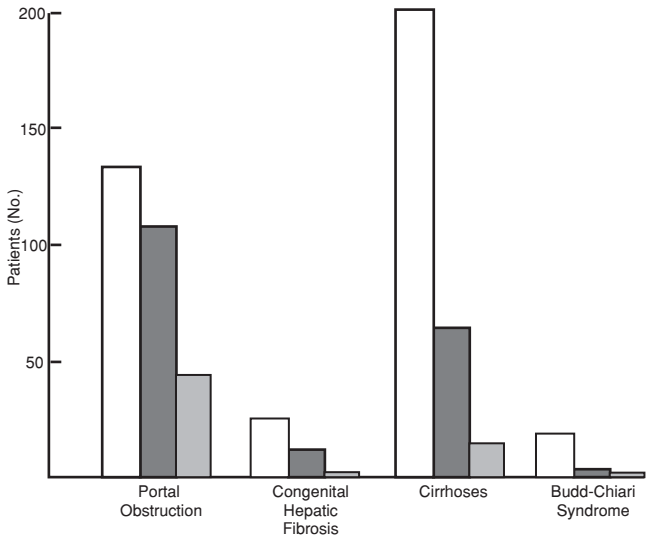

FIGURE 59-3 Relationship between the etiology of portal hypertension and risk of gastrointestinal bleeding in 380 children investigated for portal hypertension. For each etiologic group, the total number of children studied; number of children who experienced one episode of bleeding; number of children who bled before age 3 years. Reproduced with permission from Bernard O et al.[43]

room in cardiovascular shock. Immediate placement of a large-bore intravenous cannula or, if necessary, intraosseous needle in children < 2 to 3 years of age is essential so that large volumes of fluid and blood can be rapidly delivered. Children with known poor liver function should be assumed to be coagulopathic, and clotting factor supplementation should be instituted immediately. Many children will also have thrombocytopenia secondary to hypersplenism, and platelet transfusions are indicated. However, gastrointestinal bleeding in children with portal hypertension may not always be so dramatic. Small variceal bleeds or bleeding from portal hypertensive gastropathy can be much more subtle, with only small to moderate amounts of blood loss, a slow decline in hematocrit, and no overt cardiovascular decompensation. However, the propensity for these children to develop more severe bleeding must not be underestimated, and all should be admitted and carefully observed to better optimize therapy (see Figure 59-2).

Gastric lavage with saline via a nasogastric tube may be helpful in determining the extent and duration of bleeding. Perhaps the most important use of gastric lavage is to help clear the stomach of blood to allow better visualization of the mucosa at the time of endoscopy. Contraindications to gastric lavage include children in whom the procedure may increase bleeding (known large varices or intractable coagulapathy) or induce esophageal perforation (recent sclerotherapy with possible esophageal ulcerations). The choice of lavage solution is probably not particularly important. Room temperature saline is the most physiologic; iced saline lavage has not been shown to be more efficacious. Once the patient is fully resuscitated and stabilized, upper endoscopy should be performed, primarily to confirm the site and cause of bleeding and to determine if treatment is indicated. Other etiologies of upper gastrointestinal bleeding will alter management, such as portal hypertensive gastropathy, gastric or duodenal ulcers, or Mallory-Weiss tears. If variceal bleeding is confirmed, therapeutic sclerotherapy or variceal ligation may be attempted, although this is frequently technically very difficult if bleeding is still active.

In the child who continues to have uncontrollable bleeding, balloon tamponade may be the only method that can stabilize the patient until a more definitive procedure, such as liver transplant or a portosystemic shunt, can be undertaken. The Linton tube is used for small children or if bleeding gastric varices are present, and the Sengstaken-Blakemore tube is used for larger children. The length of the esophageal balloon limits the use of the Sengstaken-Blakemore tube to children weighing over 40 kg. Both are highly effective and can stop esophageal and or gastric variceal bleeding in up to 90% of patients.[48] However, experience is required to minimize the considerable risks associated with balloon tamponade, such as aspiration, esophageal rupture and ulcers, and airway obstruction. Correct positioning of the tube must be confirmed radiographically to avoid inflating the gastric balloon in the esophagus. The volume of air inflated into the gastric and esophageal balloons varies with the child's size and must be enough to create adequate pressure to compress the bleeding sites but without causing ischemic injury to the esophageal or gastric mucosa. Pressure in the esophageal balloon should be 15 to 40 mm Hg. The gastric balloon should be inflated to 30 mL and can be incrementally increased to about 200 to 300 mL depending on the size of the child. It is recommended that the appearance of the gastric balloon in relation to the size of the stomach is checked radiographically after each incremental change in volume to avoid the risk of overinflation.

Children should be intubated and sedated to minimize the risk of aspiration. The balloons can be safely left inflated only for 12 to 24 hours. As such, balloon tamponade can be viewed only as a temporizing measure.

Prevention of Rebleeding and Prophylaxis of First Variceal Bleeding

Several modalities, alone or in combination, have been investigated to control variceal bleeding. These include sclerotherapy, variceal ligation, vasoactive drugs, and portosystemic shunts. Most large controlled trials have been performed in adults, and the results have generated considerable controversy over the effectiveness of each modality.[49–53] Common principles, also relevant to pediatrics, are discussed below.

Sclerotherapy and Variceal Ligation. Obliteration of esophageal varices may be achieved by endoscopic injection of sclerosing substances or by ligation of varices, commonly using elastic bands. Prevention of rebleeding, which occurs in up to 50% of patients and incurs a mortality of 20 to 70% if untreated, is the goal of aggressive attempts to obliterate varices.[53,54]

Sclerotherapy is a well-established modality to control variceal bleeding in children and has decreased the need

for shunt surgery.[55,56] After a first variceal bleed, elective sclerotherapy, usually requiring repetition, can successfully eradicate esophageal varices in up to 90% of patients with a low incidence of rebleeding and recurrent varices. Because rebleeding often occurs within 2 to 6 weeks of the initial bleeding episode, the first sclerotherapy should be scheduled early. The short-term benefits of reducing rebleeding episodes are not to be underestimated and are particularly important while the child awaits liver transplant. Sclerotherapy or endoscopic variceal ligation should now be considered the first line of treatment for variceal bleeding. In a study comparing outcomes in children treated with a surgical shunt versus sclerotherapy, the low rate of rebleeding after sclerotherapy led the authors to conclude that the more invasive shunt surgery could usually be avoided.[57] Although some controlled trials in adults have shown that sclerotherapy reduces long-term mortality,[58] it must be remembered that sclerotherapy does nothing to decrease portal hypertension itself but only redirects blood away from the esophageal collateral circulation to other collaterals. Until the child undergoes a definitive procedure that actually decreases or eliminates portal hypertension (ie, shunt operation or liver transplant), the patient remains at risk of life-threatening variceal bleeding, often from newly formed gastric varices. Portal hypertensive gastropathy with increased bleeding risk may also be exacerbated by esophageal variceal obliteration.[59]

No consensus as to the value of prophylactic sclerotherapy has been reached. A randomized controlled trial of 100 children with esophageal varices showed that prophylactic sclerotherapy compared to observation resulted in a significant reduction in variceal bleeding in the treatment group but more congestive hypertensive gastropathy. Importantly, there was no difference in survival between the two groups.[60] In another pediatric series, 42% of children bled after prophylactic sclerotherapy.[57] In some adult studies, use of a scoring system that predicts a high risk of variceal hemorrhage has been used to justify prophylactic sclerotherapy. There is conflicting evidence as to whether the incidence of bleeding is reduced or the survival improved.[45,61]

Sclerotherapy still requires considerable operator skill, particularly in small children. Although techniques and choice of sclerosant vary among endoscopists, convincing evidence that one is better than another is lacking. Paravariceal injection of sclerosant is favored by some and intravariceal injection by others. Sclerosants used include ethanol, morrhuate, ethanolamine, and tetradecyl. We use ethanolamine 5% or diluted 1:1 with ethanol. To decrease complications, it is important to avoid a large volume of sclerosant on any single injection and to limit the number of injections, even if of relatively small volume, performed at any one time. We recommend 1 mL or less of sclerosant per site and no more than 5 to 6 mL per session in children < 10 years of age. Both practices decrease the risk of esophageal ulceration and the eventual development of esophageal strictures, perforation, and systemic absorption of sclerosant.

In recent years, the newer technique of endoscopic ligation of esophageal varices with elastic bands has supplanted sclerotherapy for variceal obliteration in adults and is now being used in children (Figure 59-4). Variceal ligation may be a more effective modality to prevent a first variceal bleed compared with sclerotherapy. In a randomized trial of 49 children comparing endoscopic ligation with sclerotherapy for bleeding esophageal varices secondary to extrahepatic portal venous obstruction, the rebleeding rate was significantly higher in the sclerotherapy group (25% versus 4%).[62] The advantages are increased efficacy with a reduced incidence of complications, such as esophageal ulceration, perforation, stricture, systemic sclerosant, and gastroesophageal reflux.[63] The disadvantage, now mostly eliminated in adults, is the need for a large-caliber endoscope that can accommodate, within its lumen, an outer cylinder that snares the varix and an inner cylinder that deploys the bands. Because many early models could deploy only one band at a time, an overtube was often used so that multiple intubations of the esophagus were avoided. Improved endoscopes have eliminated these problems for adults, and smaller-caliber endoscopes suitable for use in pediatric patients have been developed. As well, recent technical advances in pediatric endoscopes have overcome the previous limitation of only one band being placed at a time. Successful esophageal ligation in children is increasingly being described.[64–68] A high rate of eradication is reported (72–100%) and a low risk of rebleeding (< 25%). Note that in these studies, the mean age is between 7 and 8 years. Price and colleagues performed endoscopic variceal ligation in 22 children, ranging in age from 8 months to 19 years.[69] Of 18 children who did not undergo early liver transplant, 12 had complete eradication of the varices over an average of four sessions. Rebleeding between sessions occurred but not after obliteration of varices. None of the children developed esophageal stenosis or gastroesophageal reflux. However, perforation of the cervical esophagus occurred in one

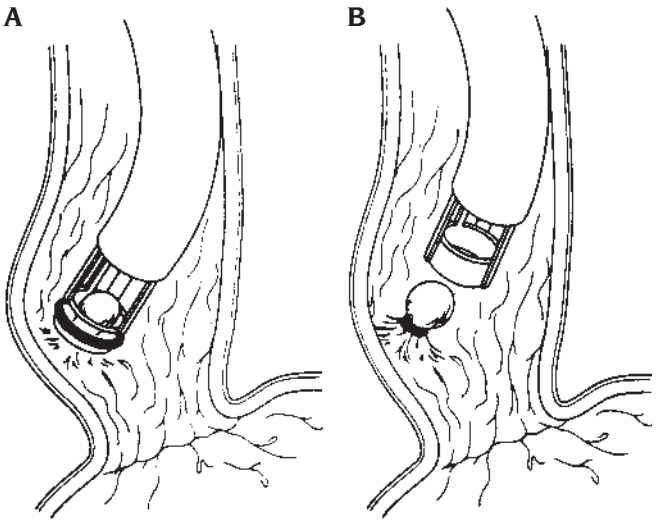

FIGURE 59-4 Endoscopic ligation of esophageal varices. The esophageal varix is drawn up into the ligation device with suction (*A*) and the base of the varixis ligated with an elastic band (*B*). From Greenfield LJ, Mulholland MW, Oldham KT, et al. Surgery, scientific principles and practice. Philadelphia: Lippincott-Raven; 1997.

child, underscoring the size limitations of the instruments needed in small children. These authors stress that the diameter of the child's esophagus should be 10 mm or more to allow for the safe passage of a 32-French instrument. They advocate limiting the technique to children over 1 year of age until endoscopic instruments can be further miniaturized. However, in a recent study using a small-diameter endoscope, 28 children, some as young as 3 months, were treated, with obliteration of varices in 26 and rebleeding in only 2.[70]

Surgical Management: Shunts, Transjugular Intrahepatic Portosystemic Shunts, and Nonshunt Procedures.

Shunts. The success of sclerotherapy and variceal ligation in controlling variceal bleeding and the availability of liver transplant have reduced the need to perform portosystemic shunts to manage uncontrollable bleeding.[57] However, portosystemic shunts are still an important modality to treat variceal bleeding in selected cases: children with extrahepatic portal vein obstruction; children with congenital hepatic fibrosis; children with well-compensated cirrhosis, in which liver transplant may not be required for several years; children with variceal bleeding, in whom liver transplant is contraindicated; and, rarely, children awaiting liver transplant to control life-threatening variceal hemorrhage. Prophylactic shunt surgery is not advocated because previous studies showed a decreased survival and an increased risk of encephalopathy compared with no treatment.[53]

Shunt procedures, including transjugular intrahepatic portosystemic shunts (TIPS), are the only modalities that effectively reduce portal pressure and thus definitively treat the underlying cause of variceal bleeding (Figure 59-5). The major disadvantages of portosystemic shunts are hepatic decompensation caused by reduced portal blood flow and precipitation of encephalopathy. Both complications occur much more commonly with nonselective (total) shunts, that is, portocaval, mesocaval, and central splenorenal shunts (see Figure 59-5). The development of selective shunts, notably the distal splenorenal shunt first described by Warren and colleagues in 1967,[71] significantly reduces both complications as forward flow of portal venous blood to the liver is preserved. The distal splenorenal shunt and its modifications are now the preferred shunt, whenever technically possible, particularly in elective situations.[72] In a pediatric series, there was no rebleeding and no encephalopathy in the 81% of children in whom the shunt was successful.[73] In the emergency setting, surgical options are more limited, and portocaval or mesocaval shunts may be the only feasible choice. Bleeding is effectively controlled, but the mortality rate may be as high as 20 to 50%.[74,75] This is most likely related to the severity of the underlying liver disease in patients undergoing these procedures. Both portocaval and mesocaval shunts are equally effective in stopping bleeding. The side-to-side portocaval shunt and the mesocaval and portocaval H-graft shunts have the theoretical advantage of preserving at least some forward portal flow, although all have a high incidence of encephalopathy and hepatic decompensation, particularly in patients with underlying cirrhosis.

The applicability of shunt procedures to pediatric patients is constrained by the small caliber of the veins to be shunted and the subsequent risk of shunt thrombosis. In two pediatric studies,[76,77] the median ages were 9 years and 8 years, respectively, although in the latter series, children as young as 2 years were shunted. These two studies reported patency rates of 9% and 80%, respectively. In children with underlying cirrhosis, complications included ascites, hepatorenal syndrome, and encephalopathy (occurring in one patient with a nonselective shunt).

Children with extrahepatic portal vein obstruction are particularly good candidates for shunts.[78] In one experience, shunt patency was 93% in a group of 92 children with a mean age of 6.5 years.[43] Innovative shunt procedures[79] designed to restore portal blood flow include bypassing the cavernoma by an autograft (using a portion of the patient's jugular vein) placed between the superior mesenteric vein and the left portal vein (the Rex shunt). In two reports, portal decompression was achieved, with concomitant decreases in spleen size, increased white cell and platelet counts, control of bleeding, and restoration of physiologic hepatopetal flow. All shunts were patent at a median of 6 months after the procedure.[80,81]

An important consideration in placing a surgical shunt is to be certain that the shunt procedure itself does not compromise a potential liver transplant procedure required in the future.[82] Mazzaferro and colleagues showed that properly performed portosystemic shunts do not preclude successful liver transplant or have an impact

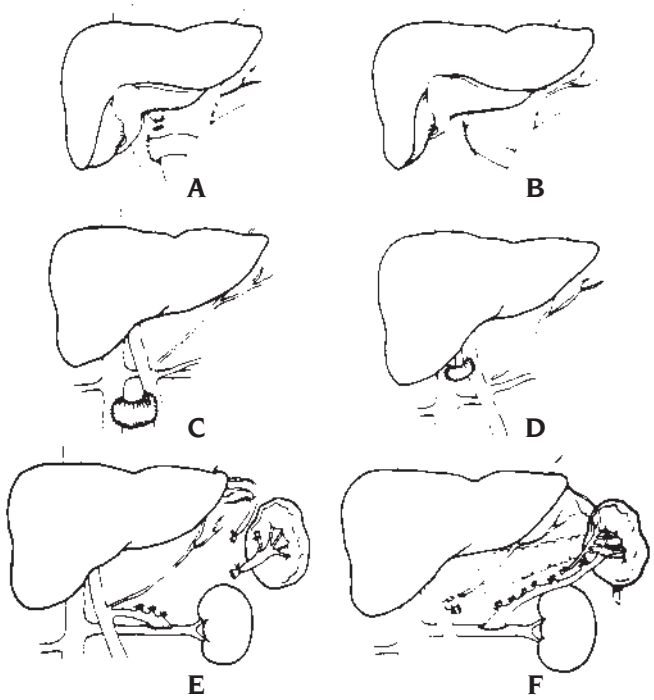

FIGURE 59-5 Portosystemic shunts used for emergency treatment of variceal bleeding: end-to-side portocaval shunt (*A*), side-to-side portocaval shunt (*B*), mesocaval interpositional H-graft (*C*), portocaval interpositional H-graft (*D*), central splenorenal shunt (*E*), and selective distal splenorenal shunt (Warren Shunt; *F*). Reproduced with permission from Terblanche J et al.[49]

on post-transplant survival.[83] A potential advantage of the mesocaval shunt, as opposed to the portocaval shunt, is that the dissection is distant from the porta hepatis and, thus, less likely to create complications for the transplant surgeon in the future.

Transjugular Intrahepatic Portosystemic Shunts. The creation of a shunt between the intrahepatic portions of the portal vein and hepatic vein (Figure 59-6) by a minimally invasive technique avoids the risks of the major surgical procedure required for a standard shunt operation. This is a particular advantage in patients who are already severely compromised by their underlying liver disease. In brief, the procedure is performed by cannulating the right jugular vein to access the right hepatic vein. The needle is then passed through the intrahepatic portion of the hepatic vein and into the liver parenchyma to enter a branch of the portal vein. An expandable metallic stent is deployed across the hepatic parenchyma to create a shunt between the hepatic and portal vein branches. The size of the stent is increased until the gradient between the portal vein and the hepatic vein is ideally < 18 mm Hg.[84] To ensure success, the procedure requires technical expertise and a very careful appraisal of possible variations in vascular anatomy.[85] In adults, there is a 10% complication rate from the procedure itself, most often intra-abdominal bleeding.[86] The major complications are shunt occlusion or stenosis (25–75%), encephalopathy (5–35%),[87] progressive liver failure, and portal vein thrombosis. The success of the TIPS procedure in controlling bleeding (and also ascites)[88] has been widely reported in adults.[89,90] In two randomized trials, one comparing TIPS versus drug therapy[91] and another TIPS versus variceal ligation plus propranol,[92] both found that TIPS-treated patients had more complications. Although in one study, the incidence of rebleeding was less, neither study showed any difference in survival between the groups.[91] It appears to be most useful as a bridge to liver transplant and for those failing sclerotherapy or variceal ligation. A TIPS procedure is preferable to an operative shunt in the transplant candidate because it is associated with less operative complications at the time of transplant. However, migration of the stent into the suprahepatic vena cava or right atrium has been associated with major technical complications during the transplant procedure.[93]

In children, the TIPS procedure is made more complicated by the small size of the liver and the diminutive caliber of the portal and hepatic veins.[84] As well, there is more likelihood of anatomic variance, particularly of the portal vein, in some pediatric liver diseases, such as biliary atresia. Despite these technical limitations, there are now several reports describing the successful use of TIPS children,[94,95] even as an emergency procedure,[96] and in an infant.[97] In experienced hands, the overall success rate of 75 to 90% is similar to reports in adults. Hackworth and colleagues used TIPS to control variceal bleeding in 12 children (age 2 to 16 years; median age 9 years) awaiting liver transplant.[98] Ten of the 12 shunts were patent at the time of transplant at a median of 53 days after placement. None of the children rebled, and one developed encephalopathy. Heymen and colleagues described nine children (5 to 15 years in age) in whom TIPS was attempted.[99] In two children, vascular anomalies precluded successful TIPS placement. Although shunt occlusion occurred in four children, TIPS revision restored patency in three. All but one patient had successful control of bleeding. These two studies emphasize the important principles of the use of TIPS in children: size (and therefore age) is still a limiting factor, anatomic variations can preclude success, and the small caliber of the stent may increase the risk of stent occlusion.

Once in place, the patency of the TIPS stent should be monitored by ultrasonography every 3 to 4 months. In adult series, shunt stenosis or obstruction occurs in 25 to

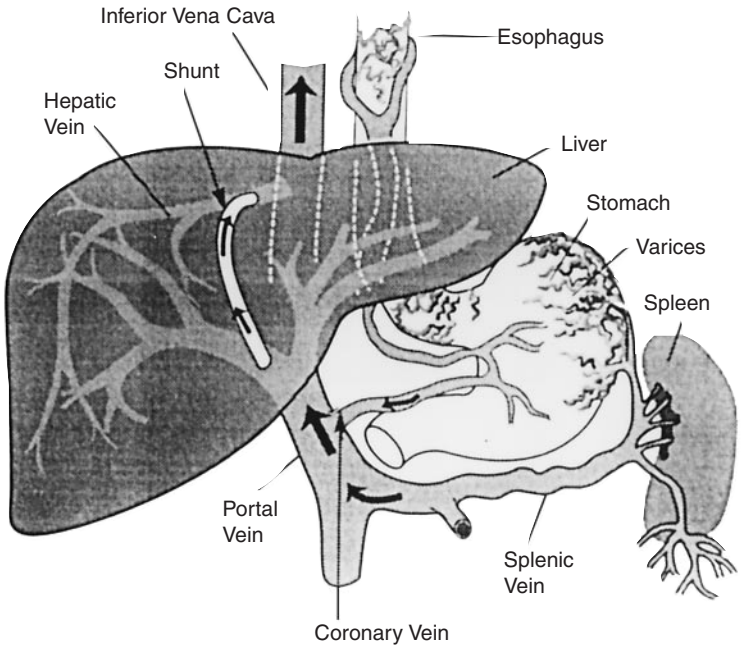

FIGURE 59-6 Anatomic location of the transjugular intrahepatic portosystemic shunt. Reproduced with permission from Vargas HE and colleagues.[33]

75% of patients.[89] Doppler ultrasonography has a reported sensitivity of 92% and a specificity of 72% in detecting shunt malfunction.[100]

At present, TIPS appears to be most useful in children who have failed sclerotherapy while waiting for transplant.[101] Shunt patency decreases markedly with time (48% patency at 1 year, 26% at 2 years in one study)[102]; therefore, the procedure should generally be reserved for children with refractory bleeding who will be transplanted within 6 to 12 months. Other more controversial indications include the use of TIPS to treat severe hypersplenism; however, a consistent improvement in white cell and platelet cell counts has not been demonstrated.[99]

In adult patients, TIPS placement has also been suggested for medically refractory ascites, Budd-Chiari syndrome, and veno-occlusive disease. These indications have not yet been explored adequately in pediatric patients. In addition, there is no proven role for placement of TIPS to prevent bleeding from portal hypertensive gastropathy.

The TIPS procedure is contraindicated whenever there is infection present in the liver, that is, hepatic abscess or cholangitis. Polycystic liver disease, hepatic neoplasm, right-sided heart failure, and severe hepatic failure with uncorrectable coagulopathy are also contraindications.[84]

Pharmacologic Therapy. The aim of drug therapy in the control of variceal bleeding is to reduce portal pressure either by decreasing flow into the splanchnic bed with vasoconstrictors or by decreasing the vascular resistance of the intrahepatic and portal circulation with vasodilators.[103]

Vasopressin, a potent vasoconstrictor, was the prototype drug used, and although it has been shown to increase the chance of stopping acute bleeding, no survival benefit has yet been demonstrated. The serious systemic vasoconstrictive side effects of vasopressin, including myocardial infraction, mesenteric ischemia, cerebrovascular accidents, and limb ischemia, are its major disadvantages.[104] To counteract these effects, some protocols combined vasopressin with nitroglycerin, a potent vasodilator, and the systemic vasoconstrictive properties of vasopressin were lessened.[33]

Somatostatin has proven to be a much safer alternative to vasopressin and can effectively decrease portal pressure by decreasing splanchnic blood flow and reducing the portal vein to hepatic vein pressure gradient with few systemic side effects.[105,106] However, its usefulness is limited by a very short half-life. More recently, a synthetic analog of somatostatin, octreotide, has been shown to have similar efficacy to somatostatin and vasopressin, with very few side effects and an acceptable half-life.[107–109] This agent is currently the preferred agent to treat children with acute variceal bleeding.[110] The advantage of beginning pharmacologic therapy early in the management of an acute variceal bleed is that the high technical expertise of sclerotherapy or variceal ligation is not required, and bleeding may be controlled enough to allow elective sclerotherapy. In one randomized study, octreotide alone was as effective as emergent sclerotherapy in stopping bleeding.[109] Octreotide dosing recommendations are to begin with a bolus of 1 μg/kg followed by continuous infusion of 1 μg/kg/h, with titration of both the bolus and constant infusion up to 5 μg/kg/h. This regimen was found to be effective in stopping bleeding in 86% of patients at a mean time of 40 hours.[110]

Nonselective β-blockers have long been used for the prevention of first variceal bleeds and rebleeding. Several randomized trials in adults have confirmed that both propranolol and nadolol have a significant benefit in both the prophylaxis of variceal bleeding and prevention of rebleeding.[53] In some trials, prevention of a first variceal bleed also increased survival.[111,112] Nadolol's advantage over propranolol is that it is not metabolized by the liver and can be given once a day.[113] Nadolol was combined with isosorbide mononitrate (nitrate vasodilators also decrease portal pressure) and compared in a randomized trial with sclerotherapy to prevent variceal rebleeding. Although the probability of rebleeding was significantly decreased in the nadolol and isosorbide group, there was no survival benefit.[114] Extrapolating from the adult doses used in the study, some pediatric patients have been treated with a starting dose of 1 mg/kg/d of nadolol, with titration of the dose until the 25% reduction of heart rate is achieved. Then isosorbide nitrate is added to reach a dose over the course of 1 week of 0.5 mg/kg twice a day.

Few studies are published in children regarding the use of β-blockers in preventing first variceal bleeds. In a nonrandomized study, 14 of 21 children had no episode of rebleeding when treated with propranolol. Success correlated with a reduction in heart rate of more than 25% of baseline and a dose of at least 1 mg/kg/d of propranolol.[115] In a study of 60 children with cirrhosis, propranolol (1–2 mg/kg/d) was most successful in preventing a first bleed (15.6% of children bled), whereas 53.3% of children bled if propranol was initiated after a first bleed.[116] However, the use of nonselective β-blockers can be limited by their side effects. This is particularly important in children with asthma or cystic fibrosis, in whom bronchospasm must be avoided.

More recently, several randomized trials of endoscopic treatment alone versus endoscopic treatment plus drug therapy for acute variceal bleeding, or to prevent rebleeding, have been published. Combined therapy was more effective for controlling bleeding[117] and for the prevention of rebleeding,[118] but there was no difference in survival between treatment groups. Similar studies have not yet been performed in children.

Transection and Devascularization Procedures. With the success of sclerotherapy, improved pharmacologic agents, and availability of liver transplant, esophageal transection and devascularization procedures are very infrequently necessary to control acute variceal bleeding. However, such major surgical procedures still have a place in treating exsanguinating hemorrhage uncontrollable by any other means. Three techniques are currently practiced[49]: staple gun transection of the esophagus, the extensive Sugiura procedure (devascularization of the abdominothoracic lower esophagus and upper gastric areas, followed by splenectomy, pyloromyotomy, vagotomy, and esophageal transection), and a more limited procedure combining esophagogastric devascularization with staple gun transec-

tion. None of these procedures have been well studied in children. However, the results in adults, particularly of the more extensive procedures, have shown a high mortality.[49] Unless contraindicated, liver transplant, even in this highly emergent situation, is most likely a better option.

ASCITES

In the child with chronic liver disease, the onset of ascites indicates that the two prerequisite conditions for ascites formation, portal hypertension and hepatic insuffiency, are worsening.[119] About 50% of patients will die within 2 years of developing ascites.[40]

The pathophysiology of ascites is discussed in detail in Chapter 5.2. In brief, intra-abdominal factors that result in a net flow of fluid (and protein) out of the mesenteric capillary bed into the peritoneal cavity are (1) decreased plasma colloid osmotic pressure, (2) increased capillary pressure, (3) increased ascitic colloid osmotic fluid pressure, and (4) decreased ascitic fluid hydrostatic pressure (Figure 59-7).[120,121] The dynamic relationship between these factors in the formation of ascites is complex. For example, hypoalbuminea (ie, decreased plasma colloid osmotic pressure) alone is not sufficient to cause ascites, and in the hepatic causes of ascites, capillary pressure increased by portal hypertension plays a key role, as supported by the observation that ascites may be ameliorated by creating portosystemic shunts.

Ascites in liver disease occurs in the context of sinusoidal and/or postsinusoidal portal hypertension and is, in part, related to obstruction of hepatic lymph flow from the sinusoids into the hepatic vein.[122] When the rate of hepatic lymph formation exceeds its drainage into the hepatic venous system, lymph accumulates in the space of Disse and then escapes into the peritoneal cavity as ascitic fluid.

Apart from the local factors leading to ascites, three theories invoke the role of systemic factors in ascites formation: (1) the underfill theory, in which decreased plasma volume increases the activity of the renin-angiotensin-aldosterone pathway, causing sodium retention[123]; (2) the overfill theory, in which renal sodium retention is the initiating event, leading to an increase in plasma volume[124]; and (3) the peripheral arterial vasodilation theory, in which the primary event is splanchnic arteriolar vasodilation secondary to portal hypertension, which causes sequestration of blood in the splanchnic bed, a decrease in circulating blood volume, and subsequent sodium and water retention.[125]

Understanding the central role of sodium retention in the pathogenesis of all three proposed mechanisms is essential for the successful management of ascites. The ability to excrete free water, independent of sodium retention, is also impaired in cirrhotic patients and may be secondary to increased plasma levels of antidiuretic hormone.[121,126]

Diagnosis

Significant volumes of ascites are easily detected on physical examination. The abdominal flanks bulge with fluid, a fluid level can be percussed, the umbilicus protrudes, and a fluid wave may be detected. In addition, the liver and spleen may become ballotable, and, particularly in young children, inguinal hernias and hydroceles may develop. Common findings on a plain film of the abdomen are diffuse abdominal haziness, separation of bowel loops by fluid, and medial displacement of the bowel.

More subtle ascites is best assessed by ultrasonography, which can detect small volumes of fluid. Confirming ascites becomes important if paracentesis is being considered to diagnose spontaneous bacterial peritonitis. The ultrasound examination is also useful to differentiate free from loculated ascitic fluid.[127]

Paracentesis is usually not indicated in the initial diagnostic evaluation of ascites in the child with known liver disease. The other causes of ascites that might occur in adults with liver disease, such as malignant or tuberculous ascites, are very rare in children. It is, however, important to know the usual composition of ascitic fluid in patients with liver disease without secondary complications. The ascitic fluid is generally clear, straw colored, or bile tinged and has a protein content of < 2.5 g/dL. The cell count is < 250 cells/mm^3 and is mostly composed of lymphocytes. The glucose and lactic dehydrogenase content mirror that of plasma.[120]

Management

Sodium restriction and the promotion of sodium excretion are the cornerstones of ascites management (Table 59-4).[128] As the excretion of fluid passively follows that of sodium, fluid restriction is generally not necessary in the initial management plan. Dietary sodium intake should be limited to 1 to 2 mEq/kg. However, only 10 to 20% of patients, generally those with a relatively normal serum sodium and a urinary sodium of > 15 mEq/24 h, will

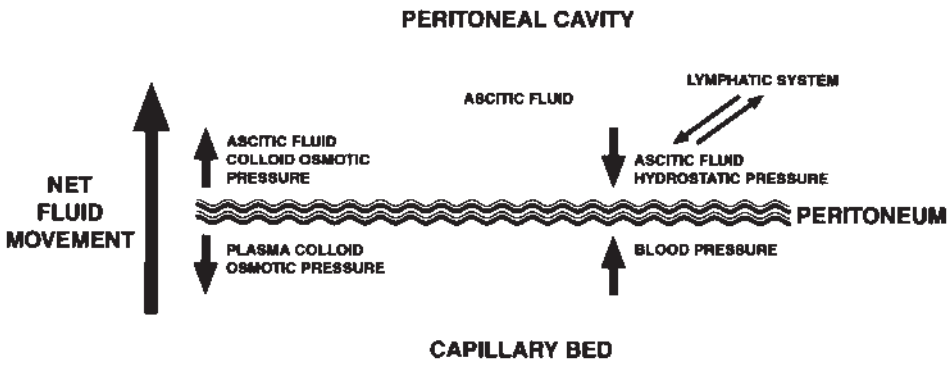

FIGURE 59-7 Formation of ascites. Factors favoring the net movement of fluid out of the capillary bed and into the peritoneal space. Adapted from Wyllie R, Arasu TS, Fitzgerald JF. Ascites: pathophysiology and management. J Pediatr 1980;97:167.

TABLE 59-4 STEPWISE MANAGEMENT OF ASCITES

Step 1. Sodium restriction: 1–2 mEq/kg/d
Step 2. Check compliance
Step 3. Spironolactone: 2–3 mg/kg/d ÷ tid
Step 4.* Spironolactone + furosemide
Step 5.* Fluid restriction
Step 6.* Intravenous albumin 1 g/kg + furosemide 1 mg/kg
*Monitor for
Hypovolemia
Hyponatremia: Na < 120 mEq/mL
→ Stop diuretics
→ Continue fluid restriction
Hypokalemia

respond to sodium restriction alone.[108] Once the urine sodium excretion falls below about 15 mEq/24 h, diuretic therapy is required.

The most logical first choice of diuretic is spironolactone because secondary hyperaldosteronism is almost always present in chronic liver disease.[129] Spironolactone is rapidly and almost completely absorbed from the gastrointestinal tract and is metabolized to a number of active compounds that competitively inhibit the binding of aldosterone to its specific receptor in the distal renal tubule and collecting system.[130]

Spironolactone therapy has proved to be safe and efficacious over many years of use. The dose should be initiated at 2 to 3 mg/kg/d per os in divided doses. At least a 3- to 4-day delay should be expected before a diuretic effect is seen.[131] If no increase in urine output occurs, the dose can be safely doubled.[120] Failure to respond to high-dose spironolactone prompts the use of a more potent second diuretic, most often furosemide. Furosemide induces not only sodium but also potassium loss in the urine so that careful monitoring of serum potassium is required. A balance between the potassium-sparing effects of spironolactone and the potassium loss induced by furosemide may obviate the need for potassium replacement when the two drugs are combined.

Hyponatremia is the electrolyte disturbance most often associated with diuretic therapy, indicating that sodium losses are exceeding water losses. Serum sodium is seldom less than 120 mEq/mL and at this level is not associated with significant complications and rarely needs to be treated. When the sodium falls below 120 mEq/mL, diuretic therapy should be suspended and fluid intake restricted.[120,128]

Monitoring 24-hour urine sodium excretion can be an important guide to therapy, particularly if ascites is failing to respond to interventions. With appropriate sodium restriction and diuretic therapy, urine sodium should certainly exceed 15 mEq/d. It has been estimated that a negative sodium balance of 120 mEq/d results in 1 L of fluid loss.[120] If the child on diuretic therapy, restricted to 1 to 2 mEq/d of sodium, still fails to diurese, water restriction of 50 to 75% of the normal daily requirement may be necessary. In patients with hypoalbuminemia, intravenous albumin infusions, which rapidly increase the intravascular colloid osmotic pressure, can be used to quickly mobilize extravascular fluid into the intravascular space. When combined with a diuretic, a brisk diuresis results. Concentrated 25% albumin can be given at a dose of 1 mg/kg intravenously followed by 0.5 to 1 mg/kg of intravenous furosemide.[120]

The most important and serious complication of aggressive diuretic therapy is hypovolemia, which occurs when water loss outstrips ascitic fluid resorption. Renal blood flow is decreased, creatinine and blood urea nitrogen are increased, and renal failure can be precipitated. Once peripheral edema has been mobilized by diuretic therapy (this occurs early in treatment), care must be taken to monitor weight, fluid balance, electrolytes, blood urea nitrogen, and creatinine. A net water loss of 200 to 300 mL/d is a useful goal,[120] but in very small children, it may be excessive. When ascites requires intensive management, such as outlined above, children should be hospitalized so that electrolyte balance, volume status, and renal function can be assessed on a daily basis. Vigorous diuretic treatment may also precipitate encephalopathy.

In children, refractory ascites is rare. Medical therapy alone is generally sufficient to reduce abdominal distention, allowing improved enteral feedings and diminished respiratory distress. However, in a recent series, if large-volume paracentesis is required, using a paracentesis needle rather than an intravascular catheter improved the efficiency of fluid removal without an increase in complications.[132] The complications of paracenteses include cardiovascular decompensation caused by rapid fluid shifts, intraperitoneal infection, and hemorrhage.

Surgical Options

Nowadays, the availability of liver transplant has made the surgical treatment of refractory ascites in children almost obsolete. The LeVeen shunt creates a drainage path from the intraperitoneal cavity to a jugular vein. Maintaining long-term patency is a problem, and in randomized trials, peritonovenous shunts, compared with medical therapy, did not improve survival.[133–135]

Controlling refactory ascites by decreasing portal hypertension through the creation of portosystemic shunts is a sound physiologic approach to the problem.[136] TIPS has become the most popular method. In adult studies, this procedure has been successful in controlling ascites in the majority of patients. However, mortality rates of 40 to 67% at 1 year and an incidence of encephalopathy of about 50% after TIPS have tempered enthusiasm for this approach. In one randomized trial in adults, mortality after the TIPS procedure was significantly higher compared with medical therapy alone.[137]

Spontaneous Bacterial Peritonitis

Spontaneous bacterial peritonitis (SBP) is defined as bacterial infection of the ascitic fluid in the absence of secondary causes, such as bowel perforation or intra-abdominal abscess. The clinical presentation of SBP can be subtle and even asymptomatic. Fever is generally present, but signs of abdominal pain and rebound tenderness are frequently absent. The child may present only with fever and irritability, the latter suggesting increasing encephalopathy. A high index of suspicion for SBP must always be maintained

in any child with ascites who presents with nonspecific deterioration.[138]

Paracentesis and ascitic fluid culture are essential to the diagnosis and appropriate antibiotic therapy of SBP. Studies in adult patients have emphasized that the presence of coagulopathy is not a contraindication to paracentesis.[139] The incidence of intra-abdominal wall hematoma is surprisingly low (< 1%), and clinically evident bowel perforation is very rare when small-sized cannulas are used.

The preferred site of paracentesis is in the avascular linear alba, 2 cm directly below the umbilicus. Care must be taken that a very large spleen is not impinging on the site. The alternative location is midway along the line drawn between the umbilicus and the anterior right iliac crest. Under aseptic conditions, a 16- to 18-gauge catheter-over-the-needle is introduced, and 10 to 20 cc of fluid is withdrawn. The ascitic fluid should be directly injected into blood culture bottles at the bedside. This technique not only increases the yield of positive cultures but also decreases the time for cultures to become positive.[140,141] Examination of the cell count of the ascitic fluid is important (Table 59-5). A polymorphonuclear (PMN) count of > 500/mm^3 has a sensitivity of 80% and a specificity of 98% for a positive culture. Cell counts of > 250 PMN/mm^3 have a slightly increased sensitivity to 85% but a small reduction of specificity to 93%. The PMN cell count is particularly important in differentiating SBP from the more sinister causes of secondary bacterial peritonitis. With bowel perforation or intra-abdominal abscesses, the PMN count will exceed 10,000/mm^3. As well, multiple organisms are cultured, the ascitic glucose concentration is decreased, and the lactate dehydrogenase concentration is increased. Differentiating primary from secondary bacterial peritonitis is crucial because secondary peritonitis is best managed by surgical intervention.[142,143]

Antibiotic therapy should be started, pending culture results, when the PMN count exceeds 500/mm^3, irrespective of signs or symptoms. When the PMN count is > 250/mm^3 or < 500/mm^3 and there is an index of clinical suspicion, antibiotics should still be started. If withheld, retapping within 48 hours is recommended.[138]

The culture results usually yield a single gram-negative organism, with *Escherichia coli* accounting for about half of the organisms.[144] Other gram-negative enteric rods, such as *Klebsiella*, are also found. Gram-positive organisms, particularly *Streptococcus* species, are seen in about 25% of cases. Anaerobic infections are very rare. Initial antibiotic therapy should cover both the likely gram-negative and -positive organisms. Cefotaxime alone has an 85% reported success rate. Avoiding aminoglycosides is important because many of these patients may already have some renal compromise. However, it should be remembered that cefotaxime alone will not cover *Enterococcus*, which is sometimes the offending organism. In this instance, ampicillin is the best choice Once the culture results are known, the antibiotic regimen can be tailored accordingly. An intravenous course of 10 to 14 days is recommended, although some studies have indicated that the ascitic fluid may become sterile within a few days.[145]

TABLE 59-5 PMN COUNTS ON ASCITIC FLUID

PMN COUNT	TREATMENT
< 250 mm^3	No treatment
> 250 < 500 mm^3	Intravenous antibiotics if clinical suspicion high or wait and retap
> 500 mm^3	Intravenous antibiotics (eg, cefotaxime + ampicillin)
> 500 mm^3	Rule out secondary peritonitis

PMN = polymorphonuclear.

Spontaneous infection of ascitic fluid is most likely a result of portal hypertension leading to an increased chance of translocation of the patient's own enteric organisms into the mesenteric lymph nodes, which drain via the thoracic duct into the systemic circulation.[146] Transient bacteremia occurs, with subsequent seeding of the ascitic fluid. Also adding to the risk are the decreased complement levels in both serum and ascitic fluid, poor reticuloendothelial system function, and defective neutrophil and phagocyte function observed in patients with severe liver disease.[142,147,148]

The first occurrence of SBP portends an accelerating risk of death from liver disease and underscores the urgency for liver transplant. In adult series, mortality during the first year after SBP is as high as 79%, usually related to complications of progressive liver failure rather than the SBP itself.[149,150] Recurrence is common—70% after the first episode in some studies.[150] Although oral antibiotic prophylaxis for SBP has been advocated by some authors for adult patients with high-risk factors for SBP (eg, elevated bilirubin, prolonged prothombin time, variceal bleeding),[151] there is no good evidence to support this concept in children. Because almost all of these children are liver transplant candidates, the risk of inducing resistant bacterial strains and fungal superinfection, both of which may have serious consequences in the post-transplant period, would seem to far outweigh any benefits.

HEPATORENAL SYNDROME

The hepatorenal syndrome is defined as functional renal failure in patients with severe liver disease. Histologically, the kidneys are normal, as evidenced by recovery of function after successful liver transplant and the successful transplant of cadaveric kidneys from patients with hepatorenal syndrome.[152] Hepatorenal syndrome most often occurs in decompensated chronic liver disease but also has been described in acute liver failure.[153] Hepatorenal syndrome is sometimes precipitated by other supervening complications of end-stage liver disease, particularly spontaneous bacterial peritonitis.[154]

About 10% of adult patients with chronic liver disease develop hepatorenal syndrome, with an associated mortality of more than 70% without liver transplant. There are few published studies of the prevalence of hepatorenal syndrome in children; however, it appears to be much less common compared with adult patients,[155] although still associated with significant mortality in the pretransplant period.[156]

Inherent in the diagnosis of hepatorenal syndrome is the careful exclusion of all other potential contributors to renal impairment. Most importantly, there must be no evidence of hypovolemia. This is particularly important in small children who may have unexpectedly large fluid losses from the intravascular compartment as a result of aggressive diuretic therapy for ascites, increased enteric losses from vomiting or diarrhea, or fluid shifts secondary to hypoalbuminemia. Measurement of central venous pressure can be very useful to determine intravascular fluid volume status. Importantly, intravenous fluid challenges will improve urine output in the hypovolemic child but will have no effect on the child with hepatorenal syndrome. Ongoing or recent hypovolemic shock, causing renal failure secondary to acute tubular necrosis, must be excluded.

Other important contributing factors to poor renal function that must be excluded are the effects of nephrotoxic drugs (particularly aminoglycosides and nonsteroidal anti-inflammatory drugs) and other intrinsic kidney diseases. Of the childhood diseases that cause chronic liver disease and have associated renal pathology, the most common are hereditary tyrosinemia, Alagille syndrome, and polycystic liver-kidney disease.

Diagnosis and Definition

The diagnosis of hepatorenal syndrome is supported by a characteristic pattern of urine electrolyte abnormalities: urine sodium of < 10 mEq/L, a fractional excretion of sodium of < 1%, and a urine-to-plasma creatinine ratio of < 10 (Table 59-6).[157,158] Although these findings are not pathognomonic for hepatorenal syndrome and, in particular, do not differentiate hypovolemia from hepatorenal syndrome, they help to exclude unsuspected acute tubular necrosis, characterized by an increased urine sodium and increased fractional excretion of sodium, as well as other causes of intrinsic renal disease. Generally, the glomerular filtration rate (GFR) is profoundly decreased, and the child is oliguric (< 1 mL/kg/h of urine output). Significant amounts of protein or blood in the urine usually exclude the diagnosis. Type 1 hepatorenal syndrome is a rapidly progressive renal failure, often associated with a precipitating event, and has a poor prognosis, whereas type 2 is a more moderate and less precipitous loss of renal function.

It is important not to rely solely on serum creatinine as an estimate of the degree of renal impairment.[159,160] For example, a creatinine level of 0.5 mg/dL in a small child may not appear worrisome, but if such a child has reduced muscle mass, this creatinine may represent a tripling of normal creatinine for age. Moreover, the rise in serum creatinine is not linearly associated with falling GFR. By the time creatinine rises above normal, GFR is already reduced by about 50%. True GFR is easily measured by plasma clearance of an isotope (eg, indium = 111 diethylenetriamine pentaacetic acid) because creatinine clearance and calculated GFR both overestimate true GFR.[161] These fundamental principles are essential to diagnose impending renal failure in children and initiate appropriate corrective action.

TABLE 59-6 HEPATORENAL SYNDROME: DIAGNOSTIC CRITERIA

Oliguria: < 1 mL/kg/d
Fractorial excretion sodium < 1%
Urine-to-plasma creatinine ratio < 10
↓ Glomerular filtration rate, ↑ creatinine
Absence of hypovolemia
Other kidney pathology excluded

Pathogenesis

The hallmark of hepatorenal syndrome is intense renal vasoconstriction with coexistent systemic vasodilation.[162] Renal blood flow, particularly to the renal cortex, is markedly reduced, despite the characteristic systemic hemodynamic changes seen in end-stage liver disease: decreased systemic vascular resistance, increased cardiac output, and decreased systemic blood pressure.[158,163] Ascites is often present, and hepatorenal syndrome is more likely to develop when ascites is resistant to diuretic therapy.[164] The mechanism of renal vasoconstriction is not yet fully understood, although several theories have been advanced. Gines and Arroyo suggest that the arterial vasodilation concept (also relevant in the mechanism of ascites generation) best explains the relationship between the opposing hemodynamic changes at the renal and systemic levels.[158] This theory proposes that portal hypertension as a result of cirrhosis causes splanchnic vascular vasodilation, with a subsequent reduction in effective systemic blood volume. The baroreceptor response is triggered, causing the activation of several systems that cause renal vasoconstriction. The splanchnic vascular system appears to be protected from the generalized vasoconstriction by the production of locally active vasodilator substances.

A variety of different mediators of renal vasoconstriction and splanchnic vasodilation have been proposed,[121] with the hope, not yet realized, that treatment could be aimed at inhibiting the causative agent.[165] The role of the renin-angiotensin axis has long been implicated in the vasoconstrictive response because cirrhotic patients are known to have high renin levels.[129] Also important is the activation of the sympathetic nervous system by the baroreceptor response to arterial hypotension. Increased sympathomimetic tone increases renal vasoconstriction and decreases GFR.[166,167] In some studies, the administration of clonidine, which decreases norepinephrine levels, has been shown to decrease renal vascular resistance.[168] Levels of antidiuretic hormone, which also cause renal vasoconstriction, are known to be elevated in cirrhotic patients.[121]

New attention is being focused on the endothelins, which are known potent vasoconstrictors that are released from many endothelial cells under conditions of stress.[169] Plasma endothelin 1 and 3 levels are two to three times higher in patients with cirrhosis and ascites, including children with biliary atresia[170] and cirrhosis,[171] compared with normal controls and are markedly increased in patients with hepatorenal syndrome.[172,173] Increased production of endothelin in the liver or splanchnic bed has been proposed. Interestingly, Nozue and colleagues reported that in eight children with cirrhosis, endothelin 1

levels were significantly higher than in normal controls, and they correlated this with increased levels of urinary sodium acetyl-β-D glucose aminidase, a sensitive indicator of renal injury.[174] After successful liver transplant in patients with hepatorenal syndrome, endothelin 1 levels fall preceding the improvement in renal function.[175] Because endothelin levels can be elevated in a variety of stress situations that may coexist in patients with hepatorenal syndrome, their causative role in mediating renal vasoconstriction in hepatorenal syndrome remains to be determined. However, preliminary reports suggest that hepatorenal syndrome may be ameliorated by infusion of endothelin antagonists.[176]

Decreased production of renal prostaglandins, which mediate renal vasodilation, may contribute to hepatorenal syndrome. This is supported by evidence that inhibitors of prostaglandin synthetase, particularly nonsteroidal anti-inflammatory drugs, decrease GFR and renal blood flow in patients with ascites. However, treatment of hepatorenal syndrome with systemic prostaglandins has not been successful.[177]

As a mediator of the excessive vasodilation evident in systemic circulation, nitric oxide has been the best studied.[178] Nitric oxide overproduction might explain both the hyperdynamic circulation so characteristic of end-stage liver disease and the splanchnic vascular vasodilation, which results in diversion of blood away from the kidney. It has also been proposed that a local increase in the production of nitric oxide within the kidney may help protect renal perfusion by counterbalancing the renal vasoconstriction that typifies hepatorenal syndrome.[179]

A direct relationship between the liver and kidney has also been invoked to explain the changes in renal circulation seen in liver failure. The proposed mechanisms are a decrease in synthesis of a liver-associated renal vasodilator factor[180] or a hepatorenal reflex that triggers baroreceptors in the liver to increase renal sympathetic nerve activity.[181]

The hepatorenal syndrome is best treated by timely liver transplant because complete recovery can be expected.[182,183] While awaiting transplant, dialysis is the mainstay of treatment. Low-dose dopamine may increase renal blood flow but does not appear to increase GFR.[184] Peritoneovenous shunts[185] or TIPS, by reducing portal hypertension, may transiently improve renal function, but neither have shown sustained benefit.[186,187]

More recently, success has been reported in the treatment of hepatorenal syndrome in adults using terlipressin, a nonselective vasopressin analog. The mechanism of the beneficial effect on renal function is thought to be secondary to the intense splanchnic bed vasoconstriction induced, which allows improvement in systemic arterial circulation and renal blood flow. Patients who receive combined therapy with albumin infusion and terlipressin, which further improves circulatory function, have the best response. In one recent study, 77% of patients receiving albumin and terlipressin alone achieved a serum creatinine level < 1.5 mg/dL.[188] This modality may be a useful bridge until transplant. Unfortunately, at this time, terlipressin is not approved for use in the United States.

Although hepatorenal syndrome is associated with increased mortality before transplant in both adult[189] and pediatric series,[156,190] once transplanted, preexisiting hepatorenal syndrome has little effect on overall survivals.[191,192]

PULMONARY INVOLVEMENT IN LIVER DISEASE

Hepatopulmonary Syndrome

The triad of hypoxemia, intrapulmonary vascular dilations, and liver disease constitutes the hepatopulmonary syndrome.[193,194] It is defined by an arterial oxygen pressure of < 70 mm Hg in room air with an alveolar/arterial gradient of > 20 mm Hg. In adults, the prevalence is between 5 and 29%,[195] with a 41% overall mortality described in hospitalized patients.[196] In children, estimates of the prevalence range from 0.5% in those with portal vein obstruction to as high as 20% in children with biliary atresia and polysplenia syndrome.[197] In other causes of childhood cirrhosis, the prevalence is between 2 and 4%.[198] Hepatopulmonary syndrome has been described in children as young as 6 months. Generally, hepatopulmonary syndrome is seen in chronic liver disease, but it has also been described in acute liver failure[199] and extrahepatic portal venous obstruction.[200] Portal hypertension is not a prerequisite for developing hepatopulmonary syndrome, although there is some evidence that the risk increases as the severity of liver disease progresses. Intrapulmonary vascular dilations may occur in patients with well-compensated chronic liver disease without demonstrating hypoxemia.[201]

There are two forms of hepatopulmonary syndrome; both are characterized by a right to left shunt within the lungs but with differing underlying mechanisms.[202] In type 1, which is more common, there is extensive dilation of the pulmonary precapillary circulation. Blood flowing through the dilated capillaries—particularly the red blood cells flowing in the central core of the stream—is less exposed to oxygen contained in the alveoli, resulting in a ventilation-perfusion mismatch. The patients with this functional shunt will be able to increase their PaO_2 to some degree when breathing 100% oxygen.

About 10% of patients with hepatopulmonary syndrome will have the much more sinister type 2 form, in which anatomic arteriovenous shunts within the pulmonary circulation, and occasionally between the portopulmonary systems, can be demonstrated on pulmonary angiography.[196,203,204] These shunts may not be in close proximity to the alveoli for oxygen exchange. Patients with these fixed anatomic shunts will not be able to increase PaO_2 when breathing 100% oxygen. The prognosis for type 2 hepatopulmonary syndrome is very poor.

Hepatopulmonary syndrome should be suspected if cyanosis, particularly of the lips and fingers, and digital clubbing are found on examination. Many patients with hepatopulmonary syndrome will have a plethora of spider nevi.[205] Because the pulmonary vascular dilations are more prominent at the lung bases, many patients will have increased dyspnea with standing (platypnea). Brain

abscesses have been described as a complication of the intrapulmonary shunts.[206]

Arterial blood gas analysis, both in room air and in 100% oxygen, is essential and should be obtained with the patient standing. The fall in PaO_2 in moving from the recumbent to the standing position (orthodeoxia) is characteristic of hepatopulmonary syndrome. The normal response to breathing 100% oxygen is a PaO_2 > 500 mm Hg. Patients with hepatopulmonary syndrome breathing 100% have a moderate shunt if the PaO_2 is > 300 mm Hg but < 500 mm Hg and a severe shunt if the PaO_2 is > 100 mm Hg but < 300 mm Hg.[207]

The site and extent of the right to left shunt can be assessed by either a technetium 99m–labeled macroaggregated albumin (Tc 99m MAA) study or by a contrast echocardiogram.

The Tc 99m MAA study uses peripherally injected labeled albumin particles with a diameter of > 20 μm.[208] Because the usual pulmonary capillary diameter is 8 to 15 μm, normally all of the labeled albumin will be trapped in the lungs. In the presence of pulmonary vasodilation, the labeled albumin particles can be detected in the kidneys and brain and the degree of shunt correlated to the isotope concentration in these extrapulmonary sites. Note that this scan does not differentiate intracardiac from intrapulmonary shunting.

The best method to evaluate the shunt in hepatopulmonary syndrome is the contrast-enhanced echocardiogram,[209] making the diagnosis in 9.1% of 88 children with biliary atresia, although only 50% had symptoms.[210] In this procedure, an agitated solution of contrast is injected peripherally. When cardiopulmonary circulation is normal, microbubbles with a diameter of > 15 μm are trapped in the lungs. If an intracardiac shunt is present, the microbubbles immediately opacify the left ventricle, whereas if there is an intrapulmonary shunt, it requires three to six ventricular contractions before the microbubbles appear in the left heart. Particularly in children who may have congenital heart disease complicating chronic liver disease (eg, Alagille syndrome or biliary atresia), distinguishing the site of the shunt is essential.

The mediator of the pulmonary vascular dilation that characterizes hepatopulmonary syndrome remains speculative. In animal models, elevated levels of endothelium-derived nitric oxide were detected in the lung homogenates of animals with the clinical features of hepatopulmonary syndrome.[211,212] This finding would appear to be supported by reports documenting increased exhaled nitric oxide concentration in adults and children with hepatopulmonary syndrome.[213,214] However, without better understanding of the underlying mechanism, pharmacologic attempts to manage hepatopulmonary syndrome have not been generally successful to date.

Presently, the only definitive treatment for hepatopulmonary syndrome is liver transplant. In the past, hepatopulmonary syndrome was thought to be a contraindication to liver transplant. However, accumulated evidence documenting successful resolution of hepatopulmonary syndrome after liver transplant has reinforced the concept that in selected patients, hepatopulmonary syndrome is, in fact, best managed by liver transplant.[195,207,215,216] Patients with large anatomic shunts who are unable to increase PaO_2 over 100 mm Hg on 100% oxygen are still not suitable candidates for liver transplant.

In selecting patients for transplant, it must be determined if the patient is safe for anesthesia and if the oxygen content of the blood supplying the graft will be adequate to support early graft function. In an analysis of several studies, Krowka and colleagues noted that 30% of patients with PaO_2 < 50% in room air died after liver transplant compared with 4% of patients with PaO_2 > 50 mm Hg.[195] In successfully transplanted patients, complete normalization of hypoxemia occurred in 82% of transplant recipients. However, the duration of use of the mechanical ventilator and intensive care unit stay tend to be prolonged in patients with hepatopulmonary syndrome.

Encouraging evidence that hepatopulmonary syndrome is reversed by liver transplant is emerging in the pediatric experience. In seven children with hepatopulmonary syndrome undergoing liver transplant, complete reversal of the syndrome occurred in all at an average of 24 ± 10 weeks. Time to extubation in this group was 58 ± 21 hours. In one pediatric study, a PaO_2 in room air lower than 60% uniformly resulted in death after transplant,[217] and in another study, there were no survivors after liver transplant if the PaO_2 was < 200 mm Hg in 100% oxygen.[197] Egawa and colleagues categorized 21 children with hepatopulmonary syndrome into three groups, depending on their shunt ratio: mild, less than 20%; moderate, 20 to 40%; and severe, > 40%.[218] An adverse significant effect on patient and graft survival was found with an increasing shunt ratio. One-year actuarial patient survivals were 80%, 66.7%, and 40% in the mild, moderate, and severe groups, respectively. Prolonged ventilator dependence, hypoxic injury to the graft, portal vein thrombosis, and intracranial thrombosis are some of the sequelae in transplanted children with severe hepatopulmonary syndrome.

PORTOPULMONARY HYPERTENSION

This phenomenon is the antithesis of hepatopulmonary syndrome and is characterized by pulmonary artery vasoconstriction (Table 59-7).[219] Why the chronically diseased liver causes two such diverse effects on pulmonary function is an unresolved conundrum. Portopulmonary hypertension has only rarely been described in children. A summary of the basic principles of the pathogenesis, diagnosis, and management is included here.[220,221]

Portopulmonary hypertension is defined as a mean pulmonary artery pressure > 25 mm Hg with a pulmonary capillary wedge pressure of < 15 mm Hg in the absence of any secondary causes of pulmonary hypertension, such as cardiac valvular disease.

On pathologic examination of the pulmonary arteries, concentric medial hypertrophy and intimal fibrosis are found.[222] The coexistence of portal hypertension appears to be a prerequisite for the development of portopulmonary hypertension.[223] In adults, the prevalence of portopulmonary hypertension in patients with portal hypertension

TABLE 59-7 COMPARISON AND CONTRAST: HEPATOPULMONARY SYNDROME AND PORTOPULMONARY HYPERTENSION

HEPATOPULMONARY SYNDROME	PORTOPULMONARY HYPERTENSION
Intrapulmonary vasodilation	Intrapulmonary vasoconstriction
Alveolar arterial gradient > 20 mm Hg	Alveolar arterial gradient usually normal
Normal mean pulmonary artery (PA) pressure	Mean PA pressure > 25 mm Hg
Perform shunt fraction study	Perform right heart catheterization
Trial of 100% O_2	Vasodilator therapy trial
Often reversible with liver treatment	May not reverse with liver transplant
Poor prognosis: PaO_2 < 300 mm Hg on 100% O_2	Poor prognosis: PA pressure > 45 mm Hg
Histology: PA normal	Histology: PA abnormal; concentric medial hypertrophy

and cirrhosis is 1 to 2%, although an 8.5% incidence was found in a recent large series of patients evaluated for liver transplant.[224] The prevalence in children is unknown.

The diagnosis may be suspected by finding abnormalities on the electrocardiogram, which are present in up to 95% of patients.[225] Right ventricular hypertrophy, right axis deviation, and right bundle branch block are common. A right heart catheterization is essential to document right heart and pulmonary pressures accurately and also allows for a trial of vasodilator therapy. Vasodilator treatment with nitric oxide, calcium channel blockers, and eprostonol has been used with varying success in adult studies.[220,226,227]

There are very few published guidelines regarding treatment of portopulmonary hypertension in children. The management of choice advocated in one article is early diagnosis followed by liver transplant as soon as possible.[228] However, experience from the adult literature recommends that patients must be carefully selected for liver transplant and that mean pulmonary artery pressure should not exceed 50 mm Hg. In one study, a mean pulmonary artery pressure of > 50 mm Hg was associated with a 100% cardiopulmonary mortality and a 50% mortality if > 35 mm Hg but < 50 mm Hg.[229] Selection of patients implies the pretransplant diagnosis of portopulmonary hypertension. However, often the diagnosis is made when anesthesia is induced at the time of transplant, thereby limiting appropriate decision making. Maintaining a high index of clinical suspicion before transplant is therefore imperative. This applies particularly to older children with cirrhosis who may wait for long periods for transplant.[230] Patients with mild to moderate pulmonary hypertension (< 40 mm Hg) will usually show resolution after liver transplant, whereas those with severe portopulmonary hypertension frequently develop progressive fatal right heart failure.

CENTRAL NERVOUS SYSTEM INVOLVEMENT

Clinically evident encephalopathy in children with chronic liver disease appears to be less common compared with adults. However, it is also possible that encephalopathy is underdiagnosed in children because its more subtle manifestations are difficult to appreciate, and there is no specific laboratory test that correlates well with encephalopathy. Irritability and lethargy are the two most common signs but may be evident in any chronically ill child. Acute changes in mental status should prompt an investigation for occult gastrointestinal bleeding (which increases ammonia production from blood in the intestinal lumen) or an intracranial hemorrage secondary to coagulopathy. Aggressive diuretic therapy, spontaneous bacterial peritonitis, and placement of a portosystemic shunt may all precipitate the development of encephalopathy.

The pathophysiology and treatment of hepatic encephalopathy are discussed in detail in Chapter 5.3, "Normal Hepatocyte Function and Mechanisms of Dysfunction." In brief, the main principle of management is to decrease gut-derived nitrogen production by restricting dietary protein, evacuating blood from the gastrointestinal tract, and administering oral lactulose or neomycin to reduce bacterial flora in the bowel. Oral lactulose is preferred, although care must be taken not to induce hypovolemia and electrolyte disturbances from increased stool losses. Oral neomycin has some systemic absorption, which has been associated with ototoxicity; therefore, extended use should be avoided.

CHOLESTATIC LIVER DISEASES: SPECIAL ISSUES

CHOLANGITIS

Ascending infection of the biliary system is most often seen in pediatric liver disease in the context of biliary atresia with a poorly functioning Kasai portoenterostomy. The major risk factors for infection are stasis in the biliary system secondary to poor bile flow and the creation of a Roux-en-Y limb, which approximates the small intestine directly to the porta hepatis. Children with biliary atresia and recurrent episodes of cholangitis may have intrahepatic bilomas, which can be diagnosed on ultrasonography.

Although cholangitis in children with biliary atresia most often occurs when there is evidence of impaired bile flow, generally within the first year after the Kasai procedure,[231] it has also been described late after surgical repair in children with no evidence of biliary obstruction.[232] Under these circumstances, direct ascending infection through the Roux-en-Y limb occurs. However, although these children have a normal serum bilirubin, biliary stasis in the small intrahepatic bile ducts may still be a contributing factor.

Primary and secondary sclerosing cholangitis and the variants of congenital hepatic fibrosis and choledochal cysts with multiple intrahepatic biliary cysts are other intrahepatic cholestatic liver diseases of children in whom cholangitis can occur. In sclerosing cholangitis, the development of jaundice and recurrent cholangitis should prompt the radi-

ographic examination of the biliary system, either by endoscopic retrograde cholangiopancreatography or percutaneous cholangiography, to rule out a dominant stricture that could be stented. In general, obstructive lesions to the external biliary system, such as stones in the common bile duct, cause cholangitis but do not cause chronic liver disease if treated appropriately. These disorders are discussed in Chapter 50.3, "Disorders of the Biliary Tract: Other Disorders."

Cholangitis is diagnosed in the child with cholestatic liver disease who presents with fever and elevated bilirubin and/or serum transaminases from baseline. The alkaline phosphatase or γ-glutamyltransferase will also be elevated. Abdominal pain is only variably present. The most common organisms are gram-negative enteric organisms, such as *E. coli*, *Klebsiella*, *Pseudomonas*, and *Enterococcus*. Blood cultures are frequently negative; therefore, it is important to initiate appropriate antibiotic treatment without waiting for culture results when the clinical index of suspicion is high. If the child fails to defervesce after 72 hours or has frequent recurrences, percutaneous liver biopsy with culture of the liver tissue may be indicated. A common initial antibiotic regimen is ampicillin and cefotaxime given for 10 to 14 days intravenously.

Prophylaxis of recurrent cholangitis is of variable efficacy. In one survey, 73% of physicians used antibiotic prophylaxis after the Kasai procedure.[233] Oral administration of trimethoprim-sulfamethoxazole is most conveniently used.[233] Long-term intravenous antibiotic prophylaxis in children via central venous catheters is occasionally indicated, although the risk of inducing multiply resistant bacterial organisms or fungal colonization must be considered.

There is no definitive effective treatment for recurrent cholangitis in the child with biliary atresia except liver transplant. As such, recurrent cholangitis is an indication for listing children with biliary atresia for transplant.[234] There is some evidence that children with biliary atresia who suffer recurrent episodes of cholangitis develop cirrhosis more quickly than those without cholangitis.[235]

Pruritus

Intense pruritus can be a complication of cholestatic liver disease, causing misery to children and their caregivers alike. The well-being of the child is overwhelmed by the intense need to itch. Severely afflicted children cannot sleep, do not eat, and are constantly irritable. Although the etiology may be multifactorial, accumulation of bile acids appears to be important because pruritus is most often associated with cholestasis and is particularly problematic in progressive familial intrahepatic cholestasis syndromes. Another contributing factor appears to be increased opiate tone because opiate antagonists have some therapeutic benefit.[236,237]

Treatment is often poorly effective.[238,239] Cholestyramine is generally not helpful because there are already unusually low concentrations of bile acids in the intestinal lumen. Choleretics, such as phenobarbital, can be tried. Antihistamines are the first line of treatment but are seldom adequate alone. Ursodeoxycholic acid, by altering bile composition, will often help reduce pruritus and should be used in doses of 25 to 30 mg/kg/d in divided doses.[240] Rifampin has also been successfully used in doses of 10 mg/kg/d.[241] Rifampin may alter bile acid composition via 6-hydroxylation of bile salts. Rifampin interacts with the nuclear receptor pregnane X and induces cyp3A, which can hydroxylate bile salts (see Chapter 5.1, "Bile Formation and Cholestasis").

Other modalities that are less well studied in children are intravenous naloxone,[242] plasmapheresis, charcoal absorption,[243] and phototherapy. The opiate antagonist approach appears to be very promising. Naloxone has been shown to be efficacious but is limited in its application by poor oral bioavailability and short half-life. Oral preparations of opiate antagonists, such as naltrexone and nalmefene, may help some patients.[244,245] A recent report suggests that dronabinol (a cannabinoid) may also be beneficial.[246]

Partial biliary diversion procedures have been successful in treating some children with progressive familial intrahepatic cholestasis syndromes and intractable pruritus.[247–249]

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CHAPTER 60

LIVER TRANSPLANT

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Successful pediatric liver transplant evolved in the 1980s and became established in the 1990s. The success of this complex procedure has led to a significant increase in the number of children undergoing liver transplant worldwide and has radically changed the prognosis of many babies and children dying of end-stage liver failure.

Liver transplant was first performed in the United States and Europe in 1963, but the first successful pediatric liver transplant was not performed until 1967, in a young girl with a malignant hepatic tumor. There were rapid advances in adult transplant throughout the 1970s, particularly after the introduction of cyclosporin A in 1978,[1] but technical difficulties and donor shortages meant that pediatric liver transplant remained hazardous. By 1986, when most adult units claimed a 1-year survival rate of 80%, average 1-year survival rates in children were only 60%.[2] Since then, there have been considerable advances in both medical and surgical management, with international 1-year survival rates from pediatric liver transplant in excess of 90% and 5- to 10-year survival rates of 80%.[3]

The improved survival rates are related to improving pre- and postoperative management in association with the development of innovative surgical techniques to expand the donor pool. These techniques have not only reduced deaths on the waiting list and improved survival overall but also have extended the range of indications for liver transplant to include semielective liver replacement, transplant for inborn errors of metabolism, and unresectable hepatic tumors. As short-term survival has improved, interest in research has focused on evaluating quality of life in long-term survivors.

By 2002, 4,252 children had undergone liver transplant in Europe,[4] with more than twice that number of children transplanted in the United States.[5] In Europe, 40% of transplants were performed in children under 2 years of age, 50% in children aged 2 to 12 years, and 10% in children aged 12 to 15 years (Figure 60-1).[4]

Despite its recorded success, liver transplant remains a complex procedure with large resource implications and a 10% mortality rate; thus, careful consideration should be given to the selection of potential recipients and the exclusion of other therapies.

INDICATIONS

Liver transplant is now accepted therapy for acute or chronic liver failure (Table 60-1; see Figure 60-1).

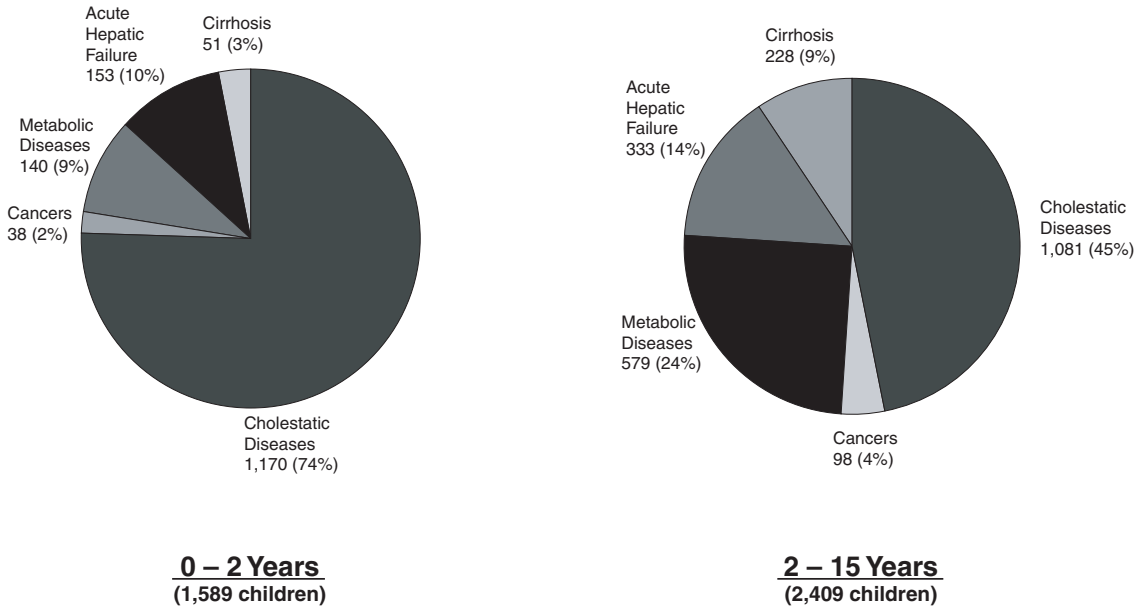


FIGURE 60-1 Primary indications of liver transplant in 3,998 pediatric patients, 1988 to 2001. Adapted from the European Liver Transplant Registry, 2002.

TABLE 60-1 INDICATIONS FOR LIVER TRANSPLANT IN CHILDREN

CHRONIC LIVER FAILURE
Cholestatic liver disease
Biliary atresia
Idiopathic neonatal hepatitis
Alagille syndrome
Progressive familial intrahepatic cholestasis
Nonsyndromic biliary hypoplasia
Metabolic liver disease
α_1-Antitrypsin deficiency
Tyrosinemia type I
Wilson disease
Cystic fibrosis
Glycogen storage type IV
Chronic hepatitis
Autoimmune
Idiopathic
Postviral (hepatitis B, C, other)
Cryptogenic cirrhosis
Fibropolycystic liver disease ± Caroli disease
Primary immunodeficiency
ACUTE LIVER FAILURE
Fulminant hepatitis
Autoimmune hepatitis
Halothane anesthesia
Acetaminophen poisoning
Viral hepatitis (A, B, C, or NA-G)
Metabolic liver disease
Fatty acid oxidation defects
Neonatal hemochromatosis
Tyrosinemia type I
Wilson disease
INBORN ERRORS OF METABOLISM
Crigler-Najjar syndrome type I
Familial hypercholesterolemia
Organicacidemia
Urea cycle defects
Primary oxalosis
HEPATIC TUMORS
Benign tumors
Unresectable malignant tumors

CHRONIC LIVER FAILURE

Cholestatic Liver Disease

Chronic liver failure secondary to cholestatic liver disease is the most common indication for liver transplant in children (see Figure 60-1). Of diseases of this type, biliary atresia remains the main indication for liver transplant in children worldwide. Despite professional education on the importance of early diagnosis and management of this condition, many children continue to be referred for treatment too late to benefit from a palliative Kasai portoenterostomy. Children who have an immediately unsuccessful Kasai portoenterostomy or who develop intractable nutritional or hepatic complications[6] should be referred for urgent transplant. Approximately 60% of children with biliary atresia will have a successful Kasai portoenterostomy; in these children, cirrhosis and portal hypertension will develop at a much slower rate, and their need for liver transplant will depend on the rate of development of hepatic complications.[7]

The outcome of cholestatic liver diseases such as Alagille syndrome and progressive familial intrahepatic cholestasis is more variable. Many children will have compensated liver disease for some time or will be well maintained on supportive management. Liver transplant is indicated if decompensated cirrhosis and/or intractable portal hypertension develop, if malnutrition and growth failure are unresponsive to nutritional support, or if there is intractable pruritus that is resistant to maximum medical therapy or biliary diversion.[8]

Some infants who present with giant cell hepatitis or neonatal hepatitis of unknown etiology develop persistent cholestasis and rapid progression to cirrhosis and portal hypertension and become candidates for liver transplant in the first 2 or 3 years of life.

Metabolic Liver Disease

α_1-Antitrypsin deficiency is the most common form of inherited metabolic liver disease presenting in childhood in Europe and the United States. Although 20 to 40% of children in any population develop persistent liver disease progressing to cirrhosis, only a minority (approximately 20 to 30%) require liver transplant in childhood.[9]

Tyrosinemia type I is an autosomal recessive disorder of tyrosine metabolism, with a clinical presentation that includes both acute and chronic liver disease and multiorgan failure with cardiac, renal, and neurologic involvement. The management of this disorder has changed dramatically since the introduction of 2(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexenedione (NTBC), which prevents the formation of toxic metabolites and produces rapid clinical and biochemical improvement. The widespread use of this drug in tyrosinemia has altered both the natural history of the disease and the indications for transplant.[10,11] Prior to the introduction of NTBC, liver transplant was indicated for acute or chronic liver failure but, more importantly, for hepatic dysplasia or hepatocellular carcinoma. Liver transplant is now indicated only for those children who have a poor quality of life (ie, those who do not tolerate the restrictive low-protein diet and the frequent bloodletting and amino acid monitoring) or do not respond to NTBC or in whom hepatic malignancy is thought to have developed.[11] Routine monitoring of children with tyrosinemia type I being treated with NTBC includes ultrasonography, computed tomography (CT), or magnetic resonance imaging (MRI) to detect the development of nodules and/or early hepatocellular carcinoma in association with regular α-fetoprotein levels. A persistent or sustained rise of α-fetoprotein may indicate the early development of hepatocellular carcinoma, which should be confirmed by the detection of hepatic dysplasia on liver biopsy.[12,13]

Wilson disease is a rare indication for liver transplant in childhood. Early diagnosis and therapy with penicillamine should be curative, but many children will present with established cirrhosis or fulminant liver failure. Liver transplant is indicated for those children who present with advanced liver disease (Wilson score > 6) or fulminant liver failure or who have progressive hepatic disease despite penicillamine therapy or other therapy, such as trientene and zinc.[14,15]

The short-term survival for children with cystic fibrosis has improved with increased attention to nutrition and

appropriate management of pulmonary disease. Liver disease develops in approximately 20% of children, mainly in boys,[16] and is becoming an increasingly common indication for liver transplant.[17] Referral for liver transplant and the timing of transplant are particularly difficult for children with cystic fibrosis. Many children present with compensated liver disease but with bleeding esophageal varices from portal hypertension.[18] In these children, management of portal hypertension by conservative means (sclerotherapy or band ligation of esophageal varices or insertion of a transhepatic portal systemic shunt) may be sufficient to control symptoms and improve quality of life for some years.[18]

Liver transplant in cystic fibrosis is indicated if there is evidence of hepatic decompensation (falling serum albumin or prolonged coagulation unresponsive to vitamin K), severe malnutrition unresponsive to nutritional therapy, or severe complications of portal hypertension that are unresponsive to medical management, such as ascites or uncontrolled variceal bleeding.[17,19] Careful assessment of pulmonary function is essential because severe lung disease (< 70% predicted lung function) may indicate the necessity for heart, lung, and liver transplant.[17,19] In some children, the choice of timing of the liver transplant may be advanced by the recognition of rapidly deteriorating pulmonary function. The management of pulmonary disease is particularly important before transplant and should include vigorous physiotherapy, intravenous antibiotics, and deoxyribonuclease.

Most children with glycogen storage disease type I should not require liver transplant and can be managed appropriately with medical and nutritional treatment. Transplant is indicated only for children who develop multiple hepatic adenomas or in whom metabolic control has affected the quality of life. Children with glycogen storage disease types III and IV are more likely to progress to cirrhosis with portal hypertension and require transplant because of hepatic dysfunction.[20]

The rare disorders of bile acid metabolism that present with persistent cholestasis may now respond to oral bile acids, reducing the need for transplant.[21,22]

Chronic Hepatitis

Autoimmune Liver Disease Types I and II. Liver transplant is a rare indication for children with autoimmune liver disease type I or II, who usually respond to immunosuppression with prednisolone or azathioprine. Liver transplant is indicated for those children who have not responded to immunosuppression despite alternative therapy such as cyclosporin A, mycophenolate mofetil, or tacrolimus and for those children who present with fulminant hepatic failure.[23] Fulminant hepatic failure is more likely in children with type II autoimmune hepatitis, who have a worse prognosis and an increased requirement for liver transplant.

Chronic Hepatitis B or C. Although chronic hepatitis B or C is a major indication for transplant in adults, it is less common in children, many of whom will not develop symptomatic liver disease in childhood. Recurrence of hepatitis B or C is likely in 90% of patients transplanted for chronic disease but not for fulminant hepatitis. Prevention or recurrence of hepatitis B is less likely with prophylactic treatment of hepatitis B immunoglobulin and/or lamivudine.

Fibropolycystic Liver Disease. Fibropolycystic liver disease is an unusual indication for liver transplant in childhood because liver function remains normal despite the development of severe portal hypertension. Liver transplant is indicated for those children in whom hepatic decompensation occurs secondary to recurrent cholangitis or portal hypertension or if hepatic enlargement affects the quality of life. Because the disease may be associated with infantile polycystic kidney disease in some children, both liver and kidney replacement may be required.[24]

Primary Immunodeficiency. As bone marrow transplant for primary immunodeficiency becomes increasingly successful, it has been recognized that many children with these diseases have associated liver disease. The most common immunodeficiency is CD40 ligand deficiency (hyper-immunoglobulin M syndrome), in which recurrent cryptosporidial infection of the gut and biliary tree lead to sclerosing cholangitis. In this group of children, it is important to consider bone marrow transplant before the development of significant liver disease or to consider combined liver and bone marrow transplant if necessary.[25]

Timing of Transplant for Chronic Liver Failure. The timing of liver transplant for children with chronic liver failure may be difficult because many children will have compensated liver disease for some years. Although it may be possible to predict biochemical decompensation by studying serial estimates of lidocaine metabolite formation and excretion,[26] this has not proved universally to be of value. The most useful guide to the timing of liver transplant is provided by a variety of parameters that include (1) a persistent rise in total bilirubin > 150 μmol/L (> 9 mg/dL), (2) prolongation of the prothrombin ratio (international normalized ratio [INR] > 1.4), and (3) a persistent fall in serum albumin to < 35 g/L.[27] Serial evaluation of nutritional parameters is a useful guide to early hepatic decompensation. Progressive reduction of fat stores (measured by triceps skinfold or subscapular skinfold) or protein stores (measured by midarm circumference or midarm muscle area) despite intensive nutritional support is a good guide to hepatic decompensation.[28] Recently, the development of the PELD Score (PELD = pediatric end-stage liver disease) has confirmed these observations.[29]

An important consideration in timing liver transplant is psychosocial development. Children with chronic liver disease may have both social and motor developmental delays that increase with time unless reversed following early liver transplant.[6,30,31]

Children with severe hepatic complications such as chronic hepatic encephalopathy, refractory ascites, intractable pruritus, or recurrent variceal bleeding despite appropriate medical management should be referred immediately for transplant. In some children, hepatopul-

monary syndrome secondary to pulmonary shunting develops and is an important indication for liver transplant.[32] It is essential that transplant is performed prior to the development of severe pulmonary hypertension because this will preclude successful liver transplant.[33]

For children with chronic liver disease to benefit from transplant, it is essential that this procedure be considered before the complications of liver disease adversely impair the quality of the children's lives and before their growth and development are irreversibly retarded.

ACUTE LIVER FAILURE

Liver transplant is indicated for acute liver failure secondary to a wide range of primary etiologies (see Table 60-1). It is good practice to refer children with acute liver failure early to a specialist unit with facilities for transplant so that the child may be stabilized and sufficient time can be given to find an appropriate donor organ.

Fulminant Hepatitis

The management of children with fulminant hepatitis is first to assess prognosis and the necessity for liver transplant and then to prevent or treat hepatic complications while awaiting a suitable donor organ or regeneration of the native liver. The factors known to imply a poor prognosis for children with fulminant hepatitis are as follows:

1. Non–A–G hepatitis
2. Development of grade III or IV hepatic coma
3. Reduction in hepatic size in association with falling transaminases and increasing bilirubin (> 300 µmol/L or > 16 mg/dL).
4. Persistent severe coagulopathy (> 50 seconds over control; INR > 4)[34]

In infants with fulminant hepatitis, coagulopathy may be more severe than encephalopathy, and both are not required prior to listing for liver transplant.[35]

It is essential that all children who have reached grade III hepatic coma or who have a persisting coagulopathy without evidence of irreversible brain damage from cerebral edema or hypoglycemia be listed for liver transplant. It may be difficult to exclude underlying brain disease. Cerebral CT or MRI may demonstrate cerebral infarction, ischemia, or hemorrhage, whereas electroencephalography (EEG) may indicate reduced voltage of brain waves, but none of these techniques are infallible. Although intracranial pressure monitoring has been demonstrated to improve selection for liver transplant by excluding children with persistently raised intracranial pressure, it has not influenced survival.[36] The technique may be impossible in children with prolonged coagulopathy and is associated with significant morbidity unless coagulopathy can be adequately corrected without inducing cerebral edema. Measurement of cerebral blood flow is not useful because it may be reduced in hepatic failure; measurement of cerebral perfusion pressure may be a more sensitive indicator for selection. EEG may demonstrate a reduction in voltage of electrical activity and ultimately brain death, but these results should be interpreted cautiously in ventilated patients or those treated with thiopental or other phenobarbitals because the EEG tracing may be affected by these drugs.

Acetaminophen overdose is a common indication for liver transplant in adults in the United States but is less common in pediatric practice. Of 73 children in one study who developed overdose, only 8% required liver transplant.[37] Children are more likely to require liver transplant if the overdose was taken with another drug (eg, LSD [lysergic acid diethylamide] or Ecstasy [3,4-methylenedioxymethamphetamine]) or alcohol and if they present with a persistent coagulopathy (INR > 4), metabolic acidosis (pH < 7.3), and rapid progression to hepatic coma grade III.[38]

Metabolic Liver Disease

Metabolic liver disease such as Wilson disease and tyrosinemia type I may present with acute liver failure, whereas fatty acid oxidation defects and/or mitochondrial disease occur in the neonatal period. In contrast to fulminant hepatitis, the clinical presentation is subacute, and liver failure develops in the presence of an underlying cirrhosis. Selection for liver transplant is based on response to medication and the presence of advanced liver disease or severe coagulopathy. Jaundice or encephalopathy may not be obvious.[14]

Neonatal hemochromatosis is a rare disorder of iron handling. The presentation is within days or weeks of birth, with severe coagulopathy, encephalopathy, and hypoglycemia. The diagnosis is based on the clinical features and presence of extrahepatic iron deposition. Recent experience has shown that medical management using an antioxidant "cocktail" may be beneficial for those children with a milder phenotype, particularly if the cocktail is started shortly after birth.[39,40]

Inborn Errors of Hepatic Metabolism

A number of inborn errors of metabolism are secondary to hepatic enzyme deficiencies that do not lead to liver disease. Liver function is normal, but the enzyme deficiency leads to severe extrahepatic disease. The purpose of liver transplant in this group of diseases is to replace the missing hepatic enzyme to prevent or reverse extrahepatic disease. Selection is based on the child's quality of life, on medical management, and on the potential mortality and morbidity of the primary disease related to the risks and outcome following liver transplant.

The timing of transplant depends on the rate of progression of the disease, the quality of life of the affected child, and the development of severe irreversible extrahepatic disease.[41]

Crigler-Najjar Syndrome Type I. Crigler-Najjar syndrome type I is an autosomal recessive disease in which there is an absolute deficiency of glucuronyl transferase, which leads to high levels of unconjugated bilirubin and the eventual development of structural brain damage secondary to kernicterus. Current management includes many hours of phototherapy daily to reduce the level of unconjugated bilirubin.

Liver transplant is curative, and children should be selected for transplant prior to the development of irreversible brain damage and at a time when continuous pho-

totherapy is affecting the quality of their life. The recent development of auxiliary liver transplant (see below), in which only part of the liver is transplanted, may be the most appropriate transplant operation for these children because it allows the possibility of gene therapy in the future.[42]

Familial Hypercholesterolemia. Children with homozygous or heterozygous familial hypercholesterolemia are prone to premature development of coronary artery disease owing to a deficiency in the number of receptors needed for the metabolism of cholesterol on hepatocytes. Liver transplant should be performed before there is irreversible coronary artery disease, but recent progress with gene therapy for this condition suggests that auxiliary liver transplant or gene therapy may be more appropriate treatment strategies at present.[42,43]

Organicacidemias. These rare disorders include propionicacidemia or methylmalonicacidemia, in which there are abnormalities in the metabolism of propionate and methylmalonate, respectively. Children with these diseases are at risk of recurrent metabolic acidosis, developmental delay, and irreversible brain damage. Liver transplant is palliative treatment for these conditions because the enzyme deficiency affects all parts of the body. Isolated case reports have indicated that orthotopic liver replacement was necessary to provide adequate enzyme supplementation, but it is possible that auxiliary liver transplant may be sufficient for mildly affected patients.[42]

Four main disorders of the urea cycle lead to neurotoxicity with accumulation of ammonia and glutamine. The defective enzymes are (1) carbamyl phosphate synthetase, (2) ornithine transcarbamylase, (3) argininosuccinic acid synthetase (deficiency causes citrullinemia), and (4) argininosuccinate lyase. Medical management of these conditions is reduction of ammonia by withdrawing dietary protein and treatment with oral sodium benzoate (0.1–0.25 g/kg/d) and/or phenylbutyrate (0.25–0.6 g/kg/d).

Liver transplant in these urea cycle defects is considered for patients in whom dietary and medical management is ineffective. Although liver transplant corrects hyperammonemia, it does not completely correct the amino acid abnormality or reverse any preexisting neurotoxicity,[44] thus indicating that transplant should be considered prior to irreversible neurotoxicity.

Primary Oxalosis. In this rare autosomal recessive disorder, there is a deficiency of the hepatic enzyme alanine–glycoxylate aminotransferase, which leads to overproduction of oxalate with deposition in the cornea, brain, cardiac muscles, bones, and kidneys, leading to renal failure and systemic oxalosis. There is considerable controversy over the best management of this condition, but it is agreed that liver transplant is required before the development of renal failure or severe systemic oxalosis. In those infants who present with renal failure, combined liver and kidney transplant is required.[45–47] Because the enzyme deficiency results in overproduction of oxalate, auxiliary liver transplant is not suitable.

Hepatic Tumors

With the increasing success of liver transplant, children with either benign or malignant tumors are considered for liver transplant. Benign tumors include hemangiomas or hemoangioendotheliomas, adenomas, and focal nodular hyperplasia. These tumors are selected for liver transplant only if they cause hepatic dysfunction or are associated with an unacceptable increase in liver size and hepatic resection is impractical. Persistent heart failure in hemangioendotheliomas is best treated by selective hepatic embolism.

Malignant hepatic tumors such as hepatoblastoma or hepatocellular carcinoma that are either unresectable or refractory to chemotherapy are considered for liver transplant as long as there are no extrahepatic metastases.[48] It is important to carry out a search for extrahepatic metastases that includes CT of the chest and abdomen and regular monitoring of serum α-fetoprotein. A careful assessment of cardiac function is essential to exclude the cardiotoxic effects of chemotherapeutic drugs such as daunorubicin. It is best to time the transplant between chemotherapy treatments to prevent relapse or recurrence, and elective living-related transplant is particularly helpful in this situation. Children with rhabdomyosarcomas may not be considered for transplant because of the extent of the tumor and the early presence of extrahepatic metastases.

PRETRANSPLANT EVALUATION

The pretransplant evaluation of the patient (Table 60-2) is particularly important and should include the following:

1. Assessment of the severity of the liver disease and the possibility for medical management

TABLE 60-2 PRETRANSPLANT ASSESSMENT

Nutritional status
Height, weight, triceps skinfold, midarm muscle area, midarm circumference
Identification of hepatic complications
Ascites, varices
Cardiac assessment
Electrocardiography, echocardiography, chest radiography, cardiac catheterization
Respiratory function
Oxygen saturation,* ventilation-perfusion scan,* lung function tests†
Neurologic and developmental assessment
Electroencephalography, Bailey developmental scales, Stanford-Binet intelligence scales
Renal function
Urea, creatinine, electrolytes, urinary protein-to-creatinine ratio, chromium EDTA
Serology
Cytomegalovirus; Epstein-Barr virus; varicella-zoster virus; herpes simplex virus; hepatitis A, B, and C; HIV; measles
Hematology
Full blood count, platelets, blood group
Radiology
Ultrasonography of liver and spleen for vascular anatomy, wrist radiography for bone age and rickets
Dental assessment

EDTA = ethylenediaminetetraacetic acid; HIV = human immunodeficiency virus.
*If cyanosis present.
†In cystic fibrosis.

2. Assessment of the technical feasibility of the operation
3. Consideration of any contraindications
4. Psychological preparation of the family and child

The severity of liver disease should be assessed by evaluating the following:

1. Hepatic function. Listing for liver transplant is based on evidence of deterioration in hepatic function as indicated by albumin (> 35 g/L), coagulation time (INR > 1.4), and cholestasis, as evidenced by a rise in bilirubin (150 µmol/L, 8 mg/dL). Portal hypertension should be established by estimating the size of the spleen and portal vein by ultrasonography and by diagnosing esophageal and gastric varices by gastrointestinal endoscopy.
2. Renal function. Many children with acute or chronic liver failure will have abnormalities of renal function, including renal tubular acidosis, glomerulonephritis, acute tubular necrosis, and/or hepatorenal syndrome. Assessment of renal function is important to provide a baseline for the nephrotoxic effects of immunosuppressive drugs post-transplant and to consider the necessity for perioperative renal support.
3. Hematology. Baseline information on full blood count, platelets, and coagulopathy is obtained. Determination of blood group is essential for organ donor matching.
4. Serology. Previous evidence of varicella, measles, or infection with hepatitis A, B, or C viruses; cytomegalovirus (CMV); or Epstein-Barr virus (EBV) is important information for postoperative management. Donor grafts are matched by CMV status if possible.
5. Radiology. The rapid development of Doppler ultrasonography techniques has greatly improved the pretransplant assessment of vascular anatomy and the patency of hepatic vessels. It is unusual now to require MRI or angiography for these assessments. Evidence of retrograde flow and/or a reduction in the size of the portal vein (< 4 mm at the porta hepatis) suggest advancing portal hypertension and are indications for early transplant.[49] Children with biliary atresia have an increased incidence of abnormal vasculature, the hypovascular syndrome, which consists of an absent inferior vena cava, preduodenal or absent portal vein, azygous drainage from the liver, and polysplenia syndrome.[50] It may be associated with situs inversus, dextrocardia, or left atrial isomerism. Because these abnormalities may increase the technical risk of a liver transplant, it is important to diagnose these before transplant.[51]
6. Cardiac and respiratory assessment. Liver transplant is associated with significant hemodynamic changes during the operative and anhepatic phases. It is important, therefore, to have baseline information on both cardiac and respiratory function. Electrocardiography, echocardiography, and oxygen saturation will provide most of the necessary information. It is also important to determine the presence of congenital cardiac disease. Children with biliary atresia have an increased incidence of congenital cardiac disease, particularly atrial and ventricular septal defects, whereas peripheral pulmonary stenosis is a known feature of Alagille syndrome. Cardiomyopathy may develop secondary to tyrosinemia type I and the organicacidemias, although children with malignant tumors who have received chemotherapy need particular cardiac assessment. Cardiac catheterization is required in some cases to determine whether cardiac function is adequate to sustain the hemodynamic effect of liver transplant or if cardiac surgery is required preoperatively. If the cardiac defect is inoperable, liver transplant occasionally may be contraindicated. A small percentage of children with end-stage liver disease develop intrapulmonary shunts (hepatopulmonary syndrome). Clinical signs include cyanosis, digital clubbing, and reduced oxygen saturation. The diagnosis can be confirmed by bubble echocardiography, ventilation-perfusion scans, and/or cardiac catheterization.[32]
7. Neurodevelopmental assessment. Because the aim of liver transplant is to improve quality of life, it is important to identify any preexisting neurologic or psychological defects not only to consider whether they would be reversible post-transplant but also to evaluate the necessity for corrective management.[30,31]
8. Dental assessment. Advanced liver disease has an adverse affect on all aspects of growth and development, including dentition. Pretransplant dental problems include hypoplasia with staining of the teeth and gingival hyperplasia. Because gingival hyperplasia is a significant side effect of cyclosporine immunosuppression, it is important to establish good dental hygiene in the patient prior to transplant.[52]

CONTRAINDICATIONS FOR LIVER TRANSPLANT

With increasing experience, there are fewer contraindications to transplant. Although historically considered difficult, age < 1 year and size < 10 kg are no longer contraindications for transplant. Portal vein thrombosis increases the technical risk of the surgery, but it can now be managed with venous or prosthetic grafts. Vascular abnormalities such as the hypovascular syndrome are no longer considered contraindications. Although infection with human immunodeficiency virus (HIV) was a contraindication, the improvement in long-term prognosis with antiviral drugs means that this disease can be controlled before transplant. The following contraindications remain:

1. Severe systemic sepsis (in particular, fungal sepsis) at the time of operation. It is important that the operation be deferred until the infection has been appropriately treated.
2. Malignant hepatic tumors with confirmed extrahepatic metastases.[53]
3. Severe extrahepatic disease that is not considered reversible following liver transplant. This includes severe cardiopulmonary disease for which there is no possibility of corrective surgery or severe structural brain damage with a poor prognosis.
4. Multiorgan failure, especially owing to mitochondrial cytopathy,[54] because it has been shown that unless the

mitochondrial defect is confined to the liver, liver transplant is not curative.

5. Alpers disease and sodium valproate toxicity, related disorders in which defects in the respiratory chain have been identified in some patients. Liver transplant is contraindicated in the presence of these diseases in the same way as in the case of mitochondrial cytopathies because of the progression of neurodegeneration despite transplant.[55]
6. Recurrent disease. Hepatitis B and C have a recurrence rate of 90 to 100% post-transplant but can now be treated with antiviral agents before and after transplant.[56,57] Autoimmune liver disease recurs in 24% of cases, as does primary sclerosing cholangitis. Although liver transplant is not contraindicated for these conditions, the rate of recurrence must form part of the counseling of families. Autoimmune hemolytic anemia in association with giant cell hepatitis is a rare and fatal disease in which there is a 100% recurrence rate post-transplant, and transplant is not recommended.[58]

PREPARATION FOR LIVER TRANSPLANT

Immunization

Most units consider live vaccines to be contraindicated after liver transplant because of the risk of dissemination secondary to immunosuppression. It is therefore better to complete normal immunizations before transplant. This includes diphtheria, tetanus, polio, Pneumovax for protection from streptococcal pneumonia, and *Haemophilus influenzae* type b vaccine for protection against *H. influenzae*. In children older than 6 months, measles, mumps, rubella, and varicella vaccinations should be offered in addition to hepatitis A and B vaccination.

Management of Hepatic Complications

It is important to ensure that specific hepatic complications are appropriately managed while the patient waits for transplant.

Recurrent variceal bleeding should be managed as described (see Chapter 59, "Treatment of End-Stage Liver Disease"), with sclerotherapy or esophageal varix ligation. Difficult or intractable variceal bleeding may require the insertion of a transjugular intrahepatic portal systemic shunt.[59]

Sepsis that includes ascending cholangitis and spontaneous bacterial peritonitis should be treated with broad-spectrum antibiotics, whereas in children awaiting transplant for acute liver failure, prophylactic antifungal therapy is essential.

Ascites should be managed with diuretics and restriction of salt. Intervention with hemodialysis and hemofiltration should be considered if acute renal failure or hepatorenal failure develops.[60]

Nutritional Support

The importance of nutritional support has been demonstrated with studies indicating that nutritional status at liver transplant is an important prognostic factor in survival.[61,62]

The main purpose of nutritional therapy is to prevent or reverse the malnutrition associated with liver disease and to minimize fat malabsorption and ongoing catabolism. A high-calorie protein feed (150 to 200% estimated average requirement) may be effective (Table 60-3). It is possible to provide this high-energy intake with standard feeds using calorie supplements, but a moderate liver feed that can be adapted more easily may be better for infants. Because many of the modular or supplemented feeds are unpalatable, they are best given by nocturnal nasogastric enteral feeding or continuous enteral feeding. Occasionally, enteral feeding is not tolerated owing to severe hepatic complications such as ascites and intractable variceal bleeding; in these circumstances, parenteral nutrition is necessary.[63]

Psychological Preparation

Liver transplant is a major undertaking for the child and family; thus, psychological counseling, information giving, and preparation of the child and family are paramount using a skilled multidisciplinary team with play therapists, psychologists, and schoolteachers. Particular care is required when counseling parents and children with inborn errors of metabolism who are not dying of liver failure. These families must be aware of the risks and complications of liver transplant and must make informed decisions with regard to potential mortality and to the necessity for long-term immunosuppression compared with medical treatment available for their children's conditions.

Parents of children who develop acute liver failure may be too stressed to fully appreciate the implications and consequences of liver transplant. In these families, counseling—in particular, counseling of the child—should continue postoperatively.

TABLE 60-3 NUTRITIONAL SUPPORT IN INFANTS AND CHILDREN UNDERGOING LIVER TRANSPLANT*

	PREOPERATIVE	POSTOPERATIVE
Carbohydrate (g/kg/d)	Glucose polymer 15–20	Glucose 6–8
Protein (g/kg/d)	Low-salt protein 3–4	Whole protein 2.5–3
Fat (g/kg/d)	40–60% MCT 8	80–90% LCT 5–6
Energy intake (EAR)	120–150%	120%

Adapted from Kelly DA and Mayer ADM.[64]

EAR = estimated average requirement; LCT = long-chain triglyceride; MCT = medium-chain triglyceride.

*Best provided as a modular feed in infants and as calorie supplements in older children.

LIVER TRANSPLANT SURGERY

The organization of liver transplant is complex and involves a large multidisciplinary team. The process involves four stages: organ procurement, the donor operation, the "back-table" operation, and the recipient operation.

Organ Procurement

Organ donation and procurement are handled regionally or nationally, depending on geographic variation, but all countries have a national network (United Networks for Organ Sharing [UNOS] in the United States, United Kingdom Transplant Support Service Authority [UKTSSA] in the United Kingdom, and Eurotransplant in Europe). Once patients are accepted by the transplant team, they are listed and prioritized according to the severity of liver disease. All countries recognize a priority system, which allows patients with acute fulminant hepatic failure the greatest prioritization. Many countries use a system that subsequently and sequentially prioritizes patients in intensive care, in hospitals, or at home. In the United States, a scoring system (PELD) is used to prioritize patients who do not have acute fulminant hepatic failure. The majority of liver grafts are retrieved from heart-beating donors, although there are increasing donations from living-related donors. The procurement coordinator is responsible for establishing the suitability of potential cadaveric organs, for coordinating the procurement team, and for the donor operation. Liver grafts are matched by size, blood group, and (for CMV-negative children) CMV status. There are no absolute age limits, but, in general, malignancy (except localized brain tumors), uncontrolled bacterial sepsis, and HIV positivity remain absolute contraindications for acceptance as donors.[64]

The development of reduction hepatectomy has extended the size range applicable to young children, but the donor shortage has led to replacement of reduction hepatectomies with split-liver grafts.

Donor Operation

It is usual to retrieve the liver from a cadaver donor as part of a multiorgan operation in which liver, kidneys, heart, lungs, small bowel, corneas, skin, and bone may also be removed. It is important to maintain appropriate hemodynamic stability and ventilation while paralyzing agents are given to prevent spinal reflexes and broad-spectrum antibiotics are used to prevent infection. The liver is evaluated to identify abnormal arterial anatomy, which is particularly important for split-liver grafting. The porta hepatis is dissected, and the common bile duct is divided. The common hepatic artery, the superior mesenteric vein, and the hepatic veins are identified. Once the cardiothoracic organs are mobilized, heparin is administered to achieve full anticoagulation, and the abdominal organs are perfused with ice-cold preservation solution and packed with ice slush to achieve rapid cooling. Liver dissection is completed once the cardiothoracic organs have been removed. The hepatic artery is resected with a patch of aorta at the origin of the celiac trunk. The portal vein is divided at the confluence with the superior mesenteric and splenic veins. The infrahepatic vena cava is divided above the origins of the renal veins, whereas the suprahepatic vena cava is divided at the junction of the right atrium. Once the liver is removed, the hepatic artery and portal vein are flushed with preservation solution, and the bile duct is rinsed. The liver is hermetically sealed in a plastic bag, immersed in preservation solution, and transported, packed in ice.

Back-Table Operation

The back-table operation is particularly important for reduction hepatectomies and split-liver grafting. It is usually performed at the recipient hospital at the same time as the hepatectomy of the recipient. If a whole-liver graft is being used, the back-table operation is straightforward, but in the majority of pediatric liver transplants, either a liver reduction or a split-liver graft is performed.

Some units recommend that liver reduction or splitting be performed in situ as part of the donor operation, which has the advantage that the surgery is performed in a well-perfused functioning liver, without the risk of warm ischemia. However, this increases the operating time at the donor hospital, which may compromise other donor organs. The principles of the liver reduction are based on the segmental anatomy of the liver.[65] The liver has eight segments, and although it is possible to use a single-segment liver graft for neonates, in practice, the liver is divided along the plane of the falciform ligament to produce a left lateral segmental graft (segments 2 and 3) drained by the left hepatic bile duct (Figure 60-2). The common bile duct, portal vein, and hepatic artery are all preserved with the left lateral segment. For a split-liver graft, the portal vein is preserved with the left graft, and the hepatic artery, common bile duct, and inferior vena cava are preserved with the right graft. At implant, an arterial anastomosis is required for the left split graft.[66–69] Cur-

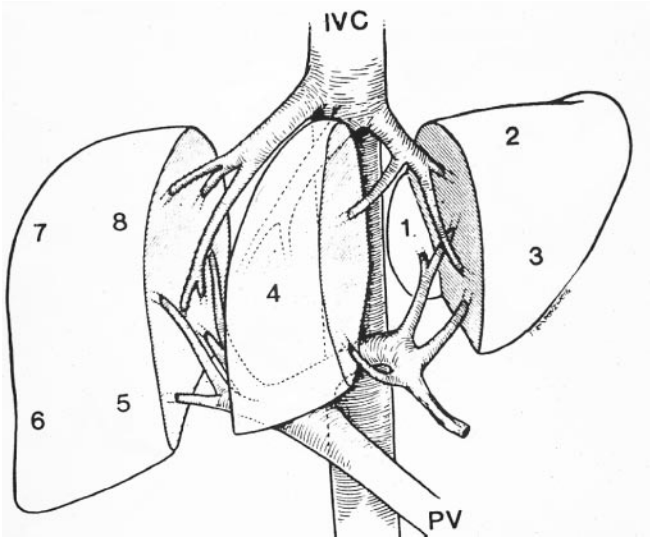


FIGURE 60-2 Schematic diagram of liver demonstrating eight segments. The left lateral segments 2 and 3 are most commonly used for reduction hepatectomy, split livers, living-related transplant, or auxiliary transplant.

rently, split-liver grafting is performed only using high-quality donor livers because of concerns related to primary graft dysfunction.

Recipient Operation

Management of the recipient operation has been much improved by an understanding of coagulation disorders, improved monitoring, and sophisticated hemostatic techniques that have reduced transfusion requirements and allowed better hemodynamic stability. Constant monitoring of electrolytes and blood gases and coagulation is essential. Thromboelastography, if available, is helpful to assess coagulation. Adequate supplementation of bicarbonate, electrolyte solutions, coagulation products, and platelets is essential.

In adults and older children, venovenous bypass is used, in which blood is diverted from the portal and intrahepatic cable circulation to the superior vena cava, reducing portal hypertension and intestinal congestion when the portal vein is clamped. This technique also improves venous return and renal perfusion when the vena cava is clamped during the hepatectomy phase.

In patients who have had no previous abdominal operations, the hepatectomy is straightforward. The liver is mobilized, with division of adhesions and ligamentus attachments. The porta hepatis is dissected, and the bile duct, hepatic artery, and portal vein are divided. In children with biliary atresia following a Kasai portoenterostomy, the hepatic dissection is more difficult, and there is a higher risk of gastrointestinal perforation.

It is important to carry out graft implant quickly to minimize warm ischemic injury that occurs as soon as the liver graft is removed from the cold preservation fluid. If a whole graft is being implanted, the suprahepatic vena cava, infrahepatic vena cava, and portal vein are anastomosed to the equivalent recipient vessels. If a left lateral segment graft is being performed, a triangular incision is made on the anterior aspect of the recipient vena cava to anastomose the graft left hepatic vein. A similar procedure is carried out for a split-liver graft. Once the venous anastomoses are complete, the liver is flushed with a warm rinsing solution, and the venous clamps are removed. The arterial anastomosis is carried out once the liver has been perfused from the portal vein. The donor celiac artery is anastomosed to the bifurcation of the recipient hepatic and gastroduodenal or splenic arteries. A donor arterial graft may be required to act as a conduit in children with small or aberrant vessels. The biliary anastomosis is the last to be performed. In adults and larger children without biliary pathology, the donor recipient ducts may be joined together to form a duct-to-duct anastomosis. Children with biliary atresia and those weighing less than 40 kg require a choledochojejunostomy using a Roux-en-Y loop of recipient jejunum.

Once the anastomosis is completed, the operative field is examined for hemostasis, and perfusion of the liver is established. If the liver graft is too large to permit abdominal closure without compromising hepatic venous outflow, then either a Silastic patch may be inserted or the abdomen may be left open temporarily until the liver has reduced in size.

Living-Related Liver Transplant

The shortage of suitable organ donors for young infants led to the development of living-related liver transplant.[70] This technique not only improves the supply of liver grafts for small children, it also allows optimal timing and reduces the stress of waiting for a suitable organ. In addition, the graft is obtained from a healthy individual with minimal preservation time.

The operation has received wide acceptance in Japan, where cadaveric transplant has not been possible until recently. The graft obtained from a live donor is usually a left lateral segmental graft. The liver parenchyma is divided along the line of the falciform ligament, keeping the blood supply intact. The bile duct, hepatic artery, portal vein, and hepatic vein are divided, and the graft is flushed with cold preservation fluid. Microvascular techniques are required during implant to reduce the risk of thrombosis.[71]

Although excellent results have been obtained,[72] particularly in Japan, the case for living-related transplant remains controversial because there are potential risks to the donor. Even in healthy individuals, partial hepatectomy has an appreciable morbidity, with an estimated risk of mortality of 1 in 250. It is important that donors are not only carefully evaluated for anesthetic risk, blood group compatibility, liver size, and anatomy but also that they are fully informed about the dangers of the procedure and, more importantly, the prospect of finding a cadaver graft for their child. Current organ donor shortages mean that most units will continue to offer living-related transplant.

Auxiliary Liver Transplant

In auxiliary liver transplant, part of the donor liver (usually segments 2 and 3) is implanted beside or in continuity with the native liver. The main purpose of this form of liver transplant is to ensure that the native liver is retained in the event of graft failure or for the future development of gene therapy. This operation is advisable for patients with metabolic liver disease secondary to hepatic enzyme deficiency in whom the liver is functioning normally but for whom liver transplant is considered because of the development of severe extrahepatic disease. Auxiliary transplant is now accepted therapy for Crigler-Najjar syndrome type I[42] and also for propionicacidemia and ornithine transcarbamalase deficiency.[73]

The role of auxiliary liver transplant in the management of fulminant hepatic failure is more controversial. The rationale for using this technique in this condition is that, with time, the native liver may regenerate. Two recent studies in adults demonstrated that the native liver regenerates in approximately half of the patients.[74,75]

POSTOPERATIVE MANAGEMENT AND COMPLICATIONS

Postoperative Management

Immediate postoperative management is based on ensuring hemodynamic stability, respiratory function, and fluid balance. Most patients remain in the intensive care unit for 24 or 48 hours until liver function is satisfactory, with good

hepatic artery and portal vein flow on Doppler ultrasonography. Infants with severe malnutrition owing to chronic liver disease or patients with hepatic coma secondary to fulminant hepatic failure may require a more prolonged period of intensive care.[6]

The aim of fluid management is to maintain circulating volume with crystalloid while replacing wound losses with colloid. It is important to ensure that the urine output is > 1 mL/kg/h and that central venous pressure is satisfactory (> 5–6 mm Hg). To prevent postoperative hepatic artery thrombosis, hemoglobin should be maintained between 8 and 10 g/L.[76] Immunosuppression is started immediately postoperatively, and the protocol will vary with center and experience. Standard protocols now include (1) cyclosporine microemulsion (Neoral), prednisolone, and azathioprine or (2) tacrolimus combined with low-dose steroids. Mycophenolate mofetil is a new immunosuppressive agent that may replace azathioprine in time (Table 60-4).[77,78] Recent studies that induce immunosuppression using the recently developed interleukin-2 antibodies, which selectively block the interleukin-2 receptors on T cells, reduce nephrotoxicity but are not yet in established practice.[79] Sirolimus, which is a macrolide antibiotic that prevents T-cell proliferation, is also sparing to the kidneys because it does not inhibit calcineurim. Its use in children is anecdotal.[80]

Broad-spectrum antibiotics should be prescribed for 48 hours unless there is persistent infection. Fluconazole or liposomal amphotericin is advisable in children with acute liver failure or in those who have a second laparotomy. Low-dose cotrimoxazole or trimethoprim is used for prophylaxis against *Pneumocystis carinii*, and oral nystatin and amphotericin are used to prevent oral and esophageal candidiasis for 6 to 12 months.

Most units use prophylaxis for CMV infection for CMV-negative recipients of a CMV-positive donor organ. Acyclovir (1,500 mg/m^2 IV) or ganciclovir (10 mg/kg IV/d) usually prevents CMV infection in the short term.[81] Although there is no proven prophylaxis for EBV, many units use either acyclovir or ganciclovir for this purpose.

Other medications include ranitidine (3 mg/kg), sucralfate (2–3 g qds), or omeprazole (10–20 mg IV bid) as prophylaxis against stress ulceration.

TABLE 60-4 POSTOPERATIVE IMMUNOSUPPRESSION

	TROUGH LEVELS*	
TIME OF INTRODUCTION (MO)	CYCLOSPORIN A (MICROEMULSION) (5 MG/KG BD)	TACROLIMUS (0.15 MG/KG)
0–1	180–230 ng/L	10–15 ng/mL
1–3	100–160 ng/L	8–12 ng/mL
3–12	70–110 ng/L	5–8 ng/mL
>12	60–90 ng/L	3–5 ng/mL

TIME OF INTRODUCTION	IMMUNOSUPPRESSANT
3–12	Prednisolone 2 mg/kg
12	Azathioprine 2 mg/kg
—	Mycophenolate mofetil 1–3 g/d

*Whole-blood monoclonal assay.

Because vascular thrombosis is higher in children than in adults, prophylaxis with antiplatelet drugs such as aspirin (3 mg/kg/d) and dipyridamole (25–50 mg tds) may be useful. Antihypertensive medication is usually necessary secondary to immunosuppressive treatment with steroids, tacrolimus, or cyclosporin A. Nifedipine (5–10 mg, 4 to 6 hourly) or atenolol (25–50 mg/d) may be required.[64]

Some children will require parenteral nutrition perioperatively, but the majority will begin enteral feeds between days 3 and 5. It is important to maintain adequate calories (see Table 60-3) and to encourage normal feeding.

Postoperative Complications

Early postoperative complications include primary graft nonfunction, surgical complications (eg, intra-abdominal hemorrhage), vascular thrombosis, and venous outflow obstruction.

The most common cause of primary graft failure is primary nonfunctioning of the graft or thrombosis of the hepatic artery or portal vein.[82] Primary nonfunctioning of the transplanted liver occurs within 48 hours. The cause is unknown and may be related to donor factors. The presentation is with prolonged coagulation (INR > 3), raised aminotransferases (> 5,000–10,000 IU/L), a rising bilirubin, and, ultimately, a rise in serum potassium (> 6 IU/L). Primary graft function may occasionally be secondary to hyperacute rejection, the diagnosis of which can be made only by liver biopsy. The only appropriate management is retransplant.

Hepatic artery thrombosis occurs in approximately 10% of pediatric liver grafts, and its frequency has decreased considerably following the introduction of reduction hepatectomy or living-related transplant because of the increased size of the donor vessel.[83] The development of microsurgical techniques for hepatic arterial reconstruction has been additionally beneficial (Figure 60-3).[84]

Portal vein thrombosis is a less common complication, but its incidence has not been altered with reduction or split-

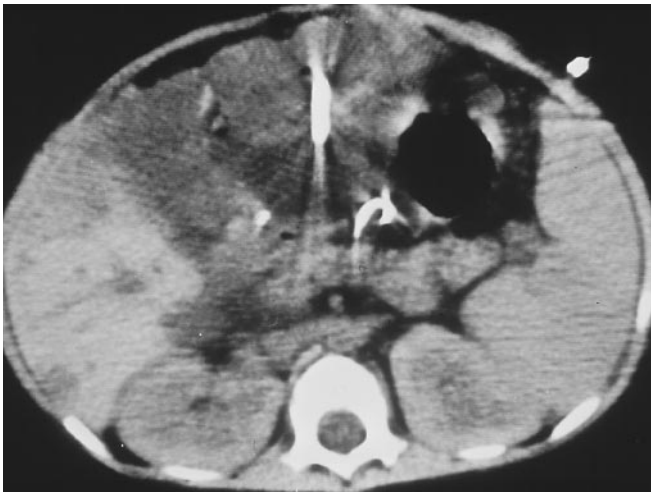


FIGURE 60-3 Hepatic artery thrombosis is a potentially life-threatening complication following liver transplant. This computed tomographic scan demonstrates a large area of infarction with abscess formation following thrombosis of the hepatic artery 7 days post-transplant. The child was successfully retransplanted.

liver techniques, although there are some advances in the management of this complication with innovative surgery.[85]

The diagnosis of hepatic artery or portal vein thrombosis is made by Doppler ultrasonography and is confirmed by angiography. Both complications may be treated by emergency thrombectomy and the use of anticoagulants or infusion of thrombolytic agents such as streptokinase. If hepatic perfusion is not adequately re-established, retransplant is required. Hepatic artery ischemia ultimately results in biliary complications, such as leaks and strictures, or hepatic abscesses.[86]

Hemorrhage from the cut surface of the liver is an occasional complication. It should be managed conservatively unless there is persistent bleeding or hemodynamic instability. Abdominal tamponade from hemorrhage may decrease blood flow and lead to renal failure.

Many factors may predispose the patient to postoperative renal failure within the first week. Patients with impaired renal function preoperatively may be further compromised by intraoperative cardiovascular instability or inotropic support. In addition, the administration of nephrotoxic immunosuppression such as cyclosporine or tacrolimus may precipitate renal failure.[64]

Oliguria (< 1 mL/kg) is common and may be managed conservatively with fluid replacement or a furosemide challenge (1–2 mg/kg IV). The development of anuria with an increase in urea, creatinine, and potassium necessitates renal dialysis or hemofiltration.

Rejection

Acute cellular rejection may occur between 7 and 10 days postoperatively. The incidence of acute rejection varies. It is less common in infants (20%) but increases to 50 to 60% in older children and adults.[87,88] Clinical features include fever, irritability, abdominal discomfort, and, occasionally, ascites. The diagnosis is confirmed by detecting a rise in bilirubin, alkaline phosphate, γ-glutamyl transpeptidase, and aspartate and alanine transaminases. Histologic confirmation is essential. Acute rejection is indicated by demonstration of a mixed inflammatory infiltrate, including eosinophils in portal tracts. There is usually a subendothelial lymphoid infiltration of blood vessels (endotheliitis) and inflammation and infiltration of the bile ducts.[89] Most units will treat acute rejection initially by using intravenous methylprednisolone in doses varying from 20 mg/kg/d for 3 days to 45 mg/kg in total, in association with an increase in baseline immunosuppression. If there is insufficient histologic or biochemical response, treatment with methylprednisolone may be repeated, but if the rejection is unresponsive to steroids, then it is usual to convert to a more potent immunosuppressive drug such as tacrolimus or to add other agents, such as muromonab-CD3, mycophenolate mofetil, or sirolimus.[90]

Cyclosporin A (Sandimmune) has been replaced by the partially water-soluble cyclosporine microemulsion Neoral. Several studies in both adult and pediatric patients post-transplant have indicated that Neoral is very well absorbed, with a peak absorption at 2 hours and a half-life of approximately 8 to 12 hours[77,91–93] in patients immediately post-transplant and in stable patients in the long term. The incidence of side effects with Neoral is similar to the range of side effects with Sandimmune, namely, gingival hyperplasia and hirsutism, whereas the incidence of hypertension and nephrotoxicity is less. It is possible that monitoring C2 levels (2 hours after dosing) may be more effective than trough level monitoring for preventing rejection and reducing side effects.[94]

Experience with tacrolimus (FK506) is increasing, and long-term prospective therapeutic studies comparing tacrolimus and Neoral are in progress. Preliminary results with tacrolimus indicate that it is an extremely effective immunosuppressive drug in the prevention of acute rejection.[78,90] It does not cause hirsutism or gingival hyperplasia, but there has been an increase in serious neurologic side effects, lymphoproliferative disease, and hypertrophic cardiomyopathy in children taking high doses of tacrolimus.[95]

Many units consider prednisolone withdrawal at postoperative intervals ranging from 3 to 12 months, which has proved easier to accomplish using tacrolimus therapy.[96]

Chronic rejection occurs in less than 10% of children after transplant.[88] Clinical features include gradual onset of jaundice, pruritus, and pale stools, indicating biliary obstruction. The diagnosis may be confirmed by detecting biochemical changes that include a relative increase in bilirubin, alkaline phosphatase, and γ-glutamyl transpeptidase compared with aminotransferases. Liver histology demonstrates extensive damage and loss to bile ducts (vanishing bile duct syndrome with arterial obliteration and fibrosis). There may be a response to an increase in immunosuppression (eg, a change to tacrolimus[97] or the addition of mycophenolate mofetil). Nonresponse to medical management requires retransplant.[98]

Biliary Complications

The range of biliary complications after transplant includes biliary leaks and strictures, which have increased with the use of reduction hepatectomies.[86] Biliary strictures may develop secondary to an anastomotic stricture related to edema of the bile ducts or hepatic artery ischemia. Biliary leaks may be secondary to leakage from the cut surface of the reduction hepatectomy or from hepatic artery ischemia. The majority of biliary leaks settle with conservative management, but large leaks that cause biliary peritonitis, biliary abscesses, or sepsis should have surgical drainage and reconstruction. The management of biliary strictures should initially be conservative, with ursodeoxycholic acid (20 mg/kg) used to allow edema to settle. Persistent strictures leading to biliary dilatation should initially be managed radiologically, using percutaneous transhepatic cholangiography. The dilated biliary tree is cannulated, and external biliary drainage is established. Once sepsis and edema of the biliary tree have reduced, biliary dilatation may be performed using balloons and biliary stents. Surgical reconstruction is required for anastomotic or recurrent biliary strictures if interventional radiology is unsuccessful.

Other Complications and Sepsis

Persistent drain losses may be due to preoperative ascites or secondary to rejection, sepsis, hepatic obstruction, or bacterial peritonitis. This troublesome complication leads to acidosis and coagulopathy owing to loss of bicarbonate and coagulation factors in the ascitic fluid. It is best to treat the primary cause if possible and to manage the condition conservatively with fluid restriction and diuretics.

Infection is still the most common complication following liver transplant.[6,99] Bacterial infections are most common immediately after transplant and are related to the high doses of immunosuppressive drugs and central line infections. The main bacteria identified are *Streptococcus faecalis* and *Streptococcus viridans*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Postoperative fungal infections are more likely in patients undergoing transplant for acute liver failure or in children undergoing laparotomies for technical complications post-transplant.[100] The most common fungal infection is with *Candida albicans*, but aspergillosis may occur in 20% of patients with fungal infections.

More recently, vancomycin-resistant enterococcus has become a significant pathogen following both liver and small bowel/liver transplant.[101] Risk factors for developing vancomycin-resistant enterococcus are recurrent central line infections treated with vancomycin therapy, but it is important to differentiate between patients who are colonized and those who have systemic infection. The mortality of patients with systemic infection is high but improved with new agents such as quinupristin and linezolide.

Late Complications Post-transplant

Late complications may occur at any time after transplant. They include CMV or EBV infection, side effects of immunosuppression, post-transplant lymphoproliferative disease (PTLD), late biliary strictures, and hepatic artery or portal vein thrombosis. Chronic rejection may occur at any time, particularly as a result of nonadherence.

Infection with CMV occurs between 5 and 6 weeks following liver transplant. The risk of CMV disease is highest in CMV-negative recipients who receive an organ from a CMV-positive donor.[81,102,103] Although prophylaxis with intravenous ganciclovir (5 mg/kg/d) and intravenous immunoglobulins is more effective than prophylaxis with ganciclovir alone, approximately 20% of patients will develop primary infection. Treatment with intravenous ganciclovir and intravenous immunoglobulin is usually effective.[104]

The development of primary infection with EBV is a significant problem in pediatric transplant. Because two-thirds of children undergoing liver transplant are likely to be EBV negative before transplant and 75% of this group will develop primary infection after transplant,[79] it is essential to diagnose primary EBV infection to reduce immunosuppression and thus prevent the development of lymphoproliferative disease.[105]

There is a well-known association between the development of primary EBV infection and the subsequent development of post-transplant lymphoproliferative disease.[105] EBV stimulates lymphocyte proliferation, which ranges from benign hyperplasia to malignant lymphoma. The clinical features are varied and include symptoms of infections, mononucleosis (tonsillitis and lymphadenopathy), isolated lymph node involvement, and EBV infiltration in the liver, gut, and iris, ranging from isolated organ involvement to malignant lymphoma.[106]

Considerable efforts have been devoted to the early diagnosis of EBV infection and PTLD. It is now possible to measure EBV polymerase chain reaction (PCR) prospectively and to reduce immunosuppression when high levels are achieved.[106–108] Serologic confirmation of EBV infection (EBV IgM antibodies) is usually a late feature, and it may be more appropriate to measure EBV PCR sequentially. Patients who develop gut PTLD may present with diarrhea, weight loss, and gastrointestinal bleeding. The diagnosis should be confirmed histologically by biopsy of the appropriate tissue (liver, gut).[107] Characteristic histology includes polymorphic B-cell proliferation or lymphomatous features of nuclear atypia necrosis. Immunofluorescent staining of heavy- and light-chain immunoglobulins may differentiate monoclonal from polyclonal infiltrates, which has significant prognostic implications. Confirmation of EBV involvement may be obtained by using in situ hybridization techniques to demonstrate EBV-encoded small nuclear ribonucleic acid.[108] It was initially felt that the incidence of PTLD was higher with tacrolimus than with cyclosporine, but this may be due to use of inappropriately high levels of tacrolimus when the drug was initially released.[109] First-line treatment for PTLD is reduction of immunosuppression. Acyclovir (1,500 mg/m^2/d) or ganciclovir (5 mg/kg/d) may also be prescribed, but there is no clear evidence that either is effective. Use of rituximab, a monoclonal antibody, and human leukocyte antigen–matched T-cell therapy directed against EBV are under investigation and may prove effective.[110,111] As reduction of immunosuppression leads inevitably to graft rejection, balancing treatment for PTLD and rejection may be difficult. Under these circumstances (or if lymphoproliferative disease becomes overtly malignant), chemotherapy is required.[112]

Late biliary strictures may be due to hepatic artery ischemia or thrombosis and may lead to recurrent cholangitis, hepatic abscess, and the development of secondary biliary cirrhosis. Although they may be treated radiologically, as described above, retransplant may be required for the development of biliary cirrhosis.[113]

Portal vein stenosis owing to anastomotic stricture may lead to portal hypertension with varices and splenomegaly. Initial treatment is radiologic by venoplasty, but surgical reconstruction with an intrahepatic mesoportal shunt may be required.[85,114]

Gastrointestinal perforation is an infrequent complication after liver transplant. It is related to previous abdominal surgery and to malnutrition.[114,115] Some units take this potential complication so seriously that an elective laparotomy is planned at 7 and 14 days post-transplant.[116]

SURVIVAL

Current results from international centers indicate that 1-year survival following liver transplant may be in excess

of 90%.[3–6] Long-term survival (5–10 years) ranges from 60 to 80%.[3,117] Preliminary results suggest that patients who undergo elective living-related transplant have a higher 1-year survival rate (94%) than those of equivalent hepatic status receiving cadaveric grafts (78%).[118]

Many different factors influence survival. Initially, age at transplant was considered a significant risk factor, and transplant was contraindicated in infants aged under 1 year and weighing less than 10 kg.[119] Reduction hepatectomy and living-related transplant have not only reduced the waiting-list mortality in this group of children[120] and extended liver transplant to this young age group,[6] they also have demonstrated that equivalent survival may be achieved in infants transplanted under the age of 1 year compared with that of older children (Figure 60-4).[121]

It has previously been demonstrated that protein malnutrition at the time of liver transplant has a significant influence on both morbidity and mortality.[61,62] The degree of malnutrition, in addition to the severity of liver disease, has been demonstrated to have a significant effect on short-term survival,[29,122,123] and a number of studies have demonstrated improved survival for children with transplants and metabolic liver disease compared with those with chronic liver disease or fulminant hepatitis (see Figure 60-4).

In general, outcome is not related to diagnosis, although children with fulminant hepatic failure are less likely to survive the initial transplant.[113] An important aspect contributing to improved survival has been the increase in surgical and medical experience,[124] particularly with the development of innovative surgery.

There has also been an improvement in the rate of retransplant for technical problems or graft failure secondary to chronic rejection.[113,125] It is clear that although the rate of retransplant has fallen with increased surgical experience, survival following retransplant is considerably less. Children receiving more than one graft have a 50% 1-year survival compared with 90% in children receiving only one graft. This may be related to the factors contributing to the necessity for retransplant, such as primary graft nonfunction, technical problems, and the development of multiorgan failure.

In some instances, survival may be affected by the recurrence of the original disease. Recurrence of hepatitis B virus (HBV) infection is 100% in patients who were positive for HBV deoxyribonucleic acid (DNA) or hepatitis B e antigen at the time of their initial operation.[126] Recurrent HBV disease is associated with chronic hepatitis or cirrhosis (79%), submassive necrosis (9%), or fibrosing cholestatic hepatitis (25%).[56,127] The recurrence rate of chronic hepatitis B post-transplant has been much reduced by therapy with the nucleoside analog lamivudine.[128]

Chronic hepatitis C is a rare indication for transplant in children, but recurrence is inevitable in those children who were infected preoperatively before screening for hepatitis C virus became available[129,130] and in those who were infected perioperatively.[131–133] The outcome for these children is varied, with the majority developing nonspecific hepatitis. However, a minority develop rapidly progressive liver failure. Treatment for hepatitis C infection has improved with the combination of interferon and ribavirin, which achieves a 45% sustained remission rate overall.[134]

It is now clear that there is recurrence of autoimmune hepatitis both immunologically and histologically post-transplant in 25% of cases. It may be more severe than the original disease,[135] and it is important to ensure that immunosuppression with steroids is continued in this group of patients. A variant of autoimmune liver disease, giant cell hepatitis with autoimmune hemolytic anemia, has also been demonstrated to recur post-transplant.[58] Recurrence of malignant hepatic tumors in children transplanted for hepatoblastoma or hepatocellular carcinoma is directly related to the presence of extrahepatic metastases at the time of surgery.[48,53]

DE NOVO AUTOIMMUNE HEPATITIS

Several studies have documented the development of autoantibodies (antinuclear antibodies, smooth muscle antibodies, and, rarely, liver-kidney antibodies) post-transplant in both children and adults in recipients without autoimmune disease pretransplant,[136,137] which is associated with a graft hepatitis and progressive fibrosis. The hepatitis resolves with steroid therapy or azathioprine.[138,139]

LONG-TERM RENAL FUNCTION

The calcineurin inhibitors cyclosporine and tacrolimus both cause nephrotoxicity, and 4 to 5% of patients develop severe

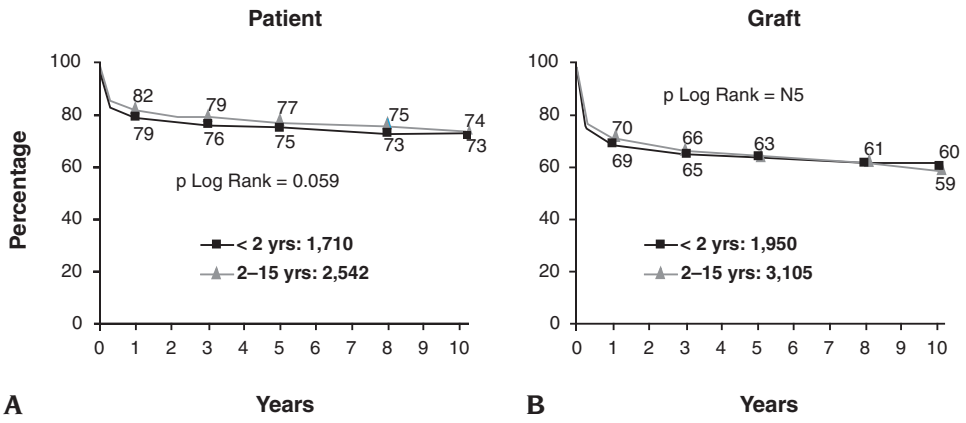


FIGURE 60-4 Patient (*A*) and graft (*B*). Survival of children following liver transplant less than and greater than 2 years at transplant, 1988 to 2001. Mean follow-up was 10 years. The survival of children older than 2 years (n = 2,542) for all indications was similar to those children under 2 years (n = 1,710) (*A*). There was no difference in graft survival (*B*). Adapted from the European Liver Transplant Registry, 2002.

chronic renal failure long term, requiring renal transplant. The use of low-dose calcineurin inhibitors or renal-sparing drugs such as mycophenolate mofetil or sirolimus for maintenance immunosuppression prevents significant renal dysfunction.[139,140] Acute postoperative hypertension is seen in 65% of children but persists long term only in 28%.[141]

HYPERLIPIDEMIA

As children survive longer, it is important to remember that both cyclosporine and sirolimus increase serum lipids, particularly cholesterol, and high levels may require transfer to tacrolimus or mycophenolate mofetil.[90]

TRANSPLANT TOLERANCE

It is now thought that 20% of adults will develop tolerance to the graft and can be withdrawn from immunosuppression.[142] Complete withdrawal of immunosuppression in children has not been documented.

QUALITY OF LIFE AFTER TRANSPLANT

It is now anticipated that children who survive liver transplant will achieve a normal lifestyle despite the necessity for continuous monitoring of immunosuppressive treatment. Children transplanted for certain metabolic liver diseases such as α_1-antitrypsin deficiency, Wilson disease, and tyrosinemia type I may have both phenotypic and functional recovery. Children with Crigler-Najjar syndrome type I have functional recovery of enzyme activity following either orthotopic or auxiliary liver transplant, whereas children with organicacidemias will have only palliation of their defect because the enzyme defect is not restricted to the liver (propionicacidemia or methylmalonicacidemia).[143] Children transplanted owing to urea cycle defects and organicacidemias may require protein restriction particularly during acute illness, reflecting the partial cure of their disease.

An important aspect in achieving normal quality of life is nutritional rehabilitation after transplant. Although early studies evaluating growth in children after liver transplant indicated that almost two-thirds did not achieve their growth potential,[144] recent studies have demonstrated that with appropriate nutritional support, 80% of survivors will achieve normal growth patterns and body habitus.[6,145] Nutritional rehabilitation begins in the first year, with a return to normal muscle and fat stores within 6 to 12 months after transplant.[6,145] Initially, weight gain may be excessive owing to the effects of steroids, increased appetite, and salt and water retention, but most children regain normal weight within 12 months, and the weight is maintained for the long term.

Linear growth may be delayed between 6 and 24 months, which is directly related to steroid dosage and preoperative stunting.[146] Children who were particularly growth retarded or stunted before transplant (height standard deviation score < –1) initially have rapid catch-up growth but may not achieve their genetic potential,[147,148] whereas children who were less stunted (height standard deviation score > –1) have slower catch-up growth but may eventually achieve normal height.

The growth-suppressive effects of corticosteroid therapy after liver transplant have been clearly demonstrated. Centers that discontinue steroids post-transplant or institute alternate-day steroids early have reported increased catch-up growth at an earlier stage.[149]

Future growth may depend on the etiology of the pretransplant disease. For instance, 50% of children who were transplanted for the hepatic complications of Alagille syndrome and who were growth retarded before transplant did not achieve normal height.[150]

Additional factors in the etiology of post-transplant growth failure may be behavioral feeding problems and the difficulties in establishing normal feeding. Before transplant, many children will have been fed unpalatable feeds, often by nasogastric tube, and may have missed their normal developmental milestones for chewing, swallowing, and feeding. A significant proportion of these patients will have difficulty establishing normal feeding regimens post-transplant and will require nocturnal enteral feeding for 1 to 2 years.[146]

An important aspect of long-term survival is the development of puberty. A long-term study from France has demonstrated that there are no differences between the genders in attaining puberty and developing secondary sexual characteristics.[148] Girls develop menarche, and successful pregnancies have been reported for females receiving both cyclosporine and tacrolimus immunosuppression.[151]

Side Effects of Immunosuppression

The side effects of immunosuppression are well known (Table 60-5). Hypertension is common with steroids, cyclosporine, and tacrolimus but tends to be short term and related to the intensity of immunosuppression. Growth failure and stunting related to steroids may have a significant effect on the ability to regain normal height. Hirsutism and gingival hyperplasia are recognized side effects of cyclosporine that are dose related and, although

TABLE 60-5 IMMUNOSUPPRESSIVE COMPLICATIONS POST-TRANSPLANT

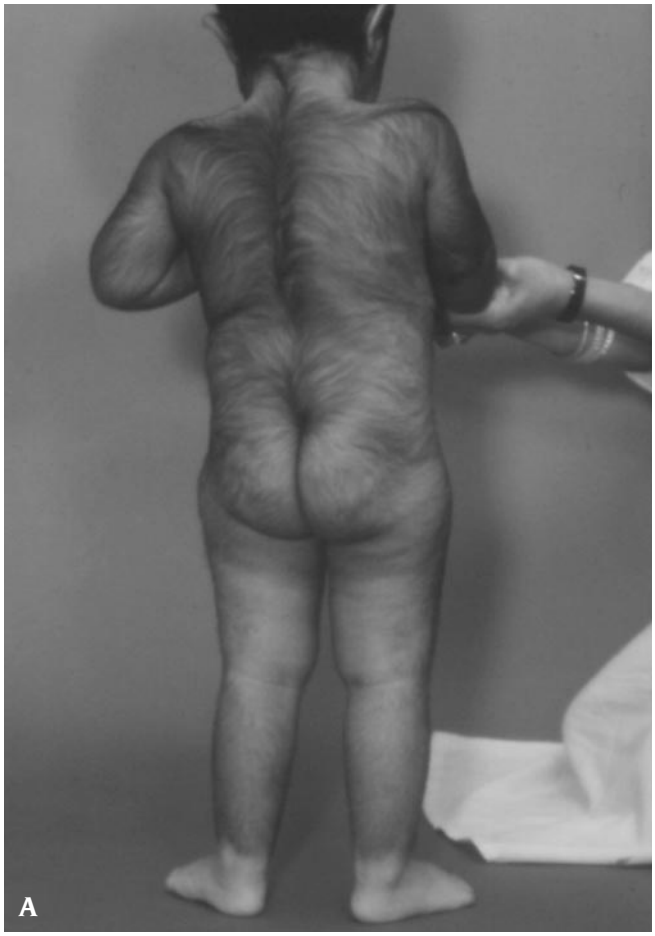
Steroids
Stunting
Hypertension
Cushingoid facies
Salt and water retention
Weight gain
Cyclosporin A
Hirsuitism
Gingival hyperplasia
Cyclosporin A/tacrolimus
Nephrotoxicity
Hypertension
Neurotoxicity
? Lymphoproliferative disease
? Skin cancers
Tacrolimus
Hyperglycemia
? Cardiomyopathy

cosmetic, have an important effect on quality of life, particularly in adolescence (Figure 60-5). There remains a long-term risk of PTLD, skin cancer, and other tumors.

Psychosocial Development

It is of particular concern that neurodevelopmental outcomes following liver transplant should be normal. Previous studies have demonstrated that there is an initial deterioration in psychosocial development in the first year after transplant, as noted by a deterioration in social skills, language development, and eye/hand coordination,[152] presumably related to the stress of the operation, the high doses of immunosuppression, and prolonged hospitalization. It is now known that most children will achieve normal psychosocial development within 1 to 2 years but that the rate of improvement is related to the age at the onset of liver disease and the age at the time of transplant.[153] Risk factors for persistent developmental delay include malnutrition at the time of transplant, length of hospital stay, and age at transplant, with younger children at particular risk of developmental delay.[31] In a longer-term study of a small group of children, 80% achieved normal height and weight measurements over a 5-year period, and all of the children attended school, but 30% had special educational needs. Psychological testing indicated that 75% of this group were functioning normally.[153–155]

The stress of liver transplant on family structure and dynamics is well known, although most parents indicate improved psychological symptoms following successful liver transplant. A longer-term study indicated that 20% of marriages dissolved and that 30% of families were considered to be functioning outside the normal range.[156]



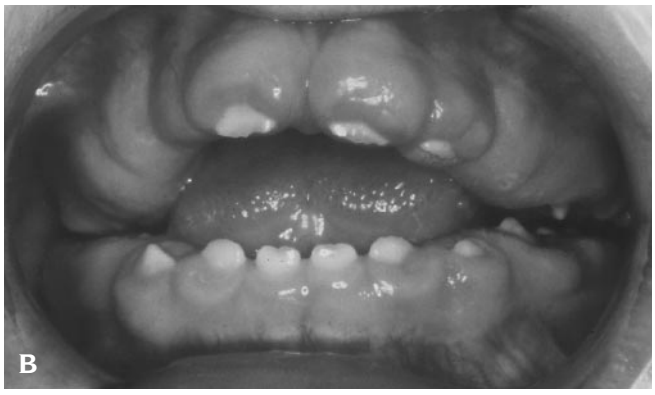


FIGURE 60-5 Quality of life of children following liver transplant is an important aspect and may be affected by cosmetic side effects such as hirsutism (*A*) or gingival hyperplasia (*B*) secondary to cyclosporine.

NONCOMPLIANCE WITH THERAPY

Noncompliance with immunosuppressive therapy is less common in liver transplant recipients than in renal transplant recipients,[157,158] which may be related to the median age at renal transplant (14.3 years) compared with 2.5 years for liver transplant. It is possible that children who were grafted at a young age are more likely to accept medication through their adolescence, or there may be insufficient long-term data as yet.

SUMMARY

Liver transplant for acute or chronic liver failure or for metabolic liver disease is an effective therapy that restores good quality of life to over 80% of recipients. Considerable advances in medical and surgical expertise and immunosuppression have improved not only survival but also the quality of life for the majority of liver transplant recipients. It is essential to encourage both child and family to return to a normal life as far as possible, although continued counseling and support by a multidisciplinary team are essential. The long-term outlook for children receiving liver transplant in the twenty-first century is likely to be limited by organ donor shortages, the side effects of immunosuppressive drugs, and the potential development of post-transplant lymphoproliferative disease or other tumors. It is hoped that advances in molecular genetics will lead to effective gene therapy or hepatocyte transplant and reduce the need for solid organ transplant.[159,160]

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CHAPTER 61

GALLBLADDER DISEASE

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The role and importance of the gallbladder are summarized wittily in the following short poem:

> Chemo Memo
>
> Man's liver is a brownish blob
> That does a most prodigious job.
> It manufactures gall, or bile
> And normally keeps some on file
> Stored neatly in a pear shaped sac.
> From there the liver's yields attack
> The food man eats, to change its state
> By methods man can't duplicate,
> Or even halfway understand.
> He ought to treat this outsize gland,
> With due respect and loving care
> To keep it in top-notch repair,
> Because to get along at all
> Man needs an awful lot of gall[1]

The essential functions of bile or "gall" manufactured by the liver and stored in the gallbladder are to facilitate absorption of fats and fat-soluble vitamins from the diet and to secrete heavy metals such as copper from the body. Gallstones were recognized in ancient Egypt, but it was many centuries before the functions of the gallbladder and the relationship of gallstones to disease were understood. Up until the nineteenth century, it was (mistakenly) believed that the removal of the gallbladder was incompatible with life. The first cholecystectomy was not performed until 1882 in Berlin, and it was a great success.[2]

The gallbladder is a vesicular structure, usually found under the right lobe of the liver between the porta hepatis and inferior tip of the liver lying in the gallbladder fossa. It is covered by the same peritoneal covering as the liver.[3] The liver, bile ducts, and gallbladder arise from the primitive foregut. The cranial portion develops into the hepatic parenchyma and the intrahepatic ducts, and the more caudal section evolves into the gallbladder and extrahepatic bile ducts.[4] This chapter reviews the epidemiology, genetics, pathogenesis, diagnosis, and treatment of common gallbladder diseases with particular reference to children. Gallstone disease is the most expensive gastrointestinal disease in adults in the United States today[5]; therefore, most research has concentrated on adults. The literature and clinical practice pertinent to children, extrapolating from adult studies where necessary, are reviewed.

PHYSICAL CHEMISTRY COMPOSITION OF BILE

Bile is an aqueous solution composed of bile salts, cholesterol, phospholipid, water, electrolytes, and heavy metals, as well as proteins, immunoglobulin A, vitamins, and toxins.[6] The functions of bile are myriad and in part are dependent on the function of individual components. Bile salts in the intestine act as detergents, which solubilize and aid the absorption of dietary lipids and lipid-soluble vitamins. Bile is the major route of elimination of a large number of compounds, including cholesterol, bilirubin conjugates, drugs, and heavy metals, especially copper. Cholesterol elimination from the body occurs mainly in bile either as cholesterol or as bile salts, which are products of cholesterol metabolism. Bile also contains secretory immunoglobulin A, which may play a role in prevention of infection. Bile flow resulting from the active transport of bile acids into the canalicular lumen is termed bile acid dependent, and bile flow due to the transport of glutathione and bicarbonate is bile acid independent.[7] The bicarbonate and electrolyte concentrations of human bile differ little from serum.[6,8]

A constellation of transporters, which are found on hepatocytes and cholangiocytes, modulates bile composition. Transporters allow the uptake into hepatocytes of molecules such as cholesterol and bile salts and their subsequent export into the lumen of canaliculi. These transporters are discussed in the section dealing with gallstone formation.

EPIDEMIOLOGY

In adults living in the United States, gallbladder disease is one of the most expensive digestive diseases, and annual costs of treatment were estimated to total more than $6 billion dollars in 2001.[5] Treatment of children accounts for only a fraction of this budget because gallbladder disease is uncommon in childhood. Ascertaining the true prevalence of gallbladder disease in either adults or children is difficult because most patients with gallstones are asymptomatic. Studies of hospitalizations, surgical records, and autopsy series underestimate the prevalence of gallstone disease. The introduction of ultrasonography allowed non-

invasive screening for gallstone disease and therefore a more accurate estimate of prevalence. A study of gallstone prevalence in 1,570 children between the ages of 6 and 19 years in Bari, Italy, found ultrasonographic evidence of gallstones in just two asymptomatic girls, aged 13 and 18 years—a prevalence rate of 0.13%.[9] One of the two girls had a family history of gallstone disease, but the other had no recognized risk factors. In contrast, approximately 20.5 million adults in the United States are estimated to have gallstones based on extrapolation of the findings of 14,238 ultrasound examinations performed between 1988 and 1994 as part of the Third National Health and Nutrition Examination Survey (NHANES III) study.[10]

Within the United States, there are striking differences in cholesterol gallstone prevalence between ethnic groups. Native Americans have the highest rate of all groups in the United States. For example, the Pima Indians, a tribe in Arizona, were found to have a gallstone prevalence rate of 48.6%.[11] This rate was established in Pima Indians using an age- and sex-stratified random sample of 600 members of the tribe, by review of medical records, and by performing oral cholecystography in those without a history of cholecystectomy or a previous abnormal cholecystogram. In those aged 15 to 24 years, the prevalence was 5.9% in females and 0% in males. However, there was a dramatic increase in prevalence in females aged 25 to 34 years to 73.2%. In males aged 25 to 34 years, the increase was only to 4.4%. Prevalence rates in men did not approximate those of women until they were older than 55 years.[11] To investigate the reasons for this dramatic increase in gallstone prevalence in Pima Indians with age, bile composition and bile acid pool size of a group of 66 children and adolescents aged 9 to 21 years were studied. Bile cholesterol saturation increased during puberty in both genders but was 15% higher in females than in males. In males but not females, the bile acid pool increased. Hence, the metastable bile found in prepubertal children is altered by the increasing cholesterol concentration during puberty, and cholesterol precipitation and gallstone formation can occur.[12]

There are other medical conditions in children that are associated with a recognized increase in the prevalence of gallstone disease. These diseases include cystic fibrosis and hemolytic diseases, which increase the risk of pigment gallstones and are discussed in more detail later in this chapter.

As noted, the proportions of bile constituents change at puberty in both children and adolescent Pima Indians and also in white children.[13,14] Before puberty in the Pima Indians, bile cholesterol saturation (expressed as a percentage) is saturated but metastable in both girls, 116 ± 7%, and boys, 99 ± 7%. After puberty, there is a significant increase in this index, an increase that is higher in females, 156 ± 16%, than in males, 124 ± 2%. Lithogenic bile was present in 71% of females older than 19 years but only 13% of females younger than 13 years.[13] Two series have shown lower cholesterol-to-bile salt excretion ratios in white children than in adults.[15,16] Mean biliary cholesterol saturation index in 10 healthy American children with an average age of 2.3 ± 0.6 years was 0.72 ± 0.04 compared with 1.08 ± 0.09 in healthy adults[15] and 0.57 ± 0.12 in 11 Swiss children.[16] It is important to note that in these studies of bile composition in children, bile-rich duodenal aspirates were used.

In another study, gallbladder bile composition was investigated in 18 children from whom gallbladder bile was aspirated at the time of laparotomy for nonhepatobiliary disease. Significant differences were noted between infants and children in this study. Infants displayed more dilute bile than children, but bile was more saturated with cholesterol. The cholesterol saturation index in all infants was greater than 1, whereas in almost all children, it was less than 1. Infants also had a significantly shorter nucleation time, 11.6 days, compared with children, who had a nucleation time of 28.6 days, which is similar to that of healthy adults. Total biliary lipid of infants, 3.3 ± 3.8 g/dL (mean ± SD), is significantly lower than in children, in whom the total content is 9.1 ± 3.0 g/dL. Interestingly, in all bile samples, even those supersaturated with cholesterol, cholesterol was not present in the vesicular phase.[17] It is not clear why infant gallbladder bile is so different, but potential factors are a milk-only diet and the poor concentrating ability of the gallbladder.

FORMATION AND CONTENT OF GALLSTONES

For gallstones of any type to develop, some or all of the following need to occur: alterations in the proportion of bile constituents, nucleation, changes in gallbladder motility, or infection. Infection appears to be important only for brown pigment gallstone formation. The development of each of the three major gallstone types, namely cholesterol, black pigment, and brown pigment stones, is reviewed. The location of gallstone types differs with type; cholesterol and black pigment gallstones are almost always found in the gallbladder, but brown pigment gallstones are more commonly found in the extrahepatic ducts and even in the intrahepatic ducts.[18,19] In brief, the development of cholesterol gallstones requires hypersecretion of cholesterol into bile.[20] Black pigment stones are associated with a large number of diseases, all of which elevate bilirubin concentration in bile. Brown pigment stones, which are rare in both children and in the Western world, occur in the presence of obstruction and subsequent infection.

Cholesterol Gallstones

Cholesterol gallstones are a frequent problem in adults and, hence, extensively studied. For cholesterol gallstones to form, there must be (1) hypersecretion of cholesterol into bile, (2) decreased motility of the gallbladder, (3) increased mucin production by the gallbladder, (4) increased conversion of primary bile salts to more hydrophobic bile secondary bile salts, and (5) increased rate of formation of cholesterol crystals.[18,20] Once this pathway starts, it often leads to a self-perpetuating cycle as hypersecretion of cholesterol itself decreases gallbladder motility. This leads to a repeat of the cholesterol gallstone formation pathway, with the net result of more cholesterol gallstones.

How does cholesterol get into bile? Cholesterol is secreted into bile directly or in the altered form of bile salts, and these pathways are the routes of elimination of cholesterol from the body.[20] There are four potential fates

for cholesterol in hepatocytes. Cholesterol can be (1) stored in the hepatocyte as either or both the free and the ester form, (2) returned to serum as low-density lipoproteins, (3) secreted into bile, or (4) converted into bile salts. The latter two account for cholesterol elimination and are most relevant to bile formation and composition. Cholesterol is secreted into bile via adenosine triphosphate (ATP) binding cassette (ABC) twinned sterol half-transporters ABCG5 and ABCG8.[21–23] Cholesterol conversion to bile salts starts with 7α-hydroxylase, an enzyme found only in hepatocytes, the activity of which is regulated by bile salt concentration. As illustrated in Figure 61-1, bile salts are transported into bile by the bile salt export pump known as both BSEP and ABCB11[24] and phospholipid by ABCB4, previously known as multidrug-resistance protein (MDR)[35] at the canalicular membrane.

In Figure 61-2, the physical chemical pathways of vesicle and micelle formation are illustrated. In the canalicular lumen, cholesterol and phosphatidylchloline vesicles are formed and then dissolved by bile salts to form mixed micelles. As bile ducts get bigger, bile becomes more concentrated, and more phosphatidylcholine is solubilized by bile salts.[26] The cholesterol concentration of micelles increases, and, eventually, in the gallbladder these cholesterol-rich vesicles fuse and may lead to cholesterol crystal formation. Patients with cholesterol gallstones have higher cholesterol-to-phosphatidylcholine ratios than patients without gallstones.[27] Such people are secreting cholesterol in excess of the ability of bile salts and phosphatidylcholine to form micelles; thus, the cholesterol saturation index will be greater than 1, and bile will be metastable.[18] The importance of phosphatidylcholine secretion in prevention of cholesterol gallstone formation is illustrated by the discovery that defects in the *ABCB4* (*MDR3*) gene are associated with symptomatic intrahepatic and gallbladder cholesterol cholithiasis. A study of six adults with recurrent cholesterol gallstones postcholecystectomy revealed mutations in *ABCB4* in all six; three had homozygous missense mutations, and two were heterozygotes and one had homozygous nonsense mutations. Two of the six also displayed hepatic bile supersaturated with cholesterol and a low phospholipid concentration.[28] *ABCB4* (*MDR3*) defects are also associated with progressive familial intrahepatic cholestasis type 3 (see Chapter 55.6, "Biliary Transport")[29] and with intrahepatic brown pigment gallstones.[19]

For the actual formation of cholesterol gallstones, nucleation or aggregation of submicroscopic cholesterol crystals must occur.[30] Both pro- and antinucleating factors are described; immunoglobulins,[31] *N*-aminopeptidase,[32] and fibronectin[33] have been identified as pronucleating factors and apolipoprotein A-I[34] as an antinucleating factor. Once a nucleus has formed, further cholesterol monohydrate crystals attach, as can mucin, calcium, and even unconjugated bilirubin.[18] Cholesterol stones can vary in size from a few millimeters to a few centimeters and can be solitary or multiple.

Black Pigment Gallstones

Black pigment stone formation requires excess bilirubin in bile.[35] Excess secretion of conjugated bilirubin into bile occurs in (1) hemolytic diseases, (2) disorders of dysfunctional erythropoiesis, and (3) diseases that cause bilirubin

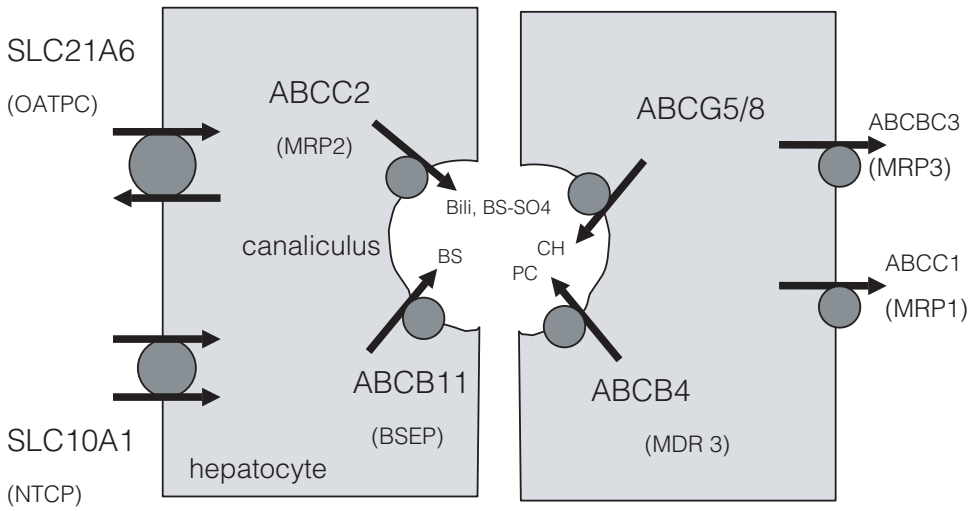

FIGURE 61-1 Transporters on the hepatocytes that have a role in modulating the composition of bile. A hepatocyte couplet is shown in this figure, and the canaliculus is in the center. The major canalicular transporters of biliary lipids are shown; the bile salt export pump (BSEP) (ABCB11) exports bile salts (BS). The half-transporters ABCG5/8 together transport cholesterol (CH). Multidrug resistance–associated protein (MRP) 2 (ABCC2) exports bilirubin conjugates (bili) and sulfated bile salts (BS-SO4). Multidrug-resistance protein (MDR) 3 P-glycoprotein (ABCB4) transports phosphatidylcholine (PC). On the basolateral surface of the hepatocytes, organic anion transporting polypeptide C (OATPC) (SLC21A6) and Na+-dependent taurocholate cotransporting polypeptide (NTCP) (SLC10A1) transport bile salts into the hepatocytes, MRP3 (ABCC3) allows monovalent bile salt efflux, and MRP1 (ABCC1) allows organic anion efflux, probably including unconjugated bilirubin. Courtesy of Dr. Richard S. Kwon, Brigham and Women's Hospital, Boston.

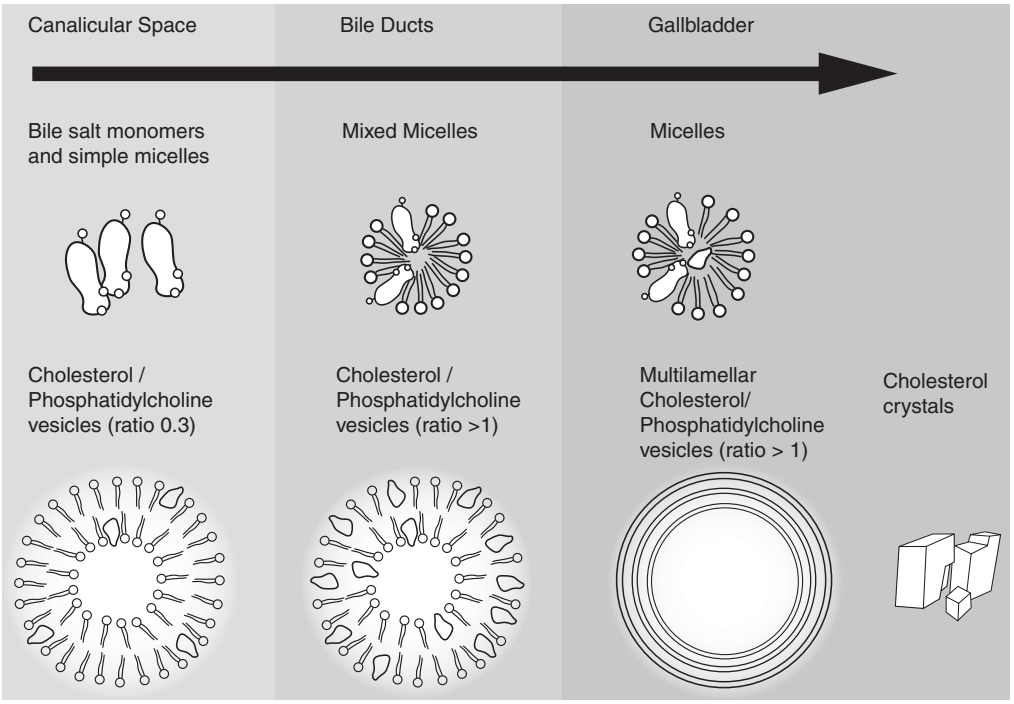

FIGURE 61-2 Pathway of cholesterol crystallization in bile. Cholesterol is hypersecreted into bile in the form of vesicles of cholesterol and phosphatidylcholine, as shown on the left of the diagram. As bile becomes more concentrated in bile ducts, the concentration of simple bile salt micelles also increases and the micelles preferentially extract phosphatidylcholine from vesicles, as shown in the middle column, creating thermodynamically unstable cholesterol-rich vesicles. In the gallbladder, as shown on the right, these vesicles fuse and aggregate to form a cholesterol-rich template for formation of cholesterol crystals. Reproduced with permission from Donovan JM.[20]

to undergo enterohepatic circulation.[36,37] When excess conjugated bilirubin is present in bile (ie, bilirubin supersaturation),[18] it can be deconjugated by β-glucuronidase or by other nonenzymatic means. This unconjugated bilirubin precipitates as an insoluble calcium salt known as calcium bilirubinate. Black pigment stones also contain calcium hydrogen bilirubinate, calcium carbonate, and calcium phosphate; small amounts of cholesterol (less than 20% by weight); and mucin in a glycoprotein matrix.[20] Free radical polymerization of calcium bilirubinate and possible oxidation in the presence of gallbladder mucin leads to the formation of black pigment gallstones.[20]

Brown Pigment Gallstones

Brown pigment stones are laminated and contain bacterial cytoskeletons,[38] calcium bilirubinate salts, cholesterol, and fatty acids.[20] These stones can occur in the presence of cholesterol or black pigment stones, which are causing obstruction and hence infection. Intrahepatic brown pigment stones are associated with decreased expression of MDR3 and phosphatidylcholine transfer protein as well as increased 3-hydroxy-3-methylglutaryl coenzyme A reductase activity.[19]

Genetics

Formation of cholesterol gallstones in humans is likely due to the interaction of environmental factors with genes, although none have been definitively identified in humans. Investigations in mice have led to the identification of *Lith* genes, which are candidate genes for cholesterol gallstone disease. The genetics of cholesterol gallstone disease is an example of a complex genetic trait, and a special investigative technique called quantitative trait locus (QTL) analysis is required.[39] Mice, which share similar hepatocyte and cholangiocyte transporter proteins to humans, are used as models for human gallstone disease. Environmental factors, especially diet, can be readily controlled in mice. To identify cholesterol gallstone genes by QTL analysis, mice strains that are gallstone resistant and others that are gallstone susceptible were identified. These mice were crossed and selectively back-crossed, and the progeny were phenotyped and genotyped. By this means, gallstone candidate genes are linked to polymorphic genetic markers, which differ between inbred mouse strains. The next step is to identify a chromosomal region that contains potential *Lith* genes by QTL analysis and to use the murine genome map to analyze these regions for genes, which could play a potential pathophysiologic role in cholesterol gallstone formation. Candidate genes, which colocalize with the QTLs, are studied in a specially bred mouse strain that expresses them, and the effects on cholesterol homeostasis and gallstones are investigated. Using this process, there are now nine potential *Lith* genes identified.[40]

The initial QTL experiments identified two potential *Lith* genes in mice. *Abcb11* is a strong candidate gene for *Lith 1*[41] and *Abcc2* for *Lith 2*.[39] *Abcb11* is also known as *Bsep* and is a member of the ABC family. It is found on the canalicular surface of hepatocytes and actively exports monovalent bile salts. There appears to be a gain of function so that bile salt secretion rates, along with those of cholesterol, are elevated in *Lith 1* congenic mice. ABCC2, also known as multidrug resistance–associated protein (MRP) 2, is an organic anion transporter also found on the canalicular membrane, which exports bilirubin and glutathione conjugates.[42] Other candidate *Lith* genes are involved in mucin regulation and cholesterol crystallization.[43] Human correlation of these candidate genes is actively being investigated.

For black pigment gallstones, the underlying disease (eg, sickle cell disease) leading to gallstone formation often has a genetic basis. It is interesting to note that pop-

ulations with a very high rate of cholesterol gallstone disease, such as the Pima Indians, develop pigment gallstones very rarely. Brown pigment stones are formed in the setting of infection, so genetic causes are less likely to be a major factor. However, as mentioned, gene expression differs in liver tissue from those with intrahepatic brown pigment stones.[19]

DISEASES ASSOCIATED WITH GALLSTONES IN CHILDREN

Cholesterol Gallstones

Cholesterol gallstones in children and adolescents have been associated with female gender, a positive family history, and parity. In a series of 96 patients under 25 years of age, the ratio of females to male with cholelithiasis was 4:1.[44] Unusually for a series of younger patients, only 2% of patients in this study had hemolytic disease. In another series of 50 children and adolescents with gallstones, 7 were young women who had adolescent pregnancies.[45] Three of the 50 patients in the latter study only had obesity as a risk factor for gallstones.[45] Finally, family history is significant in adults with cholesterol gallstones and in postpubertal Pima Indians.[13]

Pigment Gallstones

Black pigment gallstones are generally secondary to an identifiable risk factor. The many diseases associated with pigment gallstones in children are outlined in Table 61-1. These diseases can be categorized into three broad groupings.

Hemolytic Processes. Diseases resulting in hemolysis commonly lead to the formation of black pigment stones. An example is sickle cell disease, in which the prevalence of cholelithiasis increases with age. In an ultrasonographic study of children with major sickle hemoglobinopathies, gallstone prevalence was negligible in children younger that 6 years, but prevalence increased in the midteen years.[46] Interestingly, the severity of hemolytic disease as measured by hemoglobin levels, reticulocyte counts, and blood transfusions is not associated directly with gallstones.[46] Overall, gallstones are such a prominent feature of sickle cell disease that cholecystectomy is the most common surgical procedure performed for these patients.[47] Complication rates are equally high for both open and laparoscopic cholecystectomy in patients with sickle cell disease. Hospital stays are shorter in those who have a laparoscopic cholecystectomy, and preoperative transfusions decrease complications further.[47] Children with hereditary spherocytosis are also at increased risk of black pigment gallstone, and laparoscopic cholecystectomy for those with gallstones can be safely combined with laparoscopic splenectomy.[48] Although more common in adult populations, the presence of artificial cardiac valves can lead to red cell destruction and subsequent pigment gallstone formation in children.[49]

Enterohepatic Circulation of Bilirubin. Crohn disease, distal small intestinal resection, and cystic fibrosis

TABLE 61-1 DISEASES ASSOCIATED WITH GALLSTONES IN CHILDREN

DISEASE	GALLSTONE TYPE	PREVALENCE	AGE RANGE (YR)	REFERENCE
HEMOLYTIC PROCESSES				
Sickle cell hemoglobinopathies				
Homozygous HbSS	Pigment	26% (11/42)	2–18	46
Heterozygous HbSC	Pigment	20% (3/15)	2–18	46
β-Thalassemia anemia	Pigment	11.8% (2/17)	2–18	98
	Sludge	29.4% (5/17)	2–18	98
Hereditary spherocytosis	Pigment	3/50 children with gallstones	0–20	45
Cardiac valve replacement	Pigment	Case reports	Children and adults	52
PROBABLE ENTEROHEPATIC CIRCULATION OF BILIRUBIN				
History of abdominal surgery in infancy (especially ileal resection)	Pigment	29% < 1 yr 21% of 1–5 yr 5% of 6–21 yr	0–21	54
Cystic fibrosis	Pigment	13.2% (25/189)	3–22.5	52
Crohn disease	Pigment	Case reports	Children and adults	50
MISCELLANEOUS				
Prolonged parenteral nutrition > 4 wk	Pigment	13% (11/84)	Infants and children	53
	Sludge	3.6% (3/84)	Infants and children	53
Treatment of childhood cancer	Not reported	0.42% (16/6050)	10 yr after diagnosis of primary cancer	58
Post–heart transplant (cyclosporine immunosuppression)	Pigment and mixed type	3.2% (10/311); 8/10 transplanted < 3 mo of age	5 d to 3 yr at time of transplant	56
Down syndrome	Radiolucent gallstones (chemical analysis not reported)	4.7% (6/126)	1 mo–19 yr	57
Pregnancy	Cholesterol	7 of 50 children with gallstones	0–20	46
	Cholesterol	8/12 female patients younger than 18–26 yr with gallstones	18–26	99

HbSC = sickle cell trait (heterozygous); HbSS = sickle cell anemia (homozygous).

are examples of diseases that may result in enterohepatic circulation of excess unconjugated bilirubin from the intestine to the liver.[36,50,51] A proposed mechanism for enterohepatic circulation of unconjugated bilirubin in these disease processes is that malabsorption of bile salts from the terminal ileum leads to spillage of excess bile salts into the large intestine. These bile salts are accompanied by bilirubin conjugates, which are hydrolyzed in the large intestine by bacterial glucuronidases. The unconjugated bilirubin is further converted into either calcium salts or into urobilinoids. In the presence of large amounts of bile salts, passive nonionic diffusion of unconjugated bilirubin from the colon and return to the liver are promoted. Unconjugated bilirubin is taken up into hepatocytes, reconjugated mainly with glucuronic acid, and secreted again into canalicular bile.[36,37]

Children and adults with cystic fibrosis exhibit increased prevalence of gallstones. In a study of 189 patients (children and adults) with cystic fibrosis performed in Italy, 25 or 13.2% were found to have gallstones.[52] Gallstones have also been noted in children requiring parenteral nutrition. Those especially vulnerable are infants who have also undergone ileal resection and had multiple abdominal procedures.[53] Children with hepatobiliary disease are also at increased risk of gallstones.[54] Bile salt malabsorption leading to the interruption of the enterohepatic cycling of bile salts is the likely mechanism in the latter two diseases.

Miscellaneous. Other risk factors for black pigment gallstones are medications, especially furosemide in infants[55] and cyclosporine in cardiac transplant patients.[56] Bronchopulmonary dysplasia, independent of furosemide use, and gram-negative sepsis are also risk factors in infancy.[54] Children with Down syndrome are at increased risk of cholelithiasis, although the etiology is not understood.[57] Survivors of childhood cancers are at increased risk, although, again, the cause of this increased risk is not known.[58]

CLINICAL FEATURES OF GALLBLADDER DISEASE

The presentation of gallbladder disease is dependent on the underlying disease process. It can be difficult to gauge the contribution of chronic acalculous cholecystitis to chronic abdominal pain. Pain in patients with acute cholecystitis or acute biliary colic is usually clearly related to the presence of gallstones. It is also not clear why most individuals with gallstones are asymptomatic for long periods, whereas others have recurrent episodes of biliary colic. It is also unclear why others can present with disease outside the gallbladder as manifestations of gallbladder disease, for example, pancreatitis or cholangitis, although stone size appears to play a role. The clinical features of gallbladder disease are reviewed systematically.

Congenital Anomalies

These are generally asymptomatic, although they may predispose the patient to stasis and subsequent cholelithiasis.[59]

Acute Gallbladder Diseases

Biliary Colic. Gallstones can lead to pain, which patients describe as a steady, intense pain in the right upper quadrant or epigastrium. Some report that the pain radiates to the shoulder. Patients often vomit and classically are restless as they try to achieve a comfortable position. These episodes are usually referred to as biliary colic. Most patients have bouts of biliary colic that last for hours and can recur at random times.[60] Acute episodes of biliary colic present in a similar fashion in children, although a high index of suspicion is needed in young children. Adults can also complain of "dyspeptic" symptoms such as gas and heartburn during these episodes.

Acute Cholecystitis. If the gallbladder is inflamed and gallstones are present during these painful episodes, this is termed acute cholecystitis. Patients with acute cholecystitis can present with fever and right upper quadrant pain and may be Murphy sign positive. They often display a leukocytosis. If there are no gallstones present but the gallbladder is inflamed, this is termed acute acalculous cholecystitis, and this generally occurs in children with other major medical problems or infections. The clinical features are reviewed further in the section discussing acute acalculous cholecystitis.

Chronic Gallbladder Diseases

Chronic Gallstone Disease. Biliary colic often recurs; however, the acute episodes are short in duration rather than ongoing. A myriad of gastrointestinal complaints have been attributed to the presence of gallstones, such as indigestion, abdominal discomfort, and heartburn. It is important for patients to be aware that cholecystectomy will resolve the abdominal pain associated with episodes of biliary colic but may not resolve all gastrointestinal complaints.

Chronic Acalculous Cholecystitis. Patients with chronic acalculous cholecystitis, sometimes referred to as gallbladder dyskinesia, can present diagnostic and therapeutic challenges. These otherwise healthy children, often female, can complain of abdominal pain for months to years. The pain is often worse after eating and is localized to the right upper quadrant. Examination and routine workup are generally normal, and it is not until a hepatobiliary scintigraph scan is performed that the diagnosis is made. Excellent symptomatic relief has been reported in children with chronic alcalculous cholecystitis postcholecystectomy.[61]

Sphincter of Oddi Dysfunction. Those with sphincter of Oddi dysfunction (SOD) have complaints similar to those of patients with gallbladder dyskinesia, but cholecystectomy will not provide relief. When SOD is present, there is effectively an obstruction at the level of the sphincter, which may be caused by fibrosis or inflammation or by elevated sphincter tone. Endoscopic retrograde cholangiopancreatography (ERCP) with manometry provides the diagnosis, and sphincterotomy is the treatment of choice. In 50 healthy adult volunteers, normal basal pressure of

the sphincter of Oddi was 14.8 ± 6.3 mm Hg, and no pressure was greater than 40 mm Hg.[62] There are no comparable studies in normal children. SOD can lead to bile duct dilatation without evidence of choledocholithiasis pre- or postcholecystectomy. SOD has also been associated with acute recurrent pancreatitis and biliary colic–type pain in the absence of gallstones.

Gallstones can also lead to pancreatitis, the clinical features of which are reviewed in Chapter 64.1, "Pancreatitis: Acute and Chronic." Gallstones can also lead to obstruction of the common bile duct, causing pain, jaundice, and, if there is infection, fever.

ASSESSMENT OF FUNCTION AND IMAGING OF THE GALLBLADDER

Plain Abdominal Films

Plain films are not the investigation of choice for suspected gallstones. However, radiopaque stones, which account for 10 to 15% of all stones, can be seen in the gallbladder, common bile duct, and, occasionally, even the intrahepatic ducts and may be noted when the film is taken for other purposes.[63] A rare condition called "milk of calcium cholelithiasis" is associated with dramatic findings on abdominal radiographs. In this condition, bile is primarily composed of calcium carbonate, which is normally found in bones and teeth and is radiopaque. The gallbladder is often clearly seen, filled with multiple calculi. Surgery is curative, and the etiology is unknown.[59]

Ultrasonography

Ultrasonography of the gallbladder and bile ducts has permitted noninvasive and safe imaging of the gallbladder, gallstones, and biliary tree to be performed. Although now firmly established as the investigation of choice, ultrasonography was regarded with some suspicion in the 1970s. Indeed, the authors of one textbook stated that: "Neither we nor others have been convinced that this modality [ultrasonography] equals, let alone surpasses, currently available techniques."[3] Ultrasonography of the gallbladder is best performed on a patient who has fasted for 4 or more hours. Using a transducer placed on the anterior abdominal wall, the gallbladder is generally found on the undersurface of the liver between the right and left lobes. Rarely, the gallbladder can be found within the liver or underneath the left lobe.[64] Normal gallbladder size varies with fasting status and age. In infants less than 1 year of age, gallbladder length is usually between 1.5 and 3 cm. As shown in Figure 61-3, in older children, gallbladder length is usually 3 to 7 cm.[64,65] The diameter of the common bile duct varies with age. The following are the normal ranges of the common bile duct diameter: less than 1 mm in neonates, less than 2 mm in infants, less than 4 mm in children, and less than 7 mm in adolescents.[64,65] Gallbladder wall thickness increases when inflamed and is found to be greater than 3 mm in 50 to 75% of patients with acute cholecystitis.[64]

In the course of an ultrasonographic examination, the gallbladder is examined for evidence of stones, sludge, masses, distention, and pericholecystic fluid. Sludge, which is actually viscous bile, layers in the dependent part of the lumen and does not form acoustic shadows (Figure 61-4). Sludge moves slowly when the patient changes position.[64] Gallstones are usually mobile within the gallbladder lumen, unless impacted in the cystic or common bile ducts, and are brightly echogenic with posterior acoustic shadows (Figure 61-5).[65] Stones as small as 1 mm can be identified.[63] If gallstones are within the common bile duct, there is usually accompanying ductular dilatation. False-positive and -negative identification of gallstones can occur if the gallbladder is packed full of stones or nearby air-filled bowel loops create acoustic shadows.[64] Gallbladder polyps can be mistaken for gallstones, although they are not as mobile. Overall, ultrasonography

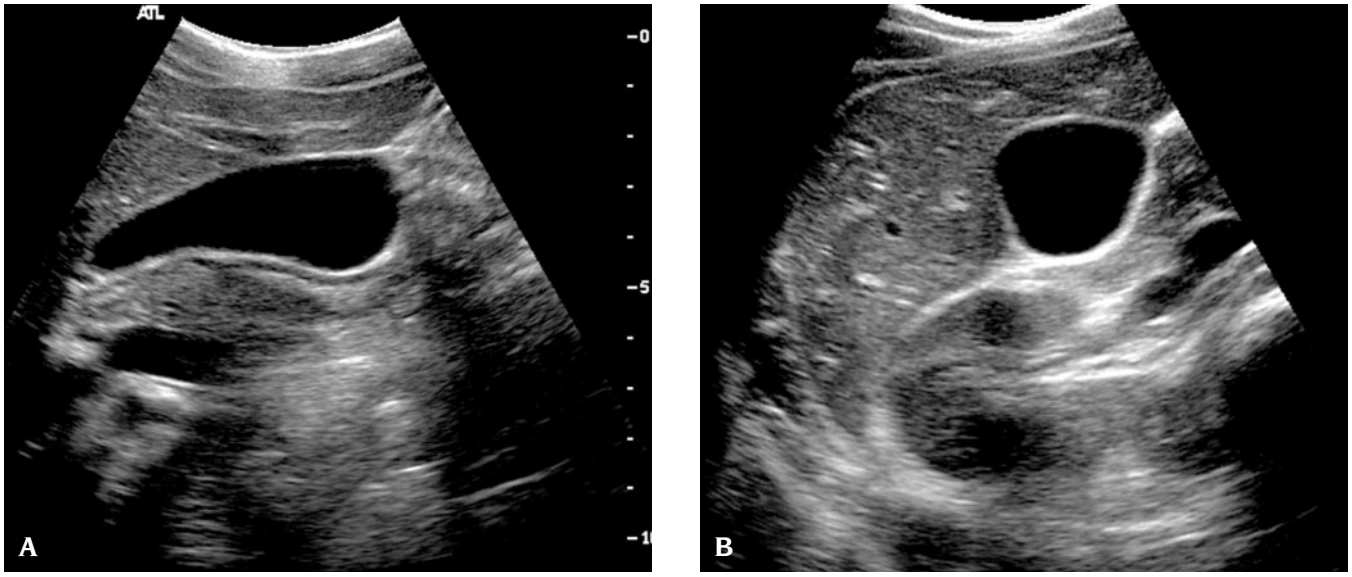

FIGURE 61-3 Ultrasonographic image of a normal gallbladder. A normal gallbladder of a healthy child is shown in two views. *A* shows the longitudinal view of the gallbladder, and *B* shows a cross-sectional view. Courtesy of Dr. Harriet J. Paltiel, Children's Hospital, Boston.

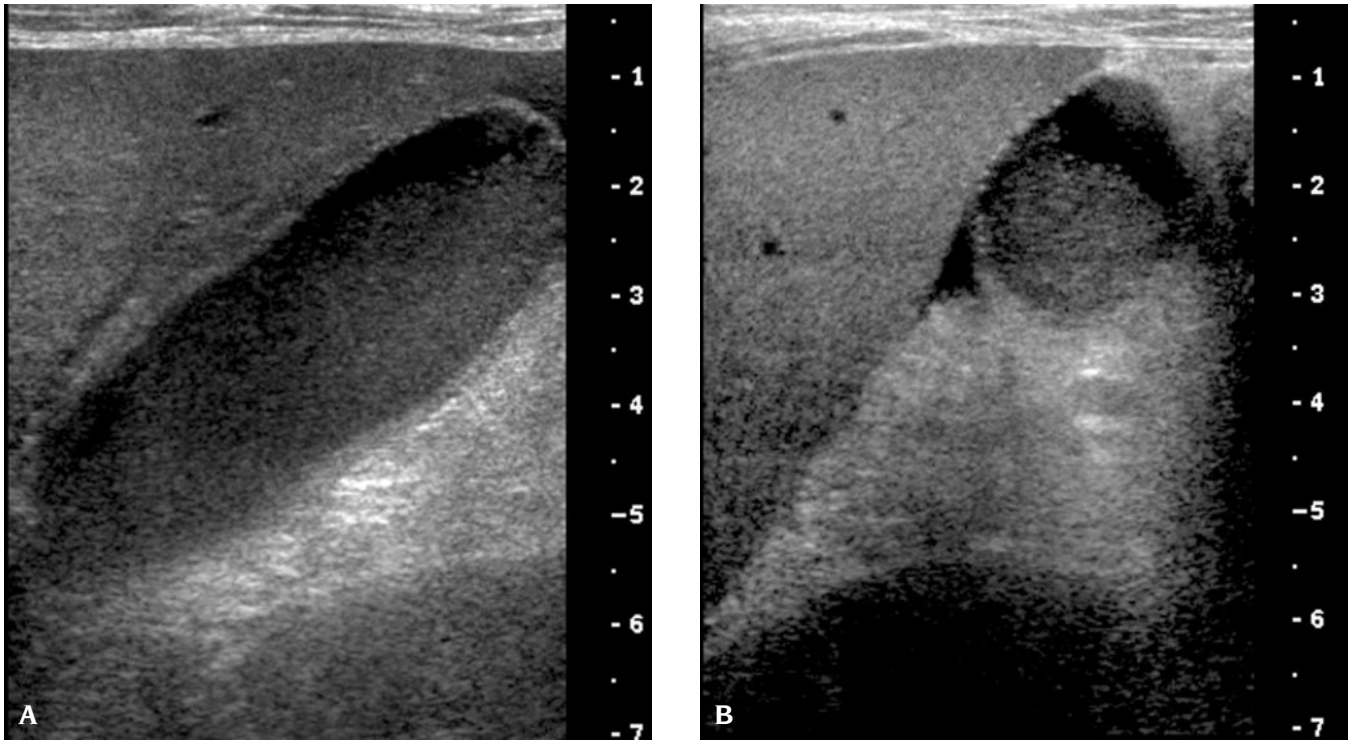

FIGURE 61-4 Ultrasonographic image of biliary sludge. *A* illustrates a child's gallbladder filled with sludge. Sludge does not form acoustic shadows (contrast this with the shadows created by gallstones in Figure 61-5). Sludge moves slowly within the gallbladder when the patient moves during the examination, and the sludge can be seen in a different position within the gallbladder in *B*. Courtesy of Dr. Harriet J. Paltiel, Children's Hospital, Boston.

has a greater than 95% sensitivity and specificity for detecting gallstones.[66]

Cholecystitis on ultrasonography is often associated with a thickened gallbladder wall (> 3 mm), an enlarged gallbladder, gallstones or sludge, and a positive Murphy sign.[64] In addition, signs of potential complications of acute cholecystitis should be sought, such as gangrene, which is suggested by irregularities in the gallbladder wall, or emphysema and perforation, which is suggested by pericholecystic fluid.[65] In chronic cholecystitis, the gallbladder may have all of the above findings or may even appear normal. In adults and very rarely in children, adenomyomatosis or strawberry gallbladder, which is due to diffuse or focal prominence of Rokitansky-Aschoff sinuses (hyperplastic mucosa extensions into the muscular layer), can cause chronic pain.[67] Hydrops of the gallbladder, also referred to as acute alcalculous cholecystitis, is characterized on ultrasonography by a distended gallbladder without evidence of obstruction or bile duct dilatation. The bile is usually anechoic.

Computed Tomography and Magnetic Resonance Imaging

Other radiologic investigations have a role in the diagnosis of specific complications. Computed tomography (CT) is used to image the pancreas, which may be inflamed sec-

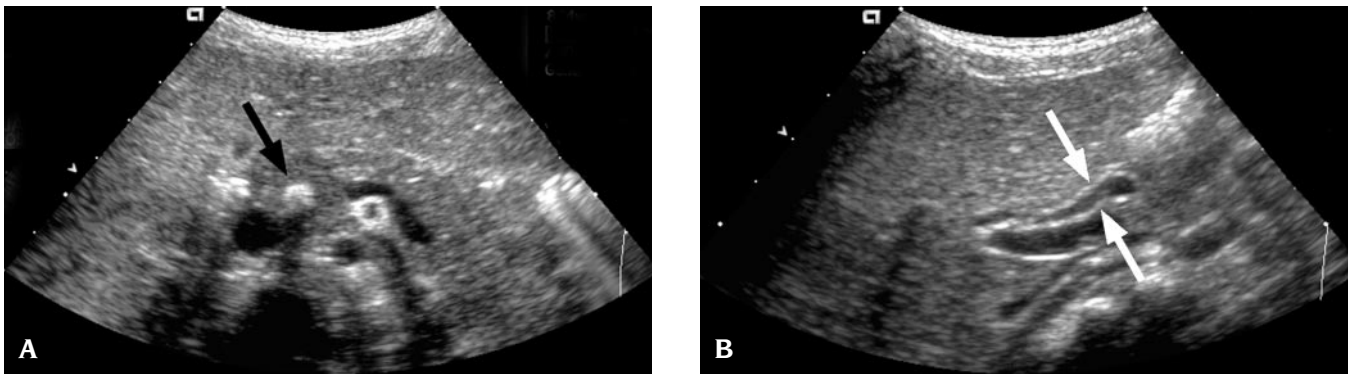

FIGURE 61-5 Ultrasonographic images of a gallstone in the common bile duct. *A,* A transverse image of the pancreas demonstrates an echogenic focus (*black arrow*) in the distal common bile duct with distal shadowing in keeping with a stone. *B* is a longitudinal oblique image of the liver hilum demonstrating dilation of the common bile duct (*between two white arrows*) down to the level of the pancreas. Courtesy of Dr. Harriet J. Paltiel, Children's Hospital, Boston.

ondary to gallstone obstruction of the pancreatic duct, and is also useful for imaging the biliary tree when complications of acute cholecystitis are suspected. It is not as reliable as ultrasonography for detection of gallstones, which may exhibit the same radiographic density as bile and therefore may not be seen on CT.[63] Magnetic resonance imaging of the pancreas and biliary tree (magnetic resonance cholangiopancreatography) is superior to CT for investigation of the anatomy of the hepatobiliary tree and the pancreatic duct. However, ERCP is still the investigation of choice to define the extrahepatic and pancreatic duct systems because there is not only excellent imaging, but therapeutic interventions, such as retrieval of impacted stones from the common bile duct and sphincterotomy, are also possible. ERCP is discussed further under surgical procedures.

Radionuclide Hepatobiliary Scintigraphy

Radionuclide imaging or scintigraphy plays a role in both the diagnosis of biliary disease and assessment of function. For acute cholecystitis in adults, hepatobiliary scintigraphy (HBS) has both a higher specificity and sensitivity. In a meta-analysis of 27 studies containing almost 3,000 patients, the sensitivity of HBS is 90% and the sensitivity is 97%.[66] The rates for ultrasonography diagnosis are 91% and 79%, respectively. However, the main role of HBS is when ultrasonographic findings are equivocal for acute cholecystitis. A positive test of cholecystitis by HBS is based on a lack of gallbladder visualization. An intravenously administered radioactive pharmaceutical, usually technetium 99m hepatic iminodiacetic acid, is secreted from the liver into the hepatic bile ducts but is unable to enter the gallbladder if the cystic duct is obstructed. The isotope instead enters the small intestine.[68] If the gallbladder is not seen in the first 60 minutes, there are two options: delayed imaging at 4 hours or the more popular administration of morphine. Morphine administration increases pressure at the sphincter of Oddi and forces bile into the gallbladder if the cystic duct is patent. The morphine protocol is more specific, equally sensitive, and completed within 90 minutes.[68] Despite the increased specificity of HBS, ultrasonography is still the test of choice because it allows for evaluation of gallbladder anatomy as well as other abdominal organs. Ultrasonography is also less invasive and less expensive and involves no exposure to radioactivity.

Radionuclide imaging also can play a role in the diagnosis of both acute and chronic alcalculous cholecystitis. A prospective study comparing ultrasonography and radionuclide imaging in adults with acute alcalculous cholecystitis found a sensitivity of 70% and a specificity of 100% for radionuclide scanning in association with ultrasonography.[69] Gangrene and perforation, both complications of acute acalculous cholecystitis, can be identified on HBS by spill of radionuclide into the peritoneal cavity.[68] HBS is useful for diagnosis of chronic alcalculous cholecystitis, also referred to as gallbladder dyskinesia or spasm. Cholecystokinin (CCK) is administered during the study. Poor contractility of the gallbladder in response to CCK is the hallmark of chronic acalculous cholecystitis. If the gallbladder ejection fraction is less than 35% in a patient with a clinical history suggestive of chronic alcalculous cholecystitis, there is a greater than 90% positive predictive value.[68] False decreases can occur in patients with diabetes and celiac disease and in pregnancy, as well as in those receiving morphine and octreotide. Sphincter of Oddi spasm or bile duct dyskinesia has clinical symptoms similar to those of chronic alcalculous cholecystitis and an abnormal response to CCK—sphinchter contraction rather than dilatation. However, the best test for this condition is sphincter manometry. Finally, HBS is useful postcholecystectomy to detect bile leak complications.

Miscellaneous Imaging Techniques

The other available modalities to investigate the anatomy of the biliary tree are used much more rarely nowadays. Oral cholecystography has been superseded by ultrasonography for the detection of gallstones, and T tube cholangiography is performed infrequently in this era of laparoscopic cholecystectomies. Highly specialized interventions such as transhepatic cholangiography for investigation of strictures are still performed in the proper clinical setting.

Role of ERCP

ERCP in children is not a trivial procedure because general anesthesia is generally required in young children and infants.[70] The techniques of this procedure are reviewed in detail in Chapter 67.5, "Endoscopic Retrograde Cholangiopancreatography." The roles of ERCP in gallbladder disease in children are to remove common bile duct stones in choledolithiasis, measure sphincter of Oddi pressure, and, occasionally, delineate anatomy. Children undergoing ERCP because of biliary pathology have been reported to have higher complication rates than do adults. In one reported series of 15 such children, 6 presented with pancreatitis and 1 with bleeding post-ERCP.[70] A second group emphasized the role of ERCP with sphincterotomy and stone removal in children with common bile duct stones.

ERCP is also used to perform manometry at the sphincter of Oddi to make the diagnosis of SOD. A sphincterotomy can be performed at the same time if the diagnosis is confirmed.

Choice of Imaging Methods

The best imaging method for a child with suspected gallbladder disease depends on the suspected condition. The imaging methods of choice for a number of different gallbladder diseases are outlined in Table 61-2.

SURGICAL MANAGEMENT OF CHOLELITHIASIS

Laparoscopic cholecystectomy is currently the procedure of choice in children requiring cholecystectomy.[71] There is evidence to indicate that laparoscopic cholecystectomy patients have a shorter hospital stay and less analgesic requirements than their open cholecystectomy counterparts.[72] Open cholecystectomy is reserved for the few children in whom laparoscopic cholecystectomy is a relative contraindication or when it cannot be completed safely.

TABLE 61-2 IMAGING METHODS OF THE HEPATOBILIARY TRACT FOR DIFFERENT CLINICAL SITUATIONS

	IMAGING METHOD OF CHOICE					
DISEASE PROCESS	ULTRASONOGRAPHY	RADIONUCLIDE IMAGING	CT	MRI/ MRCP	ERCP	MISCELLANEOUS
Cholelithiasis	+++					
Acute cholecystitis	+++	++ (may need delayed imaging and/or intravenous morphine)				
Acute acalculous cholecystitis	++	+				
Chronic acalculous cholecystitis	+	+++ (with CCK)				
Sphincter of Oddi spasm	+	++ (with CCK)			+++ (sphincter manometry)	
Biliary leak postcholecystectomy	+	+++	+			
Bile duct obstruction	+++	++	++	+++	+++	Depending on site of obstruction, intraoperative cholangiogram or transhepatic cholangiogram may be required

CCK = cholecystokinin; CT = computed tomography; ERCP = endoscopic retrograde cholangiopancreatography; MRCP = magnetic resonance cholangiopancreatography; MRI = magnetic rseonance imaging.

Laparoscopic cholecystectomy is performed under general anesthesia with the patient in the supine position. An insufflation needle is introduced into the peritoneal cavity, with great care to prevent inadvertent visceral or vascular injury,[73] or a trochar may be introduced under direct vision to prevent damage to adjacent structures. After insufflation of the peritoneal cavity with carbon dioxide, a camera is introduced that allows the introduction of additional working ports under direct vision. Calot triangle is exposed by retracting the gallbladder up and over the liver[71] and freeing pericholecystic adhesions. Blunt dissection of the cystic duct is begun at the neck of the gallbladder, and the cystic duct–common duct junction is delineated. Clips are carefully applied to the cystic duct prior to its division before turning attention to the cystic artery, which is then clipped and ligated. The peritoneum of the gallbladder is then incised and is then freed from the gallbladder bed using hook cautery,[71] and the gallbladder is removed.

Children may also display gallstones in the common bile duct choledocholithiasis. Preoperative findings suggestive of this are listed in Table 61–3. Waldhausen and colleagues recommend routine use of intraoperative cholangiography.[74] They ultimately identified common bile duct stones in 18 of 100 patients studied and found preoperative suspicion of choledocholithiasis in 20 patients. The suspicion was based on finding one or more of the following abnormalities: ultrasonographic evidence of a dilated common or intrahepatic bile duct or identification of a specific common duct stone (6 patients), biochemical abnormalities consisting of elevated liver function tests (9 patients), elevated amylase and lipase (10 patients), or conjugated hyperbilirubinemia (2 patients). Fifteen of the 20 patients suspected preoperatively were confirmed to have evidence of choledocholithiasis based on intraoperative cholangiography or ERCP; no stones were found in five. Three additional children in their series were found to have cholangiographic evidence of choledocholithiasis, which had not been predicted on preoperative evaluation. They concluded that intraoperative cholangiography should be performed routinely and ERCP should be reserved for patients in whom common duct stones are identified and the surgeon prefers not to perform laparoscopic or open common duct exploration. However, Newman and colleagues do not recommend routine intraoperative cholangiography but rather preoperative blood work, ultrasonography, and ERCP as indicated.[75] In their series of 117 children undergoing cholecystectomy, 14 were suspected to have common bile duct stones, of which 8 patients were confirmed at the time of ERCP. One child was found at the time of surgery to have a common bile duct stone not identified preoperatively. Therefore, 7 children were incorrectly identified in this series of 117 children (6 false-positives and 1 false-negative) as having or not having stones in the common bile duct on the basis of preoperative examination and bloodwork. These authors do not recommend routine intraoperative cholangiography because only 9 of 117 children actually had stones in the common bile duct, and 8 of these were suspected preoperatively.[75]

TABLE 61-3 PREOPERATIVE FINDINGS SUGGESTIVE OF COMMON BILE DUCT STONES

Jaundice
Elevated canalicular enzymes
Pancreatitis
Dilated common bile duct on ultrasonography
Dilated intrahepatic bile ducts on ultrasonography

Laparoscopic cholecystectomy is associated with 3 to 4 times the risk of injury to the biliary tree compared with open cholecystectomy.[76] The key factor is prevention of this serious complication by correctly identifying the cystic duct and artery. If a biliary injury is recognized intraoperatively, it is recommended to convert to an open surgical approach to repair the injury.[76] If injuries of this nature are not recognized immediately, however, they may present days or months later with the triad of fever, pain, and jaundice. ERCP can play a vital role in the diagnosis and treatment of these complications.[77] If the common bile duct or one of its major branches is transected, a hepaticojejunostomy by an experienced hepatobiliary surgeon is the standard of care.

Contraindications to Laparoscopic Cholecystectomy

Recently, absolute contraindications for laparoscopic cholecystectomy have dramatically decreased as operative skills, experience, and equipment have developed. Hemodynamic instability, irreversible coagulopathy, and third-trimester pregnancy remain as absolute contraindications.[78] Situations that require special expertise but do not preclude laparoscopic cholecystectomy are acute gallstone pancreatitis, prior upper abdominal surgery, and second-trimester pregnancy.[79]

Medical Management of Cholelithiasis

The advent of laparoscopic cholecystectomy has essentially eliminated medical management of gallstones. Moreover, because medical therapy targets cholesterol gallstones only, children were generally unsuitable candidates, and, indeed, no reported studies have been performed in children. Nonsurgical therapy consists of either administration of bile salts or extracorporeal shock wave lithotripsy. Ursodeoxycholic acid and chenodeoxycholic acid are two bile salts that have been used. Both of these bile salts decrease cholesterol secretion into bile so that bile becomes desaturated of cholesterol.[80] Chenodeoxycholic acid in therapeutic doses leads to diarrhea in 50% of patients and dissolved gallstones in only 14.5% of adult patients treated for 2 years.[81] Ursodeoxycholic acid treatment is associated with less diarrhea and better stone dissolution rates than chenodeoxycholic acid, up to 40% at 2 years,[80] but is rarely used for this indication now. Extracorporeal shock wave lithotripsy has been used in association with administration of bile acids to disintegrate stones. This strategy did lead to dissolution of both solitary and multiple stones in 91% of patients, but the gallbladder remains in place, and stones can therefore recur.[82]

NONGALLSTONE DISEASES OF THE GALLBLADDER

Acalculous Cholecystitis

Acalculous cholecystitis, an uncommon condition in children, can be divided into two distinct clinical entities: acute, with symptoms of less than 1 month in duration, and chronic, with symptoms lasting more than 1 month.[83] Acute acalculous cholecystitis is characteristically associated with a concurrent systemic infection or recent surgery or trauma, whereas the chronic form is not. The condition appears to develop when the gallbladder either contracts or cannot empty its contents. Specific risk factors are prolonged fasting, parenteral nutrition, and sepsis. Children with acute alcalculous cholecystitis present with fever, right upper quadrant pain, and vomiting and exhibit abdominal tenderness on examination.[83,84] Leukocytosis and elevated bilirubin are also common. Ultrasonography diagnosis of acute acalculous cholecystitis requires gallbladder wall thickness greater than 3.5 mm, hydrops, sludge, and pericholecystic fluid.[83] These children often have associated illnesses such as infections like *Salmonella typhi*[85,86] or infective endocarditis[87] or a systemic disease such as Kawasaki disease.[83] A history of recent surgery or trauma is common. Treatment of acute acalculous cholecystitis involves serial examinations, gallbladder ultrasonography, and cholecystectomy when indicated by deteriorating clinical or ultrasonographic findings.[84] The histopathologic findings of gallbladders resected from children with acute acalculous cholecystitis range from edema and focal hemorrhage to gangrene.[83] For children who can be managed conservatively by active observation and antibiotic administration, the gallbladder will eventually return to normal function.

In contrast, children with chronic acalculous cholecystitis are generally female, are otherwise healthy, have a history of right upper quadrant pain that may be associated with nausea and vomiting, and have normal serum white cell counts and bilirubin.[83] The gallbladder is normal on ultrasonography, and the test of choice for diagnosis is a CCK-stimulated HBS.[60] This test measures the gallbladder ejection fraction, which is decreased with chronic acalculous cholecystitis. In adults, less than 35% is considered diagnostic; a comparable figure is not known in children.[68] Two reported series of children have shown good reduction in symptoms in children postcholecystectomy. In one study, following laparoscopic cholecystectomy, 70% of children had complete resolution of symptoms and 18% had partial relief.[61] In a second study, cholecystectomy, either laparoscopically or open, led to complete resolution of symptoms.[83]

Gallbladder Masses

Gallbladder masses are, fortunately, very rare in children so are discussed only briefly.

Benign Masses of the Gallbladder. Benign masses of the gallbladder are extremely rare in children and are usually polyps.[88,89] There are four main types of polyps: cholesterol ones, which arise from infiltration of lipid-laden macrophages into lamina propria; inflammatory polyps composed of granulation and fibrous tissue; adenomyoma, which arise when adenomyomatosis is confined to the fundus of the gallbladder; and adenomas.[90] Lipomas can also be found rarely in the gallbladder.[90,91] Adenomas of the gallbladder are usually solitary and may be associated with gallstones. It is not clear if they are premalignant, like adenomas of the colon. Patients with polyps of all types can present with symptoms and signs suggestive of biliary

colic, and ultrasonography may not be able to distinguish polyp type. Endoscopic ultrasonography may play a role in increasing preoperative identification of the polyp type. In a study of 182 Chinese patients aged 15 to 84 years, the sensitivity of transabdominal ultrasonography at detecting polyps was 90.1%. Of the 182 patients, 172 had polyps at the time of surgery, of which 159 were benign lesions and 13 were malignant.[91] These authors suggest surgical resection of polypoid lesions greater than 1 cm in diameter and of solitary lesions and in those older than 50 years.[91] Gallbladder polyps have been reported in three patients with Peutz-Jeghers syndrome in Germany, and conservative management is recommended unless there are symptoms suggestive of biliary colic.[92]

Malignant Tumors of the Gallbladder. Gallbladder carcinoma arises in those with a history of acute or chronic cholecystitis. It appears that chronic inflammation is necessary for this tumor to develop; it is, therefore, rare in childhood and adults under 50 years. Incidental adenocarcinoma of the gallbladder is found in 0.1 to 0.5% of adults undergoing laparoscopic cholecystectomy for gallstones and 1 to 2% of those undergoing open cholecystectomies.[93] For most patients with gallbladder cancer, however, the prognosis is poor because only 5% survive 5 years after diagnosis.[93,94] Approximately 80% of gallbladder carcinomas are adenocarcinomas and are associated with abnormal expression of *TP53*, the tumor suppressor gene.[95] Squamous cell adenocanthoma accounts for the remaining 20% of gallbladder cancers.[94] Radical cholecystectomy, ERCP placement of stents into the hepatic and common bile ducts if necessary, and palliative radiotherapy all play a role. For those found to have incidental adenocarcinomas confined to the mucosa and with lymph node metastases at the time of cholecystectomy, the cholecystectomy itself can be curative.[94]

Congenital Abnormalities of the Gallbladder. Congenital anomalies of the gallbladder are also rare, and although they may predispose the patient to inflammation and cholelithiasis, they are often found only incidentally during abdominal imaging or surgery.

Absent Gallbladder. The gallbladder can be absent, without any apparent symptoms, or can be associated with other conditions, such as extrahepatic biliary atresia. In biliary atresia, the gallbladder either fails to vacuolize from a solid state during development or postnatally becomes obstructed or obliterated. Because the mechanism of biliary atresia is unknown, the cause of the absent or "rudimentary" gallbladder also remains speculative. The gallbladder may appear to be absent in cystic fibrosis, in which recurrent attacks of cholecystitis can lead to fibrosis and/or atrophy of the gallbladder.[96]

Double Gallbladder. There are a number of forms of this anomaly. There can be a true duplicate set of gallbladders, each with its own cystic duct, which arise from an outpouching of the hepatic or common bile duct. If the pouch derives from the cystic duct, there are two gallbladders but only one cystic duct. A bilobed gallbladder appears to be similar, with two gallbladder fundi but only one cystic duct, but this anomaly arises from a duplication of the single bud, which normally develops into the gallbladder. All of the above anomalies are rare and are usually asymptomatic.[97]

Miscellaneous. The gallbladder can be abnormally folded during development, and the appearance on cholecystograms of the folded gallbladder is that of a type of bonnet or "phrygian cap," for which the condition is named.[97] The gallbladder empties and functions normally.[2] The development of ducts can also be abnormal, and 20% of people have an aberrant insertion of the cystic duct into the common hepatic duct.[97] The cystic artery, which usually arises from the right hepatic artery, can arise aberrantly from the left. These anomalies are of great importance during cholecystectomy. Finally, the position of the gallbladder can vary; in situs inversus, the gallbladder is in a normal relationship with the liver, but the liver is now on the left side of the abdominal cavity. The gallbladder can appear to be buried in the liver or it can be excessively mobile, which increases the risk of torsion. The latter two anomalies are important for radiologic interpretation.

CONCLUSION

Gallbladder disease in children is an uncommon, although not rare, problem. Clinicians are reporting increased numbers of cholecystectomies in children. It is not yet clear if this represents a true increase in the incidence of gallstones in children or more ready access to laparoscopy. However, the current epidemic of childhood obesity is likely to lead to an increased frequency of cholesterol gallstone disease either in adolescence or early adulthood, and we will all become more familiar with this disease.

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CHAPTER 62

CONGENITAL ANOMALIES

Dominique M. Jan, MD

Congenital abnormalities of the pancreas are rare. These anomalies are more commonly discovered at endoscopy, at surgery, or by imagery. This chapter focuses on the congenital malformations of the pancreas, which could lead to clinical findings.

EMBRYOLOGY OF THE PANCREAS

Human pancreatic development has been known since the work of Streeter in 1942.[1] The pancreas forms as a result of the fusion of the two buds, which arise from the dorsal and ventral aspects of the distal foregut. Owing to craniocaudal development, the dorsal pancreas develops ahead of the ventral pancreas. The ventral bud forms the dominant pancreatic and the bile duct. The ventral pancreas rotates clockwise around the duodenal axis. The rotation of the duodenum is the result of duodenal growth. In the normal course of development, between the 8 and 12 mm stages (the fourth to sixth week), the common duct and the right portion of the ventral bud are carried dorsally around the circumference of the duodenum to lie adjacent to the dorsal pancreatic bud. This rotation is the result of duodenal growth, during which all enlargement is on the ventral side only. Thus, the dorsal pancreatic bud forms the anterior part of the head of the pancreas, the body, and the tail of the pancreas.[2] The ventral pancreatic bud forms the posterior part of the head of the pancreas and the posterior part of the uncinate process. The ventral bud is divided into right and left portions; the left portion atrophies, whereas the right portion is moved posteriorly by its connection to the bile duct. The dorsal and the ventral duct systems fuse so that most of the longer dorsal duct drains into the proximal part of the ventral duct to form the main pancreatic duct (duct of Wirsung). If the proximal portion of the dorsal duct remains, it forms an accessory duct (duct of Santorini). The fusion of the ducts occurs during the second month of development. Failure of the dorsal and ventral ducts of the fetal pancreas to fuse leads to pancreas divisum.

More recently, tissue culture and recombination experiments have shown that commitment to a pancreatic fate occurs before morphologic evidence of pancreatic development as early as embryonic day 5-8 in mice and stage 11 in chickens.[3] Experiments in mouse and chicken embryos have revealed that permissive signals secreted by adjacent mesodermal structures (notochord, aorta, and cardiac mesoderm) are important for the pancreatic program. The dorsal foregut (giving rise to the pancreas) is in close contact with the notochord. The ventral foregut is in close contact with the cardiac mesoderm (Figures 62-1 and 62-2).

Initial bud formation relies on different genes dorsally and ventrally. The expression of the homeobox gene *Hlxb9* is necessary to the dorsal bud formation.[4] The gene is active by controlling the expression of signaling molecule produced in the notochord (fibroblast growth factor 2 and activin-βB).[5] The mesenchymal tissue adjacent to the buds is important for all of the embryologic processes, expansion, branching, and differentiation.

The only gene clearly demonstrated to be causal to congenital anomalies of the pancreas formation is the gene coding for the homeodomain protein PDX1. It is expressed in the endoderm of the foregut at the regions where the ventral and the dorsal buds will form. In PDX1 null mutant mice, the ventral bud forms but fails to grow, and the dorsal bud grows but never forms a functional pancreas. Mutation in this protein has been identified in a patient with pancreas agenesis.[6]

Annular pancreas is usually sporadic, but familial descriptions have been reported with an apparent autosomal dominant transmission. Inactivation of a signaling molecule Sonic hedgehog (Shh) has been described as a potential mechanism that can lead to annular pancreas.[7] Shh is expressed in the endoderm in the regions lateral to the PDX1-expressing endoderm.

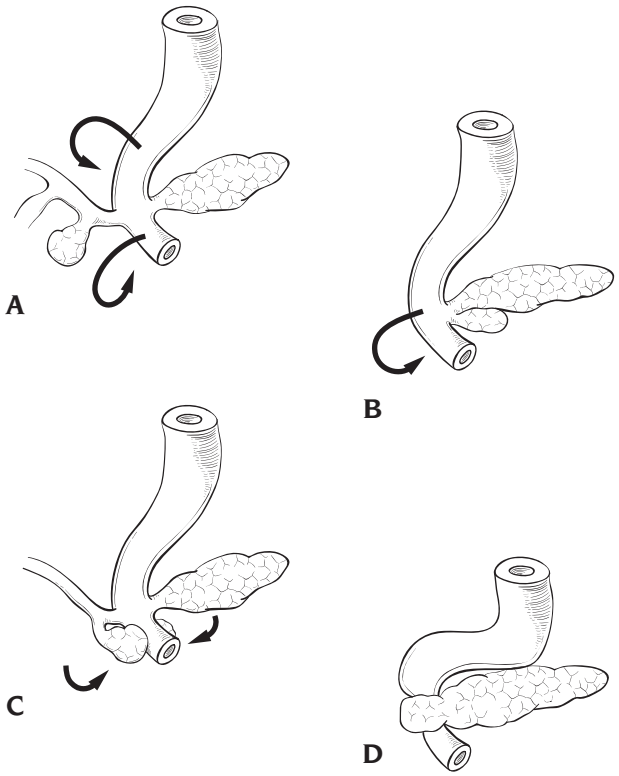

FIGURE 62-1 Embryology of the pancreas. *A*, Eight to 12 mm stage; *B*, fusion of the ducts; *C*, malrotation of the right portion of the ventral bud; *D*, annular pancreas.

Pancreas divisum, which is the most common anomaly of the pancreas, affects 5 to 6% of the population. Inappropriate fusion of the ventral and pancreatic buds has been reported in mice heterozygous for null alleles of Shh.[7]

Aberrant localization of pancreatic tissue affects 0.6 to 2% of the population.[8] The most common sites of pancreatic heterotopias are the stomach, duodenum, and Meckel diverticulum. Because inhibition of Shh leads to pancreatic ectopia in chickens, this signaling molecule may be involved in this anomaly in humans.[9]

ANNULAR PANCREAS

Annular pancreas is the complete encirclement of the second part of the duodenum in a ring-like fashion by a thin, flat band of pancreatic tissue. The annular tissue is histologically normal, containing both acini and islet cells. The anomaly may be associated with partial or complete duodenal obstruction. The annular pancreas occurs with a frequency of 1 in 20,000 births but is more commonly found in neonatal duodenal obstruction (8–21%).[10]

Pathogenesis

Annular pancreas may be the result of a hypertrophy of the normal pancreas tissue by failure of atrophy of the left ventral bud. The fixation of the ventral pancreas before the onset of rotation leads to formation of the annulus.[11] This theory and its chronology are supported by most of the fetal evidence reported. The ventral origin of the annulus has been confirmed immunohistochemically.

Pancreatic tissue often penetrates the muscularis of the duodenum. A large duct is usually present that connects to the Wirsung duct.

Annular pancreas is associated with a number of other congenital malformations in more than 75% of children.[12] This suggests that the defect of annular pancreas is the result of an early embryologic malformation.

Clinical Presentation

Symptomatic annular pancreas may present at any age, from birth through adult life. Approximately one-third of the cases are symptomatic during the neonatal period and half during the first year of life.[12,13] The age at presentation is determined by the degree of duodenal obstruction and by coexistent malformations.

The diagnosis of annular pancreas associated with duodenal obstruction may be suggested antenatally by polyhydramnios. The diagnosis of duodenal obstruction may be confirmed by antenatal ultrasonography.[14]

In the newborn period, the diagnosis is based on the typical appearance of a double-bubble sign of gastric and duodenal obstruction on supine and upright radiographs of the abdomen. Contrast studies are not necessary for the diagnosis unless the differential diagnosis of volvulus with malrotation is discussed.

In children beyond the neonatal period and in adults, symptoms differ because they are more likely recurrent vomiting secondary to partial duodenal obstruction and chronic gastric distention, pain as the result of mild pancreatitis, or peptic ulcers, as have also been described in patients with annular pancreas.[9] In older patients, different investigations may help the diagnosis. Upper gastrointestinal studies are useful, and ring-like smooth symmetric narrowing of the duodenum is observed in the majority of symptomatic children. Contrast-enhanced computed tomography allows direct visualization of the annular pancreas. More recently, magnetic resonance cholangiopancreatography (MRCP) has been used to visualize the pancreatic ducts and the bile duct and can demonstrate the pancreatic duct of the annular portion of the pancreas.[15] In teenagers and adults, diagnosis has also been achieved using endoscopic ultrasonography.[16]

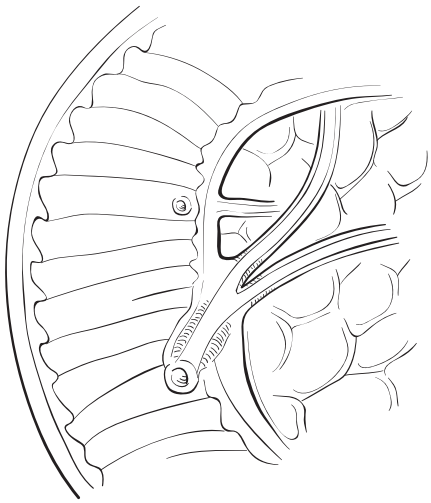

FIGURE 62-2 Normal patterns of the pancreatic ducts.

Management

Surgical management of annular pancreas is mostly concerned with relief of duodenal obstruction. The definitive diagnosis is made at the time of the laparotomy. Direct dissection of the annular ring is not recommended. Such attempts may be technically difficult, and the division of the annular pancreatic duct is associated with a high risk of pancreatic peritonitis or postoperative pancreatitis, fistulae, and late fibrosis. A large duodenoduodenostomy is recommended as a bypass operation. However, the morbidity is high with duodenal functional obstruction owing to the remaining dilated proximal duodenum. Duodenoplasty of the proximal duodenum and diamond-shaped duodenoduodenostomy may reduce the complication rate. The long-term prognosis is excellent.[13]

ECTOPIC PANCREATIC TISSUE

Pathogenesis

Ectopic pancreas is defined as the presence of pancreatic tissue lacking anatomic and vascular continuity with the main body of the pancreas. Islands of pancreatic tissue may be found in many sites and have been reported in 0.5 to 1.5% of autopsies.[17] Ectopic pancreatic tissue has been located most commonly in the pylorus, duodenum, Meckel diverticulum, and, less frequently, in the colon, appendix, gallbladder, and anomalous bronchoesophageal foregut fistulae.

Ectopic pancreatic tissue has been observed in knockout mice for homeobox gene *Cdx2*. Inhibition of Shh signaling also leads to ectopic pancreas in chickens.[18] The pathogenesis remains unclear because many theories are not demonstrated. A hypothesis regarding ectopic migration of pancreas precursor to in situ error of stem cell differentiation is still controversial. This theory is supported by the expression of PDX1, a marker of pancreatic progenitor cells, in the antral stomach, duodenum, and small bowel, the main localizations for ectopic pancreas.

Clinical Presentation

In most cases, ectopic pancreas remains asymptomatic. It is an incidental finding during surgery for another indication. Because the ectopic pancreas may occasionally cause complications, management remains controversial. Some recommend no further investigations and management; others recommend local resection as ileal resection of the Meckel diverticulum to avoid any further bleeding.

The most common clinical findings are gastrointestinal hemorrhage secondary to mucosal ulcerations close to the pancreatic tissue,[19] pain secondary to pancreatitis and obstruction in prepyloric localization in newborns, intussusceptions, and exceptional malignant transformation of the ectopic tissue in papillary neoplasm.[20,21]

Diagnosis

The diagnosis could be made endoscopically or radiographically in antral or prepyloric localizations. In other localizations, diagnosis is made at the time of surgery. A definitive diagnosis is made histologically.

Management

The treatment of symptomatic ectopic pancreas is surgical. Excision is indicated as Meckel diverticulectomy or antral or ileal resection.

PANCREATIC AGENESIS AND HYPOPLASIA

Complete agenesis of the pancreas is a lethal condition that has been reported in the literature. Lack of insulin leads to intrauterine growth retardation, hyperglycemia, coma, and a rapid fatal issue.[22]

Partial anatomic pancreatic agenesis is unlikely to be symptomatic because the endocrine and exocrine functions are normal. Agenesis of the dorsal pancreas has been described in diabetes and pancreatitis.[23] Magnetic resonance imaging can assist in making the diagnosis.

Agenesis of the acinar tissue occurs in Shwachman syndrome, autosomal recessive disorder with exocrine pancreas deficiency, growth retardation, skeletal anomalies, and bone marrow dysfunction.[24] The prognosis is poor until bone marrow transplant.

DUCTAL ANOMALIES

Any variation of the normal patterns of the dorsal and ventral ducts leads to a number of common ductal anomalies. Some anomalies are pure anatomic variations without any clinical significance, for example, the absence of the duct of Santorini or the absence of a connection between the accessory duct and the main duct. Two ductal anomalies have been implicated in the pathogenesis of clinical disease: the failure of the fusion of the dorsal and ventral ductal systems, which results in a ductal pattern known as pancreas divisum,[25] and the pattern of the junction with the common bile duct.

PANCREAS DIVISUM

Pathogenesis

The ducts remain separated, and the dorsal pancreas, which is the main drainage of the pancreas, empties into the duodenum via the smaller accessory papilla. Multiple variants of the divisum anomalies have been described anatomically or after endoscopic retrograde cholangiopancreatography (ERCP).[26] The small accessory duct leads to functional obstruction of the pancreas and pancreatitis (Figure 62-3).

Clinical Presentation

The importance of the abnormalities lies in its presumed relationship with documented pancreatitis not attributable to alcohol, infection, or biliary tract disease. Because there is a large gap between the anatomic incidence and the frequency of these documented cases of pancreatitis, the causal relationship remains controversial. Pancreas divisum was identified in 7.4% of all children with pancreatitis and 19.2% of children with relapsing or chronic pancreatitis.[27]

Diagnosis

Diagnosis of pancreas divisum depends on ERCP in children older than 3 years and/or MRCP to demonstrate the

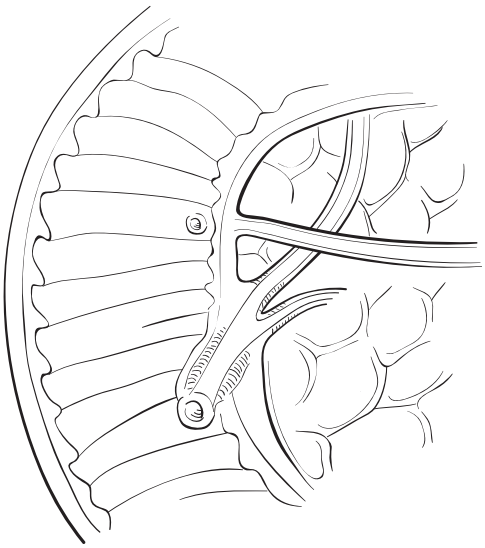

FIGURE 62-3 Ductal anomalies—pancreas divisum.

visualization of the duct of Santorini draining the pancreas. To confirm the diagnosis, the accessory papilla should be cannulated to visualize the duct of Santorini.[28]

Management

Because the causal relationship between pancreas divisum and pancreatitis is controversial, management is the subject of many discussions. Despite medical management of the episodes of pancreatitis, the visualization of a pancreas divisum leads to endoscopic treatment. Beneficial results could be expected by endoscopic enlargement of the accessory papilla. Surgical or endoscopic intervention is directed toward relief of ductal obstruction by a transduodenal sphincteroplasty.[29]

COMMON CHANNEL SYNDROME

Pathogenesis

Anomalies of the junctions of the common bile duct and the main pancreatic duct may be clinically significant. The extrahepatic bile duct is developed from the ventral pancreatic duct. Initially, the junction is extraduodenal and moves toward the duodenum wall. A failure of this progression toward the duodenum results in an abnormally long common pancreatobiliary channel. The junction remains outside the duodenum wall and is therefore not surrounded by the normal sphincter mechanism. The long common pancreatobiliary channel usually exceeds 10 mm in length compared with the normal estimated length of 5 mm in children. This may permit reflux of the pancreatic enzymes containing trypsin into the common duct, with resulting damage to the ductal wall. Occasionally, bile may reflux into the pancreatic duct as well.

Pancreaticobiliary reflux has been confirmed by dynamic MRCP after secretin stimulation.[30]

Clinical Presentation

Reflux of ductal contents in either direction may lead to two different clinical situations: choledochal cyst and pancreatitis. Common channel syndrome (Figure 62-4) is identified in 75% of the children with choledochal cyst. Recurrent pancreatitis is observed in 70% of choledochal cysts even after surgical disconnection of the bile duct from the pancreatic duct. Pancreatitis without choledochal cyst is related to bile reflux in the pancreatic duct with ductal ectasia.

Diagnosis

Abdominal ultrasonography is often diagnostic of choledochal cyst and is an indication for further imaging studies.

MRCP is noninvasive and can be performed in children without the use of contrast agents. It demonstrates the choledochal cyst, the common channel, and pancreatic ductal ectasia. Invasive imaging studies are mandated only if there is no clear evidence of congenital malformation and if the bile duct dilatation can be related to choledocolithiasis.

Percutaneous transhepatic cholangiography and ERCP are the most effective imaging studies to demonstrate the common channel.

In children less than 3 years of age, MRCP or percutaneous transhepatic cholangiography can be performed safely under general anesthesia. In older children, MRCP or ERCP can be discussed, but ERCP should be avoided in episodes of acute pancreatitis.

Management

Radical excision of the choledochal cyst and reconstruction by a Roux-en-Y hepaticoenterostomy is the treatment of choice for choledochal cyst.

Good results can be achieved with hepaticoenterostomy.[31] Postoperative complications are uncommon. Cholangitis and pancreatitis are the most frequent complications and could lead to imaging reassessment for anastomotic stricture or hepatic duct or pancreatic duct calculi. Malignancy of the biliary enteric anastomosis has been reported even many years after radical excision.[32]

Pancreaticojejunostomy and/or endoscopic sphincteroplasty may be curative in exceptional common channel syndrome without choledochal cyst but with pancreatic ductal ectasia.

FIGURE 62-4 Ductal anomalies—common channel syndrome.

OTHER CONGENITAL ANOMALIES

Congenital Pancreatic Cysts

Congenital pancreatic cysts are uncommon. Unilocular or multiple congenital cysts are lined by an epithelium. The presentation is variable, including asymptomatic cysts, abdominal masses, and pancreatitis. The cysts may be associated with other anomalies, such as polydactyly and anorectal malformations. Multilocular cysts may be an isolated pancreatic lesion, as well as part of von Hippel-Lindau disease, with hereditary cerebellar cysts, hemangiomas of the retina, and kidney and liver cysts.[33]

Enteric Duplication Cysts

Intrapancreatic or juxtapancreatic gastric or duodenal duplications with ductal communications are uncommon. Symptoms may include failure to thrive, abdominal pain, and episodes of pancreatitis. The lesion may be recognized by ultrasonography, upper gastrointestinal studies, and MRCP.

Exploration is necessary to excise the lesion and to alleviate recurrent episodes of pancreatitis. Excision of the duplication is performed close to the duodenal wall without pancreaticoduodenal resection.[34]

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CHAPTER 63

TUMORS

Assad Butt, MB, BS, DCH(Lon), MRCP, CPCH

Pancreatic tumors and lesions that may mimic tumors rarely occur in childhood. They can present major diagnostic and therapeutic challenges. Pancreatic cancer in adults is the fifth leading cancer in the United States and has the poorest survival rate of the major malignancies, accounting for more than 25,000 deaths annually. In contrast, childhood pancreatic cancer represents less than 5% of all malignancies affecting children under the age of 15 years.[1,2]

Recent advances in genetics and immunohistochemistry have resulted in better understanding of the underlying pathogenetic mechanisms and biologic behavior of these lesions. Improvements in imaging techniques, including the application of newer modalities such as endoscopic ultrasonography (EUS) and magnetic resonance cholangiopancreatography (MRCP), have helped with better localization, improved noninvasive characterization, and tissue sampling of lesions.[3–5]

The World Health Organization classification of pancreatic tumors is currently one of the most widely accepted. This classification is based on the nature of the tissue of origin and relative malignant potential of the tumor (Table 63-1).[6] Although not all of the tumor subtypes have been reported in children, this classification provides a useful framework for considering the differential diagnosis and discussion of pancreatic tumors in children.[7]

PRIMARY EPITHELIAL EXOCRINE TUMORS

Benign and malignant exocrine tumors of the pancreas can be either cystic or solid and can arise from either ductal or acinar tissue. There is a spectrum of biologic behavior and tumor aggressiveness between different tumor groups.

CYSTIC TUMORS

Pancreatic cystic tumors are an important group clinically because other cystic lesions of the pancreas may mimic them. Pseudocysts are the most common cystic lesions encountered in childhood, accounting for about 75% of pancreatic cystic lesions. They occur either secondary to trauma or chronic pancreatitis resulting from other causes and are discussed in detail in Chapter 64, "Pancreatitis." Their diagnosis may be confused with cystic neoplasm and may result in inappropriate management.[8] The investigation and differential diagnosis of cystic neoplasia are considered below.

SEROUS CYSTADENOMA

Two types of serous cyst adenoma exist: serous microcystic adenoma and serous oligocystic adenoma. Both rarely occur in children.

Serous microcystic adenomas have a female predominance and are more commonly found in the pancreatic body and tail.[9] One-third of these lesions are asymptomatic and found incidentally, whereas the remainder have symptoms related to pressure of the tumor on adjacent structures.[7] The lesions are benign tumors consisting of numerous small cysts arranged around a central stellate scar. They are lined by epithelial cells and show evidence of ductular differentiation.[10] No specific tumor markers identify this lesion. Radiographs may show calcification in some patients. Computed tomography (CT) reveals a well-demarcated multiloculated cyst, on occasions demonstrating the central stellate scar.

Surgical excision of the tumors is usually unnecessary unless the patients are symptomatic. However, the histologic diagnosis must be confirmed.[7]

Serous oligocystic adenomas are benign tumors with an equal sex predominance that occur more rarely in children than the serous microcystic adenoma. The etiology is unknown; however, it is of interest that two reports in children have isolated cytomegalovirus from adjacent pancreatic tissue.[11,12]

Most tumors are located in the head and body of the pancreas and are composed of a few relatively large cysts showing evidence of ductular differentiation. Infants usually present with a palpable abdominal mass. Resection is needed if lesions are symptomatic.

MUCINOUS CYSTADENOMAS

This benign cystic pancreatic tumor is closely related to a spectrum of mucinous cystic tumors that includes mucinous cystadenocarcinoma and consequently has a definite malignant potential. There is a strong female preponderance. The majority of tumors are located in the tail of the pancreas.[9] The tumors usually consist of a solitary large unilocular cyst composed of mucin-secreting epithelial cells. Because of the malignant potential, wide surgical excision, including the associated capsule, is always recommended.[10]

TABLE 63-1 CLASSIFICATION OF PANCREATIC TUMORS

PRIMARY	EXAMPLE OF TUMOR TYPE
EPITHELIAL	
Exocrine	
Benign	Serous cystadenoma, mucinous cystadenoma (premalignant), mature cystic teratomas, papillary cystic tumor
Borderline malignant	Papillary cystic tumor
Malignant	Mucinous cystadenocarcinoma, ductal adenocarcinoma, acinar adenocarcinoma, pancreatoblastoma
Endocrine	
Benign	Insulinoma (90–95%), gastrinoma (40%), VIPoma (50%), GRFoma (67%), glucagonoma (40%), somatostatinoma (25%)
Borderline malignant	No pediatric example exists
Low-/high-grade malignant	Insulinoma (5–10%), gastrinoma (50%), VIPoma (33%), GRFoma (33%), glucagonoma (60%), somatostatinoma (75%), MEN type I (associated with gastrinoma, insulinoma, and VIPoma in decreasing frequency, respectively), islet cell carcinoma (functional or nonfunctional lesions)
NONEPITHELIAL	
Benign	Fibrous histiocytoma, juvenile hemangioendothelioma, lymphangioma, glomus tumors, myofibromatosis
Malignant	Rhabdomyosarcoma, lymphosarcoma, lymphomas
SECONDARY	
Malignant	Adenocarcinoma (local spread from stomach, intestine, biliary tract), malignant melanoma, leukemia, renal and lung tumors (hematogenous spread)
TUMOR-LIKE LESIONS	
Exocrine	Pancreatic cysts, chronic pancreatitis, ductal changes (eg, squamous metaplasia, mucinous cell hypertrophy, ductal papillary hyperplasia, adenomatoid duct hyperplasia), acinar changes (eg, focal acinar transformation), heterotopic pancreas, heterotopic spleen in pancreas, hamartomas, inflammatory pseudotumors
Endocrine	Islet cell hyperplasia, islet cell dysplasia, persistent hyperinsulinemic hypoglycemia of infancy (nesidioblastosis)

Adapted from Kloppel H et al.[6]
GRF = growth hormone releasing factor; MEN = multiple endocrine neoplasia; VIP = vasoactive intestinal polypeptide.

CYSTADENOCARCINOMA

SEROUS CYSTADENOCARCINOMA

This entity has not been reported in the pediatric literature.

MUCINOUS CYSTADENOCARCINOMA

The clinicopathologic features of this malignant tumor are similar to that of its benign counterpart, the mucinous cystadenoma. However, the tumor is invasive, tending to spread locally in the same way as ductal adenocarcinoma. The incidence is half that of mucinous cystadenomas. The prognosis is excellent if resection is complete.[9,10]

MATURE CYSTIC TERATOMAS ("DERMOID CYSTS")

These lesions are benign extragonadal germ cell cysts derived from all three germinal layers and may include hair, teeth, cartilage, sebaceous material, and sweat glands. They are rarely found in the pancreas but can occur anywhere in the substance of the pancreas or be attached to it.[13–15] Excision is the treatment of choice.

PAPILLARY CYSTIC TUMOR

Papillary cystic tumors of the pancreas are exceptionally rare neoplasms in children. Since their original description by Frantz in 1959, more than 400 cases have been reported in the English literature (mean age 23.9 years; range 2–74 years). By 1999, approximately 90 cases were reported in children (age group < 18 years).[16] Such cases represent 8 to 17% of all pediatric pancreatic neoplasms.[17] The tumors occur most frequently in young females (91% of cases), especially adolescent girls, and there may be a preponderance in Asian and black patients.[16–18] There is no conclusive evidence for the role of sex hormones in the pathogenesis of papillary cystic tumors; however, they may have an influence on tumor growth. Patients typically present with vague gastrointestinal systems such as upper abdominal discomfort or pain caused by an enlarging and often palpable abdominal mass. Because of slow tumor growth, patients often remain asymptomatic until the tumor has enlarged considerably or is detected incidentally on ultrasound imaging for an unrelated indication. There is usually no evidence of endocrine or exocrine dysfunction, and liver function and endocrine tests are normal.[16,17,19]

The tumors are slow growing and of low malignant potential. The body and tail are more frequently affected (64%) than the head of the pancreas, with a well-circumscribed tumor mass of average diameter between 8 and 10 cm.[16] Macroscopically, the tumors are round or oval, encased by a fibrotic capsule and showing intermingling solid and overt cystic-hemorrhagic areas on the surface. Microscopically, typical features are sheets and cords of polygonal cells comprising pseudopapillary structures with fibrovascular stalks or pseudorosettes.[16] Immunohistochemically, they may express various markers, including neuron-specific enolase (91/108 cases), α_1-antitrypsin (101/132 cases), vimentin (47/65 cases), progesterone (11/31 cases), and estrogen (3/56 cases), as well as other neuropeptides.[17] Most studies have not demonstrated reactivity for pancreatic hormones; importantly, this distinguishes papillary cystic tumors from nonfunctioning endocrine tumors, which may look similar on microscopic histologic assessment.[16]

Tumor invasion of adjacent organs has been reported but is rare.[20,21] The treatment of choice is complete excision of the lesion. Overall, more than 95% of patients are cured. Long disease-free intervals have been recorded after

initial resection even in patients whose tumors have either spread locally or metastasized.[21,22] More aggressive tumors are seen in older patients, tumors with a high deoxyribonucleic acid (DNA) index, aneuploidy, frequent mitotic figures, and/or nuclear atypia.[23] The origins of this tumor remain enigmatic. In a limited number of cases, chromosomal abnormalities have been found that are known to be associated with oncogenes. The chromosomal analyses have shown unbalanced translocations between chromosomes 11 and 14, loss of the X chromosome, and trisomy 3.[23] Recent studies suggest that there are abnormalities in the β-catenin or adenomatous polyposis coli (APC) gene pathways, influencing the pathways of neoplastic progression in papillary cystic tumors.[24,25] In Abraham and colleagues' study, a series of 20 papillary cystic tumors were analyzed by immunohistochemistry and molecular genetic techniques.[25] Almost all papillary cystic tumors harbored alterations in the APC–β-catenin pathway. Nuclear accumulation of β-catenin protein was present in 95% (19/20 cases), and activating β-catenin oncogene mutations were identified in 90% (18/20 cases) of the papillary cystic tumors. These findings are similar to those alterations recently identified in other nonductal pancreatic neoplasms (pancreatoblastomas and acinar carcinomas). In contrast, genetic alterations commonly found in pancreatic ductal neoplasms (ductal adenocarcinoma) were absent or detected only invariably.[25]

APPROACH TO INVESTIGATION AND DIAGNOSIS OF CYSTIC TUMORS

A precise diagnosis of all pancreatic cystic lesions is of key importance to exclude cystic pancreatic neoplasia. A more detailed discussion of non-neoplastic lesions is considered in Chapter 62, "Congenital Anomalies of the Pancreas."

Ultrasonography is the most useful initial diagnostic test and can confirm the cystic nature of the lesions. Endoluminal ultrasonography has been recently proposed for delineating small cystic lesions and obtaining tissue samples by fine-needle aspiration to aid specific diagnosis and thus help differentiate benign from potentially malignant lesions (Figure 63-1). One recent adult study suggests high sensitivity (91%), specificity (60%), and accuracy (82%) for EUS in identifying malignant or potentially malignant pancreatic cystic lesions compared with surgical histopathology.[4] The study included 34 patients (16 men, 18 women; mean age 55 years) who underwent surgery for suspected pancreatic cystic lesions based on CT and transabdominal ultrasonography. In addition to EUS, other assessments did not improve overall evaluation of the lesions. The respective sensitivity, specificity, and accuracy were (1) cyst fluid cytopathology, 27%, 100%, and 55%, and (2) carcinoembryonic antigen, 28%, 25%, and 27%. Although this approach has been applied recently to children and shows promise, it needs further evaluation.[3]

CT and/or magnetic resonance imaging (MRI) have an established role in determining the nature of a cyst and its precise relationship to the pancreatic duct and surrounding tissues.[26–28] The advent of MRCP has provided a further tool for evaluation. One recent study suggests that this modality has a distinct advantage over endoscopic retrograde cholangiopancreatography in the diagnosis of mucin-producing tumors of the pancreas and cystic lesions. MRCP provides a better image of the entire lesion, including the distal part that is obstructing the pancreatic duct.[5] Use of findings from multiple modalities can be helpful in formulating a differential diagnosis. Selective celiac and gastroduodenal angiography have been suggested as useful diagnostic tools in selected patients. [29]

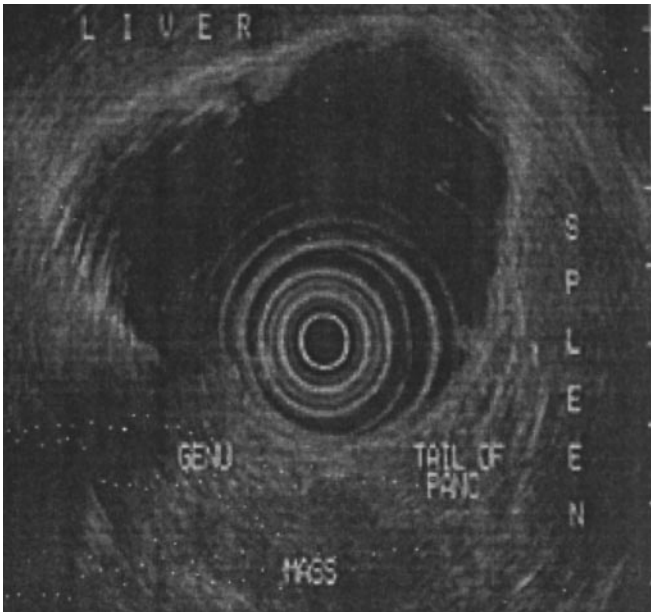

FIGURE 63-1 Papillary cystic neoplasm in a young girl as visualized via endoscopic ultrasonography. Reproduced with permission from Victor Fox, The Children's Hospital, Boston.

Despite advances in imaging techniques, definitive preoperative diagnosis is not possible in many cases (Figure 63-2). In a recent multi-institutional review of 398 cases of cystadenomas and cystadenocarcinomas of the pancreas, 93% of cases required surgery to establish a definitive diagnosis.[30] The study reported cases of serous cystadenoma

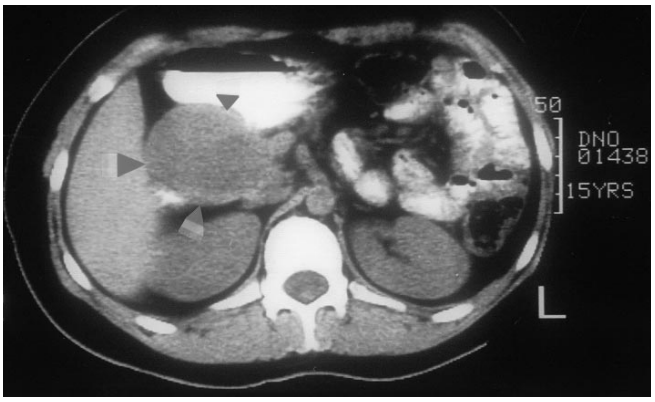

FIGURE 63-2 Appearance of a tumor in the head of the pancreas seen on an unenhanced computed tomographic scan. A definitive diagnosis of papillary cystic tumor was established postoperatively. Reproduced with permission from Professor L Spitz, The Institute of Child Health, Great Ormond Street Children's Hospital, London.

(n = 140), mucinous cystadenoma (n = 150), and mucinous cystadenocarcinomas (n = 78).[30] In approximately one-third of each group, the tumor was asymptomatic at presentation. Preoperative diagnostic accuracy was poor: 20% in cases of serous cystadenoma, 30% in cases of mucinous cyst adenoma, and 29% in those diagnosed with mucinous cystadenocarcinoma. Furthermore, even the utility of intraoperative frozen section allowed for definitive histologic diagnosis in only 50% of cases of serous and mucinous cystadenomas and 62% of cases of mucinous cystadenocarcinoma. Consequently, conservative management was warranted in only 7% of cases who had asymptomatic well-documented serous cystadenoma based on a combination of findings from spiral CT, EUS, and/or diagnostic aspiration of cyst.[30]

ADENOCARCINOMA

Adenocarcinoma of the pancreas exists in two malignant forms arising from ductal or acinar tissue. Ductal adenocarcinoma is by far the most common malignant pancreatic tumor in adults compared with acinar adenocarcinoma, which occurs in only 11%. In contrast, the incidence in children is more evenly distributed, with 53% of the carcinomas of ductal origin and 47% of acinar origin. The total number of pediatric cases reported in the English literature is very small, accounting for only 30 cases in total up to 1983.[31,32]

A combination of genetic and environmental factors most likely contributes to the pathogenesis of pancreatic adenocarcinoma. Genetic predisposition is suggested by an increased incidence in patients with hereditary pancreatitis, as well as a report of four otherwise healthy siblings of an index case of pancreatic ductal carcinoma who also developed this condition.[33]

A number of other conditions have also been linked to a potential increased risk of developing pancreatic carcinoma. These have included hamartomatous polyposis syndromes such as Peutz-Jeghers syndrome, as a direct result of functional deletion of tumor suppressor genes,[34] and celiac disease or dermatitis herpetiformis. However, an increased risk is not observed in children and adolescents with celiac disease. Epidemiologic studies have suggested an increased risk with environmental or other factors, such as men exposed to degreasing agents and women who smoke or have uterine myomas or prior oophorectomy.[35] In addition, higher social class, increased consumption of animal fat and protein, and wine consumption are potential risk factors.[35] With emergence of the field of transplant in recent years, an increased risk of developing de novo malignancies in this group should also be considered. In a series of 1,151 organ allograph recipients, 1,813 de novo malignancies were found, of which 755 involved the hepatobiliary-pancreaticoduodenal area.[36] Whereas lymphomas comprise the majority (63% of cases), pancreatic carcinomas accounted for 11% of the total.[36,37] Down syndrome shows an interesting tumor profile; whereas most solid tumors are underrepresented, pancreatic tumors are in excess.[38] Patients with cutaneous malignant melanoma have an increased risk of subsequent primary carcinoma.[39]

Ductal Adenocarcinoma

The malignant and aggressive nature of ductal adenocarcinoma relates to its ability to spread along the ducts to the capsule of the pancreas, adjacent lymph nodes, and retroperitoneal space.[31] Patients typically present with abdominal pain and weight loss. More than half of the patients present with obstructive jaundice. Additional symptoms include those of gastrointestinal bleeding, pancreatitis, and depression.[40]

The tumors are usually located in the head of the pancreas. They often generate a dense fibrotic reaction, resulting in a compact hard retroperitoneal mass. CT often demonstrates these lesions as irregular heterogeneous enhancing lesions without a visible capsule.[41] Histologically, they appear as moderately well-differentiated ductal carcinoma cells containing mucin but lacking zymogen.[7,40] Immunohistochemically, they are almost all positive for carcinoembryonic antigen.[7]

Cytogenetic studies show specific chromosomal abnormalities, including the loss of 9p, 17p, and 18q, as well as gains of 8q and 20q. Analysis of pancreatic fluid obtained at the time of endoscopic retrograde cholangiopancreatography by fluorescent in situ hybridization in 12 affected patients revealed a loss of 18q in 92%.[42] Further studies have shown that 70% of these mutations resulted in activation of oncogenes (ie, Ki-*ras*, C-*erb*B12) or in activation of tumor suppressor genes (*TP53*, *MTS1*, *Smad4*, $P16^{INK4}$).[42] One study found Ki-*ras* gene mutations at codon 12 in about 90% of cases and abnormal expression of the C-*erb* B12 oncogene in nearly 20% of cases.[43] A recent report suggests that a genetic diagnosis of ductal adenocarcinoma can be made by analysis of pancreatic fluid collected endoscopically for Ki-*ras* mutation analysis of both the supernatant and sediment of pancreatic fluid.[44] Other studies have shown alterations in those factors that may promote (*bax*) or block (*bcl-2*) apoptosis.[45] Studies have also shown an important role for tumor growth factor (TGF)-β and its signaling proteins in (1) allowing for tumor cell anchorage–independent growth,[46] (2) enhancing tumorigenicity by the TGF-β signaling inhibitor *Smad7*,[47] and (3) influencing disease progression (overexpression of type 2 TGF-β receptor may be a marker that correlates with disease progression and is associated with decreased patient survival).[48] Finally, recent studies have suggested an increased expression of inducible nitric oxide synthase in human pancreatic cancer, which correlates positively with the degree of apoptosis as expressed by the apoptotic index. These findings could, in the future, provide the basis for the development of potential therapeutic strategies.[49]

Only 10 to 20% of cases are amenable to curative surgical resection. A Whipple procedure is typically performed when the tumor is confined to the head of the pancreas. In addition to removal of the tumor, en bloc resection of the distal stomach, duodenum, and common bile duct is performed. Subsequently, continuity is restored by fashioning a pancreaticojejunostomy, choledochojejunostomy, or gas-

trojejunostomy.[40] In general, the 3-year survival rate is approximately 2%, with a mean survival after diagnosis of 4 to 6 months.[50] The core prognosis is related to a delay in diagnosis because 85% of tumors have metastasized at the time of diagnosis.[51] Palliation with chemotherapy, either single agent or in combination, and use of external beam radiation are of value for a limited duration only. A limited experience with high linear energy transfer particle (neutron or heavy iron) radiation therapy has produced significant decreases in tumor size but no improvement in patient survival.[40] A number of patients have gained some relief from intractable peritoneal pain with celiac plexus neurolysis.[52]

Acinar Adenocarcinoma

Acinar adenocarcinoma has already metastasized in most patients by the time a diagnosis is made. The tumor typically presents more commonly in males and in a reported age range from 3 to 90 years.[53,54] Symptoms at presentation are attributable to local expansion of the tumor or metastases. About 15% of patients present with a syndrome involving polyarthralgia, extrapancreatic fat necrosis, and eosinophilia.[53]

The tumor is a well-circumscribed nodular mass that occurs evenly throughout the pancreas. Ultrasonography shows a midrange echogenic mass. Dual-phase helical CT usually reveals large encapsulated lesions of lower attenuation with or without calcified and necrotic areas.[41] Histologically, acinar tumor cells are arranged in a ribbonlike configuration and contain eosinophilic cytoplasm and periodic acid–Schiff–positive zymogen granules on electron microscopy. Electron microscopic features can help distinguish acinar adenocarcinoma from islet cell tumors of the pancreas, which can look similar on routine light microscopy. In addition, immunostaining properties can distinguish between these two entities, which have also been reported to coexist as mixed acinar–islet cell carcinomas associated with insulin secretion.[55,56] Immunostaining of granules for various pancreatic enzymes is typically positive, including trypsin and lipase.

Cytogenetic studies show that these tumors typically lack those cell markers associated with ductal adenocarcinoma (ie, Ki-*ras* and *TP53*).[57] A recent study suggests that acinar adenocarcinomas are genetically distinct from their ductal counterparts, with demonstration of frequent allelic losses on chromosome 11p and alterations in the APC–β-catenin pathway.[58] It is of interest that similar genetic alterations have also been described in pancreatoblastoma (see the following section).

Treatment is directed at complete excision of the primary lesion and offers the only possible chance of cure. The prognosis is generally more favorable than that for ductal adenocarcinoma. Chemotherapy and radiotherapy are used mainly for palliation.[7]

PANCREATOBLASTOMA

Pancreatoblastoma was originally described by Becker in 1957. Subsequently, 65 pediatric cases have been reported in the literature and 4 cases in adults.[59] Although rare, it is the most common pancreatic neoplasm in childhood. It usually presents in early childhood (mean age approximately 4 years), but this tumor can occur at any age from the neonatal period to adulthood.[60] The tumor is more common in males (male-to-female ratio 1.3:1), and those of Asian descent account for approximately 50% of reported cases.[60,61] There is a reported association of pancreatoblastoma with Beckwith-Wiedemann syndrome (three reported cases).[61,62]

Clinically, most patients present with an incidental abdominal mass. Associated pain, weight loss, and obstructive jaundice are rare.[60] A number of patients have diarrhea of undetermined etiology on presentation. Cushing syndrome and the syndrome of inappropriate secretion of antidiuretic hormone (SIADH) have also been reported concurrently with pancreatoblastoma.[61]

Radiologic studies help determine tumor site and extent of disease. Ultrasonography reveals solid lobulated pancreatic masses with mixed echogenicity.[61] Fine-needle aspiration guided by ultrasonography has been useful in confirming the diagnosis.[61] CT typically reveals large firm, lobulated masses (7–8 cm in diameter) and provides more detailed information.

A review of 59 cases showed that the tumor can occur in any region of the pancreas, and typical features include hemorrhage (94% of cases), capsule formation (92% of cases), and necrosis (90% of cases).[63] All neonatal cases demonstrated cystic changes.[63] MRI, as with other pancreatic neoplasms, demonstrates high signal intensity on T_2-weighted images, with more variable intensity on T_1-weighted images.[64] Gadolinium enhancement is reported to be helpful in distinguishing pancreatoblastoma from other pancreatic tumors.[7] CT and/or MRI will usually correctly make a preoperative diagnosis of pancreatoblastoma.[65] Although a pancreatoblastoma is a malignant tumor, the course is more favorable than with pancreatic acinar cell carcinoma as a result of the presence of a surrounding capsule. Consequently, metastatic spread occurs rarely. Sites of metastases at diagnosis or at the time of recurrence are hepatic (21/69 patients), pulmonary (3/69), and bone (2/69).[59]

Serum α-fetoprotein (AFP) is elevated in nearly 80% of cases, with a median level of 1,280 ng/mL (range 30–138,000 ng/mL).[59] When elevated at diagnosis, evaluation of serum AFP levels is useful to follow the course of the disease, including monitoring for tumor recurrence. However, one reported case in the literature describes a boy with a pancreatoblastoma and a high level of AFP at diagnosis whose tumor recurred as metastatic lesions without a concomitant increase in serum AFP level above normal limits.[66] Yolk sac tumor or hepatoblastoma should also be considered in the differential diagnosis of patients with high AFP levels. One report has suggested that pancreatic AFP (yolk sac origin) can be differentiated from liver AFP (as seen with hepatoblastoma) by using lectin-affinity immunoelectrophoresis.[67]

Histologically, the tumor consists of a soft solid mass, which is usually surrounded by a fibrous capsule that

becomes infiltrated in advanced cases. The tumor is composed of epithelial tissue with acinar differentiation, squamoid cell nests, and occasional endocrine cells. Rarely, cases have pronounced mesenchymal elements, including chondroid and osteoid tissue. Recent cytogenetic studies have provided insights into the possible pathogenesis of pancreatoblastomas. One report has demonstrated an association with chromosome 11p loss of heterozygosity and insulin-like growth factor 2.[68] Another suggests that pancreatoblastomas are genetically distinct from pancreatic ductal adenocarcinomas but bear a close resemblance in molecular pathogenesis to heptatoblastomas. In addition, a link is proposed between familial adenomatous polyposis and pancreatoblastoma. [69]

Treatment is by excision, often involving pancreaticoduodenectomy. If complete resection is achieved initially or after preliminary chemotherapy for reduction of tumor size, 80% achieve complete remission (21/24 cases).[61] Of 10 children who had marginally resectable tumor or partial resection, only 5 survived.[61] Because often tumors cannot be completely resected at diagnosis, preoperative chemotherapy has been employed with some success; however, there is no consensus regarding optimal chemotherapeutic regimens. In one report, a regimen including cisplatinum and doxorubicin documented good tumor response in 9 of 10 children who received the chemotherapy, with the 6 subjects remaining disease free for a median time of 28 months after initial diagnosis.[59] However, chemotherapy provides only transient benefit and must always be followed by surgery. Local radiotherapy after surgery is recommended in patients with a marginally resectable tumor or with tumor spillage during resection.[59] Prognosis is generally better in children. Overall, about 70% of patients are well at 1 year, and tumor-free survival after 5 years is approximately 30%.[7] A tumor-free follow-up of 28 years after surgery has been reported.[70]

PRIMARY EPITHELIAL ENDOCRINE TUMORS

Pancreatic endocrine tumors are a rare and diverse group of lesions that account for a greater proportion of all pancreatic tumors in children than in adults. The tumors may be either functioning or nonfunctioning. Although many of the tumors exhibit features of multihormonality, the specific clinical syndromes that are caused by these neoplasms are predominantly attributable to the systemic effects of only one of their secretory products. (Table 63-2). Advances in our ability to detect the various hormones that are secreted in excess by these tumors have resulted in major progress in our understanding and diagnosis of these challenging tumors. The diagnosis often rests more on the identification of humoral factors than on tissue biopsy. There is a wide overlap in biologic behavior and in the spectrum of benign and malignant potential of these tumors. Descriptions of gastrin-producing tumors (Zollinger-Ellison syndrome), VIPomas (vasoactive intestinal polypeptide; Verner-Morrison syndrome), and carcinoid tumors can be found in Chapter 47, "Secretory Tumors." Glucagonomas, somatostatinomas, and growth hormone–releasing factor adenomas are listed in Table 63-2 to illustrate their main biologic features but are not discussed in the text because they have not been reported in the pediatric age range.

Recent studies have shown new insights into the factors that might influence or predict the biologic behavior of these tumors. One report has suggested that assessment of tissue mitotic rate and necrosis correlates strongly with survival.[71] Other studies have suggested allelic losses on the X chromosome in foregut endocrine tumors, including pancreatic neoplasia, shown by X chromosome loss of heterozygosity.[72,73] A study using the technique of comparative genomic hybridization, which allows for the simultaneous evaluation of the entire tumor genome, has shown a variety of types of chromosomal imbalances in 96% of cases (25/26 patients). These abnormalities discriminated to some extent between sporadic foregut and midgut tumors and reveal new distinct candidate regions in the human genome that are associated with sporadic endocrine tumors.[74]

INSULINOMA

Insulinomas are the most common functioning pancreatic endocrine tumors. Benign tumors usually account for between 90 and 95% of cases. The first description of a patient with an insulinoma syndrome was in 1902, predating the discovery of insulin by Banting and Best in 1922.[75] A large review of patients with insulinoma from 1974 included 1,067 cases (mean age 45.5 years; 68% of patients in the age range of 30 to 60 years; female predominance in a ratio of 3:2).[76] The reported incidence is of 4 cases per 1 million person-years.[76]

Clinically, patients present with symptoms of hypoglycemia, including neuropsychological symptoms such as loss of consciousness, lethargy, confusion, dizziness, blurred vision, epilepsy, and coma, occurring in up to 92% of patients.[76] Amnesia is also reported in 41% of cases.[77] Irreversible central nervous system damage owing to hypoglycemia was evident in 6.8% of patients. Symptoms related to catecholamine response, such as palpitations, tachycardia, and hypertension, were also common. In addition, patients may complain of hunger, vomiting, or epigastric pain. One study documents obesity in 47% of cases.[76]

Hypoglycemia may be a feature of a number of other clinical conditions. The presence of hypoglycemic symptoms, abnormally low serum glucose, and relief of symp-

TABLE 63-2 ENDOCRINE TUMORS OF THE PANCREAS

TUMOR TYPE	MAJOR CLINICAL FEATURE(S)
Insulinoma	Hypoglycemia, altered mental state
Gastrinoma	Gastric acid hypersecretion, peptic ulceration, steatorrhea
GRFoma*	Achromegaly
Glucagonoma*	Hyperglycemia, mild diabetes mellitus, necrolytic erythematous migratory rash
Somatostatinoma*	Hypochlorhydria, weight loss, mild diabetes mellitus, steatorrhea, cholelithiasis
VIPoma	Severe watery diarrhea, achlorhydria, hypokalemia

*These tumors have not yet been reported in the pediatric age group

toms with glucose infusion (Whipple triad) are supportive of the diagnosis.[75] The diagnosis can be aided by inducing fasting hypoglycemia, with measurement of insulin levels. Insulin levels are typically greater than 6 μU/mL despite a serum glucose level less than 40 mg/dL.[75] In the presence of hypoglycemia, an absence of urinary ketones or elevated free fatty acids supports the diagnosis. Other supportive tests include the quantification of proinsulin, which is less than 24% of serum insulin in controlled subjects and greater than 24% in 90% of patients with insulinoma.[78,79] The classic fasting period is 72 hours because previous data suggest that all patients will become symptomatic with hypoglycemia by this time.[75] However, recent evidence suggests that with current available insulin and proinsulin assays, a 48-hour fast is sufficient for a definitive diagnosis and should now become the standard.[80]

The C-peptide suppression test has been advocated as a screening test in suspected cases. In this test, injected porcine insulin fails to suppress endogenous C-peptide levels in affected patients. Despite the various means to establish a diagnosis, there is a delay from the time of presentation of a mean of 37.4 months.[75] In unusual cases in which Munchausen syndrome by proxy is suspected, quantification of C-peptide, which is produced in equimolar concentrations with endogenous insulin but is absent in pharmaceutical insulin, and measurement of serum and urine levels of oral hypoglycemic agents should be performed.

Ninety-eight percent of insulinomas are located within the pancreas.[81] Extrapancreatic sites include the duodenum, ileum, and lungs.[82] The lesions are typically small (90% less than 2 cm in diameter), solitary (83%), and nearly equally distributed between the head, body, and tail of the pancreas.[76,83] The presence of multiple tumors should raise the suspicion of multiple endocrine neoplasia (MEN) type I, as discussed later in this chapter.

One of the major challenges in the management of insulinomas is the difficulty of preoperative localization because of their small size. In different series, 20 to 60% of tumors are not visualized prior to surgery.[84] A multiinstitutional study from 1990 reported the sensitivity of localizing a tumor by various noninvasive and invasive modalities as follows: abdominal ultrasonography, 39%; CT, 33%; arteriography, 62%; and transhepatic portal venous sampling, 89%.[84] A report of 11 cases assessed by the same modalities showed similar sensitivities. In addition, newer techniques of MRI, EUS, and the arterial stimulation test with venous sampling were also reported, showing sensitivities of 30%, 50%, and 91%, respectively.[85,86] More recent experience with EUS shows promise. Evaluation of 54 endocrine tumors of the pancreas, including 29 cases of insulinomas, showed an overall sensitivity and accuracy of 93% in locating the tumor.[87] Some reports have suggested that extensive invasive investigations to achieve preoperative localization of the tumor are unnecessary because a combination of intraoperative palpation (sensitivity greater than 90%) and intraoperative ultrasonography will detect nearly all solitary lesions.[88–90]

Initial treatment of insulinoma is dietary, involving the use of frequent snacks and complex carbohydrates to prevent hypoglycemia. Pharmacologic means may be used to treat hypoglycemia in patients with inoperable or metastatic disease or in those instances in which a tumor cannot be localized. Diazoxide acts by directly inhibiting beta-cell release of insulin and by increasing glycogenolysis by inhibition of cyclic adenosine monophosphate phosphodiesterase.[75,83] Diazoxide is effective in 60% of patients but has major side effects that are usually dose related, such as sodium retention, nausea, and hirsutism.[82] Other alternatives include octreotide, which is shown to be efficacious in 40 to 60% of cases, and verapamil, which reduces insulin release by calcium channel blockade.[82]

The treatment of choice is surgical removal of the tumor. Most surgeons advocate enucleation of these firm adenomas with their pseudocapsule rather than resection. In circumstances when the tumor is not localizable intraoperatively, medical treatment with possible re-exploration at a future date is favored over blind distal resection of the pancreas because 32% of lesions are located in the head of the pancreas. Tumor infiltration is indicative of a malignant state, which warrants resection rather than enucleation. Metastases occur typically to local lymph nodes or the liver. Malignant lesions are usually greater than 2.5 cm in diameter, in contrast to a typical insulinoma, which is between 0.5 and 1 cm.[90] Complications of resection can be seen in up to 55% of cases and include pancreatitis, fistula formation, pseudocyst formation, bleeding, and infection.[84] In addition to aggressive resection, malignant insulinomas have shown variable response to antihormonal chemotherapy and radiotherapy.[7,75] Postoperatively, rises in serum glucose can be seen within 20 minutes of resection in 70% of cases.[78] Consequently, intraoperative monitoring of glucose and immunoreactive insulin has been advocated to confirm that the insulinoma has been completely excised.[91] Hypoglycemia may last for up to 20 days postoperatively and therefore needs monitoring during this time.[76]

MEN TYPE I (WERMER SYNDROME)

MEN type I is a syndrome of malignant potential that involves synchronous or metachronous development of endocrine lesions in the parathyroid, pancreas, and anterior pituitary glands. Less common are associated lesions in the gastrointestinal tract, thymus, lungs, and other sites. In contrast, MEN type II involves the thyroid gland and adrenal medulla.

MEN type I is an autosomal dominant condition that can also occur sporadically. Patients may have primary hyperparathyroidism (88–97%), multiple duodenal pancreatic endocrine tumors (81–82%), and/or pituitary adenomas. Associated skin angiofibromas and collagenomas are common.[92] Of less frequent occurrence are thymic carcinoids, adrenocortical adenomas, thyroid adenomas, and subcutaneous lipomas.[93,94] Patients with MEN type I account for 17% of all patients with hyperparathyroidism, 33% of all patients with Zollinger-Ellison syndrome, 4% of patients with insulinoma, and a small fraction of patients with VIP-secreting tumors, somatostatinomas, and glucagonoma syndromes.[95]

The occurrence of MEN type I was first linked to a tumor suppressor gene (*mu*) at chromosome 11 q13 in 1988. Further studies revealed *MEN1* gene mutations in sporadic cases of gastrinoma (33%) and insulinoma (17%) and deletions of one of the MEN alleles in gastrinomas (93%) and insulinomas (50%).[96]

Pancreatic lesions are either functioning or nonfunctioning. The latter are usually numerous and are microadenomas. Of the functioning lesions, the majority are gastrinomas, followed by insulinomas and VIPomas.

Specific treatment is determined by the type of pancreatic lesion. Tumors are often multiple, especially gastrinomas, which are typically within the duodenum. Production of multiple hormones is documented in 57% of cases. However, 80% contain one predominant hormone, most commonly insulin.[97] The significant morbidity and mortality associated with these tumors and the possibility of testing for *MEN1* gene mutations have raised the issue of screening affected members and kindred. One recent study suggested that screening facilitates the identification of individuals who carry *MEN1* gene mutations and allows one to exclude nonmutant gene carriers from further investigations. This study included 45 members from 10 *MEN1* Swiss families.[98] Prospective biochemical and radiologic screening of carriers was undertaken and revealed the following abnormalities in asymptomatic *MEN1* gene carriers: nine cases of primary hyperparathyroidism, three cases of nonfunctioning pancreatic tumors, one case of gastrinoma, one case of a nonfunctioning microadenoma of the pituitary gland, and one case of macronodular adrenal hyperplasia. Earlier detection of *MEN1*-associated tumors may allow for a reduction in future morbidity and mortality. A similar approach has also been recently applied in families presenting with familial hyperparathyroidism in which genetic screening for *MEN1* mutations was carried out with the suggestion that positive genetic screening may predict disease and allow early detection and appropriate treatment before initiation of symptoms.[99]

A number of biochemical and radiologic investigations have been suggested to detect lesions associated with MEN type I. These include serum prolactin and insulin (IGF-I) for pituitary lesions, serum parathyroid hormone and total serum calcium (corrected for albumin level) for the parathyroids, and serum glucose, insulin, proinsulin, glucagon, gastrin, and plasma chromogranin A for the pancreatoduodenal tumors. In addition, a standardized meal or secretin simulation test analyzing serum polypeptides and gastrin is recommended.[100] Studies have suggested the use of fasting human pancreatic polypeptide levels as an additional screening tool. One study of 202 patients with MEN type I revealed its sensitivity to be 95% and specificity to be 88% in the detection of islet cell tumors.[101] Advances in EUS are promising for tumor localization, but other imaging modalities have been inadequate for tumor screening.[102] The risk of metastasis appears to be related to tumor size, with a 25% risk with 2 cm lesions and a 60% risk with 3 cm lesions. However, this relationship remains controversial.[103]

A recent single-institution retrospective review of 233 patients with MEN examined outcome. This revealed that 28% of patients died of causes related to MEN type I, most commonly metastatic islet cell malignancy. The remaining patients died of causes unrelated to MEN type I, most commonly coronary artery disease and nonendocrine malignancies (14% each). The overall 20-year survival of MEN type I was 64% compared with an age- and gender-matched comparison group of 81%.[104] The authors suggest that earlier diagnosis and appropriate treatment of potentially malignant tumors may lead to a reduction in premature mortality.

ISLET CELL CARCINOMA

Islet cell carcinomas arise from functional pancreatic lesions associated with hypoglycemia (see above) or the Zollinger-Ellison syndrome (Chapter 47). They are usually associated with MEN type I, which was discussed earlier. Nonfunctioning islet cell carcinomas occur more commonly in children than in adults. Diagnosis is often delayed because these tumors present as an abdominal mass with a high incidence of metastases.[7]

There is a reported association between von Hippel-Lindau (VHL) disease, a hereditary cancer syndrome, and pancreatic islet cell tumors.[105] VHL disease is caused by germline mutations of the VHL tumor suppressor gene located on chromosome 3p25. The syndrome is characterized by the development of vascular tumors of the central nervous system and retina, clear cell renal carcinomas, pheochromocytomas, endolymphatic sac tumors, and benign cysts affecting a variety of organs, in addition to pancreatic islet cell tumors.[106–107] In one report, solid pancreatic lesions were detected in 12% of patients with VHL disease.[105] Studies suggest that larger primary tumors (greater than 2–3 cm) are associated with hepatic metastases. These may be prevented by early detection and resection.[105,108] Furthermore, analysis of germline VHL mutations may help predict the occurrence of metastatic disease. Eighty percent of such patients showed mutations in exon 3 (4/5 patients) compared with 46% (18/39 patients) without metastatic disease.[108] A malignant islet cell tumor has also been reported in a 12-year-old boy with tuberous sclerosis complex.[109]

PRIMARY NONEPITHELIAL TUMORS

A variety of benign nonepithelial tumors have been reported to occur in the pancreas in childhood (see Table 63-1).[110–114]

Malignant nonepithelial tumors such as rhabdomyosarcoma and lymphoma have all been described in children, albeit rarely.[115] Primitive neuroectodermal tumors are rarely described in solid organs. A recent case series (n = 7 cases) shows that primitive neuroectodermal tumors can sometimes arise as primary neoplasms of the pancreas, particularly in the pediatric and adolescent population (age range 6–25 years; mean 18 years).[116] Such cases demonstrate typical translocation chromosomal abnormality, that is, (t 11; 22) (q 24; q12), and characteristic immunohistochemical staining monoclonal antibody for O13 (CD 99, p30/32 MIC2).[116]

SECONDARY TUMORS

Secondary involvement of the pancreas by tumors results most commonly from hematogenous spread and includes malignant melanoma, renal tumors, lung tumors, and leukemia. Relatively few tumors invade the pancreas directly. They are usually adenocarcinomas of the stomach, intestine, or biliary tract, which are themselves rare in childhood.

TUMOR-LIKE EXOCRINE LESIONS

The most important entity in this group is pancreatic cystic lesions, which have been discussed earlier. Other lesions that may appear tumor-like include chronic pancreatitis, ductal changes (such as squamous metaplasia, mucinous cell hypertrophy, ductal papillary hyperplasia, and adenomatoid duct hyperplasia), acinar changes (such as focal acinar transformation), heterotopic pancreas, heterotopic spleen in the pancreas, hamartomas, and inflammatory pseudotumors.[7] Inflammatory pseudotumors are an important clinical group that pose diagnostic and therapeutic challenges because of their resemblance to malignant pancreatic lesions such as sarcoma. Consequently, complete surgical excision aided by radiologic surveillance appears to offer the best chances of successful management.[117]

TUMOR-LIKE ENDOCRINE LESIONS

This group of disorders comprises the entities of islet hyperplasia/dysplasia and persistent hyperinsulinemic hypoglycemia of infancy (PHHI), formally termed nesidioblastosis.

PERSISTENT HYPERINSULINEMIC HYPOGLYCEMIA OF INFANCY

PHHI, until very recently, was an enigmatic syndrome. New advances have helped define this entity at the molecular genetic and clinical levels, permitting a rational approach to classification, diagnosis, treatment, and prognosis.[118] The autosomal recessive form of PHHI is the predominant familial type, with an estimated incidence in the white European population of 1 case per 50,000 live births. However, in populations with a high rate of consanguinity, the incidence may be as high as 1 in 2,675 live births. Sporadic PHHI accounts for 95% of all cases.[118]

Remarkable progress in this field over the last 7 years has been achieved through fundamental investigations into the regulation of insulin secretion. This process is highly complex and integrated, involving regulation by nutrients, hormones, and the autonomic nervous system. These regulatory influences impinge on the pancreatic beta cell, where insulin secretion is ultimately regulated by an adenosine triphosphate (ATP)-sensitive potassium channel (K_{ATP}) that controls the polarity of the beta-cell membrane.[119] The pancreatic K_{ATP} channel consists of two protein subunits: the sulfonylurea receptor 1 (SUR 1) and the inward rectifying potassium channel (ie, Kir 6.2), whose distinct genes are located at adjacent loci on chromosome 11 (11p15.1).[120,121] Mutations in these K_{ATP} channel genes are now known to be responsible for some forms of familial PHHI.[122–124] Furthermore, other genes that are responsible for insulin secretion but are not themselves part of the channel have also been discovered, and mutations causing autosomal dominant forms of PHHI have been described.[125,126] However, the majority of cases of PHHI (greater than 95%) are sporadic, without identifiable gene mutations. Nevertheless, one study has shown that despite the inability to detect mutations with the *SUR1* gene, all five patients with sporadic PHHI investigated had absent K_{ATP} activity in their islets, attesting to the critical role of K_{ATP} channels also in the pathogenesis of sporadic PHHI.[127]

The clinical phenotype of PHHI associated with known molecular defects is summarized in Table 63-3, including a description of their management and prognosis. The

TABLE 63-3 PERSISTENT HYPERINSULINEMIC HYPOGLYCEMIA OF INFANCY: COMPARISON OF CLINICAL PHENOTYPES ASSOCIATED WITH KNOWN MOLECULAR DEFECTS

TYPE (MOLECULAR DEFECTS)	HYPOGLYCEMIA/ HYPERINSULINEMIA	ASSOCIATED CLINICAL, BIOCHEMICAL, OR MOLECULAR FEATURES	RESPONSE TO MEDICAL MANAGEMENT	RECOMMENDED SURGICAL APPROACH	PROGNOSIS
Sporadic (*?SUR1* or *Kir6.2* mutations)	Moderate/severe in first days to weeks of life; macrosomic at birth	Loss of heterozygosity in microadenomatous tissue	Generally poor; may respond to somatostatin better than to diazoxide	Partial pancreatectomy (microadenoma; 30–40% cases)	Excellent
				Subtotal greater than 95% pancreatectomy (diffuse hyperplasia; 60–70% cases)	Guarded; diabetes mellitus develops in 50% of patients; hypoglycemia persists in 33%
Autosomal recessive (*SUR/Kir6.2* mutations)	Severe in first days to weeks of life; macrosomic at birth	Consanguinity a feature in some populations	Poor	Subtotal pancreatectomy	Guarded

Adapted from Sperling MA and Menon RK.[118]

results of genotype-phenotype comparisons owing to mutations of the K_{ATP} channel have yielded mixed results. For example, for some mutations, there is a correlation between the severity of clinical disease and demonstration of parallel severity of dysfunction of the mutant channel in in vitro functional studies. The DeltaF1388 mutation has been associated with severe disease and the H125Q mutation with mild disease. In contrast, the N188S mutation, despite a phenotype associated with severe clinical disease, shows minimal channel dysfunction in vitro.[118]

In suspected cases, a diagnosis of hyperinsulinemia must initially be established. Hyperinsulinemia is generally considered to exist if the circulating insulin concentration exceeds 5 μU/mL when the corresponding glucose concentration in the same sample is less than 2 to 2.5 mM (< 45 mg/dL). Because insulin concentrations may not always be elevated at the time of hypoglycemia, an alternative test is the measurement of IGF binding protein 1 levels at the time of hypoglycemia. The secretion of this is acutely inhibited by insulin, and patients with PHHI have serum levels that are approximately 10 to 20% of the control level values.[118] This test supplements existing indirect evidence of severe hyperinsulinemia, including a brisk glycemic response to parenteral glucagons, exceeding 30 to 45 mg/dL from a baseline hypoglycemic glucose concentration and an exogenous glucose requirement greater than 15 to 20 mg/kg/min necessary to maintain euglycemia.[118]

Initial treatment includes the administration of intravenous glucose at high rates in severe PHHI in the neonatal period. Other pharmacologic measures can also be employed. Diazoxide acts to maintain patency of the K_{ATP} channel and may not respond because of the absence of functional pancreatic K_{ATP} channels seen in patients with mutations in *SUR1* or *KIR 6.2* genes.[128] Calcium channel blockers, particularly the long-acting somatostatin analog octreotide, may be more effective as medical therapy, with reports of success in as many as 50% of affected infants.[129]

Failure of medical management is common and a clear indication for surgical pancreatectomy in an attempt to avoid long-term neurologic sequelae of hypoglycemia. Surgery may be guided by the type of histopathologic lesion. Focal and diffuse histopathologic lesions characterize sporadic PHHI. Focal PHHI is characterized by focal adenomatous hyperplasia of islet-like cells, with small beta-cell nuclei packed closely together. In contrast, in diffuse PHHI, the islets of Langerhans cells throughout the pancreas are irregular in size, with hypertrophied insulin-secreting cells containing large, abnormal beta-cell nuclei.[118] It has been proposed that a histologic index of beta-cell nuclear crowding may provide a reliable discrimination between the diffuse and focal adenomatous forms of sporadic PHHI, that is, focal lesions showing more nuclear crowding than diffuse—hence intraoperatively providing the ability to distinguish and potentially guide the extent of pancreatectomy necessary.[130] Further guidance to predict between the two histologic lesions has been applied preoperatively using the technique of transhepatic retrograde pancreatic venous catheterization with measurement of insulin concentration at multiple sites.[118] In diffuse hyperplasia, uniformly high insulin concentrations without evidence of a gradient are seen. With localized lesions, a distinct gradient in insulin concentrations is observed. However, only a few centers have developed the expertise and experience to perform this catheterization procedure with a high degree of safety and success. Despite pre- and perioperative evaluations, the long-term outcome following subtotal pancreatectomy remains questionable because of persistent hypoglycemia and development of insulin-dependent diabetes in a high proportion of patients (see Table 63-3).[131]

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CHAPTER 64

PANCREATITIS

1. *Acute and Chronic*

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Clinical understanding and treatment of pancreatic diseases lag behind understanding and treatment of many other important diseases. This delay arises from a combination of factors making it difficult to study the pancreas, including its inaccessible location, hesitation of clinicians and researchers to use invasive testing methods, poorly understood pathophysiologic processes, and limited treatment options. However, a number of recent advances in cell biology and genetics have provided the critical tools to unravel some mysteries of pancreatic pathophysiology and have provided genetic susceptibility testing, which also allows for better classification of pancreatic diseases. In addition, high-quality abdominal imaging techniques, including magnetic resonance cholangiopancreatography (MRCP), now provide critical anatomic information with minimal risk. These advances, framed within the context of acinar and duct cell physiology, provide the foundation for future treatment and prevention of pancreatic diseases.

PATHOPHYSIOLOGY OF ACUTE PANCREATITIS

The pancreas is a gland serving three primary functions: production of a bicarbonate-rich fluid by the duct cells, which helps neutralize gastric acid entering the duodenum; synthesis of digestive enzymes within acinar cells with eventual delivery into the duodenum to help digest complex nutrients; and production of hormones by islet cells to regulate nutrient storage and use related to meals. Although each of the functions and cell types differs, their interrelationship remains critical for normal organ function.

Most of the pancreas is composed of acinar cells. The duct cells connect the acinar cells to the duodenum and flush the digestive enzymes out of the pancreas after acinar cell secretion. The islet cells function independently of the acinar cells, but the acinar cells must remain in close proximity to the islet cells to maintain normal gene expression profiles. This is accomplished through the insular-acinar portal system, in which blood vessels extend from the islets to the acinar cells so that insulin-rich fluid continually bathes the acinar cells.

Acinar Cells Synthesize and Store Trypsinogen

The acinar cells synthesize and store digestive enzymes until they are stimulated to secrete the enzymes into the ducts. All of the major digestive enzymes are synthesized by all acinar cells. All enzymes, except amylase and lipase, are synthesized as proenzymes (zymogens) that require activation by cleavage of an activation peptide by trypsin. Trypsinogen (the proenzyme form of trypsin) is activated by the intestinal brush border enzyme enterokinase or by another trypsin molecule (Figure 64.1-1). Trypsinogen also slowly autoactivates, possibly because trypsinogen has some trypsin-like activity.[1] Because trypsinogen is synthesized in the same cell, is stored in the same subcellular compartment as the other enzymes, and has the potential to become active trypsin, there is always the danger of premature trypsinogen activation with subsequent activation of other digestive enzymes and digestion of the pancreas itself. Trypsin not only activates itself, it also inactivates itself through autolysis, a process that is regulated by calcium.[2–4] High calcium concentrations and pH are optimal for trypsin survival in the duodenum[5] but favor autolysis within the acinar cells when calcium concentrations are low. Thus, a number of critical mechanisms are found throughout the pancreas that protect acinar cells, the duct, and the pancreas itself from premature trypsinogen activation.

Pathophysiology of Calcium Signaling

Calcium is the most important second-messenger signaling system within the acinar cells. Activation of the acinar cell through the major receptors results in increases in intracellular calcium, which, in turn, activates protein synthesis and zymogen secretion. With physiologic stimulation, intracellular calcium levels rise and slowly oscillate within well-controlled concentrations.[6b] The process begins with opening of calcium channels on the basolateral membrane, which allows calcium to enter the cell and causes release of intracellular calcium stores. Basolateral calcium is rapidly transported through the rough endoplasmic reticulum to the apical membrane, where it initiates secretion of the zymogen granules into the duct lumen.[7] With hyperstimulation, the calcium levels rise above critical levels, followed

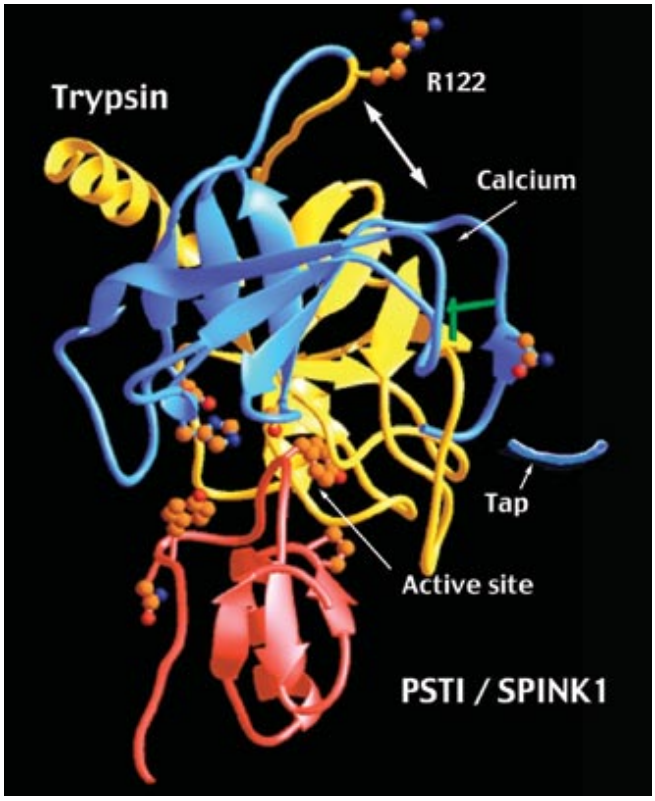

FIGURE 64.1-1 Crystallography-based model of trypsin (yellow and blue) attacking pancreatic secretory trypsin inhibitor/serine protease inhibitor, Kazal type 1 (PSTI/SPINK1-red) at the active site (*long arrow*). The critical regulatory site for trypsinogen activation is shown after cleavage of trypsinogen activation peptide (TAP). The critical regulatory region of trypsin is the flexible autolysis loop *(line with two arrowheads)* with the target amino acid residue R122 shown in the exposed conformation. The calcium binding loop is shown without calcium, which would stabilize the autolysis loop in a conformation protecting R122 from attack by another trypsin (see CD-ROM for color image).

by sustained activation of trypsin.[6b,8] The link between extracellular calcium, acinar cell stimulation, and trypsinogen activation has also been demonstrated in animal models.[9,10] Finally, exposure of the acinar cell apical membrane to bile acids (especially taurolithocholic acid 3-sulfate) causes extended calcium signals near the secretory granules, which leads to trypsin activation.[11] These observations link calcium dysregulation with acute pancreatitis.[12]

Duct Cells Normally Flush Secreted Digestive Enzymes out of the Pancreas

The ductal cells make up less than 5% of the total pancreatic mass but are responsible for the large volume of bicarbonate-rich pancreatic fluid. This function is critical for sweeping digestive enzymes released from the acinar cells out of the pancreas and into the duodenum. The intralobular ductules penetrate the acinus and include the centroacinar cells. Pancreatic bicarbonate secretion is derived from the centroacinar and proximal duct cells.

Duct cells are polarized cells that maintain a significant electrical membrane potential. The basolateral membrane contains ion channels and transporters that participate in regulating chloride, bicarbonate, sodium, and potassium, as well as the sodium-potassium adenosine triphosphatase ion pump that drives secretion. The most important transporter is the sodium-2 bicarbonate cotransporter, which uses the sodium electrochemical gradient to transport bicarbonate into the cell. The apical membrane also contains channels and transporters, with the most important being the cystic fibrosis transmembrane conductance regulator (CFTR). CFTR is both a chloride and a bicarbonate channel, and the opening or closing of this channel determines duct cell fluid secretion.[13] Mutations in the CFTR reduce the fluid-secreting capacity of the pancreas, increasing the risk of having active trypsin within the pancreas for prolonged periods of time. This is especially dangerous because calcium levels within the duct lumen are elevated, thus eliminating the trypsin autolysis protective mechanism. Unregulated trypsinogen activation in the duct and failure to flush the enzymes because of mutations in the CFTR may also lead to both acute pancreatitis and chronic pancreatitis, as is seen in patients with cystic fibrosis (CF), atypical CF, or forms of CFTR-associated polygenic pancreatitis.[14,15]

Other Protective Mechanisms

The vast majority of mechanisms that are protective against acute pancreatitis center on control of trypsin. This includes preventing trypsinogen activation, inhibiting active trypsin, destroying trypsin, or sweeping trypsin out of the pancreas. Identification of specific mutations in several genes proved the importance of several of these mechanisms. As noted above, the first protective mechanism is the synthesis of trypsinogen in an inactive form (trypsinogen) with an activation site located in the target organ, which is the duodenal lumen. Several mutations in cationic trypsin gene enhance trypsin activation,[16] and these mutations increase susceptibility to acute pancreatitis.

If trypsinogen is activated within the acinar cells, it is inhibited by pancreatic secretory trypsin inhibitor (also known as serine protease inhibitor, Kazal type 1 [SPINK1]). This peptide is a specific trypsin inhibitor and an acute-phase protein.[17] Mutations in *SPINK1*, which likely reduce its ability to inhibit trypsin, are associated with pancreatitis in children, some familial pancreatitis, and various forms of tropical pancreatitis. These mutations are common, seen in 2% of most populations.[18] Although intracellular SPINK1 is a highly effective trypsin inhibitor, the number of trypsinogen molecules greatly outnumbers the number of SPINK1 molecules, so that the inhibitory capacity is limited.[1] If more trypsinogen is activated than SPINK1 can inhibit, then other mechanisms must be employed.

Studies in hereditary pancreatitis drew attention to the trypsin self-destruct mechanism.[19] The trypsin molecule has two globular domains held together by a single side chain. This side chain is critical to trypsin regulation. In the middle of the side chain is an arginine, or target amino acid for trypsin attack. Biochemical studies demonstrate that this site is sensitive to trypsin hydrolysis, and cleavage of the site is the first step in trypsin autolysis. The importance of this site and autolysis mechanism is demonstrated by patients with hereditary pancreatitis who have muta-

tions altering the amino acid sequence at R122 (eq. R122H) and recurrent acute pancreatitis when trypsin cannot be destroyed within the acinar cells. Calcium protects trypsin from autolysis by protecting the R122 site from attack.[4,20] So trypsin autolysis only occurs in compartments with low calcium concentrations.

Summary

Some of the mysteries surrounding acute pancreatitis are beginning to be solved. This allows the construction of conceptual models that help organize knowledge about pancreatic physiology, pathophysiology, and pancreatic disease.[4] Acute pancreatitis can be thought of as an event that occurs when trigger factors drive trypsinogen activation beyond the protective mechanisms. Key molecular events occur within the acinar cell in relation to loss of calcium regulation with intracellular hypercalcemia and, therefore, elimination of trypsin autolysis protective mechanisms. Trypsin that is activated outside the acinar cell in which calcium levels are already elevated must be flushed quickly out of the duct by a CFTR-dependent mechanism or inhibited, eliminated, or corraled by other means. These factors are important in understanding susceptibility to acute pancreatitis. Once initiated, the resulting immune response and a variety of complications become the primary concern.

ACUTE PANCREATITIS

Acute pancreatitis is defined clinically as the sudden onset of abdominal pain associated with a rise in digestive enzymes in the blood or urine. The mechanism appears to involve the premature activation of trypsinogen because trypsinogen activation is among the earliest biochemical changes in experimental pancreatitis. Trypsinogen activation peptide is one of the earliest markers of acute pancreatitis in humans; endoscopic retrograde cholangiopancreatography (ERCP)-associated pancreatitis can be attenuated by pretreatment with trypsin inhibitors (eg, gabexate), and mutations that enhance trypsinogen activation, diminish trypsin inactivation, or limit trypsin clearance from the ducts are associated with acute pancreatitis. Second, acute pancreatitis is associated with a vigorous immune response, which contributes to the severity of the pathologic condition. By the time acute pancreatitis is recognized clinically, the trypsinogen activation and autodigestion phase may be over, and the inflammatory consequences dominate the clinical picture.

The prevalence of acute pancreatitis in children appears to be increasing.[21–23] The reason for this observation is unclear but does not appear to be simply related to referral bias or improved methods for diagnosis.[23]

Etiology of Acute Pancreatitis

A number of factors are able to trigger an attack of acute pancreatitis. In adults, the vast majority of cases are associated with gallstones, alcohol abuse, hypercalcemia, hypertriglyceridemia, medications, and blunt trauma. This profile appears to differ in children. Recently, a number of single and multicenter studies have investigated the etiologies of acute pancreatitis in children (Table 64.1-1). Acute pancreatitis associated with severe systemic illnesses is striking, accounting for about 20% of reported cases.[23] In some cases, acute pancreatitis may be overlooked in the intensive care unit setting.[24] However, the contribution of

TABLE 64.1-1 ETIOLOGY OF PANCREATITIS IN 1,276 CHILDREN

	STUDY (SETTING)						
	BENIFLA AND WEIZMAN (REVIEW)	DEBANTO ET AL (INPATIENT [MILD])	DEBANTO ET AL (INPATIENT [SEVERE])	LOPEZ (INPATIENT)	WERLIN ET AL (D/C RECORDS)	ALVAREZ CALATAYUD ET AL (INPATIENT)	TOTAL
Number	589	162	40	274	180	31	1,276
Age, mean (median)	9.2	9.4	6.9	NA	(12.5)	7.9	
Male-to-female ratio	1.2	0.9	1	NA	0.9	1.2	
Etiology							
Systemic (eg, HUS)	14	1.9	20	53	14	6.5	20.8%
Gallstone		7.4	2.5		12	16	3.1%
Structural/divisum	15	2.5	2.5	10	7.7		10.6%
Infectious (eg, viral)	10	2.5	2.5	5	8	19	7.7%
Medications	12	11.1	7.5	5	12	9.7	10.2%
Trauma	22	13	20	19	14	6.5	18.6%
Post-ERCP	3.1	0			5.5		1.2%
Familial	2	6.8	7.5	"A few"	3		2.4%
Cystic fibrosis		3.1	0	0.4	0.6	0.5	0.6%
Hypercalcemia	1	3.1	0		0		0.9%
Hypertriglyceridemia	1	0.6	2.5		1		0.8%
DKA		1.2	0	0.7	4.4		0.9%
Other		3.7	7.5	0.4	10	7	2.4%
Idiopathic	23	40.1	27.5	17	8	35	22.2%

D/C = discharge, DKA = diabetic ketoacidosis and "diabetic"; ERCP = endoscopic retrograde cholangiopancreatography; HUS = hemolytic uremic syndrome; NA = not available. Benifla and Weizman[51] is a review of 18 pre-1999 studies from the United States (*n* = 9), the United Kindom (*n* = 5), Canada, Taiwan, Hong-Kong, Switzerland, and Israel. DeBanto et al[21] is from a multicenter (*n* = 6) study in the midwestern United States. Lopez[22] includes outpatient (Dallas, TX), Werlin et al[23] is from a referral center in Milwaukee, WI, and Alvarez Calatayud et al[117] is from Madrid, Spain.

pancreatitis to the overall severity of these diseases cannot be calculated from these reports, and it appears that deaths owing to acute pancreatitis in children alone are now uncommon (< 2%).[21,23] Hemolytic uremic syndrome is identified as the most common of all systemic diseases, causing acute pancreatitis in children.[21,23] The mechanism of pancreatitis is unknown and likely multifactorial, noting that uremia itself is a risk factor for pancreatic injury.[25–27] Although not classified separately in most case series, acute pancreatitis after organ transplant is also common.[23]

Gallstones are an important cause of acute pancreatitis in children. Choi and colleagues reported gallstones in 16 (29%) of 56 cases in a recent report from Korea.[28] In previous studies, gallstones were often included with structural abnormalities of the pancreas and biliary system as "biliary." This etiology should not be overlooked because therapeutic ERCP may be indicated.

Structural abnormalities also increase the risk for acute pancreatitis. The most common is pancreas divisum, but other abnormalities in the pancreatic or common bile duct (eg, choledochal cysts, choledochocoeles, and partial pancreas divisum) have been identified in children with otherwise unexplained acute pancreatitis.[23]

Infectious acute pancreatitis in North America and Europe is primarily viral in etiology. This includes mumps (39% of cases of pediatric acute pancreatitis reported in a Scottish study[29]), enterovirus, Epstein-Barr virus, hepatitis A, cytomegalovirus, rubella, coxsackievirus, varicella, rubeola, measles, and influenza virus.[21,23,30–33] Children with human immunodeficiency virus (HIV) frequently develop acute pancreatitis, usually from a secondary infection such as cytomegalovirus, *Mycobacterium avium intracellulare*, *Pneumocystis carinii*, and *Cryptosporidium parvum*, as well as from HIV medications.[34,35] Bacterial infections also occasionally cause acute pancreatitis.[30] In third world countries and tropical regions, acute pancreatitis is often associated with helminth infections such as *Ascaris lumbricoides*.[36–38] These cases can be severe, complicated, and difficult to treat.[39]

A variety of mediations are associated with acute and recurrent acute pancreatitis.[31,40,41] The strongest evidence in adults includes azathioprine or 6-mercaptopurine, thiazide diuretics, sulfonamides, furosemide, estrogens, and tetracycline, with suggestive evidence for L-asparaginase, iatrogenic hypercalcemia, chlorthalidone, corticosteroids, ethacrynic acid, phenformin, and procainamide.[41] Table 64.1-2 lists medications associated with acute pancreatitis in children.[21,23] The anticonvulsant valproate is the most common medication reported in these series. Grauso-Eby and colleagues reported 4 cases (1 fatal) from their institution, plus 29 others from the literature.[42] The median age was 8.9 years (range 1–18 years), and the mean dose was 45.6 mg/kg/d (range 20–85 mg/kg/d), with approximately equal doses in fatal and nonfatal cases. Seventy-one percent were taking multiple antiepileptic drugs, and children were taking valproate for a mean of 19 months (range 1 month to 10 years).[42] However, Pellock and colleagues reviewed over 3,000 patients treated with valproate from 34 studies and found only 2 documented cases of acute pancreatitis and a similar number of cases of amlyase elevations in valproate-treated versus placebo-treated patients.[43] Furthermore, in over 2,000,000 reports to the Toxic Exposure Surveillance System compiled by the American Association of Poison Control Centers, acute pancreatitis was noted in only one of the four subjects with fatal valproate ingestion.[44] These data indicate that the association of valproic acid with acute pancreatitis is idiosyncratic but remains an important cause of pancreatitis in children. The other major causes of medication-associated pancreatitis in children are L-asparaginase and prednisone.

Trauma is a common cause of acute pancreatitis in children and is responsible for nearly 20% of cases (see Table 64.1-1). In the majority of cases, the trauma is blunt and accidental (eg, bicycle handlebar), but child abuse makes up a proportion of cases.[23] A major concern in these cases is pancreatic duct transection, which may require surgical intervention.

Post-ERCP pancreatitis is reported in several series.[21,23] Newer imaging modalities such as high-resolution computed tomography (CT) and MRCP, are, therefore, replacing ERCP as a diagnostic tool. ERCP, however, remains invaluable for therapeutic interventions.

Familial pancreatitis includes hereditary pancreatitis and other forms of pancreatitis that occur in families with an incidence that is higher than expected in the population by chance alone.[45] Atypical CF with compound heterozygous CFTR mild-variable mutations or CFTR mutations plus modifying factors, such as *SPINK1* mutations, are the most common cause of familial pancreatitis in our experience (D. Whitcomb, unpublished, 2003). Note that genetic testing was not available for cationic trypsinogen gene testing before 1996,[19] and for *SPINK1* gene testing before 2000,[46,47] and the role of CFTR testing was previously unclear.[4,48,49] There is no association between α_1-antitrypsin mutations and acute pancreatitis.[50] As genetic testing and better diagnostic testing become more widely available, the percentage of children with familial or gene mutation–associated pancreatitis will rise, whereas the idiopathic category will continue to fall.[23,51]

TABLE 64.1-2 MEDICATIONS ASSOCIATED WITH ACUTE PANCREATITIS IN TWO RECENT US STUDIES

DRUG	DEBANTO (*N* = 202)	WERLIN (*N* = 180)
Acetominophen	0	1
L-Asparaginase	6	2
Azathioprine/6-mercaptopurine	0	2
Cocaine	0	1
Fosphenytoin	0	1
Furosemide	1	0
Macrodantin	1	0
Metronidazole	1	1
Pentamidine	1	0
Phenytoin	1	0
Prednisone	6	0
Valproate	8	14
Multiple (several candidate drugs)	4	0
Unknown (or not recorded)	4	0

Adapted from Wankum P and Tobias JD[24] and Choi BH et al.[28]

An evaluation of a child with acute pancreatitis should include measurement of calcium and triglyceride levels because these causes must be addressed to prevent recurrence. Pancreatitis is occasionally seen in a number of other metabolic disorders, such as diabetic ketoacidosis[21–23] and inborn errors of metabolism[52] (Table 64.1-3).

Recurrent Acute Pancreatitis

Recurrent acute pancreatitis is seen in about 10% of children after a first episode of acute pancreatitis.[23,51] Recurrent acute pancreatitis is most commonly seen in patients with structural abnormalities, idiopathic pancreatitis, or familial pancreatitis.[23,51] A careful evaluation aimed at identifying reversible causes should be undertaken. In addition to avoiding another attack of acute pancreatitis, one should consider the prevention of chronic pancreatitis through eliminating known risk factors. Theoretically, the progression to chronic pancreatitis may be slowed by the use of antioxidants (which may also reduce the frequency and severity of recurrent symptoms in hereditary pancreatitis patients[53]), but these approaches are currently unproven.

Diagnosis of Acute Pancreatitis

The diagnosis of pancreatitis is currently based on the syndrome of sudden onset of typical abdominal pain plus elevation of amylase or lipase to at least three times the upper limit of normal levels.[21–23,30] The diagnosis of acute pancreatitis can be difficult because no readily available test confirms the diagnosis. Although there have been multiple attempts to determine the sensitivity and specificity of elevations in both enzymes in adults, the studies all suffer from the absence of a method to separately and absolutely document pancreatitis. It is clear that both enzymes can be normal when there is radiographic and clinical evidence of pancreatitis. Also, both enzymes can be elevated by other conditions unrelated to pancreatitis. The level of elevation is also not diagnostic, although the higher the level is above the upper reference limit, the more likely it is that there will be pancreatic inflammation. Levels just above the upper reference limits may still be secondary to pancreatitis, especially in patients presenting several days after the onset of symptoms.

Other pancreatic products, such as phospholipase A_2, trypsin, trypsinogen activation peptide, and elastase, are elevated in pancreatitis, but none have found widespread use in the clinical laboratory setting. Serum transaminases are elevated in some patients, and the combination of elevated amylase or lipase and elevated serum transaminases may be more predictive of pancreatitis than elevated amylase or lipase alone.

Diagnostic testing for acute pancreatitis begins with a high index of suspicion, such as unexplained abdominal pain or vomiting. Pain is usually epigastric, in the right upper quadrant, or in the left upper quadrant, with radiation through to the back. However, back pain can occur alone, and pain may localize to other areas of the abdomen. Nausea and vomiting are common and may be the dominant clinical features. Other less common clinical signs include fever, tachycardia, hypotension, jaundice, and abdominal signs such as guarding, rebound tenderness, and a decrease in bowel sounds.[51] Occasionally, the diagnosis is first suspected because of feeding intolerance when feeds are introduced in patients with systemic illnesses.

Transient fever or jaundice can be present. Jaundice or elevated transaminases should raise the possibility of biliary tract involvement. Rarely, patients present with ascites or an abdominal mass. Epigastric tenderness is a useful but nonspecific and unreliable sign.

CT and abdominal ultrasonography of the pancreas are primarily used to document pancreatitis, determine the severity or identify complication (eg, pseudocysts), determine if there is underlying chronic pancreatitis, or identify other causes of unexplained signs or symptoms (Figure 64.1-2). Ultrasonographic findings include enlargement of the pancreas, altered echogenicity of the pancreas, dilated main pancreatic duct, gallstones, biliary sludge, dilated common and intrahepatic bile ducts, pancreatic calcification, choledochal cysts, and fluid collections, either peripancreatic or cystic. A CT scan will show similar findings, except that abnormal attenuation is seen rather than altered echogenicity. The CT scan is usually done several days into a severe course of acute pancreatitis when the patient fails to improve. There is experimental evidence indicating that CT contrast given early in the course of acute pancreatitis may diminish already tenuous blood flow to ischemic areas of the pancreas and thereby extend the region of necrosis. Although this is difficult to prove in humans, most experts agree that there is no diagnostic utility for a CT scan in the early phases of acute pancreatitis.[23] MRCP may be helpful in defining abnormalities of the ductal system, but its utility in pediatric patients has not been carefully studied. ERCP should be reserved for patients with unexplained recurrent episodes of pancreatitis, prolonged episodes of pancreatitis when a structural defect or duct disruption is suspected, or in some cases of gallstone pancreatitis.

Medical Management of Acute Pancreatitis

The mainstay of current treatment of acute pancreatitis in children is analgesia, intravenous fluids, pancreatic rest, and monitoring for complications.[23,30] Full attention must be paid to fluid balances because patients are usually kept without food and may lose fluids from the vascular compartment from a capillary leak syndrome and "third spacing." Fluid

TABLE 64.1-3 PANCREATITIS IN PATIENTS WITH INBORN ERRORS OF METABOLISM

Pancreatitis caused by hyperlipidemia
Hereditary lipoprotein lipase deficiency
Apolipoprotein C-II deficiency
Familial hypertriglyceridemia and chylomicronemia
Glycogen storage disorders
Branched-chain ketoaciduria (maple syrup urine disease)
Homocystinuria owing to cystathionine β-synthase deficiency
3-Hydroxy-3-methylglutaryl-CoA lyase deficiency
Acute intermittent porphyria
Pyruvate kinase deficiency
Cystinuria
Lysinuric protein intolerance and other cationic aminoacidurias

Adapted from Simon P et al.[52]

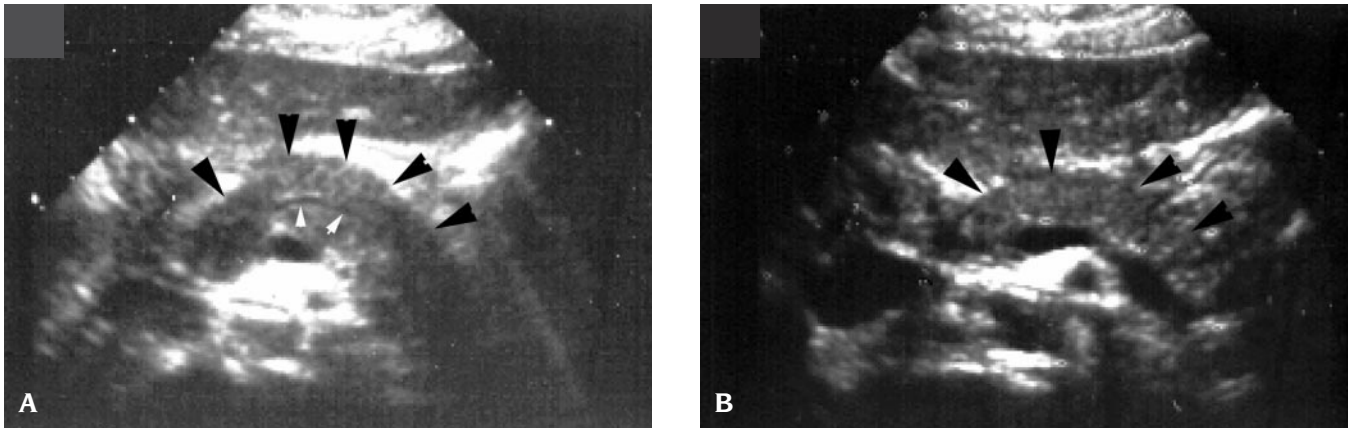

FIGURE 64.1-2 Ultrasonographic changes of pancreatitis. *A*, Sonogram of the pancreas in a 10-year-old boy who presented with epigastric pain and vomiting. He had a serum lipase 20 × upper reference limit (URL) and a serum amylase 6 × URL. The pancreas is edematous, and the main pancreatic duct is dilated. The upper edge of the pancreas is marked by *black arrowheads*, and the main pancreatic duct is indicated by *white arrowheads*. *B*, Sonogram of a normal pancreas. The same patient presented with periumbilical pain 2 months later. His serum lipase and amylase levels were normal. A sonogram showed a normal pancreas and did not visualize the main pancreatic duct, a normal finding in children. The *arrowheads* mark the upper edge of the pancreas.

losses are exaggerated if a nasogastric tube is used to decompress the stomach as a treatment for vomiting. Volume expansion early in the course of acute pancreatitis is important for both cardiovascular stability and preventing development of pancreatic necrosis. Meperidine 1 to 2 mg/kg intramuscularly or intravenously is used for pain control.[30] Enteral or total parenteral nutrition is often unnecessary in mild cases but should be instituted early if a severe or prolonged course is anticipated. Until recently, parenteral nutrition was considered the only option, but several studies show that adult patients with acute pancreatitis tolerate jejunal feedings with fewer complications than those given parenteral nutrition.[54] Antibiotics are usually unnecessary except for the most severe cases, especially if significant pancreatic necrosis is present.

Severity of Acute Pancreatitis

Acute pancreatitis can be life threatening, although death does not appear to occur as often in children (without other systemic diseases) as it does in adults.[23] Death occurs by several mechanisms, with some occurring early and others late. The early causes of death are cardiovascular collapse and respiratory failure. The cardiovascular collapse occurs with the combination of a vascular leakage syndrome with third spacing of fluid, vomiting, and having the patient receive nothing by mouth. If recognized, this complication can be treated with fluid resuscitation, guided, when necessary, by following central venous pressure. The respiratory failure is associated with an adult respiratory distress syndrome–like situation that results from leakage of fluid into alveolar spaces and inflammation. Early recognition of this complication and management of patients at high risk within an intensive care setting can be lifesaving.

Late life-threatening complications of acute pancreatitis are related to infected pancreatic necrosis and multisystem organ failure, although pancreatic necrosis appears to be uncommon in children—1 case of 380 from 7 centers (0.3%).[21,23] Judicious use of antibiotics and attention to nutrition may help limit these late complications.

Global Acute Pancreatitis Scoring Systems

Acute pancreatitis is usually divided into mild and severe forms. Originally, mild acute pancreatitis was defined as pancreatitis limited to peripancreatic fat necrosis and interstitial edema, whereas severe pancreatitis included additional features.[55,56] Other clinically based systems also have been developed, which define mild and severe according to the absence or presence of major complications requiring prolonged treatment or specific interventions or an increased likelihood of death.[57–60] Thus, mild pancreatitis includes the majority of cases, which have minimal complications, a short clinical course requiring minimal supportive treatment, and complete recovery. It is the physician's responsibility to make a rapid assessment of the patient's condition and to predict the risk of a mild or severe clinical course. To assist the physician in this decision, a number of severity criteria have been developed, including Ranson's scores,[57,58] Glasgow score,[59] the Acute Physiology and Chronic Health Evaluation (APACHE) II score,[61,62] and Pediatric score.[21] Although these criteria add little to the assessment of the experienced physician, they remain useful in reminding one to carefully consider the major systems impacted during the more severe attacks of acute pancreatitis. In general, the signs and organic symptoms of each of these systems reflect the magnitude of the inflammatory response to acute pancreatitis rather than measure the actual amount of pancreatic injury.

Ranson's criteria were developed in adults,[57,58] and several criteria (eg, age > 55 years and fluid deficit > 6 L) are not applicable to children.[21] The Midwest Multicenter Pancreatic Study Group analyzed the criteria of the Ranson and Glasgow scores, plus additional criteria, and developed a scoring system for children.[22] The seven severity factors included age (< 7 years), weight (< 23 kg), admission white blood cell count (>18.5 x 10^9/L), admission lactate dehy-

drogenase (> 2,000 IU/L), 48-hour fluid sequestration (> 75 mL/kg/48 hours), and a 48-hour rise in urea (> 5 mg/dL). If each criterion is assigned a value of 1 point, then the outcome of patients with 0 to 2 points was 8.6% severe, 1.4% death; with 2 to 4 points was 38.5% severe, 5.8% death; and with 5 to 7 points was 80% severe, 10% death.[21] The accuracy of this system was validated in three centers. Of note, young age and low weight are major risk factors. Severity was also often associated with severe systemic diseases (see Table 64.1-1, DeBanto [severe]), a pattern also noted by others.[22,23]

Complications of Acute Pancreatitis

A number of non–life-threatening complications of acute pancreatitis are recognized. These include both local and systemic complications (Table 64.1-4). Local complications include fluid collections, pancreatic necrosis (sterile or infected), pancreatic abscess, duct rupture, duct strictures, bleeding, and pseudocyst formation.

Pancreatic necrosis, which is a segmental pancreatic infarction, remains a major concern because it is associated with serious complications. This complication probably occurs in less than 5% of adult patients and less than 1% of children. The risk of pancreatic necrosis appears to increase with vascular leakage, as reflected by an elevated hematocrit in nearly all patients with pancreatic necrosis. The combination of intravascular volume depletion, inflammation, and high hematocrit therefore leads to blockage of pancreatic blood flow and infarction. The diagnosis is made by contrast-enhanced CT with demonstration of a segment of pancreatic gland without perfusion.

Complications of pancreatitis may require specific treatment. Pancreatic pseudocysts occur in children. Pseudocysts can be observed over time for spontaneous resorption or may require drainage. Currently, both endoscopic internal drainage and external drainage by interventional radiologists offer viable options to surgery. The chronicity, size, location, and complexity of the pseudocysts all contribute to decisions about the optimal choice for treatment. Abscesses often can be treated with external drainage and intravenous antibiotics, and only rarely is surgical drainage necessary. Surgery is usually necessary for traumatic rupture of the duct, although endoscopic stenting across the disrupted duct is another option.

TABLE 64.1-4 COMPLICATIONS OF ACUTE PANCREATITIS

LOCAL	SYSTEMIC
Edema	Shock
Inflammation	Pulmonary edema
Fat necrosis	Pleural effusions
Phlegmon	Acute renal failure, coagulopathy
Pancreatic necrosis	Hemoconcentration
Sterile	Bacteremia, sepsis
Infected	Distant fat necrosis
Abscess	Vascular leak syndrome
Hemorrhage	Multiorgan system failure
Fluid collections	Hypermetabolic state
Pseudocysts	Hypocalcemia
Duct rupture and strictures	Hyperglycemia
Extension to nearby organs	

Surgical Management of Acute Pancreatitis

The role of surgery in the management of acute pancreatitis is limited to débridement of infected pancreatic necrosis and cholecystectomy to prevent recurrent gallstone pancreatitis. The differentiation of infected from sterile pancreatic necrosis should be based on fine-needle aspiration for bacteriology.[63–65] Surgery in severe pancreatitis is usually deferred for at least 2 weeks to permit proper demarcation of pancreatic and peripancreatic necrosis to occur and to provide the optimal operative conditions for necrosectomy.[65] This approach decreases the risk of bleeding and minimizes surgery-related loss of vital tissue, which can predispose the patient to surgically induced endocrine and exocrine pancreatic insufficiency.

If acute pancreatitis is caused by gallstones or biliary sludge, then a cholecystectomy should be performed to avoid recurrence of gallstone-associated acute pancreatitis. It has been recommended that cholecystectomy should be performed as soon as the patient has recovered and, ideally, during the same hospital admission in mild gallstone-associated acute pancreatitis, whereas cholecystectomy may need to be delayed after an episode of severe acute pancreatitis. Endoscopic sphincterotomy is an alternative to cholecystectomy in selected cases.

CHRONIC PANCREATITIS

Chronic pancreatitis is a syndrome of destructive, inflammatory conditions that encompasses the many sequelae of long-standing pancreatic injury.[66] Thus, acute pancreatitis is an *event*, whereas chronic pancreatitis is a *process*.[4] Histologic changes from the normal pancreatic architecture include irregular fibrosis, acinar cell loss, islet cell loss, and inflammatory cell infiltrates.[67–69] Clinical diagnosis currently depends on identifying defined clinical, functional, morphologic, and histologic features that characterize the final common pathologic pathway of a variety of pancreatic disorders.[45,66]

Etiology of Chronic Pancreatitis

In adults, chronic pancreatitis is usually associated with prolonged ingestion of large amounts of alcohol (~ 70%) or appears to be idiopathic (~ 20%).[66] In children, chronic pancreatitis is usually associated with genetic conditions such as typical or atypical CF or hereditary pancreatitis or is idiopathic. The incidence of chronic pancreatitis appears to be increasing in children, perhaps reflecting better diagnostic tools or following an apparent rise in recurrent acute pancreatitis.

Current research suggests that chronic pancreatitis is better defined as a complex process beginning with acute pancreatitis and progressing to end-stage fibrosis as the result of recurrent and chronic inflammatory processes. As with acute pancreatitis, susceptibility and the rate of progression are influenced by both genetic and environmental factors. To date, genetic susceptibility factors for chronic

pancreatitis are the same as the susceptibility factors for recurrent acute pancreatitis (ie, *PRSS1*, *SPINK1*, and *CFTR* mutations). Several genetic modifiers have been reported in abstract form, but further research must be completed before their role in chronic pancreatitis can be clearly defined.

Recently, a new classification system has been developed to organize and understand the factors associated with chronic pancreatitis (Table 64.1-5). The TIGAR-O system allows multiple factors to be assessed in a single patient either as risk factors (before chronic pancreatitis develops) or as etiologies (after pancreatitis develops). This approach also allows the clinician to identify factors that can be modified to reduce risk (eg, smoking cigarettes or drinking alcohol). The common etiologies of chronic pancreatitis in adults (eg, alcohol, hypertriglyceridemia) are reviewed elsewhere.[66]

TABLE 64.1-5 ETIOLOGIC RISK FACTORS ASSOCIATED WITH CHRONIC PANCREATITIS: TIGAR-O CLASSIFICATION SYSTEM (VERSION 1.0)

TOXIC-METABOLIC
Alcoholic
Tobacco smoking
Hypercalcemia
Hyperparathyroidism
Hyperlipidemia
Chronic renal failure
Medications
Phenacetin abuse (possibly from chronic renal insufficiency)
Toxins
Organotin compounds (eg, di-n-butyltin dichloride)
IDIOPATHIC
Early onset
Late onset
Tropical
Tropical calcific pancreatitis
Fibrocalculous pancreatic diabetes
Other
GENETIC
Autosomal dominant
Cationic trypsinogen (codon 29 and 122 mutations)
Autosomal recessive/modifier genes
CFTR mutations
SPINK1 mutations
Cationic trypsinogen (codon A16V, D22G, K23R)
α_1-Antitrypsin deficiency (possible)
AUTOIMMUNE
Isolated autoimmune chronic pancreatitis
Syndromic autoimmune chronic pancreatitis
Sjögren syndrome–associated chronic pancreatitis
Inflammatory bowel disease–associated chronic pancreatitis
Primary biliary cirrhosis–associated chronic pancreatitis
RECURRENT AND SEVERE ACUTE PANCREATITIS–ASSOCIATED CHRONIC PANCREATITIS
Postnecrotic (severe acute pancreatitis)
Recurrent acute pancreatitis
Vascular diseases/ischemic
Postirradiation
OBSTRUCTIVE
Pancreatic divisum
Sphincter of Oddi disorders (controversial)
Duct obstruction (eg, tumor)
Preampullary duodenal wall cysts
Post-traumatic pancreatic duct scars

Adapted from Etemad B and Whitcomb DC.[66]

Trypsinogen Mutations

Trypsinogen mutations are responsible for pancreatitis in the majority of hereditary pancreatitis kindreds. The most common mutations include the cationic trypsinogen (*PRSS1*) R122H and N29I mutations, although a variety of other mutations are occasionally seen. Recommendations for genetic testing and genetic counseling have been published.[4,70,71] Hereditary pancreatitis caused by *PRSS1* mutations usually presents as recurrent acute pancreatitis in childhood with a median age of 10 years but a range of less than 1 year to 60 years of age.[16,72–74] The chronic pancreatitis in *PRSS1* mutation–associated hereditary pancreatitis usually follows the onset of recurrent acute pancreatitis by 10 years, although only half of the patients with recurrent acute pancreatitis develop chronic pancreatitis, and the clinical course is highly variable. Occasionally, patients will present with chronic pancreatitis without a clear history of acute pancreatitis. The most important clinical clue is a family history of pancreatitis or unexplained abdominal pain lasting for 1 to 3 days in adults from previous generations, although, in some cases, no other history of pancreatitis is seen.[75] The diagnosis is confirmed by genetic testing of the *PRSS1* gene, but about 30 to 40% of smaller hereditary pancreatitis families do not have identifiable mutations in the *PRSS1* gene.[75]

CFTR-Associated Pancreatitis

CF is the most important cause of chronic pancreatitis in children. CF is caused by severe mutations in both CFTR gene alleles ($CFTR^{sev}/CFTR^{sev}$). However, over 1,200 CFTR mutations have been identified, which are organized into five or six classes depending on the effect of the mutation on CFTR expression and function.[76] Some mutations (class IV) are mild or variable ($CFTR^{m\text{-}v}$), and compound heterozygous genotypes ($CFTR^{sev}/CFTR^{m\text{-}v}$) result in total CFTR function that is a fraction of normal (eg, 5%). These genotypes are associated with atypical CF, in which some CFTR-dependent organs are spared from severe CF-associated injury, whereas others are affected.[77,78] For example, the $CFTR^{sev}/CFTR^{m\text{-}v}$ genotypes have been associated with idiopathic chronic pancreatitis (ICP).[14,15] However, these studies also have an excess of patients who appear to be *CFTR* mutation heterozygous, whereas parents of CF patients (who are obligate *CFTR* mutation carriers) do not have an excess incidence of pancreatitis.[79]

CFTR mutation–associated pancreatitis can be divided into four mechanistic subtypes of CFTR-associated mechanisms.[4] Type 1 is CF with a $CFTR^{sev}/CFTR^{sev}$ genotype. Type 2 is atypical cystic fibrosis with a $CFTR^{sev}/CFTR^{m\text{-}v}$ genotype. Of specific interest are *CFTR* mutations that specifically block bicarbonate conductance but not chloride conductance.[80,81] These mutations may specifically target the pancreas over other organs because bicarbonate secretion by the pancreatic duct cells is central to pancreatic fluid secretion. Type 3 is $CFTR^{sev}$ or $CFTR^{m\text{-}v}$ plus a second pancreatitis modifier or susceptibility gene in a

polygenetic condition such as the CFTR^{sev}/SPINK1 N34S allele. Type 4 is CFTR^{sev} or CFTR^{m-v} plus a strong environmental risk factor such as alcohol.

Early- and Late-Onset ICP

Layer and colleagues observed that the age at onset of ICP is bimodal.[82] In early-onset ICP, calcification and exocrine and endocrine insufficiency develop more slowly than in late-onset idiopathic and alcoholic pancreatitis, but pain is more severe. In contrast, in late-onset ICP, pain is absent in 50% of patients.[82]

The etiology of early-onset ICP is being resolved. Witt and colleagues first identified the *SPINK1* N34S and other *SPINK1* mutations in children with ICP.[46] Pfützer and colleagues recently identified *SPINK1* mutations in about 25% of patients with ICP, but 87% of patients with *SPINK1* mutations developed pancreatitis before age 20 years.[47] A similar pattern was seen by Ockenga and colleagues.[83] However, 1 to 4% of representative populations have the high-risk *SPINK1* N34S allele, and the phenotype of heterozygous and homozygous genotypes is identical.[18,47] Thus, *SPINK1* mutations probably cause increased susceptibility to recurrent acute and chronic pancreatitis with homozygous mutations or as part of a polygenic condition with another genetic risk factor, such as type 3 CFTR-associated pancreatitis. Other genetic factors that increase susceptibility to pancreatitis or that modify the clinical course of recurrent acute pancreatitis toward rapid fibrosis likely will be defined in the near future.

Diagnosis

The diagnosis of chronic pancreatitis can be made by histologic or morphologic criteria alone or by a combination of morphologic, functional, and clinical findings.[69,84–87] Functional abnormalities alone are not diagnostic of chronic pancreatitis because these tests do not differentiate chronic pancreatitis from pancreatic insufficiency without pancreatitis.[66] Pancreatic insufficiency should be considered as either an end stage of destructive chronic pancreatitis or as arising from an independent condition, such as Shwachman-Diamond syndrome.[88,89]

Abdominal Imaging

Four imaging procedures are commonly used for the evaluation of pancreatic disease: CT, ERCP, endoscopic ultrasonography (EUS), and magnetic resonance imaging (MRI) or MRCP. Chronic pancreatitis with calcifications also can be identified on abdominal radiography or by transabdominal ultrasonography, and, when present, the diagnosis of chronic pancreatitis can be made with 90% confidence.[90] These techniques are used as inexpensive initial screening techniques in some centers.[90] However, abdominal radiography and transabdominal ultrasonography lack the sensitivity of CT, ERCP, and EUS.[85] EUS and MRI or MRCP are less widely available and require more technical expertise, and their precise role in evaluation of chronic pancreatitis in children remains to be fully defined.[91] At some centers, MRCP[92] (especially with secretin[93]) has replaced ERCP in most cases used for diagnosis in children, although ERCP remains a valuable tool for therapy and in some diagnostic cases.[23] Each of these newer technologies offers significant advantages and promise over ERCP or CT, but they also have limitations.

Pancreatic Function Testing

Several functional tests have been developed to diagnose chronic pancreatic insufficiency. As noted above, pancreatic insufficiency is a sign of chronic pancreatitis but is not diagnostic. The pancreas has marked functional reserve, so it must be damaged severely before functional loss is clinically recognized.[94] Invasive tests of pancreatic function (eg, the "tubed" secretin test) are the gold standard for determining exocrine pancreatic function.

Pancreatic function testing serves three purposes: to diagnose exocrine pancreatic insufficiency, to aid in the evaluation of chronic pancreatitis, and to provide a basis for rational treatment.[45,95] Mechanistically, pancreatic insufficiency reflects either impaired enzyme synthesis capacity, altered release of enzymes and bicarbonate into the intestine, or intraluminal impairment of pancreatic enzyme function or mixing.[96] Pancreatic function tests are difficult to compare between centers because they often use different stimulants and measure different parameters.[96] Furthermore, few centers perform direct testing of pancreatic exocrine secretion.

Noninvasive function tests to detect pancreatic insufficiency are also used infrequently because they are insensitive and have high false-positive rates.[95] Currently, there are two noninvasive pancreatic function tests available at many centers: fecal elastase 1 (FE-1) and "functional" MRCP. The FE-1 is an excellent test for moderate to severe chronic pancreatitis in adults[97] but is less accurate in mild to moderate chronic pancreatitis.[98] FE-1 reaches normal levels by day 3 in term newborns and by 2 weeks in infants born before 28 weeks of gestation.[99] The sensitivity and specificity of FE-1 and fecal chymotrypsin have been assessed in subjects with severe and mild steatorrhea.[100] Although FE-1 had superior performance characteristics in patients with mild steatorrhea, the ability to identify patients with moderate pancreatic insufficiency without steatorrhea remains a major limitation. The possibility of having a "functional" MRCP is very attractive because both structural and functional data could be gathered in one test. A functional MRCP protocol has been published,[101] but the sensitivity and specificity compared with those of direct testing must be demonstrated because the concentration of bicarbonate compared with the volume of pancreatic fluid is a critical discriminator in exocrine pancreatic function testing.

Genetic Testing

Genetic testing for pancreatic diseases is becoming an important part of medical practice. The results of genetic testing are highly accurate, but positive results for the major autosomal dominant, high-penetrance mutations (eg, *PRSS1* R122H) have broad implications for the patient's future health, family, employment, and insurability.[48,71,102,103] The purpose of genetic testing can be divided

into two general categories: diagnostic and predictive.[4] Diagnostic testing is done when a patient has symptoms of a disease, and a genetic test is done to determine the underlying cause(s). Examples include *CFTR* mutation testing in suspected typical or atypical cystic fibrosis or *PRSS1* (cationic trypsinogen) gene testing in suspected hereditary pancreatitis. Predictive testing is genetic testing in subjects without evidence of pancreatic disease. In general, predictive genetic testing in children is not indicated for *CFTR* or *SPINK1* mutations and is not recommended for *PRSS1* mutations unless there are first-degree relatives with a known *PRSS1* mutation, adequate genetic counseling has been offered, and the child can participate in the decision to undergo testing.[70] General guidelines are outlined in Tables 64.1-6 and 64.1-7.

Genetic testing is now commercially available for the *PRSS1* (cationic trypsinogen) R122H and N29I mutations.[66] The primary indications for cationic trypsinogen mutation testing include recurrent idiopathic acute pancreatitis, ICP, verification of a clinical suspicion in a family member of a kindred with known mutations, to help a patient understand or validate his or her condition, and to assist individuals in making lifestyle decisions (eg, reproduction, diet, smoking) based on the known risk of pancreatitis and potential pancreatic cancer.[66,71,104]

Genetic testing is also used in children with unexplained pancreatitis or episodes of pancreatitis-like pain when there is a significant concern about the possibility of hereditary pancreatitis. Identification of an established pancreatitis-associated gene mutation can be valuable in expediting an expensive and prolonged evaluation of recurrent pancreatitis in children.[45] A positive test result in a clinically unaffected person is interpreted as conferring a significant increased risk of pancreatitis, with this risk possibly diminishing with age. A negative test result in a family with a known mutation essentially eliminates the risk of this genetic form of pancreatitis. If a mutation has not been previously identified in the family, then a negative test result in an unaffected person is considered noninformative because one cannot distinguish whether the tested individual is free from genetic risk or whether he or she has inherited a different pancreatitis-predisposing gene mutation.[66] In families with hereditary pancreatitis, alcohol, emotional stress, and fatty foods are reported to precipitate pancreatitis attacks,[72] and smoking increases the risk of pancreatitis[105–107] and pancreatic cancer.[108] Testing for the purpose of encouraging mutation-positive older children to avoid these excesses is advocated by some caregivers. However, avoidance of fatty foods, alcohol, and tobacco represents excellent general medical advice and therefore provides no compelling reason for genetic testing.[71] In either case, the personal desires of older children to postpone testing or to proceed with testing to relieve their own anxieties and learn more about their own personal health must be carefully considered. Ownership of test results in children must be addressed.[66,70]

Testing for *SPINK1* mutations in individuals with early-onset chronic pancreatitis may provide important information on the predisposing causes of pancreatitis for the concerned patient.[66] However, most experts do not advocate genetic testing for *SPINK1* mutations at this time.[48,49,70] Furthermore, because less than 1% of patients with a heterozygous *SPINK1* mutation alone are likely to develop pancreatitis, the major reasons are lacking to undertake presymptomatic testing. Homozygous *SPINK1* N34S genotypes are strongly associated with chronic pancreatitis and, therefore, are likely a dominant factor in the etiology of otherwise ICP. Heterozygous *SPINK1* mutations alone are likely not disease causing[47,109] but rather act as a cofactor with other genetic mutations as part of a polygenic disorder.

There has been much interest in testing patients with ICP for *CFTR* mutations. The problem is that most panels are designed to test for the common CF-causing gene mutations, not pancreatitis-causing mutations. This area of research continues to develop, and pancreatitis-specific panels or total, low-cost CFTR gene analysis will be needed to fully interpret the CFTR genotype–pancreatitis phenotype relationship. On the other hand, pancreatitis may be the first sign of CF or atypical CF, and these children should, therefore, undergo a full evaluation for cystic fibrosis.

TABLE 64.1-6 INDICATIONS FOR GENETIC TESTING FOR *PRSS1* MUTATIONS

Recurrent (2 or more) attacks of *acute* pancreatitis for which there is no explanation (eg, anatomic anomalies, ampullary or main pancreatic strictures, trauma, viral infection, gallstones, alcohol, drugs, hyperlipidemia)
Unexplained (idiopathic) *chronic* pancreatitis
A family history of pancreatitis in a first-degree (parent, sib, child), second-degree, (aunt, uncle, niece, nephew), or third-degree (grandparent, first cousin) relative
An unexplained episode of documented pancreatitis occurring in a child who has required hospitalization and where there is significant concern that hereditary pancreatitis should be excluded
For a patient with pancreatitis eligible for an ethics committee/institutional review board–approved research protocol

Adapted from Ellis et al.[70]

TABLE 64.1-7 PREGENETIC TEST PATIENT INFORMATION FOR *PRSS1* MUTATIONS

Prior to genetic testing, patients should understand
Why the test has been suggested and provide documented informed consent
The implications of finding a pancreatitis-related mutation in the *PRSS1* gene for the health and medical care of that patient
How their genetic test result will be communicated to them and who else will be informed of their result (ie, the clinician who has requested that test, other involved pancreatic specialists, the family doctor, hospital medical records, insurance carrier)
The availability of genetic counseling after the test result is known
The pancreatic cancer risk and the possible adverse health, life insurance, and employment consequences for the patient (if not safeguarded against by state or national legislation)
The implications of a positive genetic test result for their relatives
Whether or not they wish for their test sample to be used for any research project and by what (anonymized) route this will occur

Adapted from a consensus statement of the Consensus Committees of the European Registry of Hereditary Pancreatic Diseases, the Midwest Multi-Center Pancreatic Study Group, and the International Association of Pancreatology.[70]

Modifier Genes

The reason that some patients with recurrent acute pancreatitis rapidly develop chronic pancreatitis whereas others do not likely involves mutations in disease-modifying genes.[4] This area of research is currently evolving, and several interesting candidate genes are under investigation and verification.

Treatment

The major goals in treating patients with chronic pancreatitis differ with the stage and etiology of disease. Patients with recurrent acute pancreatitis are at risk of developing chronic pancreatitis.[23,110] Efforts should be made to identify the cause of recurrent acute pancreatitis to prevent the development of chronic pancreatitis. Systematic approaches have been published,[111] and a number of risk factors can be addressed by the physician.[66] Anatomic variants can be addressed by both endoscopic and surgical interventions.

In patients with rapidly progressing chronic pancreatitis from an untreatable cause (eg, severe genetic mutations), there are two prognostic concerns. The first is development of a chronic pain syndrome, and the second is development of insulin-dependent diabetes mellitus. There are many possible etiologies of pancreatic pain.[112] The concern is that prolonged exposure of pancreatic nerves to growth factors associated with pancreatic inflammation will cause irreversible central changes so that eventual pancreatic surgery for pain will be of limited value. A second concern is the development of diabetes through destruction of the pancreatic islet cells. Although early surgical intervention with islet cell autotransplant is a consideration in these patients, the problem of islet yield, especially if delayed until chronic pancreatitis is advanced, and long-term benefit remain important issues without clear answers.

In patients with advanced chronic pancreatitis, the objectives are to treat the pancreatic insufficiency resulting from the loss of pancreatic digestive enzymes and to treat pain and diabetes mellitus if they develop. The treatment for pancreatic digestive enzyme deficiency is enzyme replacement.[113,114] The goal is to provide enzyme supplements that restore digestive function.[96] Enzymes are given with all protein- and fat-containing foods and milk products, including predigested formulas and breast milk. Microspheres, minimicrospheres, and microtablets are preferable to granules because the acid-resistant enteric coating protects the enzyme from acid degradation in the stomach and protects against the mouth and perianal excoriations that were seen previously with uncoated enzyme powders.[115,116] Enzymes should be taken about 15 minutes before each meal or snack. For prolonged meals, additional enzymes should also be taken during the meal. Parents and adolescent patients should be taught to adjust the enzyme dosage according to the anticipated amount of fat in a meal.

Generic enzymes may not be bioequivalent to proprietary enzymes.[117] Therefore, apparent treatment failures should include an investigation of the brand of enzymes that was dispensed. Another cause of failure is destruction of the digestive enzymes by gastric acid. Although enteric coating of pancreatic enzymes may protect pancreatic enzymes in the stomach, the intestine remains more acidic (1–2 pH units) in patients with *CFTR* mutations than those with other types of pancreatitis because of a loss in duodenal bicarbonate secretion.[118,119] In the past, treatment of gastric and intestinal acidity included the use of sodium bicarbonate and histamine$_2$ receptor antagonists. However, the availability, efficiency, and safety of the proton pump inhibitors have resulted in widespread use of these products. Enteric-coated products remain effective when used with proton pump inhibitors. On the other hand, patients who do not have CF and retain significant pancreatic function could theoretically benefit from a more acidic duodenal pH so that pancreatic fluid secretion is stimulated and enzymes are rapidly washed out of the pancreatic duct. In this case, gastric acid suppression would be limited, and enteric-coated enzymes are mandatory.

The dose of enzymes is usually calculated according to lipase content. A usual dose of pancreatic enzymes contains 1,000 to 2,500 U lipase/kg/meal. Adequacy of treatment is typically determined on clinical grounds. Frequent, bulky, fatty stools; excessive bloating and flatus; excessive appetite; and inadequate growth velocity are indicators of inadequate treatment. Calculation of a coefficient of fat absorption is used for clinical studies but rarely in clinical practice. Although the human FE-1 test accurately predicts exocrine pancreatic insufficiency,[120–122] it is of no value in determining the adequacy of enzyme replacements of porcine origin because the test is specific for human elastase 1.

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2. *Juvenile Tropical Pancreatitis*

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Chronic pancreatitis is mostly a disease of adults secondary to 10 to 15 years of alcoholism. Although relatively rare, hereditary pancreatitis is the most common type of chronic pancreatitis in children of the developed countries of the world. In many Afro-Asian countries, a nonhereditary, nonalcoholic form of chronic calcific pancreatitis is the most common type of chronic pancreatitis in children and young adults.[1–8] Nutritional pancreatitis, tropical pancreatitis, juvenile tropical pancreatitis syndrome, tropical calculous pancreatopathy, Afro-Asian pancreatitis, and fibrocalculous pancreatic diabetes are other terms used in the literature to describe this entity.

DEFINITION

Tropical pancreatitis is a form of chronic pancreatitis characterized by recurrent abdominal pain, pancreatic calculi, and diabetes mellitus, occurring mostly among poor children and young adults of many developing nations (Figures 64.2-1 to 64.2-3). The affected individuals are generally emaciated and may show signs of malnutrition. The notable absence of other known causes of pancreatitis, the geographic prevalence of the disease in developing nations, and the scientific plausibility of pancreatic injury in malnutrition are the keys to implicating nutritional deficiency as the most likely etiologic factor for this otherwise enigmatic disease.

EPIDEMIOLOGY

Although isolated case studies have been reported in the Indian medical literature since 1930, the first clear description of this syndrome was made in 1959 by Zuidema from Indonesia.[1] This classic article described seven malnourished Indonesian patients with pancreatic lithiasis. The youngest, a 15-year-old girl, was markedly undernourished, weighing only 33.5 kg. Her main meal at home was rice, cassava (*Manihot esculenta*), and vegetables and seldom included fish, meat, or eggs. The oldest in the group was 28 years of age. None had a history of alcohol consumption. In six patients, diabetes mellitus dominated the clinical picture. Some of them had marked swelling of both parotid glands and thinning of scalp hair resembling kwashiorkor. In one case, autopsy showed fibrosed acinar tissue and stones in the duct. Zuidema subsequently reported on 45 patients from 12 to 45 years of age with the same clinicopathologic features.[1] The diabetes of the poor in Indonesia, Zuidema concluded, was a result of severe protein malnutrition.

In 1960, Shaper observed a similar syndrome in the indigenous population of Uganda, whose diet was rich in carbohydrate but low in protein and fat.[2] The youngest patient was 10 years old. Most patients had a history of moderate to severe recurrent abdominal pain, suggestive of pancreatitis. Shaper felt that the high-carbohydrate diet associated with severe protein deficiency led to increased demands for pancreatic enzymes while potentiating the effect of protein depletion.

The syndrome of chronic pancreatitis with pancreatic calculi and diabetes has subsequently been reported by different observers from many countries, such as Uganda, Nigeria, the republic of Congo, Malawi, Zambia, Ghana, the Ivory Coast, and Madagascar in Africa; Sri Lanka, Malayasia, Thailand, India, and Bangladesh in Asia; and Brazil in South America.[1] In support of the term tropical pancreatitis, the prevalence of this disease is almost restricted to latitude 30° north and south of the equator.

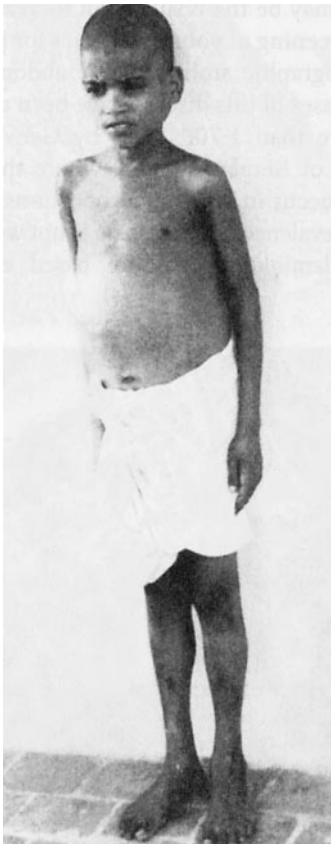

FIGURE 64.2-1 A 13-year-old boy with juvenile tropical pancreatitis. Note the emaciation and distended abdomen.

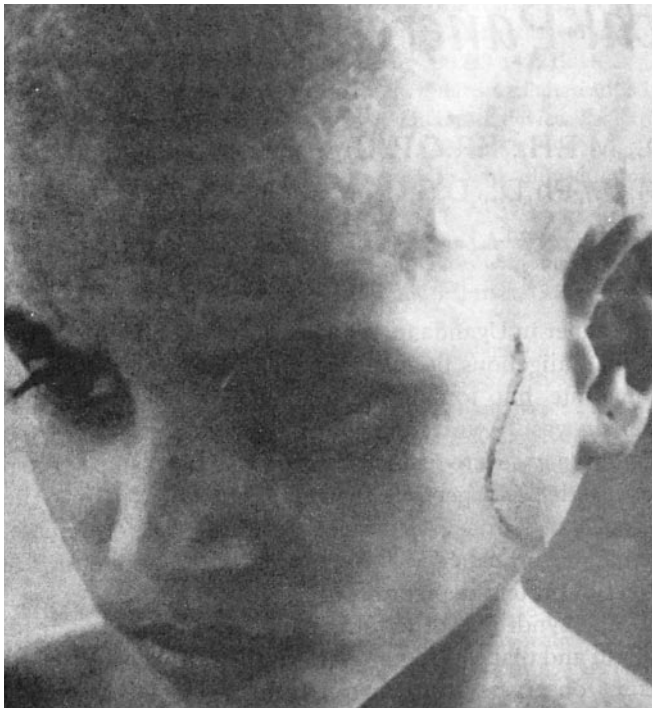

FIGURE 64.2-2 Parotid gland enlargement in the boy shown in Figure 64.2-1.

The largest series of cases of juvenile tropical pancreatitis to date is from the southwestern state of Kerala in India. This may be the result of an increased awareness and routine screening of young diabetics for pancreatic calculi with radiographic studies of the abdomen. Approximately 3,000 cases of this disease have been reported in the literature, more than 1,700 cases by GeeVarghese alone from the state of Kerala in India, where the disease was once noted to occur in endemic proportions.[5]

The true prevalence of this disease is not well established because the epidemiologic data are based exclusively on patients seen in major teaching hospitals that do not include those studied in nonteaching hospitals and outpatient clinics of private practitioners. On the other hand, the hospital data may give an erroneously high prevalence because most of the patients from villages tend to accumulate in the major teaching hospitals for treatment. The data can be further skewed because in many Afro-Asian countries, men seek medical attention more often and earlier than women. One epidemiology study in an endemic area in the state of Kerala reported a prevalence of 1 in 1,000 population.[9] In a referral diabetic center, tropical pancreatitis constituted 1% of all diabetic patients.[8] The disease currently appears to have decreased in its incidence in the state of Kerala in India, where, nearly three decades ago, it was noted to be frequent. A marked improvement in the socioeconomic status of the population in the state and an associated improvement in childhood nutrition may be reasonably assumed to be the reasons for this change in incidence.

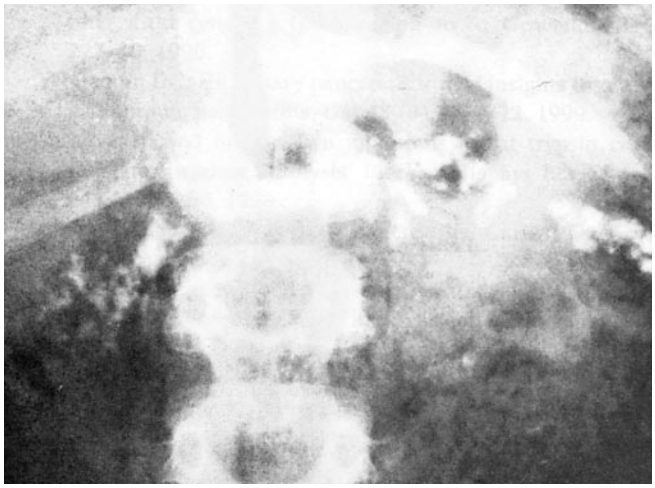

FIGURE 64.2-3 Flat plate of the pancreas in a case of juvenile topical pancreatitis. The entire main pancreatic duct and even some ductules are packed with calculi. A ductogram is seen.

PATHOLOGY

The pathologic changes in the pancreas and other organs in tropical pancreatitis have been well studied in material obtained at postmortem or surgery.[8,10,11] Because pancreatic biopsy is not done in the early stages of the disease, our knowledge of the pathology is limited to the late stages. The histologic changes in the pancreas are almost identical to those of alcoholic pancreatitis.

The size of the pancreas varies inversely with the duration and severity of the disease. In advanced stages of the disease, the pancreatic gland is as small as the little finger, and the surface is irregular and nodular. Uneven shrinkage and fibrous adhesions cause displacement of the pancreas from its normal location. The parenchyma may be replaced by fat and become indistinguishable from surrounding adipose tissue. The pancreas is firm, fibrous, and gritty to the touch, although the consistency of the organ may vary in different regions of the gland depending on the presence of fibrous tissue, cysts, or stones. Radiologic examination of the dissected pancreas often reveals multiple calculi, which are not noted in antemortem radiologic studies (Figure 64.2-4).

Homogeneous areas; varying degrees of fibrous, cystic dilatation of the gland; and pancreatic calculi of different shapes and sizes distributed throughout the duct system characterize the cut-section. The major pancreatic duct may be eccentrically placed as a result of uneven destruction of the glandular tissue. Areas of stenosis and dilatation of the ducts can be seen in the same gland. Incomplete pancreatic obstruction at the ampulla of Vater is noted in a large majority of carefully dissected cases, corresponding to the location of a solitary calculus ("sentinel stone") and/or larger stones.[5]

Pancreatic calculi vary in color, size, and shape. The larger stones are nearer the head, progressively diminishing in size toward the tail. The stones range in size from small sand particles to calculi 4.5 cm long, weighing up to 20 g. The shape of a stone is influenced by its location; may be smooth, rounded, or staghorn-like; and may be incarcerated in the main pancreatic duct and major branches.

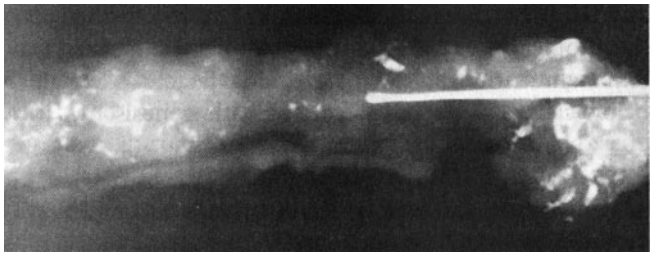

FIGURE 64.2-4 Radiologic study of the isolated postmortem pancreas. Note the numerous small radiodense areas, which are intraductal calculi. The probe is passed into the main duct to show the dilatation in relation to the shrunken pancreas.

Soft stones are formed by noncalcified protein plugs and caseous material. Sections of calcified stones show epithelial debris, fibrin, and mucinous material.

Pancreatic calculi are composed of 95.5% calcium carbonate and a small amount of calcium phosphate. Traces of magnesium, urate, and oxalate have been identified in some stones. X-ray diffraction studies of calculi have determined that calcium carbonate is found predominantly in the form of calcite and rarely in the form of vaterite.[12] Scanning electron microscopic studies and spectroscopic methods of analysis have shown that the calculi have an amorphous nidus and a cryptocrystalline periphery. The nidus is rich in iron, chromium, and nickel, and the periphery contains a number of trace elements and a preponderance of calcium.[13] These calculi are structurally and biochemically similar to stones obtained in other types of chronic pancreatitis. A nonenzymatic protein has been identified by some observers in the core of calculi. This protein, termed pancreatic stone protein (PSP), has been implicated in the pathogenesis of the disease and in calculus formation. The absence or decrease of PSP has been thought to promote nucleation of calcium carbonate and crystallization in chronic pancreatitis.[14] However, recent studies on PSP have given contradictory results, and the role of PSP in the genesis of calculi and the pathogenesis of disease is unclear.

Microscopically, the characteristic feature is diffuse fibrosis of the pancreas (Figure 64.2-5). The main duct, collecting ducts, and small ductules show marked dilatation with periductular fibrosis. Denudation of the ductular epithelium and squamous metaplasia are seen in some areas. The characteristic cellular infilitraton of the pancreas is composed of lymphocytes and plasma cells, distributed mainly around the ducts. Interlobular fibrosis is characteristic of early cases, and focal, segmental, or diffuse fibrosis is characteristic of more advanced cases. The acinar tissue shows varying degrees of atrophy and parenchymal destruction. Fibrous tissue is seen adjacent to relatively normal-looking parenchyma. As the disease advances, the islets become atrophic and are isolated and surrounded by dense fibrous tissue. In some instances, the islets appear even hypertrophied, and, as in other forms of pancreatic atrophy, a true nesidioblastosis is observed (Figure 64.2-6). Preliminary histochemical studies have identified those hyperplastic islets as B-cell nesidioblastosis. Immunohistochemistry has also shown

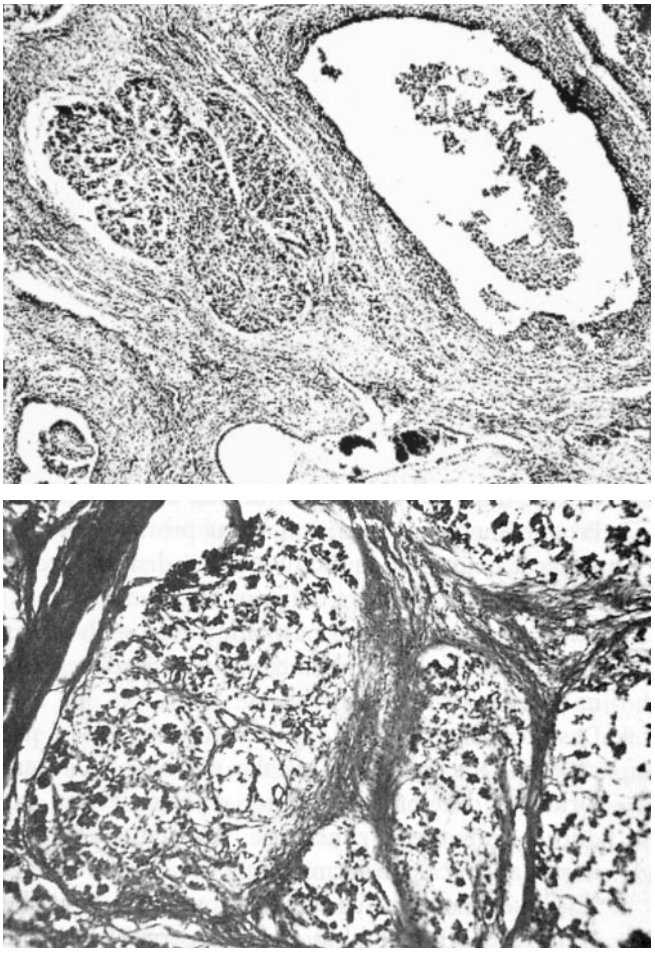

FIGURE 64.2-5 The pancreas shows extensive fibrosis, ductular dilatation, and intraductular calcium deposits. The intra- and interacinar fibrosis of the exocrine parenchyma produces the appearance of cirrhosis of the pancreas (hematoxylin and eosin).

reduced alpha cells and beta cells in the pancreas.[11,15] The vacuolation, ballooning, and glycogen infiltration of the islets characteristic of juvenile diabetes are seldom noted. The clinical significance of the islet cell hyperplasia is to be further studied.

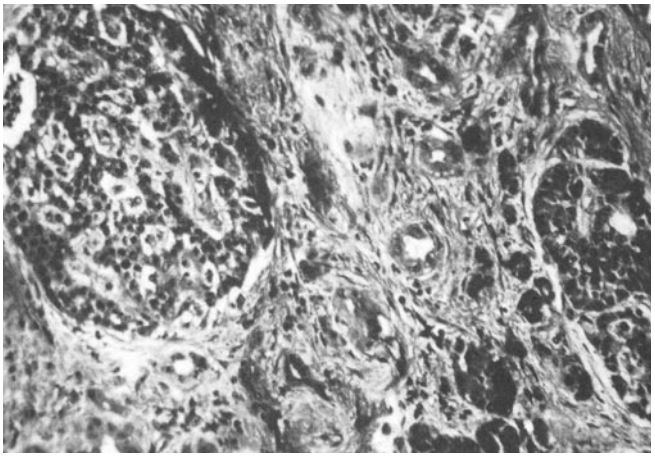

FIGURE 64.2-6 The islets show varying degrees of hypertrophy in the presence of fibrosis of the organ.

The high incidence of pancreatic carcinoma in patients of a relatively young age suggests that tropical pancreatitis is a premalignant disease similar to hereditary pancreatitis.[16,17] Other organs such as the liver and parotid glands show changes indicative of uncontrolled diabetes mellitus and/or malnutrition. The liver in the early stages shows glycogen infiltration of the cytoplasm and nuclei and fatty changes (and cirrhosis in more advanced cases). Parotid glands show hypertrophied acini, with varying degrees of round cell infiltration around the intralobular and interlobular ducts. The pathogenesis of parotid enlargement is probably a functional or compensatory hypertrophy as an adaptive mechanism to pancreatic exocrine insufficiency.[18]

ETIOLOGY AND PATHOGENESIS

The exact etiology of this disease has not yet been established. The etiologic factors proposed here are to be considered hypothetical, based on epidemiologic data, careful clinical studies, and biochemical evaluations. The hypotheses in consideration are as follows:

Malnutrition

The basis for considering malnutrition as a predisposing factor was the prevalence of the disease almost exclusively in the poor population groups of developing nations and the findings of malnutrition in many patients.[3–7] Protein malnutrition is known to cause pancreatic injury in experimental and clinical studies. In kwashiorkor, as well as marasmus, pancreatic structure and function are markedly altered.[17] Some of the histologic changes of the pancreas in kwashiorkor, such as atrophy of acinar cells, disorganization and loss of the acinar pattern, marked reduction in the amount of zymogen granules, vacuolization, epithelial metaplasia, cystic dilatation of the ducts, and an increase in fibrous tissue, mimic the histology of tropical pancreatitis.

A number of recent observations, however, speak against protein malnutrition as the sole or initiating factor of this disease[19]:

1. In India and Africa, the geographic prevalence of the disease does not correlate with that of kwashiorkor.
2. Protein-energy malnutrition, being prevalent in many tropical countries, is likely to be a denominator in most diseases affecting poor populations.
3. There are large pockets of malnutrition with relative infrequency or total absence of tropical pancreatitis in many parts of the world.
4. Despite some histologic similarities to tropical pancreatitis, the pathology of the pancreas in kwashiorkor is different. The latter disease seldom produces permanent pancreatic damage, and, more importantly, calculi formation is not a feature.
5. The disease currently is seen in some well-nourished children of affluent families, further complicating the malnutrition theory.

The advanced malnutrition noted in tropical pancreatitis studied three decades ago appears to be the consequence of the disease rather than its cause. It is thus clear that tropical pancreatitis is not secondary to isolated protein malnutrition, although nutritional factors as explained below cannot be excluded from its pathogenesis.

Free Radical Injury

Clinical protein-energy malnutrition is a complex syndrome complicated by deficiencies of a number of vitamins and trace elements, bacterial and viral infections and parasitic infestations, psychological stress, and hormonal and immunologic disturbances. The body's ability to scavenge the highly reactive free radicals is markedly impaired in malnutrition, whereas the endogenous and exogenous stimuli for free radical production are markedly enhanced. Chronic pancreatitis, alcoholic or tropical, has been hypothesized as one of the many diseases caused by unmitigated free radical injury.[20,21]

However, in view of the difficulties in studying free radical production and elimination in the pancreas, free radical injury as a mechanism of pancreatitis remains in the realm of hypothesis.

Trace Elements and Vitamin Deficiencies

Independent of their ability to scavenge free radicals, trace elements and vitamins participate in maintaining the integrity of acinar cell function and structure. Experimental studies indicate that a zinc-deficient diet results in acinar cell injury, copper deficiency induces selective and progressive atrophy of acinar cells, and selenium deficiency causes pancreatic fibrosis.[22] Vitamin A, riboflavin, folic acid, and vitamin D appear to be important for acinar cell integrity.[22] Although deficiency of these trace elements and vitamins may occur as part of the spectrum of human malnutrition, clinical pancreatic disease has not been proven to be secondary to micronutrient deficiency.

Dietary Cyanogens

The geographic distribution of tropical pancreatitis coincides with areas of consumption of cassava root (tapioca, *Manihot esculenta*), which is a source of carbohydrate for poor populations in parts of Nigeria, Uganda, Indonesia, Thailand, and the state of Kerala in India.[5]

Cassava root is known to contain cyanogenic glycosides: linamarin and lotaustralin.[23] Cyanide is normally detoxified in the body by conversion to thiocyanate, but this detoxification requires sulfur containing the amino acid methionine, which is deficient in cassava. A high-carbohydrate, low-protein diet was shown to cause pancreatic fibrosis in a recent experimental study, raising the possibility that the nutritional composition of the diet is important in pancreatic injury[24] rather than the source of carbohydrate or the presence or absence of cyanogen in the diet. Although tropical pancreatitis is prevalent in some parts of Africa and India where cassava is not consumed, it is not noted in some areas where cassava is consumed, as in the rural West African population.[25]

Genetic Factors

Many recent studies have looked for genetic abnormalities in all forms of chronic pancreatitis following the discovery of genetic mutations in hereditary pancreatitis.[26–28]

In the normal pancreas, a number of mechanisms work synergistically, preventing the premature activation of trypsinogen to trypsin. The central mechanism of acinar cell injury is autodigestion by active trypsin. Mutations of certain genes reduce the natural ability of the body to prevent the premature activation of trypsinogen. The most important mutations studied in relation to hereditary pancreatitis and, to a lesser extent, in other forms of pancreatitis are those involving the cationic trypsinogen gene (*PRSS1*), serine protease inhibitor Kazal type 1 (SPINK 1), and cystic fibrosis transmembrane conductance regulator (CFTR).[29] In light of what has been learned with regard to genetic abnormalities in hereditary pancreatitis, tropical pancreatitis is being currently evaluated for possible genetic mutations. On a small cohort of tropical pancreatitis patients, the frequency of CFTR mutations was lower than that reported in idiopathic chronic pancreatitis from the West.[30]

In two recent studies, one from India and the other from Bangladesh, it was noted that tropical pancreatitis was highly associated with the SPINK 1 *N34S* mutation.[31,32] The high prevalence of N34S mutation in patients with and without diabetes in the Indian study suggests that these two subtypes have a similar genetic predisposition.[31,32]

In conclusion, the etiopathogenesis of this syndrome remains enigmatic, and further studies are clearly needed. Its relationship to cystic fibrosis is an area that needs to be explored.

CLINICAL FEATURES

The cardinal manifestations of juvenile tropical pancreatitis are recurrent abdominal pain in childhood, followed by diabetes mellitus and pancreatic calculi by puberty and death in the prime of life. Improvement in the management of diabetes has resulted in a longer life span not noted in earlier observations. The calculated life expectancy after the onset of abdominal pain and diabetes is 35 and 25 years, respectively.[33]

The onset of the disease is insidious in early childhood, with recurrent attacks of upper abdominal or periumbilical pain before the thirteenth year (Table 64.2-1). The history is often elicited from the patient's mother, who attests to the number of school days lost. About 5% of juvenile diabetics with pancreatic calculi do not have abdominal pain.[5] The patient usually keeps the palm on the abdomen to indicate a wide area of pain as opposed to the finger tip, as in duodenal ulcer. The pain radiates to the lower end of the sternum, the left costal margin, and along the left side or posteriorly to the lumber spine. The episodes of pain last for days, not minutes or hours. The pain is usually aggravated by small amounts of food so that the patients refuse all food by mouth. In the early stages, the bouts of pain are severe and are associated with vomiting. As years pass, painful attacks become less intense but more prolonged. In an attempt to obtain relief, patients sit up, bend forward, or walk; curl up in the lateral decubitis position; clutch the skin of the abdomen; or apply hot water bottles to the area. Recent studies have noted a change in the age at onset of the disease. Only about 12% of patients report an onset before the age of 20 years.[7] The observed difference in the current series of cases from those reported earlier cannot be explained.[4,7]

TABLE 64.2-1 AGE AT ONSET OF PANCREATIC PAIN IN 100 CASES

AGE (YR)	NUMBER OF CASES
5–11	26
12–18	35
19–25	14
26–30	5
31–35	1
36–40	2
41–50	2
No pain	6
Undetermined	9
Total	100

Adapted from GeeVarghese PJ et al.[3]

An interval of several years may pass between the cessation of painful attacks and the onset of diabetes mellitus. Pancreatic pain totally disappears in a large number of patients either before or some years after diabetes develops, coinciding with "burning out the pancreas." It is uncommon for diabetes to precede abdominal pain.

Patients are often repeatedly treated with antihelminthics and antacids after the mistaken diagnosis of parasitic disease or peptic ulcer. Persistent abdominal pain in childhood of undetermined etiology has often led to diagnostic laparotomy. In the absence of demonstrable pancreatic calculi, there is no easily available test to establish the diagnosis of chronic pancreatitis at this stage of illness.

DIABETES MELLITUS

Most patients initially seek medical attention for diabetes mellitus, which becomes clinically manifest a few years after the onset of pancreatalgia. A pain-free period of 1 or 2 years and an apparent transient improvement in the clinical picture prior to the onset of diabetes are not unusual. The age at onset of diabetes from India is presented in Table 64.2-2.

The fasting blood glucose level ranges between 200 and 400 mg/dL, and postprandial blood glucose levels greater than 700 mg/dL are not rare. Pancreatic diabetes is characteristically brittle, with marked fluctuations of blood glu-

TABLE 64.2-2 AGE AT ONSET OF PANCREATIC DIABETES IN 100 CASES

AGE (YR)	NUMBER OF CASES
Below 13	2
14–15	3
16–20	19
21–25	10
26–30	9
31–35	7
36–40	4
41–50	2
Undetermined	44

Adapted from GeeVarghese PJ et al.[3]

cose values with or without insulin therapy. Episodes of hypoglycemia are characteristic and may complicate the administration of even small doses of insulin. This may be a reflection of depleted glycogen reserves in the liver or decreased glucagon release from the pancreas. Spontaneous hypoglycemic episodes have been recorded without insulin therapy. True insulin resistance, defined as a daily requirement of over 200 U of insulin in the absence of infection or ketosis reported earlier, is no longer seen with the use of purified and human insulins.[8] Metabolic acidosis is uncommon, but ketosis may be seen in less than 5% of cases.[7]

C-peptide assay shows partial preservation of beta cells, which is responsible for the ketosis resistance.[8] Diabetic retinopathy and nephropathy do occur and are related to the duration of diabetes.[5,8] Other complications of pancreatic diabetes include neuropathy, recurrent urinary tract infections, and pyelonephritis. The liver is palpably enlarged in 40% of diabetics, although the only liver functional abnormality may be elevation of alkaline phosphatase, indicating fatty liver.[5] Autonomic nervous system dysfunction occurs with similar frequency and severity, as in patients with non–insulin-dependent diabetes mellitus.[34]

EXOCRINE PANCREATIC INSUFFICIENCY

Overt exocrine pancreatic insufficiency characterized by steatorrhea is the least striking clinical feature, attributable to the very low consumption of fat in the diet. However, on a diet of 100 g of fat, more than 70% of patients develop biochemical steatorrhea.[7]

Clinical and biochemical evidence of obstructive jaundice is a well-recognized complication secondary to stenosis and compression of the common bile duct, which is tunnelled in the head of the pancreas. Pancreatic pseudocysts are less uncommon than in alcoholic or biliary pancreatitis.

DIAGNOSIS

The diagnosis of chronic pancreatic injury in the early stages of the disease in young children is seldom made. Abdominal pain in childhood is often ignored or attributed to psychogenic causes or, in the tropics, to parasitic infestations. Endoscopic retrograde cholangiopancreatography (ERCP) or computed tomography (CT) will be helpful in earlier detection of the disease. An ERCP characteristically shows a markedly dilated main duct with a radiopaque and lucent calculi. Sonograms and CT scans of the abdomen help in identifying the calculi and the dilated ducts. Cost and limited availability, however, make it impractical to use CT scans and ERCP for the routine diagnosis of tropical pancreatitis. There are no sensitive and specific noninvasive blood or urine tests to diagnose chronic pancreatitis. Even in the developed nations of the world, the diagnosis of chronic pancreatitis in adults or children is often elusive and made very late, only after ductal changes or calculi develop.

On the other hand, the picture of a well-established case of tropical pancreatitis is so characteristic that a diagnosis based on clinical features alone is suspect. The onset of diabetes mellitus with a present or past history of recurrent abdominal pain in a young individual suggests chronic pancreatitis. Extreme emaciation, bilateral parotid gland enlargement, and a distended upper abdomen are seen only in patients with established and advanced disease. A peculiar cyanotic hue of the lips has been mentioned in early reports[3,4] but is noted infrequently in the majority of patients.[7]

The diagnosis is established by demonstration of pancreatic calculi on a flat-plate radiograph of the abdomen. The most common site of pancreatic calculi on the abdominal flat plate is to the right of the first and second lumbar vertebrae. The lateral extension is up to 2 to 5 cm to the right of these vertebrae. Calculi are most numerous in the head of the pancreas. In 30% of cases, the calculi form a cast of the main duct.[5] In the lateral film, the stones are located anterior to the vertebral body but posterior to the gallbladder area.

The diagnosis of tropical pancreatitis does not depend on the demonstration of pancreatic exocrine functional abnormality. Serum amylase determination is not often useful in the diagnosis of chronic pancreatitis except in acute exacerbations. The amylase is below normal in a large number of cases. Steatorrhea is manifest only on a high-fat test diet given prior to stool fat testing. Secretin cholecystokinin stimulation tests are expensive and time consuming and are seldom performed. Limited studies done in an academic setting have shown a marked decrease in volume and enzyme output. Bicarbonate secretion is normal in some studies but markedly reduced in others. The newer diagnostic tests—bentiromide test, pancreaolauryl test, and fecal chymotrypsin assays—are likely to be of limited value except in assessing exocrine insufficiency in late stages of the disease.

MANAGEMENT

The management of tropical pancreatitis consists of alleviation of abdominal pain, treatment of diabetes, prevention of complications, and correction of nutritional problems.

The treatment of acute episodes of painful attacks is similar to the treatment of other types of pancreatitis. The measures to "put the pancreas to rest" include no feeding by mouth and the use of intravenous fluids and electrolytes. Nasogastric suction may be needed in severe cases. The treatment of pain may require repeated injections of meperidine, but there is a danger of producing narcotic addiction. The role of large doses of orally administered enzyme therapy for pain in tropical pancreatitis is not well studied. The basis of such therapy is the experimental observation that orally administered proteases suppress endogenous enzyme production through a feedback inhibition.[35,36] Enzyme therapy may not help patients with tropical pancreatitis, a disease characterized by marked dilatation of duct and ductules. Success with enzyme therapy is limited to patients with nondilated ducts with functioning acinar cells. Empiric therapy with oral antioxidants appears to be effective in the management of pain in alcoholic pancreatitis.[37] Although not well proven, in view of its simplicity, antioxidant therapy is worth a trial in all patients. Endo-

scopic papillotomy with the removal of stones and the clearance of dominant strictures and obstructions has shown good results in carefully chosen patients.

Unremitting pain is an indication for surgical treatment. The best procedure is the exploration of the pancreatic duct, the removal of stones, and longitudinal anastomosis of the split surface of the pancreas to the jejunum (Puestow procedure).[38] The relief of pain, even with surgery, may be temporary.

The treatment of diabetes is with dietary manipulation using oral hypoglycemic agents and insulin therapy. The dietary management of diabetes in pancreatitis is complicated.[8] The associated malnutrition, malabsorption, and tendency toward hypoglycemia deserve consideration in prescribing a suitable diet. A nutritious diet supplemented with vitamins and minerals is needed, and it is not advisable to restrict the carbohydrate content of the diet below 300 g. The diet may have to be supplemented with adequate protein intake, and pancreatic enzyme preparations are also advised to correct malabsorption.

If the diabetes is mild, oral hypoglycemic agents may be used, especially in the first few years after the onset of diabetes. Insulin therapy is required to control hyperglycemia in the large majority of cases. Often a combination of insulin and oral hypoglycemic agents is used to reduce the cost of therapy with insulin alone. Supplementary pancreatic enzyme therapy in a preliminary study was shown to reduce marked fluctuations of blood sugar.[39]

SUMMARY AND CONCLUSIONS

Juvenile tropical pancreatitis is a type of chronic pancreatitis that occurs in children and young adults of many developing nations. Although the etiology is not established, malnutrition is an important epidemiologic association. Other proposed etiologic factors include unopposed free radical injury, trace element and vitamin deficiencies, and dietary cyanogen toxicity. Many recent studies have identified genetic markers, and an association with SPINK 1 and N34S mutation has been noted. The occurrence of abdominal pain in childhood followed by the onset of diabetes in an emaciated teenager is the typical clinical picture, and the radiologic demonstration of calculi in the pancreatic duct is the hallmark of the disease. Patient management involves the control of diabetes with an oral hypoglycemic agent and/or insulin. Painful attacks of pancreatitis require the use of analgesics or surgery. Nutritional management should include a diabetic diet with adequate complex carbohydrates and frequent small meals, supplemented with oral pancreatic enzymes.

Tropical pancreatitis is an enigmatic disease that requires further study to explain its etiopathogenesis. It may be one of the preventable forms of diabetes in children in the tropics.

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CHAPTER 65

EXOCRINE PANCREATIC DYSFUNCTION

1. *Cystic Fibrosis*

Kevin J. Gaskin, MD, FRACP

Cystic fibrosis (CF) is an autosomal recessively inherited disorder caused by mutations of the cystic fibrosis transmembrane conductance regulator (CFTR) gene and characterized clinically by chronic suppurative lung disease and exocrine pancreatic failure. The early postmortem descriptions of CF in the 1930s recognized both the pulmonary and pancreatic components of the disorder,[1,2] establishing it as a separate entity to "celiac syndrome" (a collective term for malabsorptive disorders of children in that era). Later, di Sant'Agnese and others demonstrated that CF patients have elevated sweat salt concentrations,[3] and this has remained the mainstay of diagnosis until the present day. The CFTR gene was discovered in the late 1980s[4–7] with the subsequent demonstration that the gene product (CFTR protein) was a cyclic adenosine monophosphate (cAMP)-stimulated Cl^- channel.[8] Over the same era, there were many significant advances in clinical management, including antibiotic therapy for lung disease, microspheric pancreatic enzyme replacement therapy, and nutritional therapy, all of which enhanced median survival from 10 to nearly 40 years of age over the last half-century. This chapter addresses concepts of the pathophysiology of this disease, its genetics, and associated gastrointestinal and nutritional problems. The reader is referred elsewhere for reviews of respiratory disease[9] and reproductive tract complications.[10]

ETIOLOGY

The pathophysiologic basis of CF centers on the CFTR protein and its function in absorptive and secretory epithelial tissue. The *CFTR* gene was located on chromosome 7[4] and was subsequently cloned, and the sequence of the gene and protein product were determined in 1989.[5-7] CFTR is a membrane protein, and its secondary or domain structure, proposed by Riordan and others,[6] included two membrane spanning domains (MSD1 and MSD2), two nucleotide binding domains (NBD1 and NBD2), and a regulatory domain (R), as depicted in Figure 65.1-1. The CFTR protein functions as a phosphorylation-dependent Cl^- channel located in the apical membrane of epithelial cells, as evident from data showing (1) the expression of CFTR in cells that did not normally contain cAMP-dependent Cl^- channels and the subsequent demonstration of a Cl^- current activated by cAMP agonists,[11] (2) the demonstration of the similarity between Cl^- currents in cells expressing recombinant CFTR and in epithelial cells expressing native endogenous CFTR,[12] (3) mutations of CFTR altering Cl^- transport,[13] and, finally, (4) purified recombinant CFTR in planar lipid bilayers demonstrating Cl^- channel properties identical to those in native epithelia.[8]

CFTR is important in regulating electrolyte transport in absorptive and secretory epithelia.[14] Cholera toxin, for instance, stimulates massive chloride and subsequent fluid secretion from intestinal epithelia. The chloride secretion is mediated by a direct effect of the toxin on the cAMP-

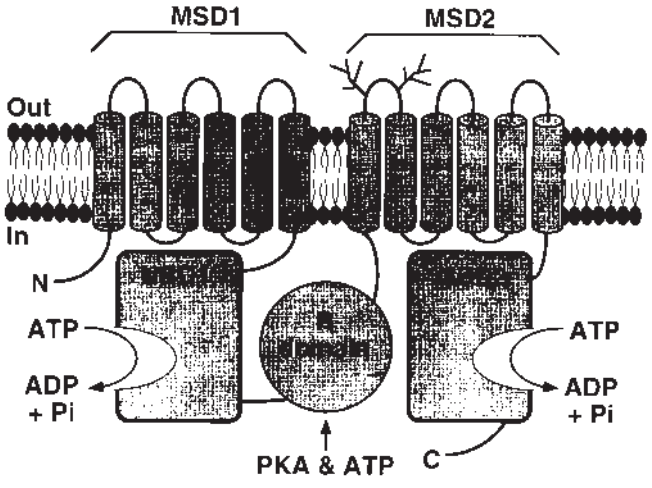

FIGURE 65.1-1 Schematic model of the cystic fibrosis transmembrane conductance regulator with membrane spanning domains (MSD1 and MSD2), nucleotide binding domains (NBD1 and NBD2), and regulatory domain (R). ADP = adenosine diphosphate; ATP = adenosine triphosphate; N = nucleotide; PKA = protein kinase A; Pi = phosphate. Reproduced with permission from Sheppard DN, Welsh MJ.[14]

dependent CFTR channel, as confirmed recently by the demonstration that the highly specific CFTR inhibitor, thiazolidinone, markedly reduces Cl^- secretion in response to cholera toxin stimulation.[15] As seen in Figure 65.1-2, a simple model demonstrates that Cl^- accumulates intracellularly following entry via the Na^+-K^+-Cl^- cotransporter in the basolateral membrane. Recycling of Na^+ occurs by the basolateral Na^+-K^+ exchanger and of K^+ through independent K^+ channels, whereas Cl^- moves down its electrochemical gradient to the lumen following activation of the cAMP Cl^- channel in the apical luminal membrane. Na^+ is transported paracellularly in response to the lumen negative voltage set up by Cl^- transport. In absorptive epithelia, for example, in the sweat duct, CFTR functions in almost a reverse fashion to the above and is involved in electrolyte and fluid absorption. In addition to its function as a cAMP-dependent Cl^- channel, CFTR as an ABC transporter (adenosine triphosphate [ATP] binding cassette protein) can regulate other membrane channels and the transport of drugs, amino acids, and peptides.[16] These functions are summarized in Figure 65.1-3 and include cAMP Cl^- channel function, facilitation of ATP release, positive regulation of outwardly rectifying Cl^- channels (ORCC), negative regulation of epithelial Na^+ channels, regulation of intracellular vesicle trafficking, intracellular compartment acidification and glycoprotein processing, and modulation of renal outer medullary K^+ channel sensitivity to sulfonylureas. Moreover, release of ATP may further enhance or regulate ORCC via purinergic receptors, and other chloride channels, including Ca^{++} activated channels, appear to be regulated by external nucleotides.

Patients with CF have impaired chloride reabsorption from sweat ducts and, in general, elevated sweat chlorides greater than 60 mmol/L. Transport defects have also been demonstrated in vivo in both respiratory and pancreatic duct epithelium. In upper airway epithelium from CF subjects, the potential difference across the epithelium of 53 mV is about twice that observed in normal controls, and

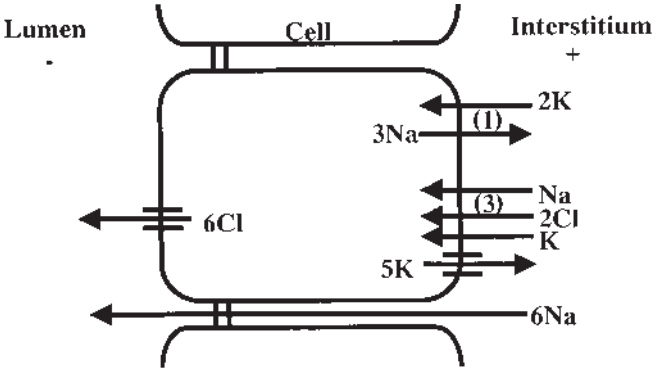

FIGURE 65.1-2 Chloride secretion via the cyclic adenosine monophosphate–activated Cl^- channel. Cl^- accumulates intracellularly via the Na^+-K^+-Cl^- cotransporter in the basolateral membrane. Na^+ is recycled via Na^+-K^+-adenosine triphosphatase and K^+ through independent K^+ channels. Cl^- moves down its electrochemical gradient following activation of the Cl^- channel and is secreted into the lumen, and Na^+ follows paracellularly owing to the lumen negative voltage set up by Cl^- transport.

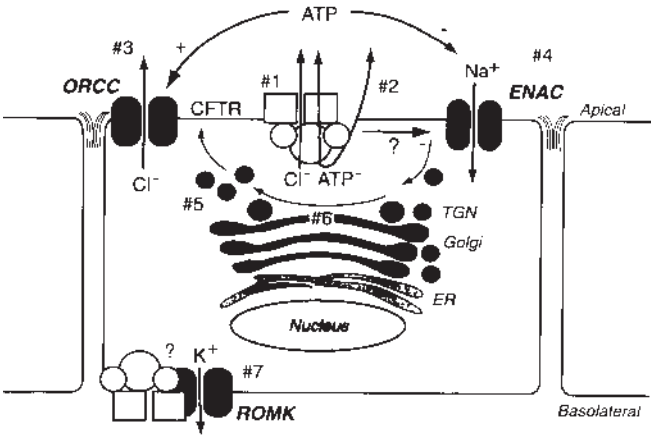

FIGURE 65.1-3 Cystic fibrosis transmembrane conductance regulator (CFTR) functions: (1) Cl^- channel function, (2) facilitation of adenosine triphosphate (ATP) release, (3) positive regulation of outwardly rectifying Cl^- channels (ORCC), (4) negative regulation of epithelial Na^+ channels (ENAC), (5) regulation of vesicle trafficking, (6) regulation of cell acidification and protein processing, (7) modification of renal outer medullary K^+ (ROMK) sensitivity to sulfonylureas. Reproduced with permission from Schwiebert EM et al.[16] ER = endoplasmic reticulum; TGN = trans-Golgi network.

this difference persists further down the airways, although perhaps of lesser magnitude.[17,18] Of interest, this difference in potential can be abolished by amiloride, a finding attributed to amiloride blocking the enhanced Na^+ reabsorption in CF airway epithelium.[19] These changes in electrolyte transport help explain the impaired fluid secretion and the relative dehydration and increased viscosity of respiratory secretions in CF, the latter having previously been attributed to an abnormality of mucus.

In the normal pancreas, the major driving force for pancreatic fluid production has been attributed to secretin–stimulated ductal bicarbonate (HCO_3^-) secretion. The conventional theory proposed that duct cell chloride secretion via CFTR increased duct luminal chloride concentration. Chloride was then exchanged with intracellular HCO_3^- via an apical cell membrane Cl^--HCO_3^- exchanger, with HCO_3^- being synthesized in the cell via carbonic anhydrase action on CO_2, the latter diffusing into the cell via the basolateral membrane.[20] Because pancreatic ductal CFTR Cl^- secretion was impaired in CF patients, Cl^- was not available for exchange with HCO_3^-, thus producing the characteristically low HCO_3^- secretion defined by in vivo pancreatic function studies.[21–23] Recently, the mechanisms of both HCO_3^- production and secretion via the Cl^--HCO_3^- exchanger have been questioned on the basis that (1) there is demonstrable Na^+ dependence of ductal HCO_3^- uptake at the basolateral cell membrane[24] and (2) there is a lack of inhibition of secretin-stimulated HCO_3^- secretion in the absence of luminal Cl^-.[25] The current model of secretion suggests that depolarization of the cell membrane potential, owing to Cl^- exit via cAMP CFTR activation, stimulates HCO_3^- entry via the basolateral electrogenically driven Na^+ HCO_3^- cotransporters and that HCO_3^- secretion into the lumen occurs predominantly via a conductive pathway. In

CF, the impaired Cl^- secretion and lack of cell depolarization inhibit HCO_3^- uptake and subsequent luminal HCO_3^- secretion. Although further work is required in this area, there is little question that pancreatic duct contents are relatively dehydrated and precipitate within the duct or ductule, leading to subsequent obstruction and proximal inflammation, with scarring and destruction of acinar tissue.[26] Furthermore, impaired HCO_3^- secretion may lead to polymerization and ductal precipitation of the zymogen granule–associated protein GP2, which normally undergoes a continuous exoendocytosis cycle to maintain the integrity of the zymogen granule.[27]

INCIDENCE, DIAGNOSIS, GENOTYPE

CF affects approximately 1 in 2,500 live births in white communities.[28] It is less frequent in African Americans, with a reported incidence of 1 in 15,300,[29] and is considered rare in Southeast Asians. The estimated carrier frequency of near 5% in white communities is extraordinarily high for a lethal gene and does suggest some survival advantage to mutation carriers. Quinton has postulated that carriers are protected against organisms causing toxigenic diarrheas,[30] as is evident by resistance to cholera toxin in heterozygotes of a CF mouse model.[31]

Classically, the diagnosis of CF is based on the clinical phenotype at presentation (lung disease, pancreatic insufficiency [PI]) and the presence of an elevated sweat chloride greater than 60 mmol/L. CF infants may have sweat chlorides in the borderline range of 40 to 60 mmol/L, and some normal adults could have values of up to 60 mmol/L. In addition, up to 20% of patients with milder disease and pancreatic sufficiency (PS; see later) may have sweat chloride values below 60 mmol/L, but average values even for this group are in the CF range at 85 mmol/L. In cases with borderline or even normal sweat chloride values, genotyping, nasal potential difference measurements, and quantitative pancreatic stimulation tests measuring HCO_3^- secretion are of value in determining the diagnosis.

CFTR is a large gene of 250 kb pairs containing 27 exons and encodes the CFTR protein of 1,480 amino acids. Over 1,200 mutations of the *CFTR* gene have now been described. The most common mutation, a basepair deletion in exon 10 resulting in a deletion of phenylalanine at position 508 on the CFTR protein, ΔF508, affects 66% of the CF chromosomes worldwide.[32] It is most frequent in northern Europeans (70–80%), is less frequent in southern Europeans (50–55%), and affects only a minority of Ashkenazi Jews (30%).[33] CFTR mutations have been categorized into five groups, as summarized in Figure 65.1-4, and are defined below[34]:

- Class I mutations reflect defective protein production, are associated with unstable messenger ribonucleic acid (mRNA), which is rapidly degraded, and involve nonsense, frameshift, and splice junction mutations.
- Class II mutations produce defective processing of the CFTR protein. ΔF508 is in this category, and improper folding of the molecule prevents trafficking to the apical membrane.
- Class III mutations involve defective regulation of the channel. Thus, although CFTR inserts correctly into the apical membrane, the mutation affecting the nucleotide binding fold impairs CFTR function.
- Class IV, mainly missense mutations involving organic residues in the membrane spanning part of the channel, insert normally into the apical membrane but have impaired conduction.
- Class V mutations lead to abnormal splicing of CFTR without alteration of the genomic coding sequence, leading to partial reduction of the normal CFTR protein but normal functional channels.

PANCREATIC DISEASE

In terms of exocrine pancreatic dysfunction in CF, patients are classified as having either PI or PS. PI patients have fat maldigestion and malabsorption as defined by a fecal fat > 7% of fat intake in 3- to 5-day fat balance studies.[35] In contrast, PS patients have normal fat digestion and absorp-

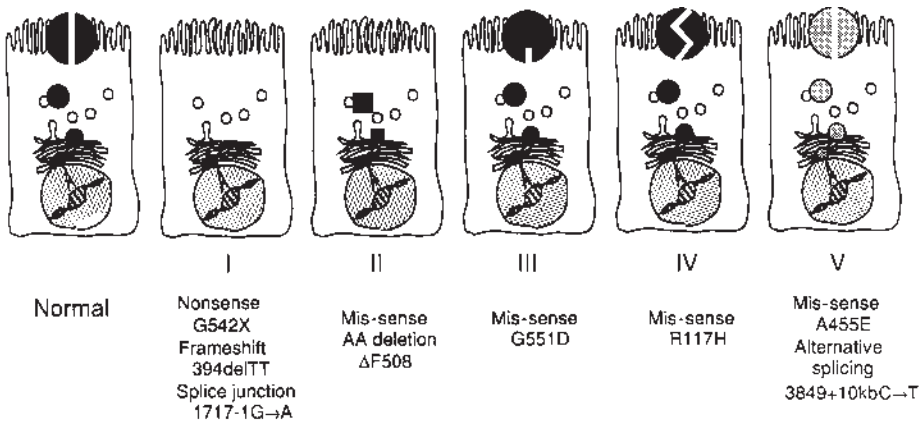

FIGURE 65.1-4 Classification of cystic fibrosis transmembrane conductance regulator (CFTR) mutations in relation to properties of CFTR protein. Class I: defective CFTR protein production: nonsense, frameshift, or aberrant splicing of messenger ribonucleic acid (mRNA); class II: defective CFTR processing: CFTR mRNA is formed, but protein fails to traffic to the cell membrane; class III: defective regulation: CFTR reaches membrane but is not stimulated by cyclic adenosine monophosphate; class IV: defective conduction: CFTR functions, but have altered properties; class V: synthesis defect: less synthesis of CFTR, but channel properties are normal.

tion, with fecal fat ≤ 7 percent of fat intake. Previously, PS patients were defined as having partial PI, partial pancreatic deficiency, or partial or normal exocrine pancreatic function.[36,37] The PS terminology reflects the fact that PS patients have sufficient endogenous pancreatic function to provide normal absorption and is based on pancreatic stimulation test studies in mainly CF patients, comparing pancreatic lipase/colipase secretion with fecal fat excretion,[38,39] as shown in Figure 65.1-5. Colipase secretion rates best delineated normal fat absorbers from those with fat malabsorption, and this is not surprising considering that the degree of lipolytic activity is dependent on the degree of colipase activity.[38] Patients with fat malabsorption had < 1% of average normal colipase activity, but those with normal absorption had a range of colipase secretion that varied from just above 1% up to within the normal control range (as depicted by clear circles in Figure 65.1-5). In large CF populations, 85 to 90% of patients are PI.[40] However, it is readily apparent in populations without newborn screening that patients may present beyond the first year of life with symptoms of malabsorption suggesting that they were PS initially and have lost pancreatic function with time. Studies of infants diagnosed by screening programs have confirmed this occurrence. At the time of neonatal diagnosis, nearly 40% were PS, but within 3 to 5 years, nearly half of these patients had developed PI.[41–43]

The occurrence of either pancreatic phenotype appears to be directly linked to the patient's genotype. In one of the first genotype–phenotype correlation studies, Kerem and colleagues demonstrated that in older CF populations, virtually all ΔF508 homozygotes and 70% of ΔF508 compound heterozygotes were PI, but over 60% of non-ΔF508 compound heterozygotes (who represented 8% of the total population) were PS, as per Table 65.1-1.[44] Subsequently, a number of non-ΔF508 mutations, including R117H and A455E, were demonstrated to be associated with the PS phenotype even if associated with a "severe" mutation (eg, ΔF508), suggesting that the mild PS mutation was dominant.[45] These findings were generally confirmed in a newborn screening population, noting that those who were initially PS and then developed PI had two "severe" mutations, but those with persistent PS had at least one "mild" mutation.[42] In regard to the genotype classification and degree of CFTR dysfunction, as shown in Figure 65.1-4, the vast majority of patients with class I to III mutations are PI, whereas those with class IV and V mutations are PS.

Among PS patients, the phenotype can vary markedly. Some will have obvious lung disease, but others will have minimal or no lung disease. Sweat chlorides may be elevated, borderline (40–60 mmol/L), or even normal, and the latter patients may have only a single mode of presentation (eg, absence of the vas deferens in males or recurrent acute pancreatitis in previously asymptomatic adults who have been subsequently identified with double mutations).[46]

Some of this phenotype variation may be explained by gene modifiers. The R117H mutation is of interest because the polythymidine tract in intron 8 (containing 5, 7, or 9 thymidines) can alter the splicing on exon 9, such that introns with fewer thymidines lead to inefficient splicing and more severe disease.[47]

Pancreatic Insufficiency

In postmortem studies of CF infants dying in the first 4 months of life, there is a marked lack of development of pancreatic acinar tissue.[48,49] Duct luminal volume as a proportion of the total volume exceeds that found in normal subjects, and the ratio of acinar to connective tissue diminishes with age. In premature infants dying from CF, secretory material obstructing pancreatic ducts is an early pathologic feature leading to duct dilatation and progressive atrophy of acinar tissue. Fibrosis can occur early, but in later childhood, patients with PI demonstrate cyst formation, calcification, and, on occasions, a grossly shrunken pancreas. These pathologic changes, which vary with age, have not been correlated with pancreatic function.

Clinical Features. CF infants with PI present with oily stools, and some parents will describe them graphically as looking like melted cheese, butter, or bacon fat. Undigested triglyceride is easily confirmed by stool microscopy

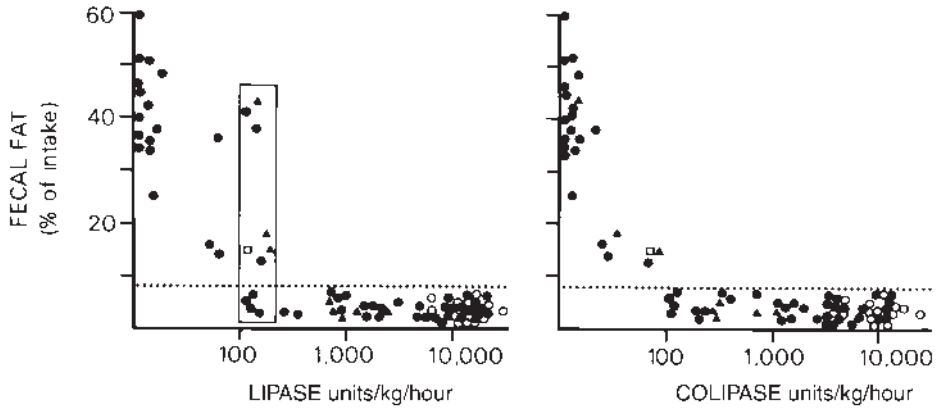

FIGURE 65.1-5 Lipase and colipase secretion rates during pancreatic stimulation with intravenous cholecystokinin and secretin in comparison with fecal fat expressed as a percentage of oral fat intake in 3- to 5-day fat balance studies. Dotted line represents 7% fat excretion. All patients with colipase < 100 U/kg/h have steatorrhea and are pancreatic insufficient, and those with colipase > 100 U/kg/h and normal fat absorption are pancreatic sufficient.

TABLE 65.1-1 ΔF508 GENOTYPING–PANCREATIC PHENOTYPE RELATIONSHIPS

	PHENOTYPE	
GENOTYPE	PI*	PS*
ΔF508/ΔF508	99	1
ΔF508/Other	72	28
Other/Other	36	64

Adapted from Kerem E et al.[44]
*Percentage of patients with pancreatic insufficiency (PI) or pancreatic sufficiency (PS) in specific genotype groups.

following staining of the specimen with oil red O or Sudan red. Up to 50% of PI patients are hypoalbuminemic with or without peripheral edema at presentation; some may have hemolysis associated with vitamin E deficiency, rectal prolapse, coagulopathy owing to vitamin K deficiency, or even raised intracranial pressure owing to vitamin A deficiency.[50,51] Macronutrient deficiency owing to severe maldigestion and malabsorption, poor feeding, or anorexia with intercurrent illness can be associated with severe failure to thrive, wasting, and even stunting. In nonscreened populations, malnutrition and hypoalbuminemia have been attributed to the lower protein content of breast milk, leading many centers to the recommendation of cessation of breastfeeding following the diagnosis of CF in infancy.

Later presentations during childhood have included patients who develop PI with oily stools and associated vitamin deficiencies, including night blindness associated with vitamin A deficiency. These older patients may have obvious chest symptoms or signs, including clubbing, and some have presented with liver disease. In a sizable proportion of patients, chest symptoms and signs predominate and are responsible for early recognition of the disease.

Diagnosis. In the context of an infant or child with a positive sweat chloride presenting with oily stools, there is little doubt that the patient is PI. However, particularly among infants from newborn screening programs, a history of oily stools may not be forthcoming. Historically, documentation of exocrine pancreatic function status has best been achieved with formal fat balance studies, with PI patients demonstrating, on average, a fecal fat excretion near 40% of fat intake, with variation from 10 to 80%.[35] Formula-fed PI infants will have fat excretions > 10% of fat intake and breastfed PI infants a fecal fat > 2 g/d.[41] In many units, fat balance studies are not undertaken owing to the natural reluctance of collectors and laboratory staff handling fecal material and the risks of contamination and cross-infection. More recently, spot stool analysis for fecal elastase 1 (FE1) has been suggested as a possible alternative, noting that FE1 was very successful in predicting the occurrence of PI.[52] However, some caution is still required with the interpretation of this test because in non-CF adults with chronic pancreatitis with moderate to severe pancreatic dysfunction but with normal fat excretion, approximately 50% registered a near-zero FE1 level, suggesting that they were PI. Moreover, with relevance to units with screening programs, FE1 has not been adequately tested in CF infants.

Treatment. Once PI is diagnosed in a CF infant, treatment should commence with oral enzyme replacement therapy (OERT). Most clinics now use the enteric-coated microspheric OERT preparations that were first marketed in the early 1980s. Prior to that time, OERT preparations consisted of dried pancreatic extract, either in powder, tablet, or capsule form. When ingested, these products were exposed to acid pepsin digestion and thus were mainly ineffective, reducing fecal fat outputs by less than 10%. The microspheric preparations were developed so that the enzyme powder was enclosed in a small bead with a pH-sensitive enteric coating, resistant to acid dissolution but readily dissolved in a pH > 6. In theory, therefore, the microspheres would dissolve only when reaching the upper small intestine. Fat balance studies on CF patients treated with these preparations have consistently shown a marked improvement in average fecal fats in older children and adolescents. Using the standard 5,000 IU capsule, patients receiving 6 to 9 capsules per day had an average fecal fat of 22%,[53] and incremental increases to 25 to 30 capsules per day produced a plateau effect, with average fecal fats between 10 and 15% per day.[54,55] In up to 50% of treated patients, fat excretion decreased to below 10% of fat intake, whereas 5 to 10% treated maintained fecal fats over 20%.[56]

Despite these improvements, during the late 1980s, additional microspheric OERT preparations were marketed containing 10,000 to 25,000 IU of lipase to aid those patients who had persistently high steatorrhea on standard-dose OERT and to reduce the number of capsules consumed by older patients in an effort to improve compliance.[57] These aims were laudable, but many patients were able to self-determine their dose of enzymes; consequently, they were consuming very large doses of lipase, often in excess of 50,000 IU/kg/d. Fat balance studies were not performed to justify these large doses, and as subsequently shown epidemiologically, the introduction of such therapy was coincidental with the emergence of a new complication of CF, namely, fibrosing colonopathy (FC).[58] This complication was a noninflammatory, colonic obstruction associated with marked intramural fibrosis, usually in the ascending or transverse colon.[59] Many patients who developed FC experienced considerable morbidity and required surgical intervention to relieve the obstruction. With doses of lipase in excess of 50,000 IU/kg/d, there is a marked risk of FC; this lessens with decreasing doses, although there is still considerable risk above 20,000 IU/kg/d.[58] Most would recommend keeping the dose below 10,000 IU/kg/d and optimally around 5,000 units/kg/d.

In patients who remain symptomatic on the standard-dose regimen, one should document the degree of steatorrhea. If it exceeds 20%, and the patient is compliant with the dose prescribed, he should be assessed for liver or biliary tract disease, giardiasis, and celiac disease. If such studies are negative, adjunctive therapy using gastric acid suppressants (eg, H_2 receptor antagonists or proton pump inhibitors) should be considered.[54] There are few patients who do not respond to such maneuvers; they may respond better to some of the acid-resistant acid lipases that are under development.

Pancreatic Sufficiency

PS patients comprise 10 to 20% of large CF clinic populations,[40] although, as indicated above, this occurrence is much higher in neonates.[41] In nonscreened populations, they usually present at a later age than PI patients[40] and may have unimodal presentations as adults, with congenital bilateral absence of the vas deferens in males at infertility clinics or recurrent acute pancreatitis. In newborn screening programs using the two-tiered immunoreactive trypsin (IRT) screening strategy, PS infants had IRT values similar to their PI counterparts.[41] However, the more recent strategy using a single IRT test and a ΔF508 mutation analysis will miss non-ΔF508 compound heterozygotes and a proportion of PS patients.[60]

Characteristically, PS patients have a mild disease with minimal pulmonary involvement and encounter nutritional, gut, or liver problems infrequently.[40] *Pseudomonas* lung colonization is reduced compared with PI patients, and the overall prognosis is vastly improved; in the United States, CF median survival of PS patients is 53 years and of PI patients 28 years. It is apparent that persistently PS patients have one or more so-called "mild" mutations, including R117H, A455E, and R347P. They demonstrate impaired Cl^- transport in their sweat, but it is less severe than that of PI patients because they have an average sweat chloride of 86 mmol/L compared with 104 mmol/L in the PI group; they consistently have higher pancreatic HCO_3^- secretion than their PI counterparts.[22] Mild chest disease occurs in 75% of PS patients, 39% have digital clubbing, and 30% have nasal polyps.[40] Interestingly, 4% had rectal prolapse, thus underscoring the validity of obtaining a sweat chloride in every child who presents with rectal prolapse even in the absence of gastrointestinal symptoms. Of equal importance, PS patients develop pancreatitis,[40,46,61] an entity that does not occur in PI patients who have lost their functioning acinar tissue. PS patients do not require enzyme therapy because, by definition, they have sufficient endogenous enzyme production to prevent malabsorption. Equally, they do not require fat-soluble vitamin supplementation. It is imperative for clinics to assess their patients for PS because treating a patient unnecessarily for years is a considerable burden to the child and an unnecessary financial burden to the health care system.

HEPATOBILIARY COMPLICATIONS

A variety of biliary tract and hepatic complications of CF have been described in CF patients, as summarized in Table 65.1-2. They include specific gallbladder diseases, including microgallbladder with or without atretic ducts, dilated or distended gallbladders and cholelithiasis, bile duct diseases with common duct or intrahepatic duct lithiasis, a disease resembling sclerosing cholangitis, and distal common bile duct stenosis. Liver complications include hepatosteatosis and the two forms of fibrotic liver disease considered pathognomonic of CF, namely, focal and multilobular biliary cirrhosis (FBC and MBC, respectively).

Pathologically, gallbladder hypoplasia with or without atretic cystic ducts has been recognized in early childhood.[48] The gallbladder wall contains small epithelial cysts, often distended with eosinophilic material; there is often mucous gland metaplasia and occasionally marked mucosal hyperplasia, but no inflammatory changes. One autopsy study of older patients demonstrated that 14% had calculi.[62] Common bile duct stenosis owing to extrinsic compression of the bile duct has been identified in postmortem specimens of infants, and in one case, the patient was mistakenly diagnosed as having biliary atresia.[63] Liver disease in CF was first recognized by Anderson and was later confirmed by Farber and Bodian.[2,64,65] Farber recognized inspissated material obstructing small bile ductules,[64] and Bodian described the entity of FBC.[65] Later, di Sant'Agnese and Blanc described the severe form of liver disease, MBC.[66] Because patients with MBC also had evidence of FBC, the authors suggested that there was a progression of the focal disease with coalescence and generation of the large nodules characteristic of MBC, as seen in Figure 65.1-6. The focal scarring histologically, as shown in 65.1-7, is associated with fibrosis of portal triads with accompanying inflammatory infiltration, bile ductular hyperplasia, and eosinophilic secretions expanding bile ductules.

Postmortem studies suggest that there is a rising incidence of liver disease with age; FBC is present in 10.6 to 15.6% of infants less than 12 months of age,[48] in 19 to 50% during childhood,[65–67] and in up to 72% of adult CF patients.[68] Similarly, MBC increased from < 1% in early childhood to up to 24% in the above adult postmortem study.[68] In addition to the fibrotic changes, almost 60% of liver specimens showed hepatosteatosis, a finding hitherto unexplained.[69] Currently, the etiology of fibrotic liver disease is also enigmatic. It is likely that the underlying CFTR transport defect impairs fluid secretion from the cholangiolar epithelium,[70] contributing to inspissation of material and obstruction in bile ductules, but it is not clear why some patients develop liver disease and others do not. A distinct possibility is that a gene modifier may influence the operation of CFTR. To date, the presence of liver disease has not correlated with the severity of lung or intestinal disease; it has been described in three patients with PS and no detectable abnormalities in pulmonary function.[71]

TABLE 65.1-2 BILIARY TRACT AND LIVER COMPLICATIONS IN CYSTIC FIBROSIS PATIENTS

GALLBLADDER
Microgallbladder
Atretic cystic ducts
Distended gallbladder
Cholelithiasis
BILIARY TRACT
Ductal stones
Common bile duct stenosis
Sclerosing cholangitis
Cholangiocarcinoma
LIVER
Hepatosteatosis
Focal biliary fibrosis (cirrhosis)
Multilobular biliary cirrhosis
± Portal hypertension
+ Liver failure

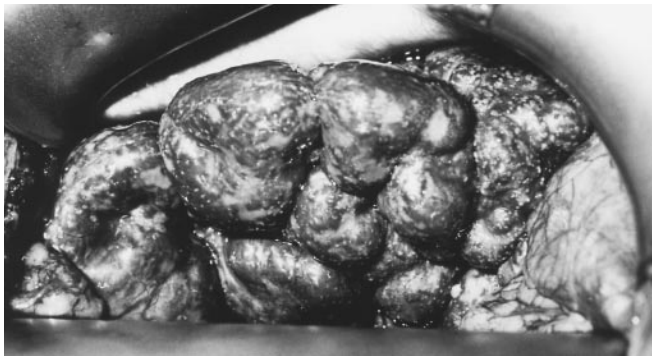

FIGURE 65.1-6 Multilobular biliary cirrhosis. Marked lobulation of the liver occurs in some patients. Focal areas of scarring are readily visible in each lobule.

It may well be that other entities, including common bile duct disease, distal stenosis, and sclerosing cholangitis, contribute to biliary tract stasis to explain the variable occurrence of liver disease.[69]

Diagnosis

The diagnosis of CF liver disease can be difficult.[72] There is little debate regarding patients with multilobular cirrhosis who have peripheral signs of chronic liver disease, spider nevi, and an obvious multilobulated firm liver, with abdominal distention and signs of portal hypertension, including splenomegaly. Such patients can be recognized clinically and confirmation provided by ultrasonographic examination of the liver. The difficulty comes when attempting to diagnose milder cases who are devoid of physical signs, except perhaps hepatomegaly. Liver function tests may be completely normal, even in subjects with MBC and portal hypertension,[72–74] and ultrasound examinations may demonstrate increased echogenicity of the liver not related to fibrosis but attributable to impacted secretions or the presence of parenchymal steatosis. In addition, although liver biopsy has been touted as being necessary to determine the presence or absence of liver disease, there is considerable sampling error, and biopsies can be normal even in those with MBC.[69] Tests of liver function, including serum bile acids[75] and hepatobiliary scintigraphy,[69] also fail to distinguish between patients with and without liver disease. Ultimately, a combination of findings (clinical, imaging, and biochemical) is used to determine the onset and progression of this complication.

Clinical Presentation

Patients with hepatosteatosis and focal biliary disease are relatively asymptomatic, although in one series, FBC patients had a higher incidence of abdominal pain.[69] Those with multilobular disease may have signs of chronic liver disease, spider nevi, liver palms, abdominal distention, and signs of portal hypertension or ascites. Some may present with esophageal variceal bleeding even prior to diagnosis of CF in late childhood and may have minimal signs of lung disease. The course of those with portal hypertension and splenomegaly is variable, but in my own unpublished series of 28 patients with portal hypertension, 10 experienced variceal bleeding over a 10-year period.

Treatment

Medical management of CF liver disease has centered around supportive measures to provide adequate nutrition, fat-soluble vitamin supplementation, and diuretic therapy for edema and ascites. Ursodeoxycholate therapy was introduced based on the theoretical possibility that it would enhance fluid secretion from the biliary tree. Initial studies were promising, reporting improvements in liver function tests and hepatobiliary scintigraphy,[76,77] but many of the studies contained small numbers of subjects with considerable heterogenicity in their underlying liver disease.[78,79] A recent Cochrane analysis concluded that there was insufficient evidence to justify the routine use of ursodeoxycholate therapy for CF patients and noted the urgent need for a prolonged multicentered collaborative trial with clinically relevant end points.[80]

The management of liver disease with portal hypertension is similar to that for non-CF populations. Patients with variceal bleeding should undergo variceal injection therapy or banding, and in many instances, their bleeding will be well controlled. If sclerotherapy fails, an alternative intervention has to be considered; possibly the best to date has been the use of reversed lienorenal shunts.[81] However, the latter procedure has to be considered in the context of the severity of the lung disease in individual patients. Often the decision is made to proceed based on the urgency of the situation.

Liver transplant is now a viable option for the unusual CF patient with synthetic liver failure or uncontrolled portal hypertension and recurrent variceal hemorrhaging. Data show survivals at least consistent with non-CF liver transplant patients, that is, 70 to 80% survival for the first 5 years post-transplant.[82] Of interest, the decline in pulmonary function was less than expected, a fact possibly related to the anti-inflammatory effects of the antirejection therapy used. Direct contraindication to transplant includes severe pulmonary disease (ie, forced expiratory volume in 1 second [FEV_1] < 50% predicted) and fungal or *Burkholderia cepacia* lung colonization.

NEONATAL LIVER DISEASE

Prolonged neonatal cholestasis occurs in CF infants, but most have considered it to be a rare phenomenon.[83,84] However, in one postmortem study, 10% of infants less than 3 months of age had FBC and 38% demonstrated histologic evidence of cholestasis[48] consistent with a clinical audit in which 35% manifested hepatomegaly/cholestasis in infancy.[72] At first glance, these disparate results are difficult to explain, but, certainly, the postmortem study included very ill septic infants from an era in which neonatal, postsurgical management would be regarded as suboptimal. Moreover, the patients were collected from a wide geographic area for which the true total incidence of CF was unknown. In preliminary results from a newborn screening program over a 14-year interval, 12 of 224 (5%) CF infants developed cholestasis, and 9 of these infants had meconium ileus (MI).[85] Prior to the diagnosis of CF, those presenting with cholestasis and acholia are often considered to have extrahepatic biliary atresia. It is thus imperative that the cholestatic infant has a sweat chloride

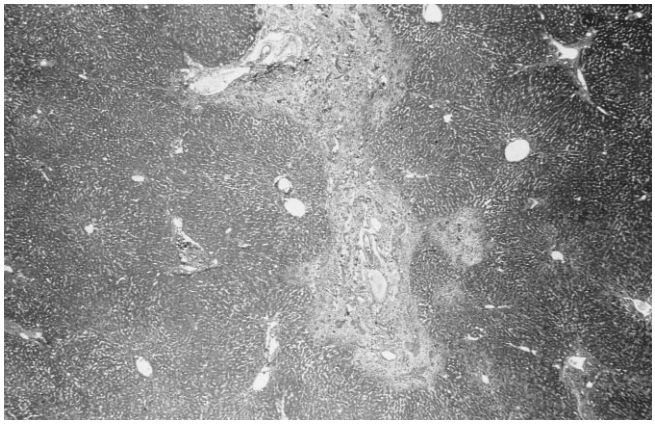

FIGURE 65.1-7 Histologic examination of a liver with focal biliary cirrhosis demonstrating fibrosis of portal triad, acute inflammatory cell infiltration, bile ductular hyperplasia, and eosinophilic secretions plugging bile ductules.

test as part of the diagnostic workup because most patients with CF do not have extrahepatic biliary atresia, although they may have prolonged jaundice for periods up to 6 months. Neonatal cholestasis in CF has been linked to MI,[85,86] but others have not found this association.[83]

GALLBLADDER OR BILIARY TRACT DISEASE

Previously, cholelithiasis was a prominent biliary tract complication of CF. With the introduction of microspheric OERT, gallstones have become virtually nonexistent, and at the Sydney clinic we have seen only 3 cases in nearly 700 patients over the last 15 years.

A few patients do experience right-sided abdominal pain unrelated to distal intestinal obstruction syndrome (DIOS); some will be tender in the right upper quadrant. Such patients may have biliary tract disease and require an ultrasound examination to exclude cholelithiasis and hepatobiliary scintigraphy to exclude distal common bile duct stenosis. Cholangiography is required to confirm the latter and may warrant surgical intervention if cholestasis ensues or the pain persists and is interfering with appetite and normal daily activity.[69]

INTESTINAL DISEASE

The gut manifestations of CF as outlined in Table 65.1-3 include esophageal, small intestinal, colonic, and rectal diseases.

Esophageal Disease

There have now been several reports of esophageal disease, particularly gastroesophageal reflux (GER) and esophagitis. One unit reported that up to 25% of patients have GER[87] and another that at least 50% of those with GER will develop esophagitis.[88] Reflux is probably a multifactorial problem contributed to by the severity and treatment of lung disease, drugs that relax the lower esophageal sphincter, and physiotherapy. Delayed gastric emptying may contribute, but the most significant feature to date is intermittent and inappropriate lower esophageal sphincter relaxation.[89]

Patients with GER may complain of heartburn, waterbrash, dysphagia, and regurgitation. Equally common are those patients whose sole symptom is anorexia; thus, patients experiencing weight loss or poor weight gain may have esophageal disease. Investigations should include a barium contrast examination and esophagogastroscopy of the esophagus and stomach, specifically looking for esophagitis, strictures, or a hiatal hernia.

Patients with esophagitis should first be treated with either H_2 receptor antagonists or proton blocking agents. Medical treatment should be prolonged for at least 6 months, and surgery should be considered only as a last resort for those unresponsive to medical therapy.

Gastroduodenal Disease

Peptic ulcer disease was considered a potentially common occurrence in CF, particularly because patients had gastric acid hypersecretion[90] and low pancreatic HCO_3^- secretion.[22] Although ulcers have been anecdotally recorded in the pre-endoscopy era, following the advent of fiberoptic endoscopy, their occurrence has been uncommon. Similarly, *Helicobacter pylori* gastritis is an unusual finding.

Small Intestinal Disease

CFTR has been localized to crypt[91] and villous cells[92,93] in the small intestinal mucosa, and small bowel biopsy specimens from CF patients demonstrate poor Cl^- transport in

TABLE 65.1-3 GUT MANIFESTATIONS IN PATIENTS WITH CYSTIC FIBROSIS

ESOPHAGUS
Gastroesophageal reflux
Esophagitis
Esophageal stricture
Esophageal varices (in association with portal hypertension)
GASTRODUODENUM
Peptic ulcer
SMALL INTESTINE
Neonatal meconium ileus (MI)
Complicated MI with volvulus or intestinal atresia, meconium peritonitis
Distal intestinal obstruction syndrome
Intussusception
Celiac disease
Giardiasis
Ileal adenocarcinoma
APPENDIX
Acute appendicitis ± perforation
Appendiceal abscess
Mucocele of appendix
Intussusception of appendix
COLON
Megacolon constipation
Pneumatosis intestinalis
Fibrosing colonopathy
Crohn disease
Colonic adenocarcinoma
RECTUM
Rectal prolapse

response to a number of known CFTR secretagogues.[94,95] Rectal suction biopsy specimens from CF patients demonstrate similarly impaired Cl^- transport,[96,97] which correlates with pancreatic phenotype, that is, samples from PI patients show undetectable transport, but those from PS patients show higher residual Cl^- transport. These findings have led to the proposal that the common small intestinal complications of CF, namely, neonatal MI and DIOS, result directly from impaired electrolyte and fluid secretion and subsequent inspissation of intestinal contents. This scenario is analogous to the genesis of exocrine pancreatic disease, in which poor fluid output leads to hyperconcentration of luminal contents, protein precipitation or aggregation, and subsequent ductular obstruction.[26,98] This hypothesis is supported by findings in the CF mouse model, in which intestinal complications at 12 to 40 days, including obstruction, perforation, fecal peritonitis, and the finding of large intraluminal "putty-like" masses, occur in animals in the absence of significant pancreatic pathology.[99]

In human CF patients, MI and DIOS are virtually exclusive to PI patients and are very rare in PS patients.[100] The CF consortium data furthermore demonstrated that MI occurred in patients with "severe" genotypes associated with PI but did not occur in patients with "mild" mutations associated with PS (eg, R117H).[101] These findings could argue that PI is a critical factor in the genesis of MI and DIOS, but they could equally reflect the severity of the underlying transport disorder in the pancreas and small intestine, as indicated by the correlation of rectal Cl^- transport with pancreatic phenotype. If so, the findings in the mouse model, which appears to have only mild exocrine pancreatic disease, could be reconciled only by the different degrees of expression of CFTR in the wild-type mouse (ie, low in the pancreas but high in the small intestine). However, to date in the small group of CF patients who are PS but have a severe genotype (eg, ΔF508/ΔF508) and poor or undetectable intestinal chloride transport, MI or DIOS has not occurred; thus, the degree of pancreatic function impairment may well contribute to the genesis of these complications.

The lack of alkalinization of intestinal contents as a result of poor pancreatic HCO_3^- secretion in PI patients can lead to protein and phospholipid precipitation in the intestinal lumen. PI patients have poor pancreatic phospholipase secretion; therefore, lecithin will accumulate in the gut lumen.[102] Although hydrolyzed by OERT to lysolecithin, OERT lacks lysolecithinase (carboxyl ester hydrolase)[103]; thus, high concentrations of lysolecithin could accumulate, which is a known hydrophobic compound capable of disrupting epithelial cells. Also, CFTR has intracellular functions that modify the production of mucus glycoproteins.[104,105] The above phenomena, including the Cl^- transport problems, PI and abnormal mucus production, in combination with the known impaired intestinal motility, could all contribute to the production of either MI or DIOS.

Meconium Ileus

Between 10 and 20% of CF neonates will present with small intestinal obstruction owing to inspissation of meconium in the terminal ileum. This phenomenon occurs in utero, and it remains enigmatic as to why only a small proportion of patients develop this complication. Approximately 10% of these cases will suffer perforation in utero and develop meconium peritonitis, an entity recognized by intra-abdominal calcification on abdominal radiographs. Most cases will present with signs of obstruction, including abdominal distention, bilious vomiting, and failure to pass meconium within the first 2 to 48 hours of life. The diagnosis may be suspected if there is a family history of CF but can be distinguished from other forms of neonatal gut obstruction by the lack of air fluid levels in erect or lateral decubitus plain abdominal radiographs (Figure 65.1-8) and a ground-glass appearance to the inspissated meconium. Nearly 50% of cases have complicated MI, in which, in addition to MI, they have malrotation with volvulus or intestinal atresia. Most will have an associated microcolon.

MI is almost invariably associated with CF. Of interest, in neonatally screened populations, the serum immunoreactive trypsin in MI patients can be within the normal range. It is thus important that the screening laboratory is informed of the diagnosis of MI to ensure that they proceed further to the mutational analysis, which would usually be performed only on infants with an initially high IRT value. Only rarely has MI been associated with non-CF conditions, and, to date, it has not been described with the pancreatic hypoplasia in Shwachman syndrome or other forms of childhood pancreatic diseases.

Diagnosis of MI is best achieved with nonionic contrast radiography. In uncomplicated MI, Gastrografin enemas

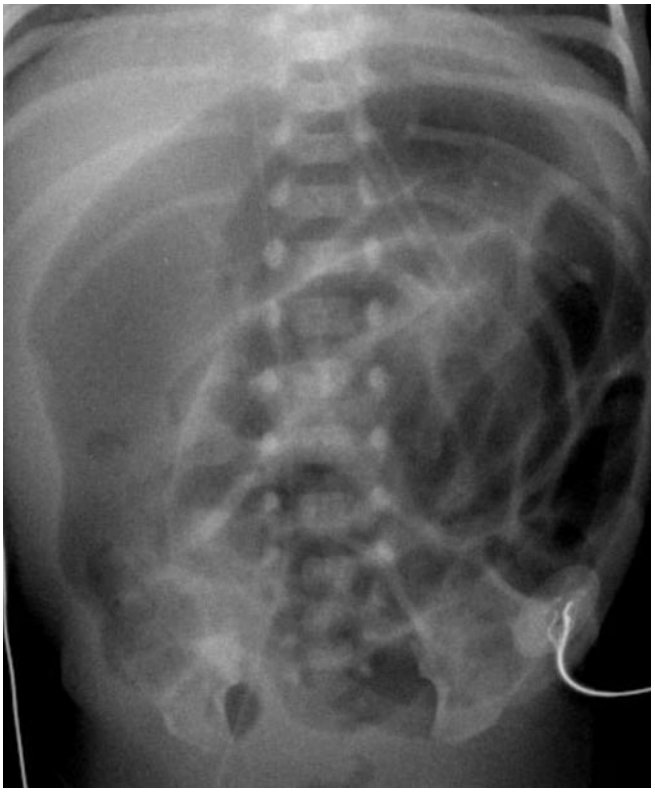

FIGURE 65.1-8 Abdominal radiograph of an infant with meconium ileus. There is marked distention of loops of small intestine without air-fluid levels. The ground-glass appearance of inspissated meconium is evident in the right iliac fossa.

can help elucidate the diagnosis, but because they are hypertonic, it may also be therapeutic in washing out the inspissated meconium. Great care is needed to avoid the perforation, dehydration, and shock associated with the use of the hypertonic enemata. Contrast radiography should be avoided in those with perforation or intra-abdominal calcification. Failure to resolve the obstruction with Gastrografin necessitates surgical intervention. The standard procedure is to milk out the inspissated material following division of the gut and to wash out the residue with saline or solutions containing *N*-acetylcysteine (Mucomyst). Some surgeons prefer the standard Bishop-Koop ileostomy, whereas others prefer a double-barreled ileostomy, and still others use the appendix stump.

Distal Intestinal Obstruction Syndrome

Beyond the neonatal period, a small number of patients present with inspissation of intestinal contents in the terminal ileum, cecum, and proximal colon, a condition hitherto referred to as MI equivalent but now renamed as DIOS. Originally, this syndrome was considered to be more common in adolescents and adults, with an incidence varying from 17 to 24% in two different studies.[106,107] However, these reports were published before microspheric OERT was available, and subsequent studies have suggested a much lower overall incidence of 5%,[108] with some 7.5 cases per 1,000 patient-years occurring in the 15- to 20-year-old age group, increasing to 35.5 cases per 1,000 in the 20 to 25 year olds.

The etiology is unclear, but DIOS rarely occurs in PS patients.[100] Patients present with palpable fecal masses in the right lower quadrant, with or without abdominal pain, or with intestinal obstruction and bilious vomiting. These symptoms and signs are nonspecific and occur also in CF patients presenting with appendiceal or periappendiceal abscesses, purulent mucoceles of the appendix, intussusception, Crohn disease, or colonic strictures. A high degree of suspicion is required when dealing with DIOS, and although fevers and localized peritonism may suggest inflammatory disease, their absence does not exclude these possibilities. Indeed, DIOS can occur simultaneously with appendiceal disease and intussusception, thus adding to the complexity of the diagnosis. The diagnosis of DIOS is initially achieved with plain erect and supine abdominal radiographs. A fecal mass should be evident in the right iliac fossa, and the erect film will help to determine the presence of obstruction. If obstruction is present, one should suspect concomitant pathology such as intussusception or appendiceal disease with an acute history; one should suspect FC, or Crohn disease with a prolonged history. Ultrasound examination is useful in these circumstances and can readily identify intussusception (Figure 65.1-9), as can a computed tomographic (CT) examination (Figure 65.1-10).

In cases of intestinal obstruction or in which washout therapy has failed, Gastrografin enemas may be useful in defining colonic pathology (ie, strictures or intussusception) and relieving impaction in uncomplicated DIOS. Colonoscopy and biopsy will be required for cases in which Crohn disease is suspected radiologically.

Treatment for DIOS has been largely empiric. In simple cases of fecal impaction without obstruction, most respond to a high-dose mineral (paraffin) oil regimen, 30 to 50 mL twice daily over a 7-day period. If the patient does not evacuate, ultrasonography and a diagnostic or therapeutic examination should be performed to exclude intussusception. Thereafter, one should consider using washout therapy with the iso-osmotic colonoscopy preparation fluid Golytely, which contains polyethylene glycol for these oil-resistant cases. Patients may require up to 5 L per 2 to 4 hours to evacuate the mass, and the procedure may need repeating daily if the mass is not completely evacuated.

In DIOS cases with obstruction (ie, with distention and bilious vomiting), it is mandatory to exclude other complications. Once eliminated, washouts per rectum should be attempted, and once the obstruction is relieved, depending on nasogastric tube output, then washout therapy from above can be considered. Persistent obstruction may necessitate diagnostic laparotomy, but this situation is unusual in the absence of intussusception or appendiceal disease.

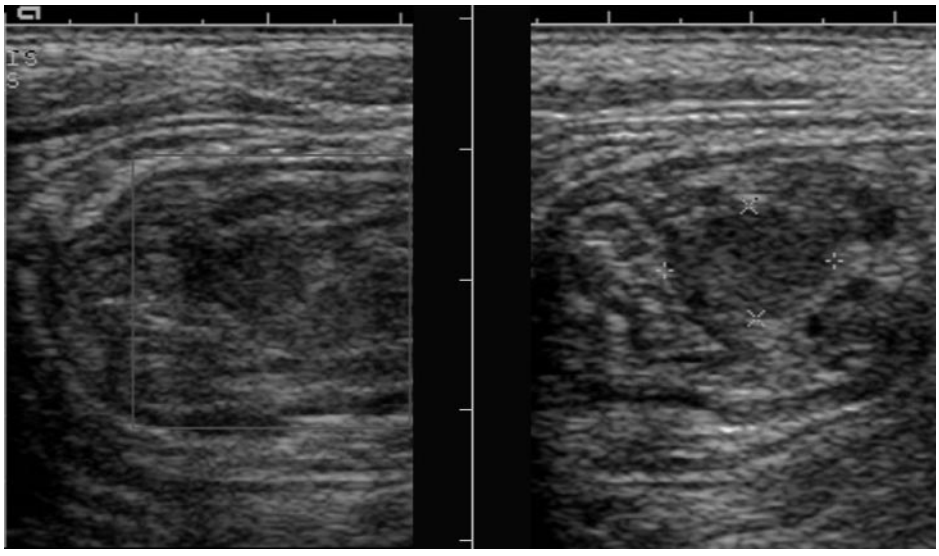

FIGURE 65.1-9 Ultrasound examination of the right lower abdomen in a patient with cystic fibrosis presenting with abdominal distention and a tender abdominal mass. The sonogram demonstrates a multilayered appearance of the intestine, representing the wall of intestine and the wall of the intussusception.

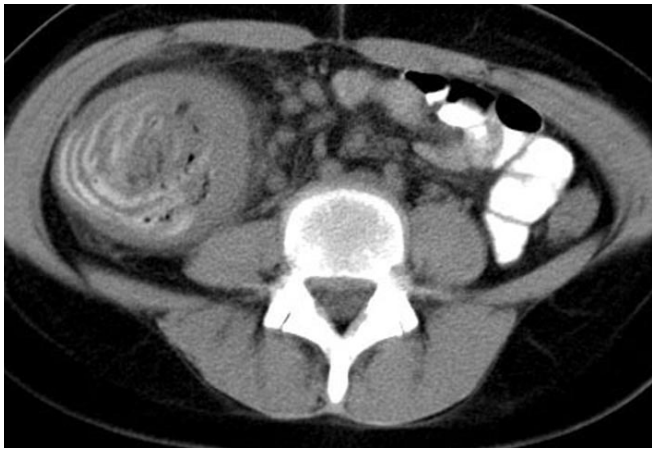

FIGURE 65.1-10 A computed tomographic examination of the abdomen of the patient in Figure 65.1-9, demonstrating the intussusception in the proximal colon.

Recurrent DIOS can be disabling for the CF patient. In such cases, novel therapies, including button placement in the appendix to allow more vigorous washouts, may be required.[109]

Appendiceal Disease

Appendiceal disease, including acute appendicitis,[110] appendiceal abscess,[111] perforation,[111] purulent mucoceles of the appendix, and intussusception of the appendix with rectal bleeding, has been described in CF patients.[112] Although less common than in the non-CF community (1 versus 7%, respectively), the diagnostic delays are of considerable concern in CF, with a high risk of perforation or abscess formation.[111] The cause for delay is unclear but may relate to the presence of a mass lesion that is mistaken for the more common DIOS or to concomitant antibiotic therapy disguising the inflammation. Fever, signs of obstruction, and localized tenderness or peritonism, with or without a tender mass in the right lower quadrant, should alert the physician to this entity, and imaging via ultrasonography or CT is essential to define such a lesion. The physical and radiologic signs may determine whether an operation is required, but the physician's suspicion will ultimately determine such action.

Miscellaneous Small Intestinal Diseases

Giardia infestation,[113] celiac disease, and Crohn disease have all been described in CF populations. Reports of giardiasis in CF are infrequent, but a recent study suggested that *Giardia* infested 28% of CF children and an even higher proportion (44%) of CF adults. These findings require confirmation but are potentially important because ongoing problems with chronic diarrhea are often misinterpreted as a failure to respond to OERT. Similarly, Crohn disease in 1 in 404 CF cases represented a prevalence approximately 11 times that observed in the non-CF control population.[114] Crohn disease should be suspected in cases presenting with abdominal pain, anemia, hypoproteinemia, and extragastrointestinal manifestations (eg, arthritis). Barium contrast imaging may demonstrate the typical segmental cobblestoned appearance, and histologic examination will establish the presence of granulomatous colitis or ileitis. Treatment should be as for non-CF patients.

The coexistence of celiac disease and CF appears to be a rare occurrence.[115] However, given that in some white communities, the incidence is as high as 1 in 75, this entity may be more common than is suspected in the CF population. It should be considered in a CF patient with ongoing symptoms of malabsorption despite compliance with optimal doses of OERT. The need for small bowel biopsy should be governed by positive serum antibody screening, specifically antiendomyseal antibodies or antibodies to tissue transglutaminase. Cases with a positive diagnosis should be managed with a gluten-free diet.

LARGE INTESTINAL DISEASE

Rectal Prolapse

Rectal prolapse (Figure 65.1-11) occurs in 10 to 20% of patients, usually prior to 5 years of age,[116] and may be the presenting feature of the disease.[117] It is far more common in PI patients; its occurrence has been attributed to large bulky stools in wasted patients prior to diagnosis or to ongoing malabsorption in patients following diagnosis. The entity has been described in PS patients[40]; thus, its occurrence cannot be entirely attributed to the presence of malabsorption or malnutrition, although the latter may contribute to the problem. All children presenting with isolated rectal prolapse should have a sweat chloride determination. In most cases, rectal prolapse is a self-resolving problem and requires no specific therapy other than ensuring compliance with OERT and providing laxatives if the patient is constipated. Persistent or recurrent prolapse can be disturbing to patients and their parents. Such patients respond well to pararectal triple saline injection therapy under anesthesia, with complete amelioration of their problems.

Fibrosing Colonopathy

This complication is a relatively new entity described in the early 1990s.[59,118] It is an intramural noninflammatory fibrosing process that affects mainly the proximal colon in CF patients up to early adolescence.[59] Pathologically and clinically, it can be associated with intestinal obstruction and may be preceded by prolonged abdominal pain with or without diarrhea and rectal bleeding. Some may also present with abdominal chylous ascites.[59,119,120]

FC may be diagnosed by imaging with contrast enemas to identify a stricture and proximal obstruction. In some, the diagnosis can be confirmed by colonoscopy and biopsy of the affected area. Surgery is required to relieve the colonic obstruction, and histologic examination of resected specimens will identify the typical elongated fibrotic stricture, which shows minimal inflammation.

Epidemiologically, the entity occurred after the introduction of high-dose lipase OERT and the consumption of large doses of enzyme in excess of 6,000 lipase units per kilogram per meal for periods exceeding 6 months.[59,119,120] Some patients were consuming greater than 50,000 lipase U/kg/d, and it is now recommended that intake does not exceed

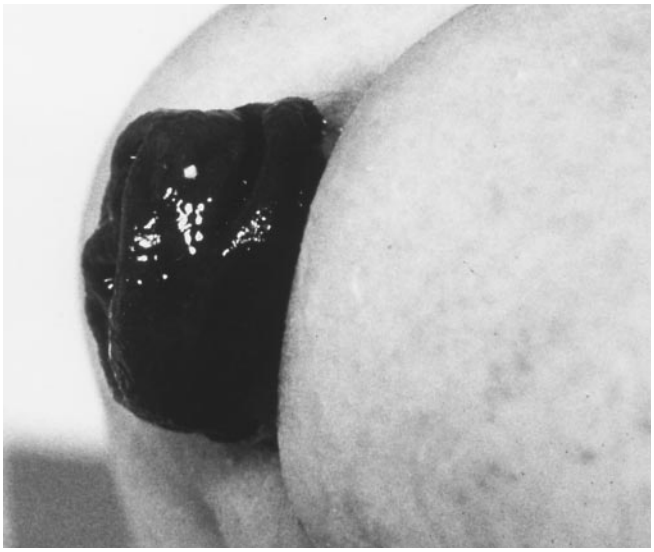

FIGURE 65.1-11 Rectal prolapse.

10,000 lipase U/kg/d or 2,500 U/kg per meal.[59,119] Since the establishment of these guidelines, the incidence of FC has substantially declined. It is important to note that although some have suggested that the entity is related to the differing copolymer content of the microspheric capsule,[121] evidence for this is tenuous because the US survey found a high odds ratio for the occurrence of FC irrespective of the capsule or microtablet used.[58]

Gastrointestinal Malignancy

Gastrointestinal malignancy has been described in adult patients with CF, with 24 cases reported from US and European CF clinic surveys.[122] The majority of cases were large or small intestinal adenocarcinomas, but esophageal, gastric, biliary, and pancreatic malignancies were described. In older CF patients, if confronted with unusual gut symptoms such as anemia, rectal bleeding, or non-DIOS intestinal obstruction, malignant neoplasms need consideration.

NUTRITIONAL PROBLEMS AND THEIR MANAGEMENT

Over the last two decades, nutritional problems have assumed a high priority in the management of CF. At least in part, the nutritional focus arose out of epidemiologic work at the CF Clinic, the Hospital for Sick Children, Toronto, which demonstrated that median survival of near 30 years was considerably better than the median survival of less than 20 years in other Canadian or US clinics.[123] This major difference was a surprising finding because the management of CF pulmonary disease was similar in the major clinics throughout North America. The same study emphasized the near-normal growth of the Toronto patients, except for a decline in weight of adolescent females, and, again, these results appeared superior to those previously published. Later data from Toronto demonstrated normal growth and lung function of their PS patients, and their PI males also had near-normal growth and superior lung function to their PI females, whose weight fell off during adolescence.[40] Apparently, within this one clinic, prognosis and pulmonary function were influenced by nutritional status. Further confirmation of these findings was made following a comparative cohort study between the Boston and Toronto clinics, which demonstrated a 10-year advantage in median survival in the Toronto clinic, despite the fact that comparisons of pulmonary function tests did not demonstrate major differences at the end of adolescence.[124]

Roy and colleagues were the first to allude to a major difference in nutritional management between the Toronto and other clinics, noting that the Toronto patients had been encouraged to consume a high-fat, high-energy diet to replace fecal losses, whereas other clinics had adhered to the universally accepted low-fat diet to avoid the social inconvenience associated with ongoing malabsorption.[125] Dietary analysis demonstrated that whereas, on average, the Toronto patients consumed over 110% of the Recommended Dietary Allowance (RDA) for energy, patients at other clinics were averaging 80 to 90% of the RDA.[126] Later, CF clinics that were changing from a low- to a high-fat diet policy observed that patients consumed over 20% more of the RDA for energy and achieved normal growth, whereas those still adhering to a low-fat, low-energy diet achieved suboptimal weight gain.[127]

Because growth failure with wasting and stunting was common in most clinics, caregivers had adhered to the concept that growth failure was somehow inherent in the underlying disorder in CF. However, the Toronto data disputed this association. Moreover, if inherent in the disease, one would not expect that nutritional rehabilitation would reverse growth failure. To date, a large number of studies have shown that nutritional rehabilitation given by a variety of methods (orally, nasogastric tube, gastrostomy) not only improves weight gain and weight percentiles but also can reverse linear growth failure with achievement of previous or better growth percentiles.[55,128–130]

Pathogenesis of Malnutrition

Protein intakes in CF children are most often very comparable to the high intakes achieved by non-CF children on Western diets of around 200% of RDA, even if a low-fat diet is used.[127] As indicated above, the major adverse effect of a low-fat diet is suboptimal energy intake and subsequent growth impairment. The patient's overall energy deficit can be influenced by other factors, including malabsorption, increased energy needs related to the underlying cellular defect, and to the occurrence of inflammatory lung disease.

Inadequate Energy Intake. Low-fat diets have been one of the main contributing factors to impaired energy intake in CF patients. Fortunately, nowadays most clinics advocate a high-energy normal fat-containing diet, with fat making up nearly 40% of the energy intake. Although, previously, patients on low-fat diets were advised to consume high-energy intakes, this was difficult on the bland high-carbohydrate diets provided. In patients on normal fat-containing diets unable to achieve required intakes for growth, the impaired intake is related to a series of interac-

tive factors, namely, lung disease, drugs given for pulmonary disease, gastrointestinal problems (especially esophagitis), liver or biliary tract disease, long-standing DIOS, and psychoemotional problems. Many of these problems can be managed with medical therapy, but if unsuccessful, invasive nutritional intervention will be required.

Energy Losses. Energy loss occurs via the gastrointestinal tract owing to maldigestion and malabsorption of nutrients. Although 40 to 50% of patients can normalize their fat absorption on optimal regimens of microspheric OERT,[54–56,131] 60% do not, and up to 10% will continue to have fat excretions of over 20% of their fat intake. Again, after investigating such patients for the presence of other complications of CF (eg, gut or liver disease), one may have to empirically treat them with adjunctive gastric acid suppression therapy, attempting to improve the alkalinity of the small intestinal milieu and thereby improve fat digestion and absorption.[54] However, such therapy may not be successful, and larger intakes will be required to avert growth failure.

Energy Expenditure. Several studies of CF patients have demonstrated increased total and/or resting energy expenditure (TEE and REE, respectively).[132–134] In one of the first studies performed, TEE, as measured by the unique doubly labeled water technique, suggested that CF infants had an average TEE that was 25% above age-matched controls.[132] Because they were infants, they were assumed to have minimal lung disease; thus, the increased energy expenditure was attributed to the underlying disease process, in keeping with in vitro studies of CF fibroblasts demonstrating increased oxygen consumption.[135] However, the degree of lung disease in an infant population is difficult to assess; thus, the authors could not conclude that the raised TEE was not in part contributed to by suppurative lung disease. A later study of older patients suggested that REE was negatively correlated with lung function in a quadratic fashion, but this study was in part retrospective.[134] In preliminary results from another center of a large group of patients between 5 and 15 years of age with normal FEV_1 (greater than 80% predicted), PI patients averaged 107% and PS patients 100% of predicted values.[136] At the completion of that study, the latter finding was confirmed, with PI patients maintaining an 11% increase in their average REE ($p < .001$) compared with their PS counterparts, thus indicating that there is an increase in REE related to the underlying severity of the genetic mutation and it occurs independently of the effect of lung dysfunction because these patients had virtually normal lung function.[137] It is of interest that the same study detected a gender difference, with CF females maintaining an average REE of 111% versus 104% for males of their respective control values. This could well explain the noted gender differences in nutritional status and growth and the differences in survival as reported by others.[123]

Malnutrition. In light of the above discussion, it is not surprising that the CF child on a low-fat, low-energy diet consuming only 80% RDA falls well short of the near 110% required for normal growth for a PI child. Furthermore, if the child has had a series of lung infections and ongoing problems with malabsorption, the energy cost of suppuration and lung disease combined with energy losses owing to malabsorption will severely impair the chance of the child even maintaining his/her present growth let alone catching up to previous growth channels.

The consequences of malnutrition in terms of macro- and micronutrients are summarized in Table 65.1-4. Inadequate protein-energy balance is associated with wasting, stunting of linear growth, and, in older children, delayed puberty. Secondary problems occur with abdominal distention, peripheral edema, and ascites with hypoalbuminemia. Fat-soluble vitamin deficiencies can occur at any age and may vary from one age to another. Whereas vitamin A deficiency commonly presents in older children as night blindness, in young infants, it can present as benign intracranial hypertension with a distended fontanel. Vitamin E deficiency often presents as a hemolytic anemia during infancy but later as a severely debilitating neuropathy with ataxia and/or external ophthalmoplegia. Vitamin D deficiency rickets rarely occurs in the hotter subtropical climates but can certainly occur in colder temperate climates, where children are not exposed to sunlight for prolonged periods annually. Overt essential fatty deficiency, which is now seen only in infants at the time of diagnosis, is usually associated with a seborrheic skin rash. A patient on a normal diet supplemented appropriately with OERT should not develop essential fatty acid deficiency.

Malnutrition in CF infants is common where neonatal screening is not operational. Infants present with failure to thrive, wasting, short stature, and/or edema. In the presence of hypoproteinemia, the infants may have a distended fontanel and hemolysis. It is significant that such findings are rare in infants from neonatal screening programs and, if present, are usually subclinical.[41,50] Both controlled and cohort studies of neonatal screening programs demonstrate nutritional and growth advantages in screened infants, although there are limited data supporting an advantage of screening on pulmonary disease and survival.[138,139]

Clinical Evaluation and Management. A summary of a nutritional assessment program is provided in Table 65.1-5. Several other research procedures, including measurement of body cell mass, body protein, and energy expenditure, are undertaken by some units, but they do not have a routine role in the day to day management of CF patients. The major routine assessments, both initially and in follow-up, are measurement of height and weight parameters. In general, maintenance of growth at a specific percentile indicates the well-being of the subject and the adequacy of nutritional intake. Equally, a falling off from a previously held percentile may indicate the inadequacy of oral intake and/or intercurrent problems, particularly chest infection and pulmonary deterioration.

At diagnosis, the other mandatory assessment is an evaluation of pancreatic function, best achieved by a 3- to 5-day fat balance study. Fecal fat values in excess of 10% of fat intake in infants (2 g/d in breastfed infants) or in excess of

TABLE 65.1-4 MALNUTRITION IN PATIENTS WITH CYSTIC FIBROSIS

MACRONUTRIENT DEFICIENCY	
Protein deficiency with hypoalbuminemia	
Edema	
Linear growth failure	
Loss of bone matrix (osteopenia with associated low calcium intake)	
Energy deficiency withweight loss	
Wasting of fat and lean mass	
Wasting of shoulder girdle and buttocks	
Stunting of growth	
Delayed puberty	
MICRONUTRIENT DEFICIENCY	
Fat-soluble vitamin deficiency	
Vitamin A	Benign intracranial hypertension (distention of fontanel in infants)
	Night blindness
	Xerophthalmia: Bitôt spots
Vitamin D	Rickets (rare in sunny climates)
Vitamin E	Hemolytic anemia (infants)
	Peripheral neuropathy
	Ataxia with spinocerebellar tract degeneration
	External ophthalmoplegia
Vitamin K	Coagulopathy
Water-soluble vitamins	Vitamin B_{12} deficiency (rare)
Essential fatty acid deficiency	Seborrheic dermatitis
Salt depletion (hot climates)	Hyponatremia
	If severe, associated with hypochloremia, hypokalemia, and metabolic alkalosis

7% of fat intake in children over 12 months indicate PI and the necessity for OERT. Serum fat-soluble vitamin levels and albumin should be measured at diagnosis and annually thereafter. The normal range of vitamin levels for infants and young children is now available, and, in general, the CF patient should be maintained within that range.[140]

Anthropometric measurements can be repeated at 2- to 3-month clinic visits, and intermittent evaluations of fat absorption may be required in the infant or child with problematic symptoms of steatorrhea. PS patients should have their absorption evaluated at least prior to school entry if asymptomatic and maintaining normal growth or as dictated by the occurrence of symptoms of malabsorption, including failure to maintain their growth percentiles. Given that there are now reports of osteopenia with low bone mineral density in undernourished CF subjects, bone mineral density should be assessed annually in subjects with suboptimal growth.[141] It is also apparent that a large proportion of CF patients (up to 30%) over 10 years of age are glucose intolerant. Although a much smaller percentage is frankly diabetic, some clinics now recommend that a fasting blood glucose be included in the annual evaluation. It remains unclear as to how to approach patients with fasting sugars above 6 mmol/L and whether a 2-hour postprandial serum glucose would be more appropriate. Currently, patients with high fasting or 2-hour postprandial glucose should be referred for evaluation by a diabetologist.

Nutritional recommendations for CF patients are as outlined in Table 65.1-6. Energy intakes should approximate 120% of the RDA but may, of necessity, be increased in patients growing suboptimally. Fat-soluble vitamins should be given on the basis of serum results because a large proportion of subjects can maintain normal levels without supplementation. Vitamin D supplementation is rarely required in sunny climates, where the patient is exposed to ultraviolet light on a daily basis out of doors. Of note, however, those patients from hotter climates require regular salt supplementation during summer months and, to avoid salt depletion and dehydration, at least a doubling of this intake when exercise is undertaken.

Specific Areas of Nutritional Concern

Infants. Recommendations for feeding CF infants have varied over the last 30 years. Prior to microspheric OERT, enzyme replacement was given to infants in the form of enzyme powder. The latter was readily activated in the higher pH of salivary secretions, and, as a consequence, oral inflammation and ulceration were frequent problems. Moreover, the breastfeeding mother experienced excoriated nipples. Both situations, either in isolation or in combination, impaired satisfactory feeding. Consequently, semielemental formula feeds were introduced with the belief that they could be used without enzyme supplementation.[142] Fecal fat analyses purportedly showed normal fat absorption, but, at least in some instances, the analysis technique did not measure medium-chain triglycerides (MCT), and given that many of the diets had high MCT content (up to 80% of fat in the formula), fecal fats were grossly underestimated. The need of OERT to hydrolyze MCT was subsequently demonstrated using the appropriate fecal analytical techniques.[143] Subsequent to the finding that OERT was required for MCT digestion, there appeared little advantage to using semielemental formulas.

TABLE 65.1-5 NUTRITIONAL ASSESSMENT OF PATIENTS WITH CYSTIC FIBROSIS AT DIAGNOSIS

CLINICAL
Height, weight, head circumference
Skinfolds for body fat
Percentile or SD scores thereof
BIOCHEMICAL
Pancreatic function: fecal fat
Serum albumin
Serum fat-soluble vitamin levels
Coagulation screen
FOLLOW-UP
Anthropometric indices (each clinic visit)
Skinfolds (annually)
Bone mineral density (at start of puberty and thereafter as indicated)
BIOCHEMICAL
Fecal fat:
In PS patients suspected of becoming PI
In PI patients for adjustment of OERT
Serum albumin (annually)
Serum fat-soluble vitamins and coagulation screen (annually)
Fasting 2-hour postprandial glucose

OERT = oral enzyme replacement therapy; PI = pancreatic insufficient; PS = pancreatic sufficient.

TABLE 65.1-6 NUTRITIONAL RECOMMENDATIONS IN CYSTIC FIBROSIS PATIENTS

ENERGY	
120% RDA, with 40% as long-chain triglyceride	
PROTEIN	
100% RDA	
FAT-SOLUBLE VITAMINS*	
Vitamin A	5,000 IU/d1–3 yr of age
	10,000 IU/d > 3 yr of age
Vitamin D	500 IU/d
Vitamin E	25 IU/kg/d infants
	100–200 IU/d older children
Vitamin K	5–10 mg, once per week
SALT SUPPLEMENTATION† (hot climates)	
0–6 mo: 0.5 g/d	
6–12 mo: 1 g/d	
1–5 yr: 2 g/d	
> 5 yr: 3–5 g/d	

*Fat-soluble vitamin therapy should be guided by vitamin A and E laboratory results and vitamin K by coagulation screen. Vitamin D is not required in sunny climates.
†Salt intakes should be doubled in hot summer months or during prolonged exercise.

In a subsequent feeding study of infants diagnosed by neonatal screening, a large group of infants achieved and maintained near-normal growth in the first 2 years of life whether breast- or standard milk formula fed.[144] Mothers provided their own choice of feeding at the time of diagnosis, and the subjects were not truly randomized to either regimen. However, in a later randomized study of nonscreened infants, those on standard milk formula achieved near-normal growth parameters.[145] There seems to be no advantage to using expensive semielemental diets in preference to breast milk or standard formula feeding in CF infants provided that the PI patient receives OERT as appropriate.

Adolescents and Adults with Growth Failure and Severe Pulmonary Disease. As respiratory disease advances in CF patients, a vicious circle occurs whereby the chest disease and its management are associated with increasing nutrient requirements in the face of severe anorexia. Weight loss, with loss of lean and muscle tissue, further impairs the likelihood of the subject responding satisfactorily to respiratory therapy, and the cycle continues. Some subjects with less severe pulmonary disease respond readily to high-energy oral supplements, including milk shakes and high-density desserts.[146] However, others with more severe disease are sufficiently anorexic to be unable to comply with these regimens. Consideration should be given in such patients to a more aggressive approach to nutritional intervention, including continuous nocturnal nasogastric tube or gastrostomy button feeds. The severity of the pulmonary disease will often preclude nightly nasogastric intubations, and permanently placed gastrostomy buttons have gained favor over the last decade. These regimens can halt weight loss and, in many instances, can be used to induce catch-up growth. However, the specific advantages of so doing have yet to be realized in terms of overall prognosis. Although initial studies indicated a slowing of the decline in lung function, they were performed on small numbers with mostly reasonably preserved lung function at the start of the studies.[128–130] In patients with advanced lung disease, the benefits are even less obvious; in fact, they may encumber a patient unduly, interfering with the quality of life in the terminal stages of the disease. These issues need to be addressed in patients with severe disease prior to commencement of such therapy, thus preventing discomfort and the emotional challenge in a patient needing palliative therapy only. Some of these patients may be suitable candidates for receiving anabolic agents. Growth hormone can increase height and weight velocity,[147] but as they increase proportionately, patients can remain significantly wasted even after 12 months of therapy. It is also uncertain as to whether the growth changes will be sustained after cessation of the therapy. Similarly, megestrol acetate produces significant improvements in appetite and weight gain, but these are not sustained after cessation of therapy. Although further treatment could be considered, adverse events, including adrenal suppression, insulin resistance, insomnia, hyperactivity, and hypertension, have been described.[148–150]

With the advent of lung transplant, the importance of nutrition needs to be re-evaluated in pre- and post-transplant subjects. Specifically, the physicians involved need to determine whether malnutrition adversely affects transplant outcome and whether the adverse effects can be overcome with nutritional supplementation.

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2. *Shwachman-Diamond Syndrome*

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In 1964, Shwachman and colleagues and Bodian and colleagues independently described a syndrome predominantly affecting the exocrine pancreas and bone marrow.[1,2] Subsequent reports of larger patient cohorts have demonstrated that the clinical phenotype of Shwachman-Diamond syndrome (SDS) affects additional organ systems, such as the skeleton and liver, and revealed that short stature is also a characteristic feature of the disease.[3–5] Furthermore, the SDS phenotype is extremely heterogeneous, and specific features of the disease change with advancing age.[3,5] After cystic fibrosis (CF), SDS is the most common inherited cause of exocrine pancreatic dysfunction. The disease has an estimated prevalence that is 20 times lower than that of CF, with an approximate incidence of 1 in 50,000 in the North American population. It is known to occur in diverse populations, including those with European, Indian, Chinese, Japanese, North American aboriginal, and African ancestry.

GENETICS

An autosomal mode of inheritance for SDS was initially suggested by the pedigree structure of several family reports[3,6,7] and has been confirmed in formal segregation analysis of a collection of 70 families with corrections for ascertainment considerations.[8] The same collection was then used to initiate investigation into the molecular basis of disease. A genome-wide scan and linkage analysis revealed significant association to disease with markers on chromosome 7 that spanned the centromere.[9] Data from all of the 15 families, each of whom had two or three patients, supported the linkage and confined the location of the affected gene to a 2.7 cM interval. Shared disease haplotypes, consistent with founder disease chromosomes, were identified in additional and unrelated families of common ethnic origins, permitting further refinement of the gene locus to a 1.9 cM interval at 7q11.[10]

Subsequently, an uncharacterized gene containing disease-associated mutations was identified within the 1.9 cM interval.[11] The *SBDS* gene (Shwachman-Bodian-Diamond syndrome) has a 1.6 kb transcript composed of 5 exons, encoding a predicted protein of 250 amino acids (Figure 65.2-1). A pseudogene (*SBDSP*) was identified in a locally duplicated 305 kb genomic segment with 97% nucleotide identity to the disease-causing gene. Gene conversion owing to recombination between *SBDS* and its pseudogene was noted to be a common event in disease alleles. Three common recurring mutations, resulting from conversion of exon 2 segments, introduce changes leading to premature protein truncation. These conversions account for 74% of alleles associated with SDS. Of the unrelated SDS individu-

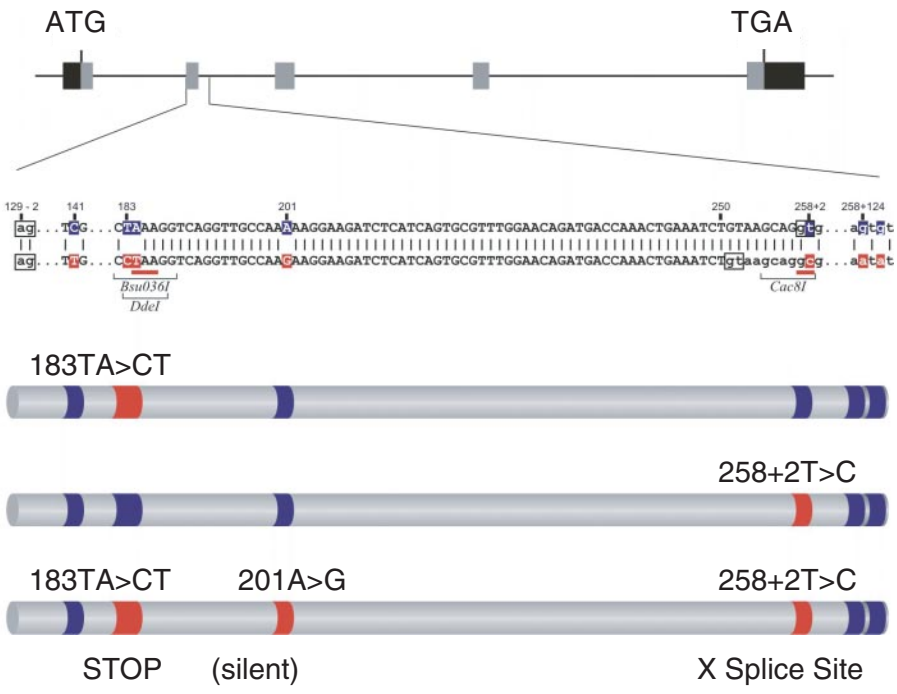

FIGURE 65.2-1 The *SBDS* gene is composed of five exons spanning 7.9 kb. A pseudogene resides in an adjoining duplicated segment. Sequence alignment of exon 2 of *SBDS* and its pseudogene *SBDPS* shows two sequence changes as well as a polymorphic change (201A→G). The most common *SBDS* mutations are derived from gene conversion events between *SBDS* and *SBDPS*. Three converted alleles are shown. These include (1) in-frame stop codon at position 184 (183 TA→CT), (2) splice site change (258+2T→C), and (3) an extended conversion mutation containing both mutant alleles and the polymorphic change.

als, 141 of 158 (89%) carried a gene conversion mutation on one allele and 60% carried conversion mutations on both alleles. Other *SBDS* mutations were identified in patients not involving gene conversion, including nucleotide substitutions leading to splicing interruption, missense changes or nonsense changes, and small deletions or insertions leading to in-frame changes or frame shifting and truncation (Table 65.2-1). These rare mutations were consistent with founder chromosome occurrence; for example, the 119delG mutation was found in two families of French Canadian descent with common haplotypes.[11]

Although the function of *SBDS* is unknown, it appears to be a member of a highly conserved family of proteins. Orthologs of *SBDS* exist in a wide variety of species, including archea, plants, and vertebrates. Ribonucleic acid (RNA) hybridization studies reveal broad but variable levels of expression in all tissues examined to date.[11] The predicted 28.8 kD protein product shows no homology to any known protein, but several lines of indirect evidence suggest that *SBDS* may be important in RNA metabolism.

CLINICAL MANIFESTATIONS

General Features

Based on retrospective analysis of small patient cohorts, affected patients have lower than average weight and height at birth.[12] Although most patients have few signs and symptoms of disease in the early neonatal period, uncommon presentations include asphyxiating thoracic dystrophy, complete bone marrow failure, or severe life-threatening infections. On occasion, poor growth in infancy has raised suspicions and even accusations of parental neglect. We are aware of a tragic example of a 2-year-old girl in whom the diagnosis of SDS was established at postmortem (P. R. Durie, personal observation, 2002). This patient died of bacterial sepsis while in foster care after being removed from her biologic parents' care owing to a presumed diagnosis of "child neglect."

More commonly, patients come to medical attention in infancy or early childhood, with one or more symptoms, which include malabsorption, malnutrition, growth failure, and recurrent infections (Table 65.2-2). Uncommonly, patients present de novo with aplastic anemia or acute myelogenous leukemia. On occasion, patients elude diagnosis until later childhood, and, rarely, the diagnosis is first established in adulthood.[13,14] As is discussed in greater detail below, a large percentage of older patients do not have severe pancreatic disease. Therefore, the absence of signs and symptoms of maldigestion does not exclude a diagnosis of SDS.

The nonspecific nature of the presenting symptoms, as well as poor awareness of the nature of the disease phenotype, can result in a delayed or even an incorrect diagnosis. The median age of diagnosis among 88 internationally ascertained patients was 1 year, with a range of 0.1 to 13 years.[5] In most case series, significantly more males than females have been diagnosed with SDS.[4,5] However, no gender differences were observed among families with more than one affected patient.[5] Given the small size of patient cohorts, the suggestion of a gender difference among isolated cases could be spurious. Alternatively, the contrasting gender differences, among singleton cases but not among multiplex patients, raises the possibility of a societal gender bias favoring clinical evaluation of males with short stature.

Although the clinical manifestations of disease are extremely heterogeneous and may change with age, all patients with SDS appear to have consistent evidence of both hematologic and exocrine pancreatic dysfunction. Other features, such as short stature, hepatic dysfunction, clinical or radiologic evidence of skeletal chondrodysplasia, and dental abnormalities, are sufficiently common to be considered primary features of the disease phenotype. Predilection to recurrent common bacterial infections, such as otitis media, as well as life-threatening deep tissue infections and sepsis, is considered to be a direct consequence of neutropenia and defective neutrophil chemotaxis. More convincing evidence is now emerging that neurologic abnormalities, learning difficulties, and psychological disorders are quite common among individuals with SDS. With further insight into disease pathobiology and evalua-

TABLE 65.2-1 MUTATIONS ASSOCIATED WITH SHWACHMAN-DIAMOND SYNDROME IN AFFECTED INDIVIDUALS FROM 158 FAMILIES

NUCLEOTIDE SEQUENCE CHANGE	AMINO ACID CHANGE	NUMBER OF ALLELES
258+2T→C*	84Cfs3	145
183-184TA→CT*	K62X	82
183-184TA→CT+258+2T→C*	K62X	8
258+1G→C*	84Cfs3	2
119delG	S41fs17	2
377G→C	R126T	2
24C→A	N8K	1
96-97insA	N34fs15	1
131A→G	E44G	1
199A→G	K67E	1
260T→G	187S	1
291-293delTAAinsAGTTCAAGTATC	D97-K98delinsEVQVS	1
505C→T	R169C	1

Adapted from Boocock GR et al.[11]
*Gene conversion mutations accounted for 235 of 316 (74.4%) alleles associated with SDS.

TABLE 65.2-2 SPECTRUM OF CLINICAL FEATURES OF SHWACHMAN-DIAMOND SYNDROME

EXOCRINE PANCREAS	HEMATOLOGIC	SKELETAL/ DENTAL	GROWTH/ NUTRITION	LIVER	PSYCHOLOGICAL/ NEUROLOGIC
Acinar cell: dysfunction, pancreatic insufficiency, pancreatic sufficiency	Cytopenias: neutropenia, anemia, thrombocytopenia, pancytopenia ↑ Hemoglobin F Marrow aplasia Myelodysplasia Acute myelogenous leukemia	Long bones: delayed maturation, metaphyseal, dysplasia, tubulation Thorax: thoracic, dystrophy, short flared rib, costochondral thickening, clinodactyly, osteopenia Teeth: caries, dysplasia, mouth ulcer	Malnutrition (at diagnosis) Short stature	Hepatomegaly ↑ Aminotransferases Histology: portal inflammation, portal fibrosis	Low intelligence Learning difficulties Pontine leukoencephalopathy

Other: dermatologic; eczema, ichthyosis; renal: anatomic anomalies, tubular dysfunction; cardiac: endocardial fibrosis.

tion of much larger populations, it is likely that other, less common phenotypic features of SDS will be shown to be directly attributable to mutations in the *SBDS* gene.

Exocrine Pancreas

Exocrine pancreatic dysfunction of varying severity appears to be a universal manifestation of SDS. Unlike CF, which is a disorder of pancreatic ductal obstruction,[15] the pancreatic defect in SDS appears to arise from a failure of pancreatic acini to develop. Histologically, the SDS exocrine pancreas shows normal ductular architecture and islets, absent or sparse acinar cells, and extensive fatty replacement (Figure 65.2-2).[2] Cross-sectional imaging may reveal a small shrunken pancreas or pancreatic enlargement owing to lipomatosis (Figure 65.2-3).[16] The suggestion that SDS is a syndrome primarily affecting acinar cells is bolstered by analysis of the results of hormonally stimulated pancreatic function studies, which reveal absent or deficient acinar function (reduced enzyme output) but preserved ductal function.[4,17] The latter is reflected by normal output of anions (chloride and bicarbonate), cations (sodium and potassium), and fluid.

Most infants with SDS have signs and symptoms of fat maldigestion owing to pancreatic failure. Stimulated pancreatic secretions of lipolytic (lipase and colipase) and proteolytic (trypsin) enzymes fall more than 98% below the mean reference values for healthy controls.[17,18] These patients show clinical evidence of steatorrhea owing to pancreatic insufficiency based on correlative evaluation with 72-hour fecal fat balance studies.[4,18] However, cross-sectional and longitudinal evaluation of older SDS patients show that a subset of affected individuals show moderate improvement in pancreatic acinar capacity.[4,5,18] Secretions of lipolytic and proteolytic enzymes are marginally improved in these "pancreatic suffi-

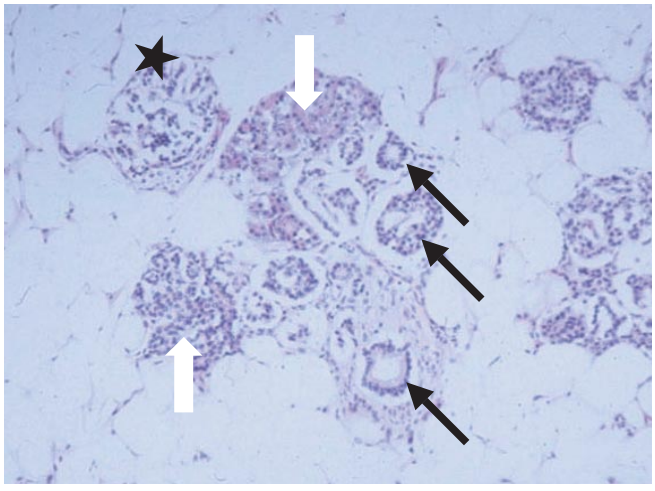

FIGURE 65.2-2 Histologic section of the pancreas from a patient with Shwachman-Diamond syndrome shows few sparse acini (*open arrows* surrounded by extensive areas of fatty replacement). Ductular architecture (*solid arrow*) and islets (*asterisk*) are normal (hematoxylin and eosin, ×50 original magnification).

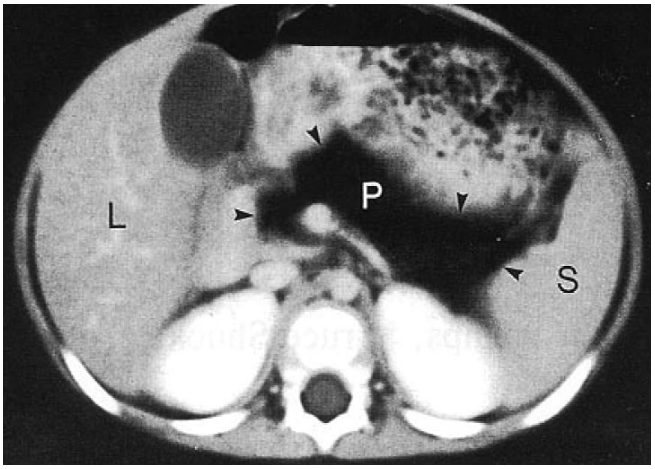

FIGURE 65.2-3 Computed tomographic scan of the abdomen after intravenous contrast injection in a patient with Shwachman-Diamond syndrome. This individual presented clinically with massive hepatomegaly and intermittent neutropenia. Marked fatty infiltration of the exocrine pancreas (P) is outlined with arrowheads. This is in contrast to the soft density of the liver (L) and the spleen (S). Reproduced with permission from Wilschanski M et al.[16]

cient" patients but are sufficient to allow normal endogenous digestion of fat and protein, respectively, without the need for enzyme replacement therapy (Figure 65.2-4). Serial evaluation by 72-hour fecal fat balance studies confirms that stool fat losses, expressed as a percentage of quantified fat intake, normalize with age in this subset of patients.

Similar conclusions have been drawn from cross-sectional and longitudinal analysis of serum cationic trypsinogen concentrations.[4,5] Correlations between serum cationic trypsinogen concentrations and the results of 72-hour fecal fat balance studies (Figure 65.2-5) demonstrate that SDS patients with pancreatic insufficiency have serum enzyme concentrations of less than 6 µg/mL (lower limits of reference range 16.6 µg/mL). In contrast, those with normal fat absorption have serum trypsinogen values exceeding 6 µg/mL. Some of these patients have values within the normal reference range (16.6–45.5 µg/mL).

Taken together, these studies demonstrate that approximately 50% of affected patients will show sufficient improvement in pancreatic acinar capacity with advancing age to no longer require enzyme supplements. However, all of these patients have evidence of pancreatic dysfunction based on quantitative intubation techniques. Therefore, the absence of signs and symptoms of steatorrhea and/or normal serum trypsinogen concentrations does not exclude pancreatic acinar dysfunction or the diagnosis of SDS.

The age-related changes observed for serum trypsinogen do not hold true for other enzymes of pancreatic origin.[19]

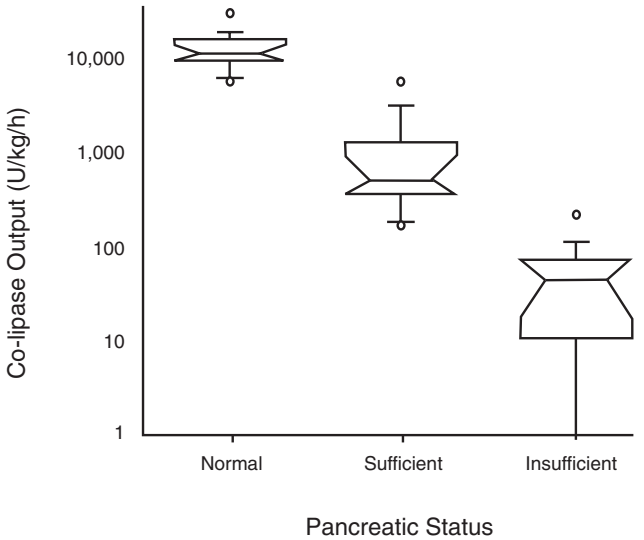

FIGURE 65.2-4 Stimulated exocrine colipase secretion controls, a pancreatic-insufficient patient, and pancreatic-sufficient patients with Shwachman-Diamond syndrome (SDS). The box plots show medians (*middle line*) and 75th (*top line*) and 25th percentiles (*bottom line*). The vertical lines extending above and below each box represent the 90th and 10th percentiles, respectively. Outliers are also shown. SDS patients with pancreatic sufficiency (based on a normal 72-hour fat balance study) have intermediate colipase secretion in comparison with healthy controls and SDS patients with pancreatic insufficiency. Similar results were obtained when total lipase and trypsin output were analyzed (data not shown). Reproduced with permission from Mack DR et al.[4]

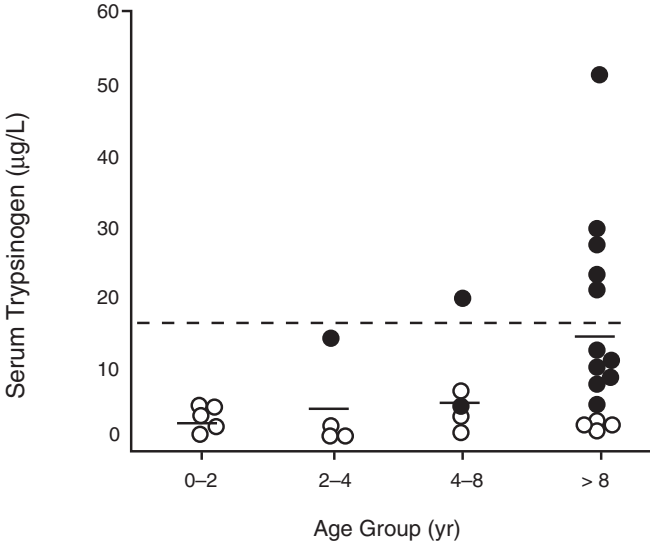

FIGURE 65.2-5 Serum cationic trypsinogen determinations grouped by age, including both pancreatic-insufficient (*open circles*) and pancreatic-sufficient (*closed circles*) patients with Shwachman-Diamond syndrome. The horizontal lines represent the mean value for each subgroup. The horizontal dashed line represents the lower reference limit (16.7 µg/mL for serum trypsinogen). Reproduced with permission from Mack DR et al.[4]

For example, we have observed discordance between serum trypsinogen and pancreatic isoamylase determinations among patients with SDS and similarly aged healthy controls (Figure 65.2-6). In controls, serum trypsinogen concentrations were normal from birth and show no age-related alterations, whereas serum pancreatic isoamylase activities were lower at birth, rising to adult values by 3 years of age. Thereafter, serum pancreatic isoamylase activity remained unchanged with advancing age. We have concluded that the age-related differences of the two pancreatic enzymes mirror their discordant maturation rate postnatally. Pancreatic trypsin synthesis and secretion are mature after birth, whereas pancreatic isoamylase secretion is low at birth and shows gradual maturation over the first 3 years of age. In contrast to the aforementioned observations in controls, all patients with SDS had low pancreatic isoamylase activities irrespective of age, serum trypsinogen level, or their pancreatic status with regard to fat digestion. The independent age-related changes of the two enzymes, which appear to be unique to the pancreas of patients with SDS, have proven to be of great value as a clinical marker of the SDS pancreatic phenotype (see "Establishing a Clinical Diagnosis").

Bone Marrow

All patients with SDS show varying degrees of bone marrow failure. Persistent or intermittent neutropenia is virtually universal among affected patients. Some patients have a variety of other isolated cytopenias involving one or more bone marrow elements,[4,5] which may include pancytopenia and even complete bone marrow failure. The prevalence and range of hematologic abnormalities among singleton cases of SDS are similar to those seen in families with multiply affected children.[5] Furthermore, the fre-

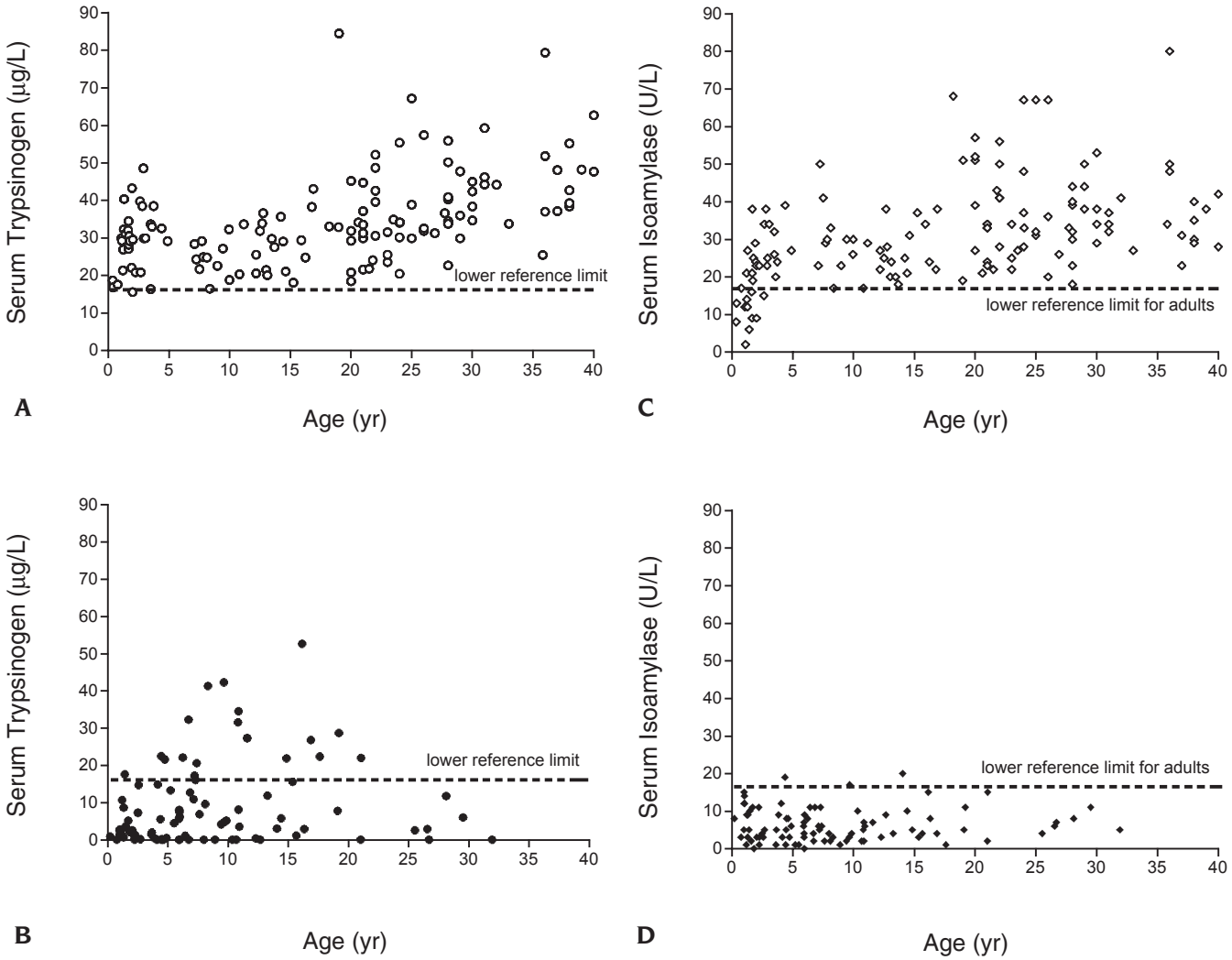

FIGURE 65.2-6 Cross-sectional serum trypsinogen levels in 23 control patients (*open circles*) and 90 patients with Shwachman-Diamond syndrome (SDS) (*closed circles*) plotted as a function of age (*A* and *B*). Twenty-two of 23 patients with SDS who were < 3 years of age showed serum trypsinogen values below the reference range, but values were within the normal range in 20% of older patients. Serum isoamylase values are plotted as a function of age in the same control patients (*open diamonds*) and patients with SDS (*closed diamonds*) (*C* and *D*). Unlike serum trypsinogen, serum isoamylase activities in control patients showed a significant age-dependent rise from birth to 3 years of age. After 3 years, intervals for pancreatic isoamylase (17–80 U/L) were similar to those previously reported for adults. All patients with SDS had low serum isoamylase activities. In 87 of 90 patients, values were below the reference range for adults. However, among patients < 3 years of age with SDS, isoamylase values tended to show overlap with values in similarly aged controls. There was no alteration of serum isoamylase with age in SDS and no association with the corresponding trypsinogen value.

quency and degree of concordance of the various bone marrow–derived cell lines in singleton cases do not differ from those observed in affected siblings.

In the largest patient cohort evaluated to date, 98% of 88 patients had chronic neutropenia, which was intermittent in 61% and persistent in the remaining patients. Iron deficiency anemia (42%) and thrombocytopenia (34%) were the next most common isolated abnormalities.[5] Abnormalities in more than one cell line were common, and pancytopenia was present in 19% of this cohort. The severity of peripheral neutropenia or other cytopenia does not appear to correlate with bone marrow cellularity. Peripheral blood counts do not necessarily predict the onset of myelodysplasia. Persistent elevation of hemoglobin F, which may reflect ineffective erythropoiesis, is present in most patients with SDS.

Neutrophil dysfunction, which is also observed in patients with SDS, almost certainly contributes to the propensity of patients with SDS to suffer from severe life-threatening bacterial infections. Chemotactic migration is significantly impaired, and some reports suggest that neutrophils from SDS patients exhibit defective bactericidal and phagocytic activity.[20–23]

The bone marrow of SDS patients shows reduced numbers of bone marrow precursors, which suggests that the bone marrow disorder is a stem cell defect.[3,24] Using long-term cultures of marrow stromal cells from SDS and unaffected subjects, Dror and Freedman demonstrated that patients with SDS also have dysfunction of the bone marrow stroma.[24] Thus, a dual bone marrow defect, namely a reduced ability of the bone marrow stroma to support and maintain effective hematopoiesis, as well as a stem cell

defect, may explain the severity of the bone marrow defect in patients with SDS.

Bone marrow testing reveals a wide range of abnormalities.[3,5,25] The bone may appear normal in some patients, especially at a young age. The majority of cases show generalized hypocellularity, isolated hypoplasia of single cell lines, or generalized maturation delay or arrest. Complete bone marrow aplasia is known to occur.[26] Myelodysplasia with or without cytogenetic abnormalities is common.[5,27] The most common clonal cytogenetic abnormalities are present in chromosome 7 (eg, monosomy 7, isochromosome 7), but abnormalities are also observed in other chromosomes.[27–29] However, cytogenetic abnormalities, including those involving chromosome 7, may not be directly related to the SDS defect because similar changes are commonly observed in other bone marrow failure syndromes. The prognostic implications of finding myelodysplasia, with or without a clonal abnormality, remain uncertain.

As is the case with most bone marrow failure syndromes, patients with SDS carry a high risk of developing leukemia. The majority of individuals develop acute myelogenous leukemia, but lymphoblastic leukemia has also been reported.[30] The true prevalence of this complication is unknown. Smith and colleagues conducted a retrospective analysis of 21 patients with SDS over a 25-year period.[27] Myelodysplastic syndrome developed in 33% of patients, five of whom developed clonal chromosomal abnormalities. Twenty-five percent of these patients developed acute myeloid leukemia, which was invariably fatal in this series of patients. Males appear to carry a 10-fold greater risk of developing leukemia than affected females.[5,7,28] In general, attempts to induce remission with chemotherapeutic agents have been unsuccessful. Stem cell marrow transplant has been successful in a small number of patients. However, the success rate has been low, with an approximate 50% rate of survival (J. M. Lipton, personal communication, 2003). Also, a high complication rate, including heart and liver failure during induction, remains a concern.

Infections

The presence of neutropenia, abnormal chemotaxis, and possibly neutrophil bactericidal dysfunction function puts patients at risk of severe life-threatening infections.[20–23] The risk of infections may be exacerbated by nonspecific immunologic disorders, including dysgammaglobulinemia and T-cell dysfunction.[31] For poorly understood reasons, infants and young children appear to carry the greatest risk of morbidity and mortality from bacterial infections. Also, younger patients appeared to be particularly susceptible to upper respiratory tract infections and otitis media. In an earlier report, Shmerling and colleagues estimated that patients carried a 25% mortality rate owing to infection.[6] However, a more recent study of 25 patients from our center, who were followed for an average period of 5 years (range 0.5–20 years), showed an 8% mortality rate from infectious complications.[4] However, better survival in this cohort could be due to increased awareness of this complication. Eighty percent of these patients experienced one or more episodes of deep tissue infections, including pneumonia, abscess, osteomyelitis, septic arthritis, or bacterial sepsis. Recurrent fevers of unknown origin are also observed in patients with SDS.[5]

Nutrition and Growth

Short stature, which is common in patients with SDS, is considered to be a primary manifestation of the syndrome. Retrospective analysis of stature at birth shows a moderate deficiency in both height and weight in comparison with healthy newborns.[12] Patients may present with malnutrition at diagnosis.[4] Contributing factors to malnutrition include poor intake owing to recurrent infections and malassimilation of nutrients owing to pancreatic insufficiency. With adequate treatment with an appropriate diet and enzyme replacement therapy, malnutrition is rarely a long-term problem. However, short stature persists, and height and weight remain significantly below the age- and sex-adjusted values for the general population. Approximately 50% of individuals with SDS are below the 3rd percentile for height and weight.[5] Mean z-scores for height and weight are –2.2 and –1.8, below the mean for age and sex, respectively (Table 65.2-3). Cross-sectional studies of growth by age show that height relative to population norms remains the same at all ages. Weight expressed as a percentage of ideal weight for height also remains normal in childhood, but limited data show a tendency for adult patients to become relatively obese. There appear to be no differences in stature or nutritional status between patients with pancreatic sufficiency and those with pancreatic insufficiency.

Skeleton and Dentition

Previous small cohort studies of patients with SDS provide an incomplete picture of the skeletal aspects of the disease.[3–6,32–35] In most studies, only a subset of patients underwent radiologic evaluation, and the lack of longitudinal information failed to provide insight into the natural history of the skeletal abnormalities. Reported abnormalities included abnormal development of growth plates and metaphyses, delayed bone age, progressive deformities, and pathologic fractures. In the perinatal period, severe restrictive respiratory failure can arise as a result of asphyxiating thoracic dystrophy.

More recently, Makitie and colleagues analyzed the radiographs of 15 patients with SDS in whom the diagnosis had been confirmed by identified *SBDS* mutations on both alleles.[36] In 10 of these patients, serial radiographs were avail-

TABLE 65.2-3 GROWTH CHARACTERISTICS OF SHWACHMAN-DIAMOND SYNDROME

AGE (YR)	MEAN HEIGHT (Z-SCORE)	MEAN WEIGHT (Z-SCORE)	MEAN WEIGHT/ IDEAL WEIGHT FOR HEIGHT (%)
0–2	–2.65	–2.62	102
2–4	–2.24	–1.84	107
4–8	–1.84	–1.54	109
8–12	–2.23	–1.45	129
> 12	–2.15	–1.47	119

Adapted from Ginzberg H et al.[5]

able for longitudinal evaluation. The results of this study suggest that skeletal changes are present in all patients with SDS, but there is variable severity and localization changes with age. Typically, infants show delayed appearance of the secondary ossification centers, but epiphyseal maturation tended to improve with age. Varying degrees of widening and irregularities of the metaphyses were common in the ribs and proximal and distal femora in early childhood. With advancing age, a number of patients showed progressive thickening and irregularity of the growth plates (Figure 65.2-7). Most patients with radiologic alterations in the metaphyses showed no clinical symptoms. Some of these irregularities were associated with asymmetric growth and can progress to severe joint deformities, particularly in the proximal and distal femur, as well as the proximal tibia. Slipped femoral epiphyses and coxa vara or coxa valga deformities will require corrective surgery. Abnormal tubulation of the long bones was also observed.

This study also suggested that the right and left limbs were affected similarly, but the legs were more severely affected than the upper limbs. This report also affirmed previous observations that generalized osteopenia may be present at all ages.[3,36] There is no evidence that the osteopenic changes are related to pancreatic insufficiency or to a deficiency of vitamin D. No phenotype-genotype correlations were observed, and patients with identical *SBDS* mutations had a range of skeletal findings.

Several studies have suggested that patients with SDS are prone to oral and dental abnormalities. However, the true prevalence and severity of oral disease await careful prospective evaluation of a larger cohort of patients. Aggett and colleagues identified dental abnormalities in 10 of 21 patients with SDS.[3] Eight of these individuals had extensive caries, and three had dysplastic teeth. Other studies have also identified cases of caries and tooth dysplasia.[4,5] There have been reports of delayed loss of primary dentition and eruption of permanent teeth. Because oral diseases, including oral caries, decay, and periodontal disease, are largely preventable, further insight into the severity and extent of the problem will allow dentists to intervene more appropriately.

Liver

Hepatomegaly is a common observation, especially in infancy.[3,5,16,37] Cross-sectional reports suggest that hepatomegaly resolves in the majority of patients by approximately 5 years of age.[3] Serum aminotransferase values are also elevated in infancy. In a larger cohort series of 88 patients, 60% had abnormal serum aminotransferase levels.[5] In most cases, values were onefold to fourfold above the upper limits of the reference range. Serial data in a subset of these patients showed a tendency for these biochemical hepatic abnormalities to resolve or improve with advancing age. Serum bilirubin levels were consistently normal. Histologic abnormalities are generally quite mild and include micro- and macrovesicular steatosis, periportal and portal inflammation and fibrosis, and, occasionally, bridging fibrosis. Progressive liver disease has not been reported in patients with SDS. There is one case report of chronic liver disease in a patient with SDS.[38] Also, affected patients have reportedly died from hepatocellular failure during induction for bone marrow transplant.

Psychological, Behavioral, and Neurologic Features

Several retrospective case studies,[4,5] clinical observations, and anecdotal expressions of concern from families suggest that individuals with SDS commonly suffer from learning and/or behavioral difficulties. At present, objective testing of patients is limited, and the prevalence and specificity of these deficits require objective prospective evaluation of a larger patient cohort. In the most extensive report to date, Kent and colleagues performed psychometric testing in affected patients, unaffected siblings, and disease controls with CF.[39] They also interviewed the patients' parents to ascertain information regarding developmental milestones, social interactions, and the impact of their child's illness on emotional and social adjustment. Psychometric testing revealed lower intelligence quotients among patients with SDS in comparison with their sibling controls and disease controls suffering from CF. Although group differences with tests of cognition and motor function were not significant, the individuals with SDS

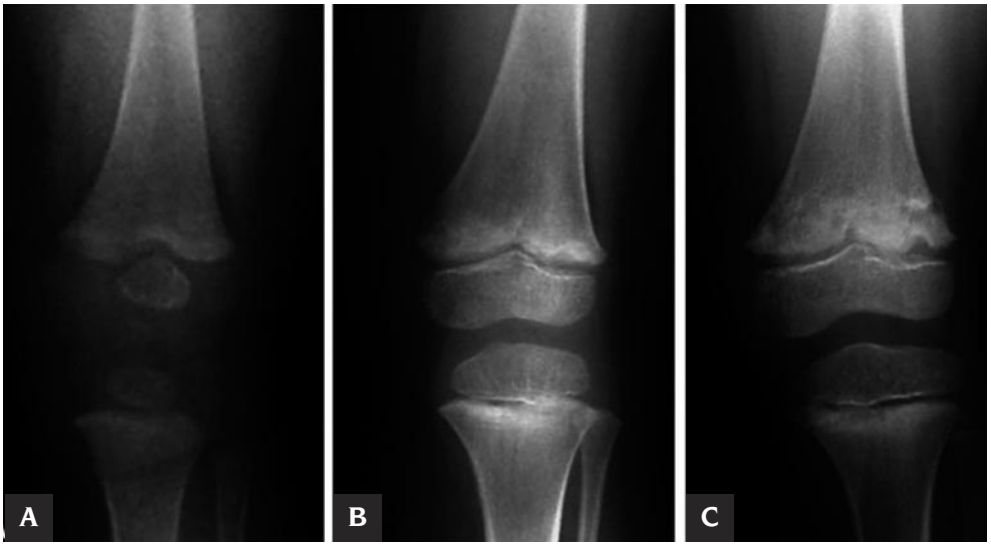

FIGURE 65.2-7 Longitudinal radiologic changes in the knee of a patient with Shwachman-Diamond syndrome. At age 5 months (*A*), secondary ossification centers are small. There is mild metaphyseal widening. At 4 years of age (*B*), secondary ossification centers are normal. The distal femoral and proximal tibial metaphyses are irregular and sclerosed. The metaphyseal changes show more severe irregularity and sclerosis at $5^1/_2$ years of age (*C*). The femoral metaphysis is more severely affected than the tibia. Reproduced with permission from Makitie O et al.[36]

tended to have the lowest scores. In this particular report, the authors found no evidence that individuals with SDS had more behavioral difficulties than the control subjects. Interim analysis of a prospective international study that is being conducted at this center suggests that children with SDS do experience significant cognitive and retention difficulties as well as problems with emotional control.

There have been isolated case reports of specific neurologic abnormalities in both children and adults with SDS.[40,41] In three patients with SDS, focal or multifocal pontine leukoencephalopathy has been identified. Histopathologically, the pons shows lesions within pontocerebellar fibers with central necrosis, calcification, and neuroaxonal spheroids. Frequently, there are extrapontine areas of demyelination. Similar neuropathologic alterations have been recognized in patients with leukemia or lymphoma after radiotherapy or chemotherapy and in immunocompromised patients, including those with human immunodeficiency virus (HIV) or acquired immune deficiency syndrome (AIDS). It is important to note that none of the patients with SDS who developed leukoencephalopathy had evidence of malignancy, nor were they receiving immunosuppressive therapy.

Other Findings

A variety of uncommon abnormalities have been described in patients with SDS. For example, dermatologic abnormalities, including ichthyosis and eczematous lesions, are commonly described in affected patients. There have also been reports of severe endocardial fibrosis, which led to death in a number of patients.[42]

Anatomic abnormalities of the renal system have been reported in individual cases, including double urethra and unilateral nonfunctioning kidney with urethral duplication.[3–5] Nephrocalcinosis and mixed amino acid urea have also been reported.[3–5] Although individual isolated cases of growth hormone deficiency and diabetes mellitus have been described, there is no evidence that these clinical conditions are directly associated with SDS.

ESTABLISHING A CLINICAL DIAGNOSIS

The diagnosis of SDS is established on the basis of characteristic clinical findings. There is no single disease characteristic or a simple biochemical test that is capable of definitively establishing or excluding a diagnosis of SDS. Furthermore, the existence of considerable disease heterogeneity and a lack of medical awareness of SDS make the diagnosis problematic. Other inherited causes of pancreatic exocrine dysfunction, including CF, Pearson bone marrow–pancreas syndrome, and Johansson-Blizzard syndrome, must be excluded. Similarly, transient neutropenia and bone marrow failure syndromes resembling SDS warrant serious consideration as alternative diagnoses. Pearson bone marrow–pancreas syndrome, Fanconi anemia, and Diamond-Blackfan anemia can be excluded by careful clinical assessment, bone marrow analysis, and/or specific laboratory tests.

With appropriate knowledge of the spectrum of the disease phenotype, the clinical diagnosis of SDS can be objectively established or excluded with a high degree of certainty in the vast majority of patients. Two clinical features of the disease, notably exocrine pancreatic and bone marrow dysfunction, appear to be consistently observed in all patients. Therefore, objective confirmation of these two phenotypic manifestation is currently considered to be an absolute requirement for establishing a clinical diagnosis.[5,43]

Pancreatic exocrine dysfunction may be identified by one or more of the following evaluations:

1. Deficit of pancreatic enzyme secretion following quantitative pancreatic stimulation testing with intravenous cholecystokinin and secretin
2. Abnormal 72-hour fat balance study (provided intestinal mucosal disease or cholestatic liver disease is excluded) plus pancreatic imaging demonstrating a small or lipomatous pancreas
3. Low serum trypsinogen in patients under 3 years of age; low serum trypsinogen or pancreatic isoamylase in patients older than 3 years of age[19]

Bone marrow dysfunction may be established by one or more of the following evaluations:

1. Neutropenia (absolute neutrophil count < 1,500 neutrophils/mm^3). Because neutropenia can be persistent, cyclic, or intermittent, it must be documented at multiple time points (at least three times over a period of 3 months or more).
2. Anemia (hemoglobin concentration below the age-related normal range)
3. Persistent thrombocytopenia (platelet count < 150,000 platelets/mm^3)
4. Persistent pancytopenia
5. Myelodysplasia with or without clonal abnormalities

Genetic analysis may be used for diagnostic confirmation. Clinical genotyping for SDS is now available in clinical molecular diagnostic laboratories in the United States, United Kingdom, and Italy, as well as at the Hospital for Sick Children, Toronto.

Other common primary features of SDS are used to provide supportive evidence of the diagnosis of SDS. These include short stature, skeletal abnormalities, and hepatomegaly with or without elevation of serum aminotransferase levels. The absence of these features does not exclude the diagnosis of SDS. Because short stature occurs in a wide variety of other conditions and is not observed in all patients with SDS, it cannot be relied on as a feature of the disease for diagnostic purposes.

BASELINE ASSESSMENT, MONITORING, AND THERAPY

Diagnostic Assessment

The basic diagnostic evaluation of patients suspected of SDS has been outlined above. Additional baseline investigations, which are summarized in Table 65.2-4, include assessment of the status of the patient's pancreas, liver, skeleton, and bone marrow. Careful nutritional evaluation should include height, weight, anthropometry, deter-

mination of serum fat-soluble vitamin levels (A, E, 25-hydroxyvitamin D), prothrombin time, and partial thromboplastin time. Baseline bone marrow biopsy should include evaluation for evidence of cytogenetic abnormalities.

We advocate a multidisciplinary approach to the assessment, education, and treatment of the patient and family. Optimally, this should include ongoing support by a hematologist and gastroenterologist with adequate knowledge of the natural history of SDS. Depending on the family's circumstances and patient's condition, the assistance of a genetic counselor, psychologist, and/or social worker may be required. A dietitian who is familiar with nutritional care and pancreatic enzyme replacement therapy of patients with pancreatic failure should provide ongoing education and monitoring for the patient and family.

Clinical Monitoring and Therapy

The assessment should be performed every 6 to 12 months and should include weight, height, anthropometric measures, bone age, pubertal development, and review of developmental progress. A complete blood count with white cell differential and platelet count should be performed every 6 months or more frequently if indicated. We recommend that serum concentrations of vitamins A, E, and 25-hydroxyvitamin D; prothrombin time; and partial thromboplastin time be performed every 6 to 12 months.

Because a significant percentage of patients suffer from skeletal and dental disorders, anticipatory monitoring is advocated. With respect to the skeleton, we recommend radiographs of the hips and lower limbs every 1 to 2 years and, when indicated, consultation by an orthopedic surgeon with experience in chondrodysplastic disorders. Parents should be advised to seek dental evaluation at an early age, and the patient's dentist should be provided with information concerning the increased risk of oral or dental disorders in patients with SDS.

Steatorrhea may resolve with advancing age, even though pancreatic enzyme secretion remains well below normal levels. In our experience, this usually occurs within the first 4 years of life.[4] Thus, periodic reassessment for evidence of maldigestion is recommended. Monitoring can be conducted by serial measurements of serum trypsinogen concentrations. Fat absorption should be assessed using a quantitative 72-hour fecal fat analysis after the individual has discontinued enzyme supplementation for at least 24 hours. At present, there is inadequate information concerning the specificity and sensitivity of the fecal elastase 2 test for assessing pancreatic function in patients with SDS.[44]

If nutrient maldigestion is identified, pancreatic enzyme replacement therapy is required. The need for fat-soluble vitamin supplements should be determined on an individual basis. Most reports of patient cohorts indicate that patients achieve normal nutritional status with therapy. In our experience, patients with SDS respond well to enzyme therapy. The same dosing range should be used as for patients with CF.[45] There is no recommended therapy for short stature. Growth velocity is usually normal, and, with rare exceptions, growth hormone levels are normal. Pubertal progress may be delayed.

Although there is no objective evidence to support the practice, experts in bone marrow failure syndromes recommend that bone marrow aspirates, biopsies, and cytogenetic studies be performed annually. They recommend more frequent evaluation if myelodysplastic or cytogenetic abnormalities are noted. These recommendations are based on the unproven assumption that anticipatory information concerning early bone marrow changes will yield a beneficial outcome to the patient.

If a patient is experiencing repeated infections in association with severe neutropenia (absolute neutrophil count < 500/mm^3), treatment with prophylactic antibiotics and/or granulocyte colony-stimulating factor (G-CSF) should be considered. There are concerns that long-term administration of G-CSF to patients with severe chronic neutropenia may increase leukemogenic risk. Although this potential risk of long-term G-CSF has not been clearly elucidated, some caregivers recommend limiting G-CSF therapy to short-term administration when a patient develops fever and/or an infection.

TABLE 65.2-4 ASSESSMENT AT DIAGNOSIS

Diagnosis*
Clinical confirmation of SDS by excluding other causes of pancreatic dysfunction and bone marrow failure
PLUS
Objective confirmation of pancreatic dysfunction
AND
Bone marrow failure
Baseline assessment
Imaging
Imaging of liver and pancreas (ultrasonography, CT, or MRI)
Skeletal survey
Bone age (older patients only)
Blood
CBC, differential, platelets; hemoglobin electrophoresis
Total and direct bilirubin, aminotransferases, alkaline phosphatase
Serum vitamins A, E, D (25-hydroxyvitamin D)
Prothrombin time, partial thromboplastin time
Serum trypsinogen and pancreatic isoamylase
Bone marrow
Aspirate
Biopsy
Cytogenetic analysis
Stool
72-Hour fecal fat balance study

CBC = complete blood count; CT = computed tomography; MRI = magnetic resonance imaging; SDS = Shwachman-Diamond syndrome.
*Genotyping may be considered for confirming the diagnosis.

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3. Other Hereditary and Acquired Disorders

Michael Wilschanski, MD

A number of rare hereditary and acquired disorders of the exocrine pancreas, apart from cystic fibrosis and Shwachman-Diamond syndrome, are discussed in this chapter.

HEREDITARY DISORDERS

Pearson Marrow-Pancreas Syndrome

This syndrome was originally described in 1979 in four unrelated children with severe macrocytic anemia, variable neutropenia and thrombocytopenia, vacuolization of bone marrow precursors, and ringed sideroblasts in the bone marrow. Pancreatic insufficiency was subsequently diagnosed associated with extensive fibrosis and acinar atrophy.[1] The bone marrow changes, including cell vacuolization and the presence of ringed sideroblasts, distinguished this condition from Shwachman-Diamond syndrome. Other differences include the presence of pancreatic fibrosis rather than lipomatosis and the absence of bone lesions. It has been shown recently that this disease results from defective oxidative phosphorylation and is associated with deletions of mitochondrial deoxyribonucleic acid (DNA).[2,3] Of the respiratory chain enzymes encoded by mitochondrial DNA, complex I is the most severely affected. Genes encompassing two subunits of complex V, one subunit of complex IV, and five transfer ribonucleic acid genes are also deleted. Oxidation of reduced nicotinamide adenine dinucleotide is abnormal in lymphocytes from these patients, but respiratory chain enzyme activities are normal in muscle mitochondria. In severely affected tissues such as bone marrow, neutrophils, lymphocytes, and pancreas, the deletions are found in over 80% of cells, whereas deletions were observed in only 50% of muscle cells. Thus, expression of the phenotype in Pearson marrow-pancreas syndrome in a given tissue may require a minimum threshold number of mutated mitochondrial DNA molecules. However, in the largest series published to date, Rotig and colleagues reported that the size and location of the mitochondrial DNA rearrangements in 21 patients did not correlate with clinical severity.[4] Interestingly, some of these patients may develop visual and muscular symptoms, which are also found in Kearns-Sayre syndrome, a mitochondrial disease characterized by a large mitochondrial DNA deletion.[5]

Johanson-Blizzard Syndrome

Johanson and Blizzard first described this syndrome whose features include pancreatic exocrine deficiency, aplasia or hypoplasia of the alae nasi, congenital deafness, hypothyroidism, developmental delay, short stature, ectodermal scalp defects, absence of permanent teeth, urogenital malformations, and imperforate anus.[6,7] More recently, other associated features were noted, including hypopituitarism,[8] diabetes mellitus,[9] growth hormone deficiency,[10] and congenital heart disease.[11] Prenatal ultrasonographic diagnosis has also recently been described.[12] The genetic defect is unknown, but the pathophysiologic basis of the pancreatic abnormality has been elucidated. Jones and colleagues performed pancreatic stimulation tests on two patients and found that the ductular output of fluid and electrolytes was preserved with decreased secretion of trypsin, colipase, and total lipase and low serum immunoreactive trypsinogen level.[13] These findings are consistent with a primary failure of pancreatic acinar development similar to that observed in patients with Shwachman-Diamond syndrome.[13] The functional exocrine disturbances are in keeping with histopathologic findings, which include absence of pancreatic acini.[14] Unlike Shwachman-Diamond syndrome, bone marrow and skeletal abnormalities are absent in Johanson-Blizzard syndrome.

Jeune Syndrome

This is a rare autosomal recessive disorder characterized by skeletal abnormalities of the thorax and extremities and nephronophthisis and is usually associated with respiratory distress in infancy. Karjoo and colleagues reported two families with asphyxiating thoracic dystrophy with exocrine pancreatic deficiency.[15] There is also one case report of pancreatic fibrosis.[16] Prenatal diagnosis has been reported.[17]

Isolated Enzyme Deficiencies

The deficiencies of the pancreatic enzymes and mucosal enterokinase are very rare but have provided insight into the physiology of digestion and the sequelae resulting from their deficiency.

Lipase Deficiency

Pancreatic lipase is involved in the essential hydrolysis of long-chain dietary triglycerides to fatty acids in the intestinal lumen. The patients present with severe steatorrhea in infancy or early childhood, but despite the maldigestion of dietary fat, failure to thrive is not a feature. It has been suggested that alternative sources of lipolytic activity prevent failure to thrive.[18] Analysis of duodenal juice revealed absent or low lipase activity and, in some cases, low amy-

lase and trypsin as well. Sheldon described this disorder in two unrelated sibships,[19] suggesting an autosomal recessive mode of inheritance. The complementary DNA encoding pancreatic lipase has been cloned[20] and the gene mapped to chromosome 10q24-q26.[21] These children respond well to exogenous pancreatic enzyme supplementation.

Colipase Deficiency

Pancreatic colipase is a cofactor that is involved in overcoming the inhibitory effects of bile salts on pancreatic lipase. Deficiency of this enzyme has been reported in two brothers, aged 5 and 6 years, and is not associated with failure to thrive.[22]

Combined Lipase–Colipase Deficiency

Ghishan and colleagues reported a patient with < 2% of control values for lipase and colipase but normal concentrations of other pancreatic enzymes.[23] Ligumsky and colleagues reported congenital combined lipase and colipase deficiency in two brothers.[24]

Amylase Deficiency

An abnormally low pancreatic amylase concentration below the age of 1 year may be physiologic; thus, the existence of amylase deficiency at this age may be developmentally related. Lowe and May reported a 13-year-old boy with absent amylase, diminished trypsin, and normal lipase concentrations.[25] Brock and Sjolund and their colleagues have reported familial selective deficiency of pancreatic amylase.[26,27] Because low serum pancreatic isoamylase has been observed in patients with Shwachman-Diamond syndrome who have normal fat digestion, this diagnosis should be considered in patients with isolated isoamylase deficiency.[28]

Trypsinogen Deficiency

There have been a few cases reported of this syndrome, which presents as severe malabsorption beginning in the neonatal period. Proteolytic enzyme activity was absent in the duodenal fluid but normalized with the addition of exogenous trypsin.[29] Normally, intestinal enterokinase activates trypsinogen, which, in turn, activates the other proenzymes. Hence, trypsinogen, chymotrypsinogen, procarboxypeptidase, and proelastase are activated to trypsin, chymotrypsin, carboxypeptidase, and elastase, respectively. Trypsinogen deficiency results in the disruption of the activation casacade because there is a lack of substrate for enterokinase. The trypsinogen gene is found on chromosome 7 and has caused renewed interest because a mutation in this gene causes hereditary pancreatitis, which is discussed elsewhere.[30]

Enterokinase Deficiency

Enterokinase (enteropeptidase) is an intestinal mucosal enzyme responsible for initiating the activation of pancreatic proteolytic proenzymes. It catalyzes the conversion of trypsinogen to trypsin, which, in turn, activates other proenzymes, including chymotrypsinogen, procarboxypeptidases, and proelastase.[31] The first case of congenital enterokinase deficiency was reported in 1969,[32] and several cases have since been documented.[33] These children present in the neonatal period with diarrhea, failure to thrive, edema, and hypoproteinemia. The diagnosis was confirmed by absent trypsin activity in duodenal fluid, which then became active following exogenous addition of enterokinase. Further confirmation of an isolated enterokinase deficiency was the finding of minimal enterokinase activity in the duodenal mucosa in the presence of normal disaccharidase activities. Fat malabsorption was present despite normal amylase and lipase concentrations in unstimulated duodenal fluid because trypsin is required for the activation of colipase and phospholipase. The patients respond well to pancreatic enzyme supplementation, which generates normal duodenal proteolytic activity. This disorder has been reported in siblings, suggesting an autosomal recessive mode of inheritance. Kitamoto and colleagues have cloned the complete complementary DNA that encodes the enterokinase amino acid sequence and mapped the gene to chromosome 21q21.[34]

ACQUIRED DISORDERS

Malnutrition

Protein-calorie malnutrition has a significant effect on pancreatic exocrine function.[35] Studies on children with kwashiorkor have shown a generalized reduction in pancreatic size and histologic evidence of acinar atrophy. Disorganization and loss of acinar pattern were noted with little evidence of inflammation or necrosis. Acinar cells were vacuolated, and the number of zymogen granules was diminished. In more severe cases, varying degrees of fibrosis and fat accumulation were observed.[36] Functional changes in the pancreas closely resemble the structural abnormalities. Barbezat and Hansen performed pancreatic stimulation tests in children with protein-calorie malnutrition.[37] There was a reduction in enzyme output in response to hormonal stimulation, but the volume output and alkalinization of the duodenal fluid were not affected. This is consistent with the histologic observation of ductal preservation, which is the source of water and bicarbonate secretion. Prompt improvement of enzyme secretion was observed with nutritional rehabilitation, although two patients had consistently low enzyme secretion.[37]

Biochemically, acute malnutrition in children has been associated with elevated cationic trypsinogen levels, which are correlated with the severity of the malnutrition.[38] However, persistently low immunoreactive trypsin levels are found in chronically malnourished children.[39] These studies suggest that in acute malnutrition, there is abnormal pancreatic cell membrane function with leakage of zymogen into the circulation, but more chronic malnutrition is associated with extensive pancreatic acinar cell atrophy and diffuse fibrosis, thus producing low trypsinogen levels. Juvenile tropical pancreatitis and its possible association with malnutrition are discussed in Chapter 64.2, "Juvenile Tropical Pancreatitis."

Surgical Resection

Exocrine pancreatic function after surgical resection has rarely been studied in children, but even with 95% pancre-

atic resection for nesidioblastosis, few develop malabsorption. Studies of pancreatic lipase and colipase secretion in children have demonstrated that malabsorption occurs only when values fall below 2% and 1% of mean normal values, respectively[40]; thus, it is not surprising that large pancreatic resections may not induce malabsorption. The necessity for pancreatic enzyme supplementation in these patients can be determined by fat balance studies.

Celiac Disease

Exocrine pancreatic dysfunction has been recognized in both children[41] and adults[42] with celiac disease. The degree of pancreatic impairment is variable, and its etiology is unclear. Some patients have primary pancreatic dysfunction, as evidenced by impaired release of pancreatic bicarbonate and enzymes into the duodenum in response to exogenous stimulation with intravenous cholecystokinin and secretin.[43] However, others have intact pancreatic function in response to exogenous stimulation but an impaired response to stimulation with liquid test meals.[44] This finding is consistent with impaired release of endogenous cholecystokinin and secretin, a concept supported by the demonstration of low serum secretin levels in response to duodenal perfusion of citric acid in untreated patients with celiac disease and normal levels after recovery of the intestinal lesion.[45] Impaired secretagogue release in untreated patients may explain the poor postprandial gallbladder emptying and diminished duodenal bile acid concentrations that, together with the impaired pancreatic enzyme release, contribute to the presence of fat maldigestion.

Carroccio and colleagues evaluated pancreatic function using fecal chymotrypsin levels at diagnosis of celiac disease.[46] They showed a lower weight increase in patients with initial low fecal chymotrypsin levels than in patients with normal chymotrypsin values. They suggested that there is a subset of patients who would benefit from pancreatic enzyme supplementation for a few months until pancreatic function returns to normal.[46] However, it is still unclear if indirect stool tests of pancreatic function can safely distinguish intestinal from pancreatic steatorrhea.

At the other extreme, profound, irreversible pancreatic insufficiency with acinar atrophy and fibrosis rarely occurs in celiac disease, but it has been reported.[47] The lesion may be related to chronic understimulation of the pancreas because of impaired endogenous secretagogue release and subsequent induction of pancreatic cell atrophy, which may be aggravated by malnutrition. In these cases, the coexistence of celiac disease and cystic fibrosis deserves consideration.[48] These patients may even have falsely pathologic sweat chloride concentrations owing to malnutrition, and personal experience suggests that mutation analysis and nasal potential difference measurements[49] may need to be performed.

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CHAPTER 66

STUDY DESIGN

1. Outcomes Research on Diagnostic and Therapeutic Procedures

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Outcomes research is the systematic study of clinical practice with a focus on clinical effectiveness and patient-centered outcomes.[1] It is designed to measure the consequences of providing medical care to determine whether and to what extent such care is beneficial. As broadly defined by the American Gastroenterological Association, outcomes research encompasses studies of (1) clinical and physiologic effects of a given medical product, procedure, or technology; (2) patient-centered end points, such as health-related quality of life (HRQOL), functional status, and patient satisfaction; and (3) medical costs relative to benefits and risks.[2] The purpose of this chapter is to provide an overview of outcomes research, highlighted by examples and pertinent studies in pediatric gastroenterology.

In recent years, outcomes research has received tremendous attention not only within the clinical research world but also from legislators and the general public. In the wake of considerable momentum over the past decade toward advancing the quality of health care, the Institute of Medicine (IOM) released its comprehensive *Crossing the Quality Chasm: A New Health System for the 21st Century* report in 2001.[3] This report highlights the importance of studying health care processes to improve medical outcomes. The IOM defines quality as "the degree to which health services for individuals and populations increase the likelihood of desired health outcomes and are consistent with current professional knowledge."[4] Outcomes research provides an important tool for amassing evidence that quality health care is being provided.

Donabedian, an early proponent of using research to advance the quality of medical care, has described a triad of (1) structure, or the characteristics of a health care setting; (2) process, or what is done to patients; and (3) outcomes, or how patients do after health care is provided.[5] His emphasis on understanding and enhancing the process of providing health care to improve outcomes can be somewhat simplistically conceptualized as "doing the right thing right."[1] Donabedian's emphasis was originally on the efficacy, effectiveness, and efficiency of health care.[6] In the past few years, this model has been expanded to incorporate safety, equity, and patient-centeredness as equally essential attributes of quality health care (Table 66.1-1).[3]

This chapter discusses these attributes of quality in the context of an introduction to outcomes research and its methodologies. Specific examples from the pediatric gastroenterology literature are used to highlight aspects of study design and their application to clinical practice. Pediatric gastroenterologists are introduced to the challenges involved in systematically evaluating their own practices with an eye to quality. Like all physicians, pediatric gastroenterologists must understand and use outcomes research to close existing gaps in the quality of care that they provide.

TABLE 66.1-1 DEFINITION OF TERMS USED IN THE EVALUATION OF MEDICAL CARE QUALITY

Efficacy	*Can* it work? (eg, in controlled trials)
Effectiveness	*Does* it work? (eg, in the real world)
Efficiency	Is it *worth* doing?
Safety	Does it *reduce risk* to patients?
Equity	Is it *nonvarying* in quality?
Patient centered	Is it *respectful* of individual patient preferences?

The first section of this chapter briefly reviews the concepts of efficacy and effectiveness, an understanding of which underlies the whole of outcomes research. In the following sections, studies of clinical outcomes, patient-centered outcomes, and health care costs are discussed. Our goal is to provide an introduction to methodology as it relates to outcomes research.

EFFICACY AND EFFECTIVENESS

Quality health care is best ensured by continual formal evaluation of the relationships between processes of providing medical care and the outcomes of those processes. Although many types of clinical research contribute to the evaluation of medical care, it is important to realize that not all clinical studies are outcomes studies. The point of distinction lies mainly in understanding the terms "efficacy" and "effectiveness."[7]

In general, efficacy studies are designed to determine if a given medical intervention can work under tightly controlled conditions. In contrast, effectiveness studies are designed to mirror daily practice and to determine if an intervention is successful in routine clinical practice. To the extent that they measure health outcomes in real-world settings, effectiveness studies are the crux of outcomes research.

Outcomes research focuses on the impact of a given medical practice on health in the real world. The data generated by outcomes research reflect changes in practice and in the natural history of the disease over time. In addition, outcomes study methodology recognizes that unpredictable and uncontrollable changes in patients' lives are important and legitimate factors in determining the ultimate effectiveness of medical care. Effectiveness studies are often less restrictive about inclusion and exclusion criteria than studies of efficacy.

Effectiveness studies, the mainstay of outcomes research, also integrate variations in clinical practice among various practitioners and in different health care settings. These characteristics of effectiveness studies are readily apparent when comparing, for example, the practices of university- or research-based health care groups with those of community-based physicians. Many outcomes studies recognize that there are physician-dependent variables in assessing the effectiveness of medical interventions.

CLINICAL OUTCOMES

In this section, we consider outcomes research that measures physiologic and clinical effects. We discuss some of the strengths and weaknesses of both experimental and observational study designs as they relate to the use of these designs in outcomes research. We also touch on the use of large administrative databases and meta-analysis as increasingly popular methodologies (Table 66.1-2).

Experimental Study Design

Experimental studies are most commonly designed as clinical trials. Clinical trials aim to isolate one factor, generally an intervention, and examine its contribution to patient health by holding all other factors as constant as possible. The gold standard for experimental clinical study design is the randomized controlled trial (RCT). An RCT is a comparative study between an intervention group and a control group. The groups are as similar as possible, except the intervention group is exposed to a health care service (eg, medication, therapy) and the control group is not. Any differences in outcomes between the two groups can then be attributed to the intervention.

Participants in RCTs are randomly assigned to either the intervention or the control group. When performed correctly, the process of randomization removes the potential for bias in the allocation of participants to either group. Randomization also optimizes the chance that the two groups will be evenly matched, on average, in their known baseline characteristics (eg, age, sex, disease status), as well as any unknown factors.

Despite their many strengths, RCTs may have a limited role in clinical outcomes research. For example, if an outcome of interest is rare, an RCT may require an enormous study population to detect any differences between intervention and control groups. If the outcome takes many years to emerge, an RCT may not be feasible. It also may not be possible to randomize certain variables, such as patient preference or physician beliefs. Other limitations of applying findings from RCTs in clinical outcomes research include the fact that RCTs are usually designed to answer specific clinical questions in well-defined patient populations and may use strict exclusion criteria to create a homogeneous study sample. These characteristics of RCTs can limit their generalizability to real-world clinical practice.

However, RCTs can be designed to provide information that is relevant to clinical practice and therefore can play a role in outcomes research. One example of a well-designed prospective, double-blind, placebo-controlled RCT in the pediatric endoscopy literature evaluated the use of oral midazolam as a premedication to conscious sedation for pediatric endoscopy.[8] Although almost all pediatric gastrointestinal procedures are performed with some type of sedation, there is a lack of consensus about the use of premedications to decrease anxiety, which many children experience before the procedure.[9] In this study, Liacouras and colleagues were specifically interested in whether the use of oral midazolam prior to intravenous (IV) line placement led to less apprehension in children before, during, and after an endoscopic procedure.[8] Investigators obtained informed consent for 123 children to be randomized to receive either oral midazolam or placebo approximately 20 minutes before IV placement for endoscopy.

TABLE 66.1-2 TYPES OF OUTCOMES RESEARCH AND RELEVANT STUDY DESIGNS

Experimental studies
Randomized controlled trials
Observational studies
Cohort
Case control
Cross-sectional
Case series
Large administrative database analysis
Meta-analysis

This RCT is a good example of an effectiveness trial that carefully investigated a study question while allowing real-world health care systems to function routinely. Once the oral medication (midazolam or placebo) was given, IV procedural sedation for endoscopy was administered in a routine manner. Importantly, the doses of procedural sedation were not dictated by the study design, and endoscopists were encouraged to use routine means to evaluate patients' levels of sedation. The results of this RCT strongly supported not only the safety but also the effectiveness of oral midazolam premedication for pediatric endoscopy. Such studies have the potential to change clinical practice and therefore can be considered outcomes research.

Observational Study Design

Observational studies often represent the best method for studying rare or remote conditions. In general, observational studies are easier and less expensive to perform in comparison with experimental studies. Observational studies may especially be useful when randomization schemes cannot be feasibly employed. They are also essential tools in outcomes research because they generally measure effectiveness rather than efficacy.

Nevertheless, there are many important disadvantages to observational studies that must be weighed in both critically reviewing the literature or when choosing a study design. In particular, because patients are not randomized, there is an unavoidable risk of both bias and confounding. Bias may be defined as any factor in a study that tends to produce results or conclusions that differ systematically from the truth. This includes errors in analytic methodology and errors of interpretation.[10] A confounder is a third factor in a study that may be associated with both the exposure and the outcome and may, in fact, be responsible for any observed associations between the two.[11]

In the following section, we describe four principal research designs of observational studies: cohort, case control, cross-sectional, and case series.

Cohort Studies. Cohort studies are observational studies that focus on factors related to the development of a disease of interest. They are especially useful for studying the incidence and natural history of disease. In a cohort study, a group of people (the cohort) who do not have the condition of interest at the time of enrolment is selected and observed over time. Suspected risk factors for the outcome are evaluated in all members of the cohort at enrolment and throughout the observational period.

By following all of the members of the cohort for development of the outcome, the relationship between risk factors and outcomes can be assessed. Cohort studies can also measure absolute risks of developing a new disease because rates of exposures and outcomes have been collected. Generally speaking, cohort studies are prospective in nature, meaning that a cohort is followed forward from a set point in time. However, cohort studies can also be retrospective and assemble subjects according to their history of exposure without consideration of outcomes, even though the outcomes may already have taken place.

Cohort studies have many significant advantages over other study designs for outcomes research. First, when a clinical trial cannot be conducted for either ethical or practical reasons, a cohort study may provide the best alternative method to study the question of interest. A cohort study may be useful for studying more than one risk factor and more than one outcome using the same study population. Cohort studies may simultaneously give descriptive information about several diseases or even help to tease out which risk factors are directly linked to which disease. Cohort studies are particularly relevant to outcomes research because they do not occur in tightly controlled research settings.

Cohort studies have some important disadvantages. Members of the cohort may need to be followed for a lengthy period of time before a sufficient number develop the outcome of interest. Furthermore, study resources may be spent following many people in the cohort who will not develop the outcomes of interest or may be lost to follow-up. Another limitation to cohort studies is that they may represent an impractical study design for investigating risk factors for rare diseases. This issue may especially be salient for pediatric gastroenterologists. For example, to study a disease with a known incidence of 1 in 10,000 children, 100,000 children will need to be followed to capture 10 cases of the disease. On the other hand, this issue can be minimized if a cohort is selected that is known to be at high risk of developing the disease.

Recently, several pediatric gastroenterology–oriented cohort studies have been designed that will ultimately improve our understanding of obesity in children.[12,13] For example, the ongoing Growing Up Today Study (GUTS) was established in 1996 to longitudinally follow the activity, dietary intake, and weight changes of children across all 50 United States.[12] GUTS participants were recruited from the offspring of participants from another cohort study, the Nurses Health Study II.

Another example of a recent cohort study in the pediatric endoscopy literature explored the relationship between clinical presentation and primary peptic ulcer disease in children.[14] Primary peptic ulcers are traditionally considered to be unusual in childhood.[15] It is well accepted that the rarity of this diagnosis has limited the ability of investigators to identify risk factors for childhood ulcer disease and to know when to perform endoscopy for diagnosis.[16] To investigate these questions, Roma and colleagues undertook a cohort study from 1990 to 1999, during which time they followed 2,550 children who had dyspeptic symptoms, including epigastric pain, periumbilical pain, bleeding, vomiting, and nocturnal waking.[14] All children then underwent upper gastrointestinal endoscopy with biopsies. Primary peptic ulcers were diagnosed in 2% (52 of 2,550) of the cohort and appropriately treated. Of the 52 patients with peptic ulcer on initial endoscopy, 25 (48%) became symptomatic after treatment and were re-endoscoped. Only 3 (0.12%) of these children were found to have a second ulcer.

In their primary analysis, the investigators found no significant differences between the clinical symptoms of those children with ulcers and those without. Therefore,

Roma and colleagues suggest that all children with dyspeptic symptoms be referred for upper gastrointestinal endoscopy to evaluate for ulcer disease.

This study highlights the types of information available from a cohort design. The results provide an estimate of overall disease prevalence in the population of interest. The clinical follow-up of patients with ulcers at the start of the study allows for an assessment of the effectiveness of treatment. Roma and colleagues' study represents an example of the importance of cohort studies to understanding pediatric gastrointestinal disease and disease management.

Case-Control Studies. A second observational study design method is that of case-control studies. Whereas cohort studies examine people who are initially free of the disease of interest, case-control studies compare people who already have the disease (the cases) with otherwise similar people who do not have the disease (the controls). Case-control studies start by evaluating the outcome (the presence or absence of the disease) and then look back into patients' histories to identify possible risk factors. Both cases and controls must be selected independently of exposure to the risk factors of interest. In this retrospective manner, case-control studies can be used to analyze whether identified risk factors were present more frequently in cases than in controls.

The advantages of performing case-controlled studies include the fact that they can be performed relatively quickly and inexpensively, even for diseases that are rare or that take lengthy periods of time to appear. To epidemiologists, case-control studies represent the basis of outbreak investigations, such as those conducted to determine the etiology of foodborne outbreaks of gastroenteritis. In addition, if the outcome is rare overall, case-control studies may require fewer subjects than cohort studies. They also simultaneously allow multiple risk factors to be investigated within the same study of a particular outcome. Because of these attractive features, case-control studies are often used as hypothesis-generating mechanisms for investigators exploring possible risk factors for rare diseases.

However, case-control studies do have a number of disadvantages, including that they are particularly subject to bias (eg, recall bias). Case-control studies must rely on studying cases that have already been identified. Misdiagnosed or asymptomatic cases or people who have already died either from the disease or other causes are missed by this type of study.

Case-control studies also depend on the identification of an appropriate control group. Controls should be selected from a population of individuals who would have been identified and included as cases had they also developed the disease. Selecting a control group that is comparable to a group of cases can be a surprisingly difficult task for investigators.

Recently, Lazzaroni and colleagues performed a carefully designed case-control study of primary upper gastrointestinal bleeding in infants.[17] Although significant upper gastrointestinal bleeding in otherwise healthy full-term infants has been described in case reports, the available data on the topic are limited owing to the rare occurrence of this disease.[18,19] There were three main aims to this study.[17] The first was to identify types of mucosal lesions in newborn babies with upper gastrointestinal bleeding. The second aim was to examine the safety and necessity of performing upper endoscopy in newborns with gastrointestinal bleeding, and the third was to identify risk factors associated with such bleeding.

Sixty-four of 5,180 infants (1.23%) born at the study center in Italy developed significant upper gastrointestinal bleeding within a mean of 26.5 hours of life. In 53 of 64 cases, an endoscopy was performed. Extensive demographic, prenatal, neonatal, clinical, and hematologic data were collected and analyzed for all cases and their mothers. The same data were collected for a group of 53 controls and their mothers, who were matched for age and sex to the 53 cases who underwent endoscopy. The controls were selected randomly from a population of full-term infants born in the same hospital.

All patients who underwent endoscopy were found to have mucosal lesions of either the esophagus, stomach, or duodenum. A comparison of the 53 patients who underwent endoscopy with the 53 controls revealed no significant differences in demographic or clinical characteristics, except that upper gastrointestinal bleeding was significantly more frequent in those infants whose mothers came from countries outside Europe. Therefore, the investigators concluded that the risk factors for upper gastrointestinal hemorrhages in infants remain obscure.

However, it is important to realize that this study was limited, albeit for ethical reasons, by the lack of endoscopy surveillance of the control group. A second limitation of this study lies in the selection of its control group. The study findings might have been strengthened or even different if a control group had been chosen that was matched by age and sex to all 64 cases of acute gastrointestinal bleeding—not just to those 53 patients who underwent endoscopy. Although case-control studies are important tools for outcomes research, the application of their conclusions for clinical practice may still be affected by limitations in study design.

Cross-Sectional Studies. Cross-sectional studies are based on a single examination of an entire population at a particular point in time. By surveying a whole population, cross-sectional studies assess the proportion of people with a certain disease (eg, the prevalence of the disease) and can be used to examine the relationship between the disease and other characteristics of the population under study. Cross-sectional studies are common in the medical literature and can produce valuable data about a wide range of diseases and types of risk factors. They are important for generating hypotheses for experimental designs.

However, as a descriptive research methodology, cross-sectional studies have several important limitations. In particular, because cross-sectional studies identify existing cases (prevalent cases) of a disease and do not capture the occurrence of new cases (incident cases), they are likely to overrepresent chronic diseases and underrepresent acute diseases. Also, people with certain diseases may either

leave the community or be located in a place where they are not surveyed.

Cross-sectional studies may also be limited by so-called "lead time bias," whereby patients are misclassified by exposures. For example, children with symptoms of peptic disease may choose to stop drinking caffeinated beverages because of their symptoms before inclusion in the study. They would therefore be counted as nonexposed. Finally, the findings of cross-sectional studies must be interpreted cautiously; the mere fact that two variables are associated does not mean that they are causally related.

An example of a recent cross-sectional study in the pediatric endoscopic literature is an investigation into the prevalence of *Helicobacter pylori* in a population of children with chronic abdominal pain, with and without the gross endoscopic finding of nodular gastritis.[20] Endoscopic findings of nodularity are reported frequently in children[21] and have been loosely associated in the past with either acute or chronic *H. pylori* infection.[22] Nevertheless, the relationship of endoscopic nodular gastritis with *H. pylori* infection in children remains ill-defined. The investigators of this study used a cross-sectional design to examine the associations between endoscopic findings and microscopic *H. pylori* infection among children with chronic abdominal pain.[20]

Bahu and colleagues prospectively included 185 children aged 1 to 12 years who presented to two pediatric gastroenterology clinics in Brazil from 1997 to 1999 with chronic abdominal pain. To improve their diagnostic yield, the investigators chose a study population at high risk for *H. pylori*.[20]

The prevalence of endoscopic nodularity in this population was 13% (95% CI 8.5–18.7), and *H. pylori* infection was identified in 27% (95% CI 20.8–34.0) of the study population. Endoscopic nodularity was found in 44% of patients with *H. pylori* infection and in 1.5% who were *H. pylori* negative. In their discussion, the authors state that their results support a significant association between endoscopic nodular gastritis and *H. pylori* infection.[20]

However, as with all cross-sectional studies, an observed association does not prove causality. In fact, because the putative exposure (*H. pylori*) and outcome (nodularity) were assessed simultaneously, it is equally possible that the nodularity in some way predisposed the patient to infection with *H. pylori*. Additionally, it may be difficult to identify confounders in cross-sectional studies. For example, in this case, both *H. pylori* and nodularity were rare in the youngest children; therefore, age may be a confounder, and there may be no true association between *H. pylori* and nodularity.

Another issue with this particular study is that it was conducted in a high-risk study population. Therefore, the generalizability of its prevalence findings to other populations for both endoscopic nodular gastritis and *H. pylori* may be limited. Finally, there was no follow-up of the patient population, as is characteristic of a cross-sectional design. Therefore, it remains a possibility that *H. pylori*–positive children with normal mucosa will go on to develop nodularity.

Case Series. Case series are generally considered the weakest study designs in the so-called "hierarchy of evidence."[23] Nonetheless, they may represent an excellent means of generating hypotheses for more robust studies to examine. A case series simply describes the presentation, and often the clinical management, of a disease in more than one patient. Patients in case series studies are generally not followed prospectively, and they are not compared with a control group. Therefore, case series are not useful for establishing a causal relationship between risk factors and disease or the clinical effectiveness of a management approach.

Case series may also be prone to selective reporting (reporting bias). Owing to small numbers and the anecdotal nature of such a report, the validity of case series findings may be difficult to establish. Similarly, a case report, as a form of case series in which only one patient is described, may suggest an association or a clinical course that is not necessarily generalizable beyond the individual case.

Nevertheless, case series and case reports are both numerous and important to medical literature. They are both used regularly to present information about patients with rare diseases and may be important for stimulating new hypotheses. In addition, a case series that describes an abnormal outcome after routine care has been administered may represent, in and of itself, a reasonable basis for re-examining that care. For example, case reports of sudden death in otherwise healthy infants receiving cisapride as a promotility agent represented sufficient basis for more outcomes studies on the use of cisapride in children with gastroesophageal reflux.[24,25]

In one recent case report, Stiffler described an 11-year-old girl with a 5-year history of persistent obscure gastrointestinal bleeding who underwent capsule endoscopy.[26] The patient described in the case report had undergone several upper gastrointestinal endoscopies, a colonoscopy, push enteroscopy, and enteroclysis prior to undergoing the capsule endoscopy—with all examinations being negative for pathology. The capsule procedure identified a small bowel narrowing with significant erosive and ulcerative changes consistent with Crohn disease.

In the discussion following the case report, Stiffler speculates that capsule endoscopy may prove to be a useful diagnostic modality in pediatric patients with gastrointestinal disease. Stiffler also explains that both pediatric gastroenterologists and their patients may prefer undergoing capsule endoscopy, which does not require sedation.

Indeed, the tactical issue of how to achieve sedation that is both safe and effective for endoscopy and colonoscopy as high-volume pediatric procedures remains a challenge for pediatric gastroenterologists. Although several studies have indicated that general anesthesia allows a safer environment for the performance of more successful traditional endoscopic procedures in children compared with conscious sedation,[27,28] others have postulated that conscious sedation can be just as safe.[29] To date, there have been no outcomes studies that have contributed to resolving this debate.[9] Instead, because most studies in the literature are based on case series at single institutions, it is difficult to compare sedation practices across investigative sites.

The decision to use one regimen over the other is generally left to the clinical judgment of individual physi-

cians, to be made on a case-by-case basis.[9] The lack of an evidence-based approach to the question of sedation for pediatric endoscopy leaves open the real possibility that individual pediatric endoscopists may make inappropriate sedation choices for their patients.

The study of patient safety is an important subset of outcomes research. Patient safety, or the concept that patients should not experience harm from health care that is intended to help them, represents one of the major steps identified by the IOM for improving the quality of health care overall.[3] The question of sedation choice and patient safety for children undergoing endoscopic procedures is a salient example of a quality gap in health care services that will best be addressed by well-designed outcomes studies in the future.

Large Database Review Studies

Outcomes research may employ large administrative databases maintained by health care providers, payers, or government agencies.[30,31] There are many advantages to such databases, which are often (but not always) considered both valid and reliable. First, they are usually electronically maintained and are therefore often computer ready for analysis and inexpensive to acquire. Many databases come packaged together with computer programs for data analysis. Such databases are also often rich in demographic characteristics, as well as diagnoses of patients. Administrative databases often reflect the health care of large populations, and findings may be generalized.

However, there are also drawbacks to using large administrative databases. For instance, they can be limited in the quality and type of clinical information available. Also, because most were developed for billing or administrative purposes, they are often lacking pertinent medical information. Moreover, large institutional databases may include incorrect or incomplete datasets, especially in regard to medical and patient care information. Inconsistencies in diagnostic coding may also limit the usefulness of these databases. As an example, variation in diagnostic codes for abdominal pain in children may make it difficult to characterize the care of or evaluate the outcomes of children with this clinical problem.

Nevertheless, nationally maintained databases are becoming important tools for outcomes researchers in pediatric gastroenterology. For example, the Kids' Inpatient Database (KID) is one of several databases developed and maintained as part of the Healthcare Cost and Utilization Project, which is a federal, state, and industry partnership sponsored by the National Institutes of Health's Agency for Healthcare Research and Quality.[32] The KID pertains to children's health issues and is the only hospital administrative dataset designed specifically to assess use of hospital services by newborns, children, and adolescents.[33] It represents the only large inpatient care database for children in the United States and contains data from approximately 1.9 million hospital discharges for children across 27 states.

Importantly, the KID includes data on all patients, regardless of payer, including children covered by private insurance or Medicaid and the uninsured. This feature of the KID highlights its ability to assess the equity of care provided to children because it includes patients who may vary greatly in personal characteristics, such as gender, ethnicity, geographic location, and socioeconomic status. According to the IOM, ensuring equitable care, or quality care that does not vary along these personal characteristic lines, is a main goal for redesigning the twenty-first century health care system.[3]

Guthery and colleagues were able to perform a population-based outcomes study using the KID to identify the principal gastrointestinal diagnoses associated with hospital use and to describe hospital use patterns associated with pediatric gastrointestinal disorders.[34] In a descriptive analysis using the KID and its associated software program, *Clinical Classification Software*,[33] Guthery and colleagues found that gastrointestinal disorders in children are a significant source of hospital resource consumption, accounting for $2.6 billion in hospital charges annually in the United States and over 1.1 million hospital days. The investigators also found that among children with principal gastrointestinal discharge diagnoses, 67.7% of discharges were from non–children's hospitals, 13.1% were from pediatric facilities, and 19.2% were from pediatric units of a general hospital. In addition, 56.7% of pediatric gastrointestinal disease discharges were from nonteaching hospitals, whereas 43.3% were from academic centers.

Guthery and colleagues concluded that care for children with gastrointestinal diseases is provided at a variety of different types of institutions, which may represent a significant source of variation in care. Further outcomes research may help to explore how this variation in care portends gaps in quality of care for children with gastrointestinal disease across the United States.

Meta-analysis

Meta-analysis has become an especially popular tool for outcomes research and, as a methodology, has been featured prominently in journals dedicated to the field of gastroenterology.[35–38] Meta-analysis employs specific statistical methodologies to retrospectively review and integrate available quantitative data across multiple studies. It is an especially useful method for assimilating data from multiple small studies that have found conflicting answers or statistically insignificant results for the same research question. In meta-analysis, the data of multiple independent studies on the same topic are compiled and analyzed across reports to increase overall statistical power. In essence, a meta-analysis allows the synthesis of the results of numerous tests so that an overall conclusion can be drawn.

There are some fundamental assumptions related to meta-analysis. First, it is important that all studies combined in the analysis have the same research question. Second, the separate studies included in the analysis should be independent of each other. Third, the studies should be of sufficient quality. If any of these assumptions can be challenged, then the use of meta-analysis is not appropriate.

However, there are a few important caveats to interpreting meta-analyses. In particular, there is a tendency in the medical literature to publish positive findings, which, in turn, introduces a bias into the data selection process for meta-analysis. An appropriately conducted meta-analysis

will also incorporate data published only in abstract form and unpublished data collected through communication with investigators.

Additionally, to test for the possibility of publication bias, a meta-analytic study design often will include a sensitivity analysis. For instance, investigators may choose to create a type of scatter plot (a so-called "funnel plot") that allows the sample size of each study to be plotted according to its estimated effect size. In a typical funnel plot, the vertical and horizontal axes represent sample size and estimated effect size, respectively. A vertical line can then be drawn on the graph defining the pooled estimate effect size, and the points from each study included in the meta-analysis will scatter around this line. If there is no publication bias, the results from small studies plotted at the bottom of the graph should have considerable variation, whereas large studies, at the top of the graph, should show less variation in their results. Thus, the graph should look like an inverted funnel. If there is publication bias against small, nonsignificant studies or if the investigator has not included all possible studies, the graph will either not assume a funnel shape (eg, be skewed or asymmetric) or will contain gaps (Figure 66.1-1).

Another possible bias to meta-analysis is that many published studies do not contain sufficient detail that allows investigators to compare the results from one study with those of another. In fact, the main drawback of meta-analysis lies in the inequality of the study designs and end points that it measures. Strict criteria for performing meta-analysis, as well as the application and utility of this outcomes study design, are the subject of many reviews.[39–41]

In one example of meta-analysis in the pediatric gastroenterology literature, Huang and colleagues recently sought to determine whether probiotic therapy improves outcomes in children with acute infectious diarrhea.[42] Although probiotics are considered a promising adjunctive therapy for children with acute diarrhea, studies on the subject have suffered from limited study power and conflicting results.[43]

In compiling data for meta-analysis, Huang and colleagues strictly focused on clinical trials of probiotic therapy in otherwise healthy children less than 5 years old with acute-onset diarrhea in the outpatient setting.[42] The selection criteria for the meta-analysis set a priori were that each trial included must have randomized its patients into intervention and control groups. Also, each trial must have performed a direct comparison between groups and reported duration of diarrhea as an outcome variable. Of a total of 29 studies on probiotic use for diarrhea that were identified and categorized by treatment setting, population characteristics, and patient comorbidities, the investigators identified 18 that met inclusion criteria for their meta-analysis. Nine were excluded either because they lacked a control group or did not report the duration of the diarrhea. An additional two studies were excluded because they used data also published in two of the included trials.

There were two other meta-analyses published in the same year as Huang and colleagues' study that also questioned the usefulness of probiotics in the setting of pediatric diarrhea. Szajewska and Mrukowicz focused on trials in children who were hospitalized and did not include abstracts or unpublished data.[44] Van Niel and colleagues used meta-analysis to look at only randomized double-blind placebo-controlled trials of probiotics in children.[45] All three meta-analyses of probiotics in children defined their outcome as the duration of the diarrhea.

As part of classic meta-analytic methodology, Huang and colleagues provided a summary table of the studies included in their analysis and concluded that probiotic therapy shortened the duration of acute diarrheal illness in children by approximately 1 day.[42] This finding was similar to both Szajewska and Mrukowicz's and Van Niel and colleagues' analyses, which detected shortened duration by 0.6 and 1.2 days, respectively.[44,45] These are three examples of well-designed, rigorously conducted outcomes studies. In their individual discussions, all three investigators appropriately questioned the real-world clinical meaning of their findings. Although probiotics may be efficacious at reducing the duration of diarrheal illness, the clinical effectiveness of this strategy that reduces symptoms by a day or less may not be convincing enough to change clinical mangement.

PATIENT-CENTERED STUDIES

In recent years, the concept that patients' perceptions of their health and satisfaction with their health are important measurable variables has emerged as a fundamental maxim. Furthermore, the more careful allotment of health care dol-

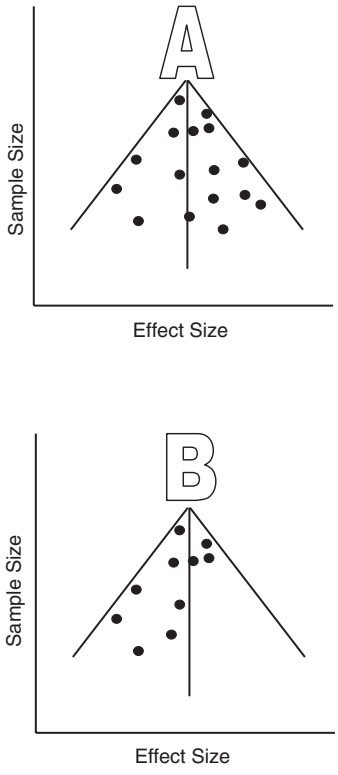

FIGURE 66.1-1 Schematic of two funnel plots for meta-analysis. Plot A shows no association between study size and effect size. Plot B depicts an asymmetric relationship, suggesting a publication bias.

lars has led to increasing demands on physicians to make informed health care decisions that truly benefit patients. In response, outcomes research has begun to focus primarily on the "patient experience"[46] of health care by extending the scope of studies beyond the end points of morbidity and mortality to include quantitative and qualitative measures of patients' perceptions of health and well-being.

Quantitative patient-centered measures may include validated instruments that are often importable across institutions and settings. Qualitative measures often rely on content analysis of open-ended patient-survey questions. The use of either type of measure in patient-centered studies may allow the evaluation of the effects of medical intervention on patients' functional status, their satisfaction, and their HRQOL.

Functional Status

Functional status is defined as a measure of the impact of health or disease condition on the ability of patients to function in various roles in society.[47] As with all patient-centered measures, it is important to recognize that the functional status of children is inherently different from that of adults. Although both may take into account the impact of health on a patient's ability to perform age-appropriate activities of daily living, as well as their moods and abilities to communicate, adult functional status may measure professional productivity, whereas pediatric functional status may be more concerned with play or school attendance.

At any age, functional status, as a measure, can be used to describe a patient at a single point in time or cumulatively over time. The latter use of functional status as a cumulative measure may allow for prediction studies of patients' likely future health gains from given treatments. A number of different pediatric instruments have been validated as measures of functional status in children with many chronic illnesses (eg, Functional Disability Inventory[48] and Functional Status II-Revised[49]). However, these instruments may not be generalizable to all patient populations.

Patient Satisfaction

Patient satisfaction can be measured in terms of patients' satisfaction with health care or with their state of health or disease condition. Although the assessment of patient satisfaction has been emphasized strongly in outcomes research, it may be a difficult measure to interpret. Indeed, the IOM does not consider patient satisfaction a useful type of outcome to measure when evaluating quality of care.[3] According to the IOM, too often "it is difficult to determine what is an acceptable level of satisfaction…[because there] is generally no standard to which to compare the results."[3] Reports of actual experiences with care may provide more clinically relevant information than satisfaction ratings. For example, it may be more appropriate to ask patients whether they were informed about the specific side effects of medications on discharge than to ask whether they were satisfied with nursing care.

When evaluating patient satisfaction in children, there is generally the added complexity of using parents' reports in lieu of their children's.[50] Nevertheless, parental satisfaction continues to be considered a relevant outcome of pediatric care. As such, several instruments have recently been developed for evaluating parents' satisfaction regarding their child's care.[51]

Health-Related Quality of Life

HRQOL is a comprehensive term that refers to the general physical and mental health status of individuals, including their psychosocial well-being, as it is affected by illness or injury and health care interventions or policies.[52] By most definitions, HRQOL is different from functional status in that it measures psychological well-being, whereas functional status is more concerned with physical ability. However, many investigators may choose to measure functional status as a component of HRQOL.[53] In either case, the term HRQOL invokes "the capacity of an individual to perform social and domestic roles so as to meet the challenges of everyday living without emotional distress of physical disability."[54]

Although, currently, there is no gold standard for measuring HRQOL, a general consensus has emerged as to what should be measured.[50] To this end, a general framework is portrayed of a classic core set of disease-related domains that should be measured in any global assessment of HRQOL (Figure 66.1-2). For the purposes of this chapter, we assume that there are at least five domains that comprise the framework for HRQOL: (1) the state of disease, (2) associated physical symptoms, (3) functional status, (4) psychological functioning, and (5) social functioning.

Each domain of HRQOL is independently measurable and can be affected by both the disease and medical intervention. However, the complex interactions between domains must also be appreciated. Also, these domains are not mutually exclusive, and individual patients may react to their diagnosis, symptoms, and treatment in frequently unpredictable ways.

There are many obstacles to measuring pediatric HRQOL, and this area of study has been slower to develop in children, in contrast to the rapid expansion of HRQOL

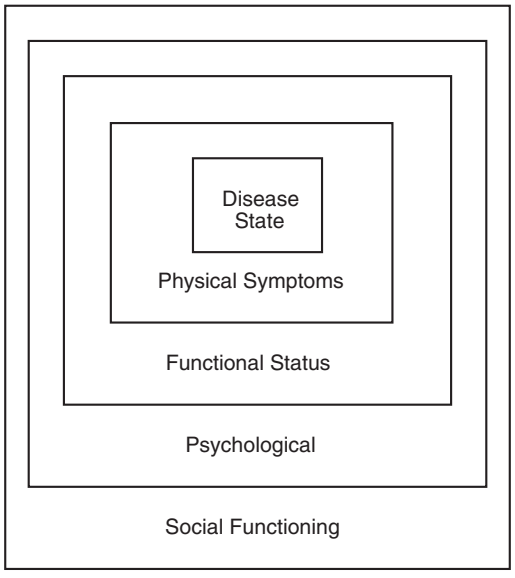

FIGURE 66.1-2 Framework for the essential domains of health-related quality of life.

research in adults.[55] Owing to inherent differences between pediatric and adult patients, most HRQOL instruments used in adult medicine are not readily applicable for use in children.[56] For example, many components of adult functional and social well-being that are measured in adult HRQOL instruments, such as economic status, job performance, and sexual satisfaction, are clearly focused on adult behaviors. Therefore, pediatric instruments, such as the Child Health Questionnaire and the Child Health and Illness Profile, have been developed to capture age-specific and -sensitive issues.[56]

Furthermore, compared with adult questionnaires, greater time and creativity are required to develop nonwritten, nonstandard means to assess HRQOL in infants and young children. Another concern that must be addressed is the incorporation and balancing of parental perceptions, which may not necessarily reflect those of their children and cannot substitute for the perspective of the child.

To date, a limited number of both general and disease-specific instruments to assess HRQOL have been developed for children.[57] Although general instruments allow physicians to assess and contrast various domains across a multiple number of disease states, they often lack the specificity required to understand the impact of a particular disease state. In contrast, disease-specific instruments are highly sensitive for a particular disease but, by definition, cannot be used to compare different disease states. Depending on research aims and feasibility, HRQOL outcomes studies in children that include a battery of both general and disease-specific instruments that measure all or some of the domains of HRQOL may be best.[58]

Patient-Centered Studies

In pediatric gastroenterology, there have been few patient-centered studies regarding interventional practices and procedures. Fortunately, there has been increasing interest in performing such research, and in recent years, there has been a substantial increase in the number of publications in this area.[59–61] In return, we are gaining greater understanding of the broader scope of the experiences that pediatric patients undergo related to their care. Pediatric liver transplant represents a particularly important example of an area of interest in which children with chronic gastrointestinal diseases are increasingly the focus of patient-centered research.

Liver transplant is now widely accepted as the definitive therapeutic procedure for end-stage liver disease in children.[62] Improvements in immunosuppression, surgical techniques, and postoperative management have all contributed to greater than 90% 5-year survival rates at most transplant centers, and morbidity and mortality rates are no longer sufficient indications of success.[63] Instead, increasing efforts are being made to evaluate the broader impact of this procedure on children by focusing on patient-centered measures, including physical and psychosocial functioning, growth and development, and a general perceived sense of well-being.[63,64]

On the other hand, evaluating patient-centered outcomes in children following liver transplant is a complex endeavor. For example, the measurement of HRQOL must factor in both pre- and post-transplant status for both the patient and the family. In many instances, parents are providing living-related grafts for transplant, and their own HRQOL as donors must also be considered. Additionally, the impact of multiple underlying diagnoses and reasons for transplant must be gauged. Post-transplant, certain diseases may recur, increasing the risk of graft failure and poor HRQOL. In other cases, extrahepatic manifestations of disease may persist despite successful liver transplant.

In one study of patient-centered factors after liver transplant, Debolt and colleagues followed 65 children for up to 5 years post-transplant and measured social, behavioral, and emotional adaptation; physical function; and family stress.[65] In this highly comprehensive study, a battery of general psychometric instruments, such as the Mental Scale of the Bailey Scales of Infant Development, the Vineland Social Maturity Scales, and the Wechsler Intelligence Scale for Children-Revised, were administered to children prior to transplant and again at 1 year of follow-up. The children's scores were compared with published data from chronically ill and medically well children. This study supported previous short-term findings showing that transplant recipients had equivalent psychological and functional status compared with both comparison groups.[66]

Debolt and colleagues' findings also indicated that post-transplant, HRQOL in children is highly dependent on immunosuppressive regimens.[65] These findings supported an earlier study by Starzl and colleagues that found that across both pediatric and adult transplant survivors, 1-year post-transplant HRQOL was significantly affected by the degree of steroid dependence.[67] Other outcomes studies more interested in physiologic factors, which may be intermediate steps to HRQOL, have demonstrated the inverse relationship between steroid doses and growth in children following liver transplant.[68] In conjunction with these more clinically oriented studies, Debolt and colleagues' patient-centered results provide tremendous incentive for pediatric hepatologists to aggressively wean post-transplant immunosuppressants.

In the last few years, greater efforts have been made to design a disease-specific HRQOL instrument to assess outcomes of children with liver transplants. In one such undertaking, Andelman and colleagues reported their design and implementation of a pediatric liver transplant–specific HRQOL instrument in children and compared its utility with that of general instruments, such as those employed by Debolt and colleagues,[65] in understanding the impact of clinical management.[69] Again, overall HRQOL in children with liver transplants was found to be similar to that of national norms and control groups of children without liver transplants when assessed by either general or disease-specific instruments.

However, Andelman and colleagues' findings also indicated a greater tendency for the disease-specific instrument to detect differences between the HRQOL of children with liver transplants and that of comparison groups without liver transplants.[69] In fact, only the disease-specific instrument detected the impact of comorbid conditions and the effect of post-transplant morbidities in transplant recipi-

ents.[70] Furthermore, administration of these disease-specific age-appropriate instruments to both children with liver transplants and their parents found different responses among children compared with their parents. These findings support a growing realization that the assessment of HRQOL in children is important and must include the perspective of the child.[71]

STUDIES OF MEDICAL EFFICIENCY

As with the growing emphasis on measuring clinical effectiveness and patient-centered end points, there is increasing focus on determining the clinical efficiency or value of medical intervention to make more informed health care decisions at the individual, institutional, governmental, and societal levels. This emphasis on clinical worth that reconciles economic analyses with clinical practice has had a direct impact on all procedural specialties, including pediatric gastroenterology. In the final section of this chapter, we examine the principles of clinical economics and studies of medical costs as essential tools for evaluating efficiency.

The basic principles of health economics involve three different dimensions of analysis, which are classically represented by three axes of a cube (Figure 66.1-3).[52] In this schematic, three approaches to economic analysis are lined up along the y-axis: cost-identification, cost-benefit, and cost-effectiveness. Along the x-axis, different types of costs and benefits can be portrayed, including those that are direct costs, indirect costs, and intangible costs. Finally, the z-axis is used to display three frequently disparate perspectives: that of the patient, the provider, and the payer. An additional perspective is that of society. The societal perspective has a global impact across all of the three axes.

One important study design for determining economic outcomes is modeling. Economic modeling involves the identification of best practices by theoretically determining the costs and benefits of all possible options for a given medical problem. Similarly to meta-analysis, the methodology of modeling involves following strict a priori guidelines that begin with the performance of a comprehensive and integrative literature review. To characterize best all of the options for medical practice, modeling may also involve conducting structured discussions with recognized medical experts on the question to be studied. Ultimately, a conceptual design model should be developed that captures a majority of differences in care processes and the likely affected health outcomes. This model can be depicted as an algorithm of competing treatment paths, which, in turn, can be comparatively analyzed—often in terms of their relative costs, risks, and benefits.

Types of Cost Analyes

There are several different types of cost analyses. Each can be useful in assessing medical costs, but each is also limited by specific considerations that must be understood prior to developing an analysis.

Cost-identification analysis (CIA) is the simplest means of determining health care costs. In this approach, analysts identify all of the costs involved in applying a certain medical intervention. The CIA is expressed as a ratio of cost per unit of treatment or service provided. This type of analysis is often used to compare the costs of one treatment versus another. For example, CIA can be used to calculate the cost of performing flexible sigmoidoscopy versus colonoscopy. However, it is important to recognize that CIA does not necessarily consider the outcomes or benefits, or which procedure may provide more information, in its calculation. Therefore, CIA is appropriate only if outcomes or benefits, such as information gained about the extent of disease, do not vary according to clinical approach. This approach is also known as a cost-minimization analysis.

The second type of medical cost analysis is cost-benefit analysis (CBA), which involves comparing the cost of a given medical intervention with the cost of its benefit. All other circumstances being equal, CBA determines a medical intervention of worth when economic benefits exceed costs. This type of analysis can express its results as either a ratio of benefit to costs (dollars over dollars) or as a net dollar amount (benefits minus costs). In using a CBA, costs and benefits are implicitly negative and positive, respectively. Another way to think of this is that a cost can be incurred or avoided, whereas a benefit is either gained or lost. These theoretical definitions open CBA to statistical manipulation. A medical cost may initially be classified as such and relegated to the denominator of a calculation. A medical cost can also be reclassified as a lost benefit and moved from the denominator to the numerator. Such reclassification (manipulation) can dramatically affect calculations when using ratios. Accordingly, CBAs that are presented as net costs or savings are preferable.

Regardless of how the results are presented, CBA is limited by its reliance on the ability to express costs and benefits in the same unit of measure. For instance, if both costs and benefits can be calculated in dollars, then CBA is possible. However, it is often difficult to express certain qualitative benefits (outcomes measures), such as HRQOL, in monetary units.

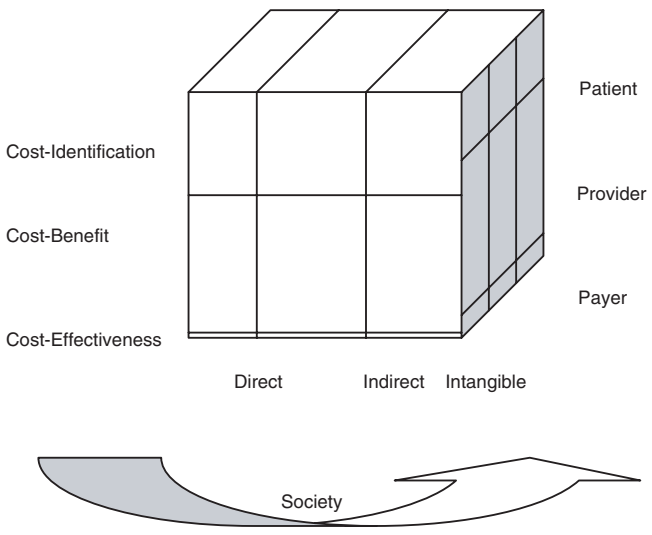

FIGURE 66.1-3 The three dimensions of economic analysis of health care. Adapted from Bombardier C and Eisenberg J.[52]

The third type of analysis is referred to as cost-effectiveness analysis (CEA). In contrast to CBA, all costs in CEA are expressed with the outcome being the denominator (dollars per outcome). This type of analysis allows costs to be expressed in terms of measured clinical or patient-centered outcomes. Accordingly, CEA is most often used to compare two different treatment options with variable or unequal end points. The option that offers less cost with the same or better outcomes is considered to be "dominant."

It is important to note that CEA is complicated by situations in which several important outcomes may result from a single medical intervention. Indeed, a CEA is expressed in terms of a single medical outcome. By definition, it excludes the incorporation of several important medical outcomes simultaneously. For example, if one were to develop a CEA for the treatment of inflammatory bowel disease in children, the results of the study might be expressed as dollars spent per prevention of remission or as dollars spent per symptom-free year of life. However, it would not be possible within the same analysis to compare the costs of remission with the costs of each symptom-free year of life.

To address this problem, analysts can rank the valued importance of the various outcomes to be measured and express the results of their analyses to each. In this weighted ranking system, the comparison of costs for each outcomes measure is called cost-utility analysis (CUA). One common strategy in CUA is to estimate people's personal preferences for different states of health and combine them into a single scale, called a quality-adjusted life-year (QALY). As a unit of "utility" that is impacted by medical intervention, QALYs are useful for measuring health improvement from health care. However, the use of QALYs has not been universally embraced because there are important ethical questions about whose values should be used to derive them.[72]

Another example of a utility-based statistical modeling technique is decision analysis, which involves analyzing decision making under conditions of uncertainty. Classically speaking, to perform a decision analysis, one needs to construct a decision model, also known as a decision tree (Figure 66.1-4). The model must include at least the following four components: (1) all possible choices that can be made in the decision making, (2) all outcomes or potential consequences of these choices, (3) the probabilities or likelihood of the outcomes of each choice, and (4) utilities or values assigned to each outcome. Utilities are often defined as a relative preference for the outcome, with 1 signaling a perfect outcome and 0 the worst possible outcome. A decision tree is then analyzed by summing the product of each probability for each outcome multiplied by its utility. The "best choice" in the decision analysis is that with the highest expected utility.

All decision analyses should include a sensitivity analysis to determine whether the "best choice" remains so if probabilities are different. There are a number of important rules to follow when performing a decision analysis. In particular, it is important to view the problem from a specific perspective (see below). Additionally, the problem must be modeled in the context of the decision and must also include an appropriate level of detail and relevance.

Types of Cost

Three commonly recognized types of cost may be considered in clinical economics: direct, indirect, and intangible. All three types of cost are highly dependent on the perspective of the analysis. The same cost might be classified as a direct cost from one perspective but an indirect or intangible cost when a different perspective is taken.

The most easily understood costs are direct costs, which are usually expressed in terms of actual dollars. Direct costs include all medical and nonmedical financial expenses encountered by both physicians and patients. Such costs include pharmaceutical costs, physician fees, and the costs of diagnostic interventions, among others. Other direct costs incurred include nonmedical costs related to the process of care, such as the cost of transportation to the hospital, the cost of special clothing needed because of an illness, and the cost of housing modifications to meet a patient's needs, among others.

Direct costs may be fixed or variable. Fixed costs of an intervention are not dependent on volume of treatment, whereas variable costs are incurred each time the treatment is provided. For example, when taking the perspective of a director of an endoscopy laboratory, the cost of endoscopy would have both fixed and variable components. Fixed costs might include the equipment used during the procedure, which must only be purchased once and can be used over and over. Variable costs might include costs of staffing and

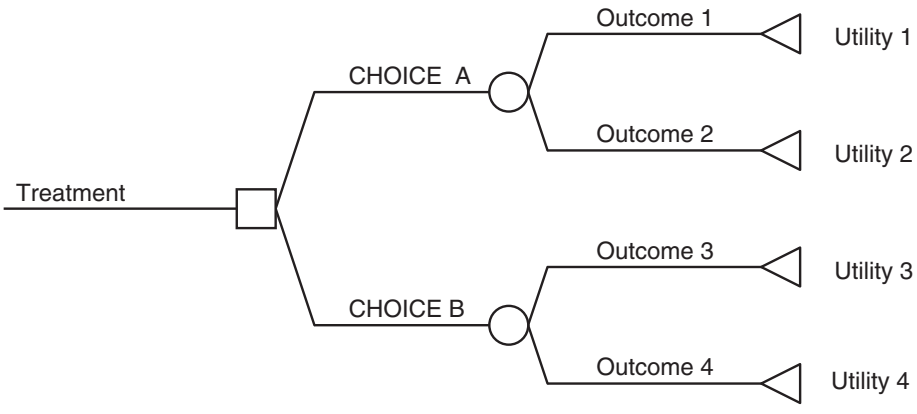

FIGURE 66.1-4 Schematic of a decision tree used in decision analysis.

disposable supplies, which vary from laboratory to laboratory based on the volume of patients undergoing procedures.

Indirect costs of health care are related to all lost opportunities in a patient's life because of illness. In general, they are the financial losses related to morbidity and mortality. For example, because of illness, adult patients may lose household income. In the case of children, indirect costs may be reflected in days away from school, which have projected financial implications for the child and for the parents. These types of costs are often difficult to calculate.

Multiple techniques have been developed for estimating the indirect costs of illness and health care in adults. Assessing the indirect costs of health care or illness in young children is more complex. Whereas the indirect cost of a complication following liver transplant in an adult may be determined by estimating the costs of time lost from the workplace, the equivalent determination in a 6-year-old child requires estimating the costs of missing part of first grade, as well as parental time lost from work.

Finally, intangible costs are those of pain, suffering, and grief. Although these costs may be very difficult to measure, they often figure prominently in decisions made by patients and physicians alike. However, few statistical techniques have been developed to determine intangible costs. Some, such as willingness-to-pay analysis, in which patients are asked to put monetary values on intangible costs, cannot be used to gain the perspective of young children, whose cognitive repertoires do not include an understanding of financial gain or loss. Therefore, although intangible costs may be among the most influential factors in the clinical decision making of pediatric gastroenterologists, they are almost uniformly absent from relevant health care cost analyses.

Perspectives of Cost

Regardless of the type of analysis and which costs are calculated, one must always be cognizant of the perspective used. In general, there are four perspectives that one might take in performing an economic analysis. These perspectives include the patient, the provider, the payer, and society. For example, indirect costs saved by spending more time following a procedure in the hospital might be important to a recuperating patient but less important to the payer, who may be more concerned with the direct costs of prolonged hospitalization. Likewise, direct out-of-pocket expenses for home nursing might be an important factor for a discharged patient recovering from a procedure but of little concern to the payer. This is true not only for adults but also for pediatric populations. Direct costs are generally incurred by parents, whereas the indirect and intangible costs might be incurred by the child and/or the parents. Factoring in all costs from the child's perspective clearly poses a unique difficulty.

Based on these definitions, a study's perspective will determine how costs and benefits are labeled and measured. For instance, early discharge following percutaneous gastrostomy tube placement can be considered at the same time a benefit to third-party payers and an incurred cost by patients and their families; parents may miss work to rehabilitate their children. Clearly, attention must be paid to perspective in interpreting medical cost literature. Although societal costs are generally used as a primary analysis and both CBA and CEA, it is important to keep in mind that the economic impact of an intervention will be reported very differently depending on the perspective of the study.

Cost Analyses

Few cost analyses involving procedures in pediatric gastroenterology have appeared in the medical peer-review literature. Although limited in number, these analyses have tackled pertinent clinical controversies from the provider's perspective of costs and benefits and have contributed important clinical and economic information about current management options.

One example of a recent cost-effectiveness study in pediatric gastroenterology was published by Deutsch and Olson in 1997.[73] In this provocative modeling study, the investigators surveyed both physician practice and associated costs involved in evaluating pediatric patients with colitis-like symptoms. Using a nationwide survey, the investigators were able to demonstrate that significant regional variation in clinical practice was associated with varying costs of care but similar outcomes. At the center of Deutsch and Olson's study was the question of whether the initial evaluation of children with colitis-like symptoms (such as culture-negative diarrhea or rectal bleeding) should consist of flexible sigmoidoscopy or colonoscopy.

By using a clinical survey that identified physician reasoning at each decision-making point, the investigators designed a clinical model for each management approach with estimated costs for each scenario. This emphasis on reasoning allowed the investigators to detect that "physician desire to know the extent of disease" was the most important factor in determining which procedure was employed initially and whether colonoscopy was performed ultimately. The dominant identified strategy in this study, taking the perspective of the providers, was that early colonoscopy was most cost effective.

Deutsch and Olson's study can also be used to illustrate the importance of perspective taken in cost-analysis studies. For example, if the perspective of the payer were taken into account, a different conclusion might have been realized because the cost of colonoscopy far exceeds that of flexible sigmoidoscopy. If the perspective of the patient was used, a CUA reveals that patient-centered outcomes, such as discomfort and preparation, would need to be factored into this analysis and possibly lead to a different relative or absolute result.

In another cost-effectiveness study, Olson and colleagues compared the clinical and economic implications of several treatment strategies in children with recurrent abdominal pain.[74] In this study, the authors investigated the cost-effectiveness of five different approaches: (1) empiric symptomatic treatment for abdominal complaints in children using antisecretory therapy; (2) empiric treatment of all patients for *H. pylori* with antisecretory and antibiotic therapy; and (3) immediate referral for diagnostic endoscopy, as well as two test-and-treat strategies using

(4) noninvasive and (5) invasive (endoscopy) methods, in which treatment with antibiotics was given only for those with documented infection. In their comprehensive assessment, Olson and colleagues created a decision analytic model that incorporated both clinical and economic outcomes of these five strategies. Followed over time, costs incurred related to treatment failures, such as eventual endoscopy, were also included.

In the economic part of the analysis, the investigators assumed the perspective of payer and included only a small portion of the patients' financial burden (the direct pharmaceutical costs). Indirect and intangible costs were not included.

It is interesting to note that when a cost-minimization approach was taken to this model, empiric antisecretory therapy (arm 1) appeared to be the most economical strategy. However, the use of this type of analysis assumed equal effectiveness. Indeed, when the high rate of recurrent pain and the need for endoscopic evaluation in arms 2 to 5 of the study were taken into account, the savings achieved by initially avoiding the invasive tests in many children were lost.

In this study, the authors believed their results to be highly influenced by parents' initial willingness to have their child undergo endoscopy. They postulated that if patients and/or their parents preferred noninvasive workups, then empiric treatment would prove to be more cost-effective. However, if patients and their parents were more willing to undergo early endoscopy, this became the more cost-effective strategy. As such, the authors suggest that evaluating the cost-effectiveness of each procedural and treatment strategy is highly dependent on patient-centered factors.

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2. Methodology (Statistical Analysis, Test Interpretation, Basic Principles of Screening with Application for Clinical Study)

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The ever-increasing amount of information on the screening, diagnosis, prevention, treatment, prognosis, and risk factors of pediatric gastrointestinal disease is both exciting and daunting. Fortunately, critical evaluation of the literature and use of information for evidence-based decision-making has evolved into a formal process. For example, the Cochrane Library publishes systematic reviews of studies.[1] Examples of available topics include the following:

- Enteral nutritional therapy for induction of remission in Crohn disease
- Glutamine supplementation for prevention of morbidity in preterm infants
- Feed thickener for newborn infants with gastroesophageal reflux
- Antituberculous therapy for maintenance of remission in Crohn disease
- Budesonide for maintenance of remission in Crohn disease
- Mechanical bowel preparation for elective colorectal surgery
- Interventions for treating collagenous colitis
- Cisapride treatment for gastroesophageal reflux in children

Similarly, the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN) Web site provides position papers on various topics.[2] However, systematic reviews are often not available to assist with decision-making about new methods of screening or diagnosing gastrointestinal disease in pediatric patients. Therefore, this chapter focuses on a straightforward way to evaluate reports of tests to screen children for risk of developing gastrointestinal disease in the future or to determine whether disease is already present.

WHAT IS SCREENING?

Screening involves the testing of apparently healthy people to find those who are at increased risk of having a disease, either now or in the future.[3] One of the best examples of a screening program involves the screening of newborns in the United States that has been in place since the 1960s. The overall goal of screening is to reduce morbidity and mortality as a result of early detection of this increased risk, although, as recently reviewed by Khoury and colleagues,[4] concerns are increasingly being raised about population screening for genetic susceptibility to conditions that have their onset in adulthood. The most useful screening tests are for diseases that result in substantial morbidity and mortality, that have a presymptomatic phase during which time the test is positive, and for which treatment or preventive strategies are available and cost effective.

In an ideal world, screening tests would be positive for everyone at risk of developing a disease and negative for everyone who was not at risk of developing the disease. They would be safe, reliable, easy to perform, noninvasive, and inexpensive. Subjects who have a screening positive test need follow-up and may be offered a subsequent diagnostic test and/or ways to prevent disease from occurring at a later stage. One of the best ways to evaluate screening tests is to randomize subjects to receive the test or not receive the test and to compare health over time, although these studies are rarely performed, and the value of screening tests is often inferred from observational studies.

WHAT ARE DIAGNOSTIC TESTS?

Diagnostic tests are usually performed in individuals who have signs or symptoms of disease or who are at risk of having disease based on a positive screening test. Again, in an ideal world, diagnostic tests would be positive for everyone who has the disease and negative for everyone who does not have the disease (ie, the test accurately discriminates between those who do and do not have the disease). Unfortunately, there are few diagnostic tests that meet these criteria. The best way to evaluate diagnostic tests is for subjects in a defined and relevant clinical population to have both the new and a "gold standard" diagnostic test and for the results of the two tests to be read independently and compared.[5,6]

WHAT IS MEANT BY DIAGNOSTIC TEST ACCURACY?

A diagnostic test is evaluated in relationship to the truth, that is, whether or not a disease is present. Four situations are possible:

- The test may be positive, and the subject has the disease (true-positive [TP]).
- The test may be negative, and the subject does not have the disease (true-negative [TN]).
- The test may be positive, and the subject does not have the disease (false-positive [FP]).
- The test may be negative, and the subject does have the disease (false-negative [FN]).

The first step to evaluating a diagnostic test is based on how good it is at correctly identifying subjects who have the disease (sensitivity) and subjects who do not have the disease (specificity). Sensitivity is calculated as the number of TPs as a percentage of all of those with the disease [TP/(TP + FN)]. Specificity is calculated as the number of TNs as a percentage of all of those without disease [TN/(TN + FP)]. The question now is what is an acceptable level of sensitivity and specificity? This varies from test to test. For example, if the test was to diagnose a highly treatable form of cancer providing that it was caught at an early stage, then a diagnostic test that missed the diagnosis in 10% or had a sensitivity of less than 90% would likely be totally unacceptable. Similarly, to expose 5% of children to toxic chemotherapy when they did not have cancer owing to a test specificity of 95% would likely be equally unacceptable. However, sensitivity and specificity alone do not tell the whole story about the diagnostic accuracy of a test.

The next step is to take into account the population that is being tested because the sensitivity and specificity of a test depend on how common the disease is in the population being tested. To understand how this impacts on the clinical usefulness of a diagnostic test, we have made up two hypothetical populations in which the results of a serologic test for immunoglobulin G to *Helicobacter pylori* (diagnostic test) were compared with the results of a gold standard test (endoscopy, biopsy, and positive histology) (Table 66.2-1). In both situations, we assumed that the sensitivity of the test is 80% and the specificity is 90%. One hypothetical population includes consecutive asymptomatic children attending a primary care clinic, 1% of whom truly have *H. pylori*, and a second hypothetical population includes consecutive children with dyspepsia presenting to a pediatric gastrointestinal specialty clinic in a hospital, 50% of whom truly have *H. pylori*. We recognize that it would be unacceptable to perform endoscopy on healthy children, as suggested in the first example. In this example, we are drawing attention to the predictive value of a positive test (PVP) and the predictive value of a negative test (PVN). Although the relationship between test sensitivity and specificity and the prevalence of the disease is typically expressed using Bayes theorem, for practical purposes, PVP can be calculated as the odds of having the disease if the test is positive [TP/(TP + FP)], and PVN can be calculated as the odds of not having the disease if the test is negative [TN/(TN + FN)]. In this example, an asymptomatic child in population 1 has a 7% chance of having *H. pylori* based on the serologic test (very unimpressive), whereas a child with dyspepsia has an 89% chance of having *H. pylori* based on the serologic test.

WHO DECIDES WHETHER A TEST IS NEGATIVE OR POSITIVE?

Many diagnostic tests produce continuous results (eg, serum albumin). In an ideal world, patients with disease would all have test results above a certain point, and healthy subjects without disease would all have test results below a certain point. In reality, test results for those with and without disease overlap, sometimes extensively. Although it is attractive to report a diagnostic test as either positive or negative, some risks are taken when

TABLE 66.2-1 COMPARISON OF THE PERFORMANCE OF A HYPOTHETICAL SEROLOGIC TEST FOR *HELICOBACTER PYLORI* IN TWO PATIENT POPULATIONS

	H. PYLORI ON ENDOSCOPY		
***H. PYLORI* SERUM IGG**	**PRESENT**	**ABSENT**	**TOTAL**
POPULATION 1*			
Positive	8 (TP)	99 (FP)	107 (TP + FP)
Negative	2 (FN)	891 (TN)	893 (FN + TN)
Total	10 (TP + FN)	990 (FP + TN)	1,000
POPULATION 2†			
Positive	400 (TP)	50 (FP)	450 (TP + FP)
Negative	100 (FN)	450 (TN)	550 (FN + TN)
Total	500 (TP + FN)	500 (FP + TN)	1,000

FN = false-negative; FP = false-positive; IGG = immunoglobulin G; TN = true-negative; TP = true-positive.

*1,000 asymptomatic children in a primary care clinic, *H. pylori* prevalence 1%: sensitivity = TP/(TP + FN) = 8/10 = 80%; specificity = TN/(TN + FP) = 891/990 = 90%; positive predictive value = TP/(TP + FP) = 8/107 = 7%; negative predictive value = TN/(TN + FN) = 891/893= 100%.

†1,000 children with dyspepsia in a pediatric gastroenterology clinic, *H. pylori* prevalence 50%: sensitivity = TP/(TP + FN) = 400/500 = 80%; specificity = TN/(TN + FP) = 450/500 = 90%; positive predictive value = TP/(TP + FP) = 400/450 = 89%; negative predictive value = TN/(TN + FN) = 450/550 = 82%.

a cutpoint is chosen to separate normal from abnormal. Figure 66.2-1 shows a hypothetical distribution of serum albumin levels in children with and without liver disease. Let us assume that 200 children were studied, 100 with liver disease and 100 without liver disease. If the cutpoint to separate normal from abnormal is placed at 4 g/dL (see Figure 66.2-1A), in this example, 15% of subjects without liver disease will be incorrectly classified as having liver disease. In addition, 15% with liver disease will be incorrectly classified as being normal. Thus, with a cutpoint of 4 g/dL, the test specificity is 85% and the test sensitivity is also 85%. If the cutpoint is moved to 3.5 g/dL (see Figure 66.2-1B), now only 2% of subjects without liver disease will be incorrectly classified as having liver disease, but 50% with liver disease will be incorrectly classified as being normal. This time, the test specificity is improved to 98%, at the expense of loss of sensitivity, which drops to 0%. Fortunately, there is an efficient way to show the relationship between sensitivity and specificity: using receiver operating characteristic (ROC) curves. ROC curves compare the TP rate with the FP rate (see Figure 66.2-1C). The goal of the diagnostic test is to have a cutoff that has the highest TP rate and the lowest FP rate (ie, is as close to the top left as possible) and as far away as possible from the line of equality (when the TP rate is the same as the FP rate). From above, in Figure 66.2-1A, using a cutpoint of 4.0 mg/dL, the TP rate [TP/(TP + FN) – sensitivity] is 85% and the FP rate [1 – (TN/(TN + FP) or 1 – specificity] is 15%. In Figure 66.2-1B, using a cutpoint of 3.5 mg/dL, the TP rate is 50% and the FP rate is 2%. Clearly, neither cutpoint is perfect, and this raises an important question: Should a cutpoint be chosen at all and, if so, based on what?

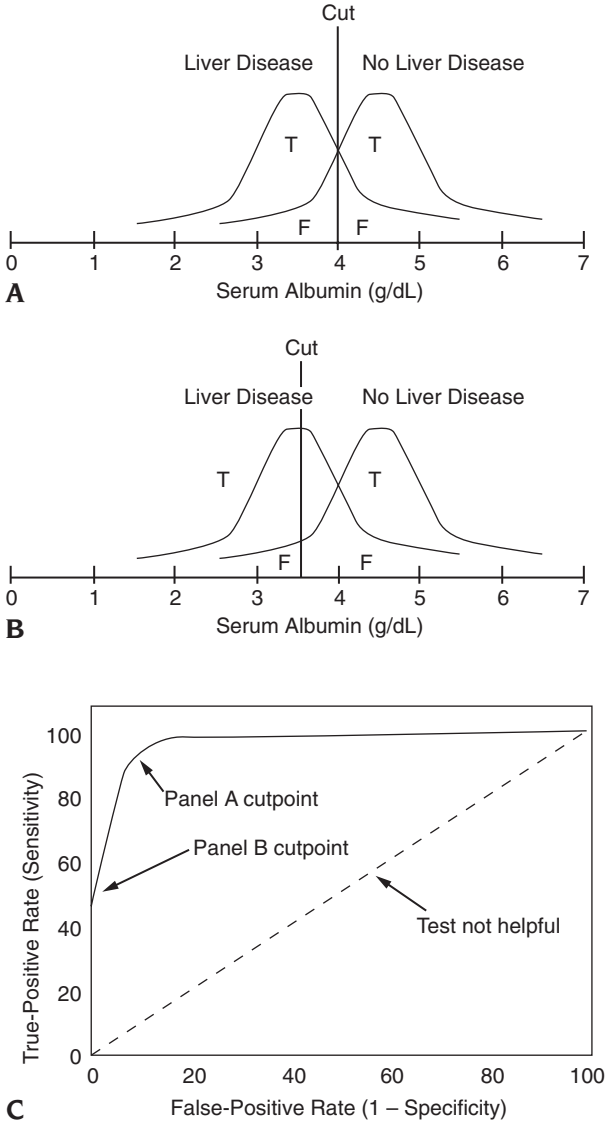

FIGURE 66.2-1 Hypothetical distribution of serum albumin levels in normal children and those with end-stage liver disease. F = false; T = true.

Along with exploring the range of cutpoints, the ROC curve provides another estimate of the overall accuracy of the diagnostic test. The estimate of the area under the ROC curve (AUC), which will be between 0 and 1, is also the probability (or chance) that a random person with the disease will have a higher test value than a random person without the disease. A diagnostic test that has an AUC of 0.5 has the same accuracy as a diagnosis that is made at random (eg, by a coin toss), meaning that the test does not help sort out who does and who does not have the disease. However, just because one diagnostic test has a higher AUC (or accuracy), it does not necessarily make it a preferred test. Cost and invasiveness must also be considered in decisions about which test is preferred.

HOW DOES ONE ASSESS THE VALUE OF A DIAGNOSTIC TEST?

Authors of studies that assess the accuracy of a diagnostic test need to convince the reader that they have done everything that they can to ensure that the test results truly represent the way in which the test will work in the population studied (ie, how useful the test will be). If the test was studied in a patient population for whom the test is not relevant (eg, a new saliva enzyme-linked immunosorbent assay–based assay to detect the presence of *H. pylori* being studied in children presenting with coughs and colds), it is very difficult to make conclusions about the way in which the test will work in children presenting with dyspepsia in whom the test is likely to be relevant. Table 66.2-2 provides a checklist of points to consider while reviewing a study of a new diagnostic test and how these points affect whether the study results are likely to be valid.

Based on this checklist, a description of the study population is critically important and should include the following:

- Demographic characteristics (at least age and gender)
- Presence of comorbid conditions
- Previous tests that have been conducted (likely nondiagnostic)
- Setting of the study (eg, primary care, tertiary care, outpatient, inpatient)
- Duration of symptoms or illness prior to testing
- Spectrum of disease and nondisease in the study population

TABLE 66.2-2 EVALUATING STUDIES OF DIAGNOSTIC TESTS

POTENTIAL THREATS TO STUDY VALIDITY IF ANY OF THE FOLLOWING ARE PRESENT	EFFECT OF THE THREAT	BEST PRACTICES APPROACH TO MINIMIZE THE THREAT OF INVALID RESULTS
POPULATION STUDIED		
Selection of only those patients who are known to have the disease	Cannot estimate diagnostic accuracy of the test	Study population is subjects for whom the test is relevant in clinical practice (eg, all patients presenting with a symptom). Population needs to be described.
Selection of patients known to have the disease and selection of another group known to be healthy normal subjects (case-control study)	Overestimates diagnostic accuracy of the test	
Systematic or unknown reasons for excluding subjects who are part of a relevant clinical population	Over- or underestimates the diagnostic accuracy of the test depending on the reasons for excluding subjects	Consecutive patients in the relevant population are studied
Some patients studied more than once	Over- or underestimates the diagnostic accuracy of the test depending on whether overrepresented subjects have or do not have the disease being studied	Each subject is studied the same number of times (usually once or at first encounter)
COMPARISON WITH A STANDARD		
Gold standard (reference) test is not done in everyone (eg, only those with positive tests have the reference test)	Cannot estimate diagnostic accuracy of the test	Gold standard test is performed on everyone (best practices) or at least a random subset of all subjects
Gold standard test is done for only those patients with positive test and a different test is done for those with negative tests	Cannot estimate diagnostic accuracy of the test	
Gold standard test is read with knowledge of the result of the diagnostic test (or the diagnostic test is read with knowledge of the result of the gold standard test)	Overestimate the accuracy of the test, particularly if either test requires subjective interpretation	The gold standard and diagnostic tests are read independently (blinded) and, if any test requires subjective interpretation, ideally should be read by more than one independent person. Results of agreement of independent reading should be reported.
TESTING		
Way in which the gold standard and diagnostic test were performed is unclear	Cannot interpret diagnostic accuracy	Way in which the gold standard and diagnostic test were performed should be described in sufficient detail to allow reader to replicate the measurements
REPORTING OF RESULTS		
No information on whether indeterminate test results occurred and/or were included or excluded in the analysis	Potential for distortion of diagnostic accuracy	All study results should be reported (positive, negative, indeterminate), and tests of diagnostic accuracy should indicate how indeterminate values were treated and effects on measures of test accuracy

Similarly, a description of the diagnostic test should include the following:

- Precise way (adequate details) in which the test was performed to enable others to reproduce the test conditions
- Cutpoint used to categorize the test as positive or negative and explanation of why that cutpoint was chosen
- The proportion of subjects who could not have the test performed or in whom the test result was indeterminate
- Test reproducibility
- Tables summarizing the sensitivity, specificity, PVP, and PVN compared with an acceptable gold standard

THE NEXT LEVEL: STATISTICAL CONSIDERATIONS

Estimates of sensitivity, specificity, PVP, PVN, and AUC will all have uncertainty associated with them. As such, researchers should also report the 95% confidence intervals associated with these values. The size of the confidence intervals and one's ability to compare the accuracy of a diagnostic test with either a null value or a "gold standard" test will depend on the study's sample size and design. In general, the fewer the patients studied in a study, the wider the 95% confidence intervals associated with the estimates of sensitivity, specificity, PVP, PVN, and AUC. However, most equations relating sample size to a desired power or confidence interval width rely on asymptomatic theory despite the fact that most studies rely on fewer than 50 patients.[7]

CONCLUSION

Improved, noninvasive diagnostic tests for pediatric gastrointestinal disease are urgently needed for a wide range of conditions, but despite the promise of exciting new

technologies (such as genetic testing and videocapsule endoscopy), there is an even more urgent need for improved quality of diagnostic studies to evaluate these new innovations. The ethical and regulatory issues relating to diagnostic testing are discussed in the next chapter (Chapter 66.3, "Ethics and Regulatory Issues").

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3. Ethics and Regulatory Issues

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Effective and safe medical care for children requires that care be appropriate to the patient's developmental and/or chronologic age.[1] Advances through research with adult subjects are not always relevant to children. Children and adults can have different manifestations of the same disease process, and age-specific differences in renal and hepatic function make it impossible to directly translate adult pharmacokinetics and pharmacodynamics to the child. In addition, children suffer unique diseases such as sudden infant death syndrome, Reye syndrome, and infantile spasms.[2]

But should children be allowed to be research subjects? Is any risk appropriate for a child? Can children truly volunteer? Can their parents "volunteer" for them? In response to such concerns, a regulatory framework that governs pediatric research has evolved.[3] Investigators working with pediatric populations must negotiate a range of federal, state, local, and institutional protections for vulnerable research participants and must also accommodate a number of methodologic challenges that are unique to pediatric research. This review summarizes the importance of pediatric research, the history of pediatric research ethics, and the fundamental regulatory requirements and provides several recommendations for pediatric research investigators.

IMPORTANCE OF PEDIATRIC RESEARCH

An often-repeated adage is that children are not small adults. Data learned from research conducted with adults may not be scalable to the child. As noted above, children have unique diseases, unique manifestations of "adult" diseases, and renal and hepatic functions that vary with developmental age. Although the focus of this chapter is children in research, it is relevant to at least note that many of the same concerns and issues are also relevant to the developing fetus. The thalidomide tragedy in the 1960s reminded the public of the importance of understanding the effects of diseases and drugs on intrauterine fetal development. Revelations about the teratogenicity of this drug generated support for a rigorous premarket drug safety screening process in the United States and continue to serve as a brutal reminder of the specific vulnerability of the developing fetus.[4]

Too often, even today, pediatric clinicians must provide clinical care without the benefit of age-appropriate, validated data.[5–7] In 2000, Conroy and colleagues demonstrated that as many as 67% of children (421/624) admitted to five general pediatric wards in Europe received drugs that were unlicensed for use in children or were being used off-label.[8] In this study, dosing issues (either amounts or frequency of doses) and use of the drug in an untested age group were the most common reasons for unlicensed or off-label uses. In 1998, McKinzie and colleagues reported a lower but still large rate of off-label drug use for children in a US emergency department: 179 of 521 children (34%) received off-label drug prescriptions, with the highest rate found in patients 3 to 11 years of age.[9] Turner and colleagues reported a significant association between the percentage of off-label drugs prescribed per child and the incidence of adverse events in five pediatric wards in the United Kingdom.[10]

Conducting pediatric research presents a number of unique challenges. Among these are (1) the need for multiple age cohorts to study different developmental stages, (2) small study populations owing to the limited incidence of many pediatric illnesses,[11,12] and (3) the resultant limited industry interest and financial support. There are also a number of pediatric-specific ethical issues to consider: (1) the acceptable risk to which one may expose a child, (2) under what circumstances a child may be a healthy control,[13] (3) at what age a researcher should discuss study participation with a child subject,[14,15] and (4) when and how a child participant should be remunerated.[16]

The US government created a number of initiatives to support pediatric research in the late 1990s. Briefly, these included the following: The Food and Drug Administration (FDA) Modernization Act of 1997 (FDAMA) required that the FDA extend drug testing to children.[17] The National Institutes of Health (NIH), in 1998, specified that children be included in all research barring a scientific or ethical reason for their exclusion.[18] The Children's Act of 2000 codified previous congressional decisions to increase money dedicated to pediatric research.[19]

Of interest, this support for pediatric research came at a time of growing concerns about the adequacy of protections of human research subjects in general and protections for children specifically.[3,20–25] The 1999 death of Jesse Gelsinger, an 18-year-old volunteer in a phase I gene transfer trial conducted at the University of Pennsylvania, and the 2001 death of Ellen Roche, a healthy volunteer in a lung physiology study at Johns Hopkins University, focused much of this concern. Federal assessments of these two tragedies raised issues about informed consent, coercion, reporting of adverse events, and financial conflict of interest, among others. These high-profile cases served as watershed events for

many in the research safety community and prompted a number of changes in the oversight of human research.[26–29]

HISTORY OF HUMAN SUBJECT PROTECTIONS

The Nuremberg Code, which provides the foundation of the current system of human subject protections, was developed following the Nuremberg Military Tribunal convened after World War II. The Code defined 10 ethical principles for research, including, among others, the voluntariness of consent and the need to minimize risk. In the years following, other complementary guidelines and principles for the protection of human research subjects were developed at the World Medical Association Declaration of Helsinki and by the Council for International Organizations of Medical Sciences (best known as CIOMS).

In the United States, federal regulations for the protection of human subjects advanced during the 1960s and 1970s in response to several sentinel events. One of these events was Beecher's review article in 1966, which focused attention on several historic but ethically controversial published peer-reviewed studies.[30] One cited study was Krugman and colleagues' work with infectious hepatitis. The investigators intentionally exposed mentally impaired children between the ages of 3 and 10 years to hepatitis virus, arguing that the children would have been exposed to the virus at some time during their institutionalization.[31] Although parental consent was obtained at admission to the institution, there were many concerns regarding coercion, inadequate consent, and inappropriate risk to this vulnerable population. Following Beecher's article, revelations about the Tuskegee Syphilis Study became public in a 1972 *New York Times* exposé.[32] These two reports together generated a public and professional demand for a regulatory framework.

The federal government responded with a series of initiatives and regulations for the protection of human subjects. *The Belmont Report*, proposed by the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (the National Commission) in 1979, provided landmark definitions in the ethical domains of respect for persons, beneficence, and justice and created the groundwork for the current Institutional Review Board (IRB) system of local peer review of human research protocols.[33] The National Commission's work ultimately evolved into the Common Rule, a set of federal regulations, which are described below.

The National Commission also identified additional protections for pediatric research participants. Among other recommendations, the Commission concluded that IRBs should consider the scientific significance of the pediatric protocol, that studies be conducted first in animals and adults before children, that the privacy and confidentiality of parents and children be protected, and that research with wards of the state, underage prisoners, or the mentally infirm be further restricted.[34] The National Commission drew distinctions between research of minimal risk and of greater than minimal risk for pediatric subjects and recommended seeking a child subject's assent and parental permission for participation. These recommendations were ultimately codified in additional regulations, which are discussed below.

REQUIREMENTS FOR FEDERALLY FUNDED RESEARCH: THE COMMON RULE AND SUBPARTS OF TITLE 45 OF THE CODE OF FEDERAL REGULATIONS, PART 46

The Common Rule is the regulatory foundation for the current system of human subject protections. This rule, found at Subpart A of Title 45 of the Code of Federal Regulations, Part 46 (45 CFR 46), "Protection of Human Subjects," is called the Common Rule because it is held in "common" by 17 federal departments or agencies, including the NIH in the Department of Health and Human Services (DHHS), the largest funding agency for biomedical research.[35] Not all research is covered by the Common Rule. The Common Rule covers only human subject research that is conducted or funded by any of the 17 agencies that have accepted the rule. Research that is not conducted or supported by 1 of these 17 agencies is not subject to this rule. Despite this legal allowable "gap" in coverage, most academic institutions that receive NIH funding voluntarily extend these protections to all human subject research conducted by the institution. This extension of the Common Rule is done with a formal agreement with the federal government.

The Common Rule (subpart A of 45 CFR 46) outlines the process of required institution-level peer review of the ethics of individual research projects through a committee called an IRB. The Common Rule provides the general framework for IRB functions, organization, and operations but allows for limited institution-level interpretation in some areas.[36] Investigators are encouraged to familiarize themselves with their institution's IRB policies and procedures.

The Common Rule includes criteria for the membership of the IRB, mandating the inclusion of both scientists and laypersons with appropriate expertise. It specifies that if an IRB reviews research to be conducted with vulnerable populations (eg, children, prisoners, or pregnant women), then that IRB must have members with knowledge specific to research and ethics in those fields.[36]

The process for reviewing research protocols draws a distinction between research protocols of greater risk to subjects that require full IRB review and those of minimal risk, which can be reviewed by an accelerated review process, termed an expedited review. Per the Common Rule, research protocols can be approved only for a maximum of 1 year, at which time investigators must reapply to the IRB for continuing review and approval. The Common Rule also requires that every approved study be monitored at a level that is commensurate with the risk to subjects involved in the study. All changes to the research protocol or informed consent document must be reported to and approved by the IRB (note that sponsors may also require review and reporting of changes). For more detail regarding the Subpart A requirements for an IRB, see Table 66.3-1.[36]

In addition to the Common Rule (Subpart A), 45 CFR 46 also has three other subparts: B, C, and D. Subparts B, C, and D were codified after Subpart A and outline additional regulations for specific vulnerable subpopulations. Subpart B, codified in 1975 and revised in 2001, addresses research with pregnant women, human fetuses, and neonates; Subpart C, codified in 1978, addresses prisoners; and Subpart D, codified in 1983, addresses children. Because Subparts B, C, and D are not followed by all of the aforementioned seventeen agencies that follow Subpart A, these subparts are not considered part of the Common Rule. Researchers should note that the NIH does adhere to all subparts.

Additional Protections for Children Involved as Subjects in Research (45 CFR 46 Subpart D)

Subpart D is of particular importance to pediatric investigators as the principal set of regulations that governs pediatric research. This subpart categorizes pediatric research, unlike adult research, into four levels of risk and benefit that trigger different levels of review, consistent with the recommendations of the National Commission (Table 66.3-2).[37] Three of the four categories can be reviewed and approved by the IRB. These include (1) research not involving greater than minimal risk, (2) research involving greater than minimal risk but presenting the potential of direct benefit to the specific child, and (3) research involving greater than minimal risk that, although not presenting the prospect of direct benefit to the specific child, is likely to produce information about the child's disease or condition. The fourth category addresses research that is greater than minimal risk, that has no prospect of direct benefit to the child, and that would not produce information about the child's disease or condition. The IRB cannot approve research in this last category; it can be approved only by the Secretary of Health and Human Services after consultation with a panel of experts.[37]

Minimal Risk in Pediatric Research. Subpart D fails to define the amount of risk that may be considered minimal for children participating in research. Some interpret minimal risk to include only those risks that a healthy child may experience in everyday activities. However, a 2002 federal advisory committee of experts to the DHHS Office of Human Research Protections interpreted minimal risk to be relative to the health condition of each specific minor. This advisory committee suggested that for a healthy child, minimal risk equated to no additional risk above and beyond the risk that a healthy child encountered on a daily basis. In contrast, chronically ill children faced substantial risk as part of their clinical care, and minimal risk for these children meant no additional risk above and beyond the risk inherent in their standard of care.[38,39]

Pediatric Assent and Parental Permission. Subpart D also amplifies the requirements of informed consent. Consistent with the National Commission's original report, subpart D requires investigators to seek parental permission rather than parental consent, drawing a distinction between the two terms to highlight that one may not consent to research conducted on another person. The IRB can require the permission of either one or both parents depending on the level of risk to the child. The permission of only one parent is adequate for research considered of minimal risk to the child participants (Section 46.404) or for research likely to yield direct benefit to the children (Section 46.405). The permission of both parents is required for pediatric research conducted under Section 46.406 or 46.407 (see Table 66.3-2) unless one parent is deceased, unknown, incompetent, or not reasonably available or when only one parent has legal responsibility for the care and custody of the child.[40] In addition, investigators must also seek the age-appropriate assent of children subjects; of note, failure to object cannot be considered an assent.[37]

TABLE 66.3-1 SUMMARY OF THE REQUIREMENTS OF INVESTIGATORS DELINEATED BY THE COMMON RULE (SUBPART A, 45 CFR 46)[36]

Requirements for IRB approval	Risk to subjects are minimized. Risks to subjects are reasonable in relation to anticipated benefits, if any, and the importance of the knowledge that may result. Subject selection is equitable. Vulnerable populations are given additional protections both in the selection of subjects and the research generally. An informed consent form and process are provided, or a waiver of all or part of the informed consent is approved. A waiver of informed consent may be obtained by an investigator from an IRB if the study involves no more than minimal risk to the subjects, if the waiver does not adversely effect the rights and welfare of the subjects, if the research cannot be conducted without the waiver, and, whenever appropriate, the subjects will be provided additional pertinent information after participation. Adequate provisions for data and safety monitoring are in place when appropriate. Adequate privacy of the subjects and confidentiality of the data are maintained.
Required elements to be provided to each subject when obtaining written informed consent	A statement that the study is research, an explanation of the purpose of the study, the length of time the subject is expected to participate, a description of the procedures, and the identification of the experimental procedures. A description of the risks and benefits. Disclosure of appropriate alternatives that might benefit the subject. A statement of how the confidentiality of the data will be maintained. A description of compensation and the procedures and treatments available for potential injury. Investigator contact information. A statement that participation is voluntary and the subject will not be penalized in any way for discontinued participation. Additional requirements must also be included whenever appropriate (eg, withdrawal from the research).

IRB = Institutional Review Board.

TABLE 66.3-2 ALLOWABLE RESEARCH WITH MINOR SUBJECTS UNDER SUBPART D, 45 CFR 46[37]

Research not involving greater than minimal risk (Section 46.404)	These studies may be permitted if the IRB finds Adequate provisions are made for soliciting the assent of the children and the permission of their parents or guardians.
Research involving greater than minimal risk but presenting the prospect of direct benefit to the individual subjects (Section 46.405)	These studies may be permitted if the IRB finds that The risk is justified by the anticipated benefits to the subjects; The relation of the anticipated benefit to the risk is at least as favorable to the subjects as that presented by available alternative approaches.
Research involving greater than minimal risk and no prospect of direct benefit to individual subjects but likely to yield generalizable knowledge about the subject's disorder or condition (Section 46.406)	These studies may be permitted if the IRB finds that The risk represents a minor increase over minimal risk; The intervention or procedure presents experiences to subjects that are reasonably commensurate with those inherent in their actual or expected medical, dental, psychological, social, or educational situations; The intervention or procedure is likely to yield generalizable knowledge about the subjects' disorder or condition, which is of vital importance for the understanding or amelioration of the subjects' disorder or condition; Adequate provisions are made for soliciting assent of the children and permission of their parents or guardians.
Research not otherwise approvable that presents an opportunity to understand, prevent, or alleviate a serious problem affecting the health or welfare of children (Section 46.407)	Allowable only if the IRB finds that the research study meets this requirement and the Secretary of Health and Human Services, after consultation with a panel of experts, determines that The research presents a reasonable opportunity to further the understanding, prevention, or alleviation of a serious problem affecting the health or welfare of children; The research will be conducted in accordance with sound ethical principles; Adequate provisions are made for soliciting the assent of children and the permission of their parents or guardians.

IRB = Institutional Review Board.

Issues of child assent and parental permission are complex. The ethical and legal controversies surrounding parents' ability to provide permission for pediatric subjects were the focus of the 2002 *Grimes v. Kennedy Krieger Institute* case.[41] This case involved the Johns Hopkins–affiliated Kennedy Krieger Institute and its 2-year-old lead abatement study in low-income housing. The judgment of the Maryland Court of Appeals discussed issues of parental consent for the participation of children subjects and attempted to define minimal risk. The court was concerned that the parents had an inaccurate assessment of their children's risks (dust lead level reporting was delayed) and benefits (the offer of a lead-reduced household may have been coercive) in this research. Regarding minimal risk, the court initially determined so-called nontherapeutic research to have a zero-risk standard but relented to define minimal risk as "the minimal kind of risk that is inherent in any endeavor."[41] Given the judgment in the *Grimes v. Kennedy Krieger Institute* case, researchers should be aware of the fluid nature of parental permission in pediatric research.[42,43]

The issue of assent is also controversial. The American Academy of Pediatrics (AAP) recommends seeking assent from children with an intellectual age of 7 or greater.[44] Researchers working with adolescents in studies in which parental consent may be inappropriate should consider state-level laws that designate some adolescents to be "mature minors" or "emancipated minors" under specific circumstances.[15] The AAP recommends several specific elements of assent, which include (1) helping the child achieve a developmentally appropriate awareness of his or her condition, (2) explaining tests and treatment, (3) making an appropriate assessment of the child's level of understanding, and (4) soliciting the child's willingness to participate.[15] Little research has been done in this area of pediatric consent. Tarnowski and colleagues examined forms that parents were asked to sign to grant their permission for their children to participate in research. The language in these consent documents was at the college graduate level rather than the required eighth grade reading level.[45] Susman and colleagues examined the comprehension level of children ages 7 to 20 years participating in cancer or obesity research. Several days following consent or assent, the subjects were found to be less likely to understand the purpose of the relevant research study, the procedures, and the risks involved than they were to understand other, more concrete elements of the study, such as the duration of their participation. When stratified into two age groups, 7 to 13 years and 14 to 20 years, there was no statistical difference in mean scores; age did not appear to affect the children's understanding of some elements of the research study in which they were participating.[46] These findings are similar to assessments of the readability of informed consent forms for adult subjects.[47] Issues of consent, permission, and assent require specific attention to comprehension if this process is to be meaningful.

Remuneration of Pediatric Subjects. Another concern, as in any research, is the issue of coercion or inappropriate influence to elicit a subject's assent or consent. The regulations speak to coercion broadly, but there are no specific recommendations on the issues of remuneration of pediatric or adult research subjects. Dickert and colleagues studied 32 IRBs and found that 37.5% had written guidelines for

investigators about adult research subject compensation. All but one institution had "rules of thumb," suggesting that institutional approaches to adult subject remuneration varied widely.[48] In terms of remuneration of pediatric subjects, Weise and colleagues found that two-thirds of 128 participating institutions permitted payment to pediatric research subjects. Only 7% (6/84) of those who permitted remuneration had written policies. The most common method of payment was money, with gift certificates and savings bonds named as other permitted and common options.[16] Although there is little formal guidance, the AAP recommends that the families of research subjects be reimbursed for direct and indirect costs of study participation. Similarly, remuneration to child subjects should be limited to a token and should be discussed with the child only at the completion of the study.[44]

Additional Protections for Pregnant Women, Human Fetuses, and Neonates Involved in Research (45 CFR 46 Subpart B)

Subpart B of 45 CFR 46 addresses research with pregnant women, human fetuses, and neonates. The specific requirements of Subpart B, revised on November 13, 2001, are described in Table 66.3-3. This revision defined permissible research with fetuses, neonates of uncertain viability, and nonviable neonates, as well as requirements for paternal consent. Note that research with viable neonates, defined as newborns until 28 days of age, falls within the scope of Subpart D.[49]

Subpart B generally requires that research involving pregnant women or fetuses must be of no greater than minimal risk or should hold the possibility of direct benefit to the pregnant woman and/or fetus. Consent is required of only the mother if the research is of minimal risk or if the research is likely to yield direct benefit to either the pregnant woman alone or both the woman and the fetus. Research likely to yield direct benefit only to the fetus requires the consent of both parents.[49]

Although viable neonates are covered in Subpart D, research on neonates of uncertain viability is covered by Subpart B. Research on neonates of uncertain viability is allowed only if there is the prospect of enhancing the likelihood of survival or if the purpose of the research is important and adds no additional risk to the neonate and the information cannot be obtained in any other way. Consent is required from only one parent.[49]

Research with nonviable neonates can be approved by the IRB only if the research will not stop the respiration or heartbeat of the neonate and, similar to research with neonates of uncertain viability, the research is important and adds no additional risk to the neonate, and the information can be obtained in no other way. For research with nonviable neonates, consent must be obtained from both parents except under limited circumstances.[49]

FEDERAL REQUIREMENTS SPECIFIC TO THE FDA

The FDA has its own set of regulations that cover any research conducted on a product that is regulated by the FDA. These regulations present special considerations for human studies that involve drugs, biologics, and devices. These regulations are similar but not identical to those of the Common Rule.

Typically, a sponsor, such as a pharmaceutical, biotechnology, or medical device company, initiates the application but does not function as the investigator. Investigators are required to assume the responsibilities of conducting the research, which include (1) abiding by the investigational plan and statement, (2) protecting the rights and safety of the human subjects, and (3) controlling any drugs under investigation.[50] In some limited cases, an individual or group of individuals may serve as the sponsor-investigator.

Although the FDA requirements for informed consent and IRB review are similar to those of the Common Rule, they are not identical. Some of the differences are listed below. Note that research studies conducted under both the DHHS and FDA are subject to both sets of requirements:

- The FDA permits the use of an investigational drug, device, or biologic for a limited number of emergency uses without prior IRB approval. The emergencies qualifying for this exemption are listed in Table 66.3-4. Each of these uses must be reported in writing to the governing IRB within 5 days, and subsequent use is subject to IRB review.[51]
- The informed consent requirements may not be waived if the risk to subjects is a breach of confidentiality.[52] This is a more stringent standard for waiver criteria than that provided by the Common Rule.
- Potential subjects are required by the FDA to be informed that the agency may review the records of the study. In addition, informed consent forms must be dated and signed by the subjects.[53]

In October 2000, the Children's Health Act of 2000 (Public Law [PL] 106-310) extended Subpart D of 45 CFR 46 (pediatric research) to all health and human services agencies, including the FDA.[19] Prior to this legislation, the FDA was one of several agencies that had agreed to abide by the Common Rule (Subpart A 45 CFR 46), but as the FDA regulated rather than funded research, it did not require the same protections for children as were required by the NIH.

Pediatric drug research was a focus of the 1997 FDAMA and the FDA Pediatric Rule. The FDAMA gave pharmaceutical companies an additional 6 months of patent exclusivity for all uses of the entire active moiety if pediatric-specific trials for on- or off-label uses of those drugs were supported.[17] In 2002, the Best Pharmaceuticals for Children Act (PL 107-109) reauthorized the pediatric provisions of FDAMA.[54] Although the FDAMA produced a regulatory pathway by which sponsors could voluntarily provide these data on drugs, it did not require sponsors to generate pediatric-specific data. Concerned that many drugs would not be evaluated in the voluntary program under the FDAMA, the FDA developed the Pediatric Rule. The Pediatric Rule served as the "stick" to the FDAMA "carrot," mandating that drug companies conduct trials in children if the on-label indication was for a substantial number of children (defined as greater than 50,000 children) or if the drug or

TABLE 66.3-3 SPECIFIC RESTRICTIONS, SUBPART B 45 CFR 46[49]

Research involving pregnant women or fetuses (Section 46.204)	All of the following must be met: Preclinical animal and nonpregnant women studies have been conducted; The risk to the fetus is caused solely by the interventions or procedures that hold out the prospect of direct benefit for either woman or fetus, or the risk is no greater than minimal risk with the prospect of important biomedical knowledge; The risk is minimized for achieving the objectives of the research; The research holds the prospect of direct benefit to the woman, the fetus, or both, or the risk is no greater than minimal risk with the prospect of important biomedical knowledge; If the prospect of direct benefit is solely to the fetus, consent is required of both the mother and father, except under limited circumstances; Both mother and father are aware of potential risks to the fetus or neonate; Children who are pregnant must adhere to the restrictions under Subpart D of 45 CFR 46; No inducements will be offered to terminate the pregnancy; Individuals engaged in the research will have no part in any decision to terminate the pregnancy; Individuals engaged in the research will have no part in determining the viability of the neonate.
Research involving neonates of uncertain viability (Section 46.205(c))	All of the following must be met: Preclinical and clinical trials have been conducted to assess potential risks to the neonates; Individuals providing consent are fully informed of the risks to the neonate; Individuals involved in the research will have no part in determining the viability of the neonate; The IRB determines that (1) the research holds out the prospect of enhancing the probability of survival of the neonate or (2) the purpose of the research is the development of important biomedical knowledge that cannot be obtained any other way and there will be no additional risk to the neonate; Informed consent will be gathered from either parent.
Research involving nonviable neonates (Section 46.205(d))	All of the following must be met: Preclinical and clinical trials have been conducted to assess potential risks to the neonates; Individuals providing consent are fully informed of the risks to the neonate; Individuals involved in the research will have no part in determining the viability of the neonate; Vital functions of the neonate will not be artificially maintained; Research will not terminate the heartbeat or respiration of the neonate; There will be no added risk to the neonate resulting from the research; The purpose of the research is the development of important biomedical knowledge that cannot be obtained by other means; The informed consent of both parents must be sought, except under limited circumstances.
Research involving, after delivery, the placenta, the dead fetus, or fetal material (Section 46.206)	Research with these materials is subject to all state, federal, and local laws and regulations. If identifiable health information of living individuals is used, those individuals are considered human subjects, and all relevant regulations apply.
Research not otherwise approvable that presents an opportunity to understand, prevent, or alleviate a serious problem affecting the health or welfare of pregnant women, fetuses, or neonates (Section 46.207)	The Secretary of Health and Human Services must consult with a panel of experts to determine if the proposed research Presents a reasonable opportunity to further the understanding, prevention, or alleviation of a serious problem affecting the health or welfare of pregnant women, fetuses, or neonates; Will be conducted in accord with sound ethical principles; Will require informed consent.

IRB = Institutional Review Board.

biologic would provide "meaningful therapeutic benefit" over existing treatment.[55] In combination, the FDAMA and the Pediatric Rule resulted in an increase in the number of pediatric trials. The Pharmaceutical Research and Manufacturers of America estimated that in 2002, 194 drugs were being studied in children.[56] In 2002, however, the Pediatric Rule was struck down when the US District Court ruled that the FDA had exceeded its regulatory authority.[57] As a result, pharmaceutical companies need only comply with the voluntary provisions of the FDAMA.

TABLE 66.3-4 ALLOWABLE EMERGENCIES BY THE FDA FOR USE OF AN INVESTIGATIONAL DRUG OR BIOLOGIC WITHOUT PRIOR IRB APPROVAL[62]

Life-threatening situations necessitating use of the test article
If the subject is unable to provide consent
Insufficient time in which to obtain consent from the subject's legal representative
No available alternative method of approved or generally recognized therapy of equal or greater likelihood of saving the subject's life

FDA = US Food and Drug Administration; IRB = Institutional Review Board.

ADDITIONAL REGULATORY AREAS

NIH Policy Guidance on the Inclusion of Children in Research

On March 6, 1998, the NIH published guidance for research conducted with children.[18] This guidance, which defines children as individuals under 21 years of age, requires that children be included in all NIH-supported research unless there are scientific or ethical reasons to exclude them. Hence, the inclusion of children in research is the rule rather than the exception. The justifications for exclusion of children in research are described in Table 66.3-5.[18]

Children's Health Act of 2000 (PL 106-310)

As noted above, this law extended Subpart D of 45 CFR 46 to all agencies of the DHHS. In addition, this broad piece of legislation provided several other pediatric research provisions. One of these provisions is the Pediatric Research Initiative, which authorizes $50 million in funding to support pediatric research by the NIH and to require the FDA to promote the use of clinical trials in pediatric populations. The legislation also creates a pediatric loan repayment program to be administered by the Secretary of Health and Human Services and the director of the NIH for professionals performing pediatric research.[19]

Institutional Requirements and State or Case Law

Although federal law provides the general framework by which human subjects in research are protected, investigators should consider local requirements. State and case laws may provide specific requirements for researchers, particularly those working with vulnerable populations or working in politically charged fields. Similarly, research institutions may have more strict internal policies in place. For example, many institutions extend the requirements of the Common Rule to include all research that is conducted by its researchers rather than restrict the Common Rule to federally supported research. Institutional recruitment and remuneration policies are specific areas that vary widely across institutions.

CHALLENGES TO RESEARCHERS

There are several areas of new and innovative research in which more regulatory guidance is needed. Such research includes broad fetus to adult longitudinal studies, tissue banking, and genetic research, among others. With broad implications for the future of pediatric research and care, it remains unclear how investigators and regulators are likely to proceed in these areas.

Other challenges result from extending human subject protections to fetuses. As discussed above, fetuses are defined as a vulnerable population protected by Subpart B of 45 CFR 46.[49] In addition, in 2002, embryos and fetuses were categorized as human subjects in the charter of the recommissioned Secretary's Advisory Committee on Human Research Protections.[58] The repercussions of these determinations remain unclear.

Another area of concern is the documentation of compliance with existing protections for pediatric populations in research. Similar to studies with adult subjects, two reviews of the pediatric research literature report that in only 52 to 61% of pediatric publications was there proper documentation of appropriate ethical review in the resulting article.[59,60] Sifers and colleagues found that parental permission was documented 41.5% of the time.[61]

Current and future guidance on human subject protections is primarily provided by the DHHS Office of Human Research Protections. Investigators should familiarize themselves with changes in federal, state, and local research policy. By working with vulnerable populations, investigators have a dual responsibility: to protect minor subjects from inappropriate risk in research and to provide pediatric populations with age-specific treatment and care.

RECOMMENDATIONS TO RESEARCHERS

1. Despite difficulties in designing pediatric research, scientifically and statistically sound studies in children can and should be conducted.
2. Consider study designs that maximize benefit to the individual child subject. Minimize risk as much as possible to risk experienced in the subject's daily life.
3. When allowable by the institution, consider remuneration strategies that are age and study appropriate.
4. Consider the most appropriate way to seek a child subject's assent if possible.
5. Review institutional, case, study, and federal policies and restrictions for pediatric research.

TABLE 66.3-5 ALLOWABLE JUSTIFICATIONS FOR EXCLUSION OF CHILDREN IN NIH-FUNDED RESEARCH[18]

The research is considered to be irrelevant to children.
There are laws or regulations barring the inclusion of children to the research.
Data are already available for children elsewhere in this research area.
A separate child-specific study in this field would be more appropriate.
Insufficient data are available in adults to perform the study ethically in children.
The study is designed to collect additional data on an existing adult cohort or
Other special cases, justified by the investigator and acceptable to the review group.

NIH = National Institutes of Health.

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CHAPTER 67

GASTROINTESTINAL ENDOSCOPY

1. *Patient Preparation and General Considerations*

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PATIENT AND PARENT PREPARATION

Anticipation and fear of potentially painful procedures provoke intense anxiety in children. These feelings may be compounded by a child's ability to sense parental anxiety. A detailed description of a procedure to the parents and to the patient in terms that are suitable to the child's stage of emotional and intellectual development may relieve some of this anxiety. The preoperative evaluation might also include a brief tour of the procedure facility. Adequate preoperative preparation may reduce anticipatory anxiety and increase patient and family satisfaction with the child's care. It may even have a positive impact on the amount of medication required for adequate sedation.[1] A child should be encouraged to bring attachment objects such as a favorite stuffed animal or blanket to provide comfort and security as he or she enters the strange environment of a hospital or procedure unit. The endoscopist should inquire about past problems with procedures or sedation, thus identifying medical and emotional risk factors that may guide decisions about the optimal procedure setting, type of sedation, and postprocedure monitoring.

A minor hurdle in providing controlled sedation to a child is the establishment of intravenous (IV) access. Premedication with oral[2] or intranasal midazolam[3] and dermal anesthesia using lidocaine preparations such as EMLA (Astra Pharmaceuticals, Wayne, PA) or Numby (IOMED, Inc., Salt Lake City, UT) can reduce the anxiety and pain of inserting an IV catheter. A topical lidocaine or benzocaine anesthetic applied to the posterior pharynx reduces gagging. Sprays work well with most children. Paste can be applied to the pharynx in infants using the tip of a finger or a soft-tipped applicator. Dimmed lights, soft music, comfortable room temperature, familiar objects, and a steady, soothing voice issuing reassuring comments are other environmental comforts that enhance successful sedation in children.[4] Parents are encouraged to accompany their child into the procedure room and during the initiation of sedation unless their own anxiety is too disruptive or stimulating for the child. They should then be escorted to a waiting area during the procedure. The continued presence of a parent during a procedure under sedation has no proven benefit, and distraction of the endoscopist and staff from their primary task is possible.

INFORMED CONSENT

A thoughtful discussion with the patient or guardian about the potential risks and benefits of a procedure should be the initial step in obtaining informed consent. This discussion should also identify the risks of sedation and include alternatives to the proposed procedure. The important elements of this discussion should then be acknowledged in writing. This process of informed consent enhances a sense of trust between patient and physician and provides legal documentation. Clinical investigators must clearly identify and obtain separate consent for any aspects of a procedure that are being conducted solely for the purposes of research. US federal regulations require that children and adolescents provide their "affirmative agreement" (assent) before they are enrolled in any research protocol.[5] Exceptions to this requirement are (1) children unable to provide assent because of age or cognitive abilities and (2) protocols or interventions that directly benefit the patient and are not otherwise available.

DIETARY RESTRICTIONS

Pre-endoscopy dietary restrictions reduce the potential for aspiration of gastric contents during sedation. Patients with large residual gastric fluid volumes with a low pH are at increased risk for aspiration injury.[6] Dietary guidelines are, therefore, primarily designed to minimize the volume of gastric acid at the time of sedation. High-risk patients undergoing deep sedation or general anesthesia (GA) are sometimes given acid-reducing and promotility medications to further reduce volume and elevate the pH of gastric fluid. Stomach contents may also be emptied through a

nasogastric tube. Conventional recommendations for a prolonged fast prior to sedation have been challenged by two studies demonstrating the adequacy of a brief fast. Ingebo and colleagues demonstrated that the duration of fasting correlates poorly with the volume of retained gastric fluid at the time of endoscopy and that clear fluids empty rapidly from the stomach in children.[7] Schriener and colleagues found no difference in gastric volume or pH in children allowed clear liquids 2 to 3 hours before induction of anesthesia compared with conventional prolonged fasting.[8]

The following presedation dietary guidelines adapted from the American Society of Anesthesiologists[9] (ASA) are recommended by the Sedation Committee at Children's Hospital, Boston:

- Children should be offered clear liquids (this includes breast milk but not other milk or formula) up to 2 to 3 hours prior to sedation to avoid dehydration.
- Infants less than 6 months may receive formula up to 4 to 6 hours and clear liquids up to 2 hours before sedation.
- For patients older than 6 months, solids and nonclear liquids should be held for 6 to 8 hours before sedation.

SEDATION AND MONITORING

Endoscopy with or without Sedation

With the exception of limited flexible sigmoidoscopy, most endoscopic procedures of the upper and lower gastrointestinal tract are associated with periods of mild to moderate discomfort and sometimes intermittent pain. The duration and severity of discomfort and pain vary with the complexity of the procedure, patient anatomy, equipment used, and the skill of the endoscopist. A procedure may be prolonged or aborted if the patient becomes uncooperative or combative owing to discomfort. Although most endoscopists prefer the use of sedation to enhance the technical success of a procedure and minimize unpleasant recall by the patient, some will withhold sedation in selected cases to reduce additional risk, time, and cost.

Gastrointestinal endoscopy has been performed safely in children without sedation.[10,11] This practice is most often advocated for young infants, less than 6 months of age. However, it is difficult to ethically justify withholding analgesic and hypnotic medications from children who undergo potentially painful or frightening procedures regardless of age.[12] The physiologic stress and emotional trauma imposed on an unsedated child during an uncomfortable procedure are difficult to measure accurately, although they may have a negative impact on future medical encounters. Therefore, most pediatricians and parents endorse the use of sedation when it can be administered safely.

Depth of Sedation

Despite more than two decades of experience with pediatric endoscopy, controversy remains regarding the preferred depth of sedation and the level of training required to administer sedative medications.[13–15] Confusion over terminology adds to this controversy.[16,17] Levels of sedation are best viewed as a continuum of states that range from minimal sedation (anxiolysis) to GA. Also, the level of sedation in a given patient may fluctuate over time. Moderate sedation, also called "conscious sedation," and deep sedation are controlled states of depressed consciousness whereby the patient maintains protective reflexes. The former describes a patient who is able to respond appropriately to physical stimulation or verbal commands, a state that is often difficult to ascertain in infants and young children. Patients under deep sedation are less arousable and respond less purposefully to pain. GA is a depressed state of consciousness in which the patient is completely unresponsive to painful stimuli and has lost protective reflexes and safe airway support. Because young children often are more anxious and less cooperative than adults, they generally require a deeper level of sedation, at times bordering on GA, to render them motionless. Some endoscopists prefer to enlist the support of an anesthesiologist or specially trained intensivist to induce the necessary level of sedation for an individual patient, while focusing their attention exclusively on the procedure. Other endoscopists will administer sedatives or supervise nurse-administered sedation.

Sedation Risk Factors

A simple anesthesia risk scale used by the ASA for preoperative assessment classifies patients according to their physical status or the presence and severity of systemic disease (Table 67.1-1). ASA class I and II patients and carefully selected class III patients may be safe for IV sedation without airway intervention. Higher risk category patients require anesthesia with complete airway control.

Among ASA class I and II patients, the endoscopist must also consider other factors when choosing an optimal approach for sedation. A history of noisy breathing, nighttime snoring, or sleep apnea should alert the endoscopist to potential difficulties with airway management. Physical findings that may compromise an airway include obesity, short neck or limited neck extension, small mouth, large tongue, tonsillar hypertrophy, deformed palate, small jaw or limited jaw mobility, and generalized or pharyngeal hypotonia. Other general factors include (1) patient age; (2) the patient's prior experience with sedation; (3) expected duration, complexity, and risks of the procedure; (4) sedation, monitoring, and resuscitation skills of the endoscopist and support staff; (5) efficient use of the endoscopist's time and personnel; and (6) cost containment. Transient nonsystemic or mild systemic conditions that

TABLE 67.1-1 AMERICAN SOCIETY OF ANESTHESIOLOGISTS' PHYSICAL STATUS CLASSIFICATION

CLASS	DEFINITION
I	A normal healthy patient
II	A patient with mild systemic disease
III	A patient with severe systemic disease
IV	A patient with severe systemic disease that is a constant threat to life
V	A moribund patient who is not expected to survive without an operation

affect airway risk must also be considered. Mild upper respiratory tract congestion in an awake patient may result in airway obstruction once sedation has been administered. Therefore, elective procedures in children with mild but unresolved upper respiratory tract infection should be deferred until the congestion has cleared.

The jaw thrust maneuver, an oral airway, or a nasopharyngeal airway may be used to acutely improve a compromised airway. However, children with any airway difficulties should have an anesthesia consultation and probable airway intubation for total control.

Sedation Monitoring

Patients who are sedated require intensive monitoring, additional skilled personnel, and immediately available resuscitation equipment. This requires that one assistant, generally a nurse who is skilled in airway management, devotes exclusive attention to patient monitoring and has no other responsibilities during the procedure.[16] A minimum of two assistants is, therefore, required when sedation is administered for endoscopy in children. One assistant supports the airway, assesses vital signs, and administers medications, whereas the other assists with biopsies or other endoscopic interventions. A third assistant may be needed occasionally to restrain an agitated child.

Essential monitoring equipment includes a transcutaneous pulse oximeter, an electrocardiogram monitor, and a blood pressure monitor, preferably an automated device. Improved technology now permits reliable expired CO_2 or capnography recording in nonintubated patients.[18,19] Although this highly accurate measurement of ventilatory function is now a standard of care for anesthetized patients, its role in monitoring nonanesthetized patients remains to be decided.[9] The endoscopy room must be equipped with a continuous source of pressurized 100% oxygen and additional suction outlets. Resuscitation equipment, including an anesthesia bag with large and small masks, medications, and equipment for airway intubation, and a defibrillator must be immediately available.

Medications

For many years, diazepam, a benzodiazepine, and meperidine, a narcotic, were the principal medications used for endoscopy sedation. Although some centers still rely on these medications, others have converted to newer derivatives, midazolam and fentanyl, respectively. Midazolam, roughly equivalent in potency to diazepam, causes minimal pain during IV infusion and provides excellent amnesia. Midazolam alone causes minor respiratory depression but, similar to other benzodiazepines, significantly potentiates the respiratory depression of narcotics.[20] Fentanyl has 1,000 times the potency of meperidine and offers rapid onset and a short duration of action.[21,22] Less urticaria, less nausea, and fewer dysphoric reactions seem to occur with fentanyl than with meperidine.

Midazolam combined with fentanyl produces in children, as in adults, rapid-onset anxiolysis, hypnosis, amnesia, and analgesia, with a relatively brief duration of action and a comfortable margin of safety against complete loss of consciousness. Following an initial dose, both midazolam and fentanyl are titrated using incremental doses every 3 to 5 minutes until an adequate level of sedation is achieved. Although rarely needed, reversal agents, flumazenil[23] and naloxone, are available for midazolam and fentanyl, respectively. Routine use of flumazenil for children undergoing endoscopy has not shown great benefit.[24] The recommended doses for these medications are listed in Table 67.1-2.

A newer IV sedative-hypnotic agent, propofol, has stimulated considerable interest given its rapid onset and brief duration of action with minimal side effects.[25–29] Propofol is typically used to induce a state of anesthesia. In low intermittent or continuous doses, however, it can be used to achieve deep sedation without GA. Because propofol is associated with a rapid transition to apnea and GA, specialists who are highly skilled in airway management, such as anesthesiologists or intensivists, have generally administered it to children. Yet there is growing interest among adult endoscopists for gastroenterologist-administered or supervised nurse-administered propofol.[30] Although analgesia is less important than sedation for most endoscopic procedures, propofol provides none. Therefore, narcotics or other analgesics should be added to propofol for particularly painful procedures.

The dissociative agent ketamine is another potent sedative that has been administered extensively by nonanesthesiologists to immobilize children. Ketamine possesses a number of properties that are desirable for pediatric endoscopy, including reliable deep sedation, amnesia, and analgesia without cardiopulmonary depression.[31] Enthusiasm for this drug has been tempered by concerns about nightmares and hallucinations, as well as laryngospasm. The former is less of a problem in children than in adults and can be attenuated by premedication with midazolam. The latter problem may result from heightened airway sensitivity and increased secretions.

TABLE 67.1-2 MEDICATIONS FOR SEDATION

MEDICATION	DOSE
Midazolam	Oral, rectal, or nasal presedation: 0.5 mg/kg (maximum dose 20 mg) IV initial dose 0.05 mg/kg (maximum 2.5 mg), then titrate additional doses at 3-min intervals to maximum cumulative dose 0.3 mg/kg or 15 mg, whichever is less.
Fentanyl	IV initial dose 1.0 μg/kg (maximum 50 μg), then titrate additional doses at 5-min intervals to maximum cumulative dose 5 μg/kg or 250 μg, whichever is less
Reversal agents	
Flumazenil	IV 0.01 mg/kg (maximum 0.2 mg); repeat at 1-min intervals to maximum of 0.05 mg/kg or 1.0 mg, whichever is less
Naloxone	IV 1 μg/kg, followed by 2 μg/kg if no response after 90 s, followed by 4 μg/kg if no response after additional 90 s, to a maximum cumulative dose of 400 μg

Clinical Studies

Few studies have prospectively examined the outcomes of sedation protocols for children undergoing endoscopy,[32–35] and comparison between studies is difficult owing to differences in study design. Sedation studies for endoscopy have often relied on potentially biased reporting by participants rather than use blinded observers. Nonstandardized behavioral rating scales have also been used. Sedation study methodology must incorporate blinding, validation, and reliable behavioral measures to provide useful comparative data.[36,37]

Several studies have examined the outcome of IV sedation for children undergoing endoscopy. Balsells and colleagues, at the Cleveland Clinic, reported satisfactory safety and efficacy of IV sedation in a retrospective review of 2,711 total endoscopic procedures (2,026 patients) over a period of 12 years.[38] In this group, only 96 (3.5%) procedures were performed under GA. The combined major and minor complication rate was only 0.3%, with no deaths, cardiorespiratory arrests, or aspiration events. Colonoscopy was completed to the cecum in 80% of cases. Chuang and colleagues, at the Children's Hospital of Philadelphia, reported their experience with IV sedation in 614 children undergoing upper gastrointestinal endoscopy.[35] The study included 150 infants under 1 year of age, and less than 1% of patients had incomplete procedures owing to inadequate sedation. Only 19 (3.1%) patients had prolonged oxygen desaturation that was easily corrected with supplemental oxygen, and there were no major cardiovascular or respiratory complications. Similarly, in a prospectively entered endoscopy database at Children's Hospital, Boston (V. L. Fox, unpublished data, 1994), 22 of 663 (3.3%) children undergoing upper endoscopy with IV sedation developed clinically significant oxygen desaturation sufficient to interrupt the procedure or require supplemental oxygen. Seven patients (1.7%) required supplemental oxygen postendoscopy, and two patients (0.5%) needed transient assistance with oxygen delivered by anesthesia bag and mask. No cardiopulmonary arrests or aspiration events occurred.

Gilger and colleagues detected a high incidence (79%) of cardiac dysrhythmias in a prospective study of 34 children receiving conscious sedation for endoscopy.[39] However, all of the arrhythmias were transient, and none required intervention. Sinus tachycardia was the most common, and most arrhythmias were temporally associated with transient oxygen desaturation. Twenty-two patients (65%) developed transient oxygen desaturation, and three patients (8.8%) required supplemental oxygen to correct desaturation.

Some pediatric endoscopists advocate GA for the majority of their patients. Anesthesia renders the child completely motionless and free of pain, discomfort, or memory of the events. It shifts the burden of responsibility and liability of sedation to the anesthesiologist, freeing the endoscopist to focus exclusively on the procedure. Modern pediatric anesthesia may provide superior sedation with respect to some outcome measures. Lamireau and colleagues performed a limited prospective study comparing cardiovascular and respiratory changes and operator satisfaction in two groups of 18 children (age range 3 months to 6 years) who underwent esophagogastroduodenoscopy under either IV sedation or GA.[40] Patients receiving GA desaturated less frequently, had a more stable heart rate and mean arterial pressure, and had higher operator satisfaction scores than patients receiving IV sedation. Some pediatric endoscopists fear that such deep sedation, however, may allow for serious mechanical trauma from the endoscope in an unresponsive patient, particularly during colonoscopy. Few pediatric data support this fear, although one perforation was reported in a recent study of 136 children undergoing colonoscopy under GA.[41] Stringer and colleagues prospectively evaluated 250 colonoscopies in 215 children, all performed under GA over a 3.5-year period during which they reported no colonoscopy-related complications.[42]

Squires and colleagues, at the Children's Medical Center of Dallas, prospectively evaluated the efficacy, safety, and cost of IV sedation compared with GA in 226 children undergoing endoscopy.[43] IV sedation was used in 103 and GA in 123 patients. No serious complications occurred in either group. Five (4.8%) cases with IV sedation and none with GA were incomplete owing to inadequate sedation. Procedure room time tended to be longer for children undergoing upper gastrointestinal endoscopy with IV sedation compared with GA, and no significant time difference was seen with colonoscopy. The average charge for the GA group was more than double that of the IV group.

INFECTION CONTROL

Patients and staff must be protected against infections that may be transmitted or acquired during an endoscopic procedure.[44] Although the rate of transmitted infection is estimated to be very low (1 in 1.8 million gastrointestinal endoscopic procedures[45]), the risk is always present, and most exposures are preventable by adhering to well-established protocols for equipment reprocessing.[46] Microorganisms may be spread by contact with contaminated equipment, blood, or other body fluids or tissues. Universal precautions should be practiced when handling blood or tissue samples to prevent contact with human immunodeficiency virus (HIV), hepatitis B virus, hepatitis C virus, and other serious bloodborne infections. Use of needleless injection catheters will reduce the risk of needlestick exposure during administration of IV medications. Endoscopy staff should wear protective gloves, moisture-resistant gowns, and eye protection for personal protection and to prevent cross-contamination.[47] Face shields are superior to standard eyeglasses for protection against splash contamination of eyes and other mucous membranes.

Although single-use disposable accessories have become commonplace, concerns about cost containment and the growing burden of medical waste sustain interest in reusable accessories. Once reusable equipment has been cleaned, the level of disinfection is determined by the type of item. Critical use items are devices that enter sterile tissue or vascular spaces, such as needles, biopsy forceps, and electrocautery snares. These items require sterilization,

defined as a process that kills all microbial organisms, including bacterial spores. This is usually accomplished by exposure to heat or ethylene oxide gas. Although endoscope water bottles are not truly critical use items, they are easily resterilized and should be filled with sterile water to prevent waterborne microbial contamination. Semicritical items are devices that contact mucous membranes and nonintact skin, such as endoscopes. These items require high-level disinfection but not sterilization. A variety of chemical germicides are used for high-level disinfection. Glutaraldehyde has been used most commonly for disinfecting gastrointestinal endoscopes, but peracetic acid[48] and hydrogen peroxide have also been used.

There are three important steps in endoscope reprocessing: (1) mechanical cleaning, (2) disinfection, and (3) rinsing and drying. Because biologic debris may be retained inside an endoscope,[49] mechanical cleaning by hand is an essential first step. High-level disinfection may be achieved with immersion in 2% glutaraldehyde solution for a minimum of 20 minutes at a room temperature of 20°C. Then the endoscope must be rinsed free of residual germicide and dried to prevent proliferation of any residual microbes during storage. A final rinse with 70% alcohol facilitates this drying stage. Automated endoscope washers may be used, but they require vigilant surveillance for contamination. Most documented transmitted infections have resulted from a breach in disinfection protocol or a contamination of automated equipment.[50]

ANTIBIOTIC PROPHYLAXIS

Antibiotic prophylaxis (Table 67.1-3) should be used for patients with valvular heart disease, biliary obstruction, or pancreatic pseudocyst. If endoscopy-induced bacteremia or contamination occurred, subsequent infection may be life threatening. Prophylaxis is used to prevent peristomal infection for patients undergoing percutaneous endoscopic gastrostomy.[51] Because there are few data measuring the rates of endoscopy-related bacteremia and infection in children, recommendations for prophylaxis have been extrapolated from adult experience. A low rate of transient bacteremia occurs during most types of endoscopic procedures.[52] Stricture dilation and variceal sclerotherapy may result in higher rates of bacteremia. Bacteremia with colonoscopy in adult patients has been reported in the range of 4 to 5%,[53,54] and infectious sequelae are rarely reported.

There have been two prospective studies in children assessing the rate of bacteremia during endoscopy. Byrne and colleagues studied 75 patients (50 esophagogastroduodenoscopy and 25 colonoscopy).[55] They detected a single episode of bacteremia after esophagogastroduodenoscopy owing to group D *Streptococcus*. El-Baba and colleagues studied 108 patients (68 esophagogastroduodenoscopy, 29 colonoscopy, 11 sigmoidoscopy).[56] Four episodes of bacteremia immediately followed the endoscopy, and all were thought to represent skin contaminants.

Indications for antibiotic prophylaxis must take into account both the rate of bacteremia or bacterial contamination and the vulnerability to severe infectious sequelae for a particular lesion or clinical condition. The American Society for Gastrointestinal Endoscopy (ASGE)[57] and the American Heart Association (AHA)[58] have independently issued updated guidelines for antibiotic prophylaxis. The ASGE guidelines recommend prophylactic antibiotics only for patients with high-risk lesions who undergo procedures with higher rates of bacteremia or contamination. Patients with cardiovascular disease and a history of endocarditis, prosthetic cardiac valve placement, system-pulmonary shunts, complex cyanotic congenital heart disease, and synthetic vascular grafts placed less than 1 year prior, are considered to be at high risk for infectious complications. Procedures associated with a higher rate of bacteremia are esophageal stricture dilation, sclerotherapy for esophageal varices, and endoscopic retrograde cholangiopancreatography with biliary obstruction. In other situations, antibiotic prophylaxis either is not recommended or is left to the endoscopist to choose on a case by case basis. Biliary obstruction and pancreatic pseudocyst are conditions with increased risk for infectious complications following endoscopy and warrant antibiotic prophylaxis independent of endocarditis risk. Infectious sequelae are less likely to occur when adequate drainage has been established. The AHA guidelines tend to be more aggressive than the ASGE guidelines in recommending prophylaxis for moderate-risk cardiac lesions. Major changes in the AHA guidelines include a simplified clinical outcome-based stratification of endocarditis risk categories (Table 67.1-4), elimination of the follow-up dose of antibiotic for oral/dental or low-risk procedures, and elimination of erythromycin as the preferred alternative antibiotic for penicillin-allergic patients.

TABLE 67.1-3 ANTIBIOTIC PROPHYLAXIS

ENDOCARDITIS PROPHYLAXIS
High risk
Ampicillin 50 mg/kg (maximum 2 g) and gentamicin 1.5 mg/kg (maximum 120 mg) IV or IM 30 min before the procedure, followed by ampicillin 25 mg/kg (maximum 1 g) IV or IM or amoxicillin 25 mg/kg (maximum 1 g) PO 6 h afterward
Low risk
Amoxicillin 50 mg/kg (maximum 2 g) PO 1 h before procedure
PENICILLIN ALLERGY
Vancomycin 20 mg/kg (maximum 1 g) IV infused slowly over 1 h beginning 1 h before procedure
Use vancomycin in place of ampicillin or amoxicillin and add gentamicin for high-risk patients
PERCUTANEOUS GASTROSTOMY
Cefazolin 25 mg/kg (maximum 1 g) IV or IM 30 minutes before the procedure
ERCP (for biliary obstruction or pancreatic pseudocyst)
Ampicillin/sulbactam 50 mg/kg (maximum 2 g) IV 30 min before the procedure or cefazolin 25 mg/kg (maximum 1 g) IV or IM 30 min before the procedure

ERCP = endoscopic retrograde cholangiopancreatography.

PROCEDURE COMPETENCE AND TRAINING

Endoscopy in children should be performed by physicians with technical and cognitive skills that are sufficient to complete the task with a high rate of success and minimal

TABLE 67.1-4 CARDIAC CONDITIONS ASSOCIATED WITH ENDOCARDITIS

ENDOCARDITIS PROPHYLAXIS RECOMMENDED
High-risk category
Prosthetic cardiac valves, including bioprosthetic and homograft valves
Previous bacterial endocarditis
Complex cyanotic congenital heart disease (eg, single-ventricle states, transposition of the great arteries, tetralogy of Fallot)
Surgically constructed systemic pulmonary shunts or conduits
Moderate-risk category
Most other congenital cardiac malformations (other than above and below)
Acquired valvular dysfunction (eg, rheumatic heart disease)
Hypertrophic cardiomyopathy
Mitral valve prolapse with valvular regurgitation and/or thickened leaflets
ENDOCARDITIS PROPHYLAXIS NOT RECOMMENDED
Negligible-risk category (no greater risk than the general population)
Isolated secundum atrial septal defect
Surgical repair of atrial septal defect, ventricular septal defect, or patent ductus arteriosus (without residua beyond 6 mo)
Previous coronary artery bypass graft surgery
Mitral valve prolapse without valvular regurgitation
Physiologic, functional, or innocent heart murmurs
Previous Kawasaki disease without valvular dysfunction
Previous rheumatic fever without valvular dysfunction
Cardiac pacemakers (intravascular and epicardial) and implanted defibrillators

complications. The necessary skills are generally acquired through formal training programs in pediatric gastroenterology. Pediatric endoscopy is also performed by pediatric surgeons who are trained in flexible endoscopy and adult gastroenterologists who offer expertise in advanced diagnostic and therapeutic techniques.

The North American Society for Pediatric Gastroenterology and Nutrition (NASPGN) has issued revised guidelines for training in pediatric endoscopy.[59] These guidelines suggest a two-tiered program of training for basic and advanced skills and include a table of minimum threshold numbers (eg, 100 cases each for diagnostic esophagogastroduodenoscopy and diagnostic colonoscopy) that are recommended before competence can be assessed for various procedures. The threshold numbers were adapted from previous ASGE guidelines. The techniques for hemostasis of variceal and nonvariceal bleeding, dilation of strictures, and placement of enteral feeding catheters are listed under advanced training.

The ASGE has also issued updated guidelines for training in endoscopy.[60] The ASGE guidelines were revised after objective evaluation of trainee skills failed to substantiate the previously recommended threshold numbers for assessing procedure competence. Recent data indicate that much higher threshold numbers are required for the average trainee to attain competence. The current ASGE guidelines distinguish between standard and advanced types of procedures and have abandoned threshold numbers in favor of serial assessment of cognitive and technical skills for individual trainees. In contrast to NASPGN guidelines, ASGE guidelines include endoscopic hemostasis, stricture dilation, and enteral access under standard procedures. Technical competence is defined as achieving 80 to 90% technical success with a specific skill such as intubation of the pylorus during esophagogastroduodenoscopy or intubation of the ileum during colonoscopy. Expert endoscopists are more likely to perform at a level of 95 to 100% technical success. Both the NASPGN and ASGE encourage a formal program of endoscopic training with periodic review of each trainee's progress led by a training director with advanced skills in endoscopy.

QUALITY IMPROVEMENT

Quality assurance is a term used to describe programs that identify and rectify problems leading to unacceptably poor performance or outcomes. In contrast, quality improvement is a term applied to programs that evaluate performance with the goal of continuous improvement beyond minimum standards. Endoscopy units are ideally suited to systematic data collection and multidisciplinary peer review, both core elements of a quality improvement program.[61] The procedure record and pathology reports, combined with clinical outcomes, provide a wealth of data that can be easily monitored and evaluated. Computerized reports may facilitate data collection and analysis. Once important clinical indicators are identified, an individual endoscopist or an endoscopy center can be assessed and compared with internal and external standards of care. Some examples of useful clinical indicators include (1) appropriateness of indications for specific procedures, (2) technical performance or successful procedure completion, (3) adequacy and yield of histopathology samples, (4) sedation- and procedure-related complications, and (5) beneficial clinical outcomes. Data can be used for reporting to hospital officials in charge of renewing clinical staff privileges and to agencies that issue institutional accreditation such as the Joint Commission on Accreditation of Healthcare Organizations.

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2. Upper Gastrointestinal Endoscopy

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In the late 1960s, endoscopic equipment saw the development of the fully flexible endoscope. This and further improvements, with smaller diameter and videoendoscopes, made it accessible to pediatrics, allowing its use in almost all ages and weights.[1–4] In 1972, a working group of the European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) convinced Olympus to develop the first pediatric endoscope. A new dimension was introduced 10 years later with the appearance of therapeutic endoscopy.[5] In the meantime, with advances in sedation and the development of specialized care units, it became increasingly distinct from adult endoscopy.[6–10] At present, endoscopy plays a primary role in the diagnosis and management of pediatric gastrointestinal disorders. As endoscopy has become part of the everyday life of the pediatric gastroenterologist,[11,12] guidelines for training minimums to ensure competence have been issued by the North American Society of Pediatric Gastroenterology and Nutrition,[13,14] the Joint Advisory Group, and, more recently, by the endoscopy steering group of the British Society of Paediatric Gastroenterology, Hepatology and Nutrition.

Before the Procedure

An important part of the procedure actually takes place before the endoscopy itself. The need for an upper examination being established, preoperative assessment will provide information on both sides. The endoscopist will inquire about past problems with procedures or anesthesia, as well as a personal and family history, to adjust any aspect of the procedure in consequence (eg, administration of antibiotics, discontinuation of some medications). Examination of the child will reveal unusual bruises suspicious of a coagulopathy, physical attributes such as retrognathia or micrognathia, a short neck that can make intubation difficult, or a loose tooth needing to be removed. From the patients' point of view, they and their parents can address questions and concerns while benefits and potential risks are explained to them. It is essential that they are taken through all aspects of the procedure, not only for legal reasons but also to reduce their anxiety. When possible, a brief visit to the day-care unit and endoscopy suite should be proposed. The rooms are child friendly, with toys, decorations, and videotapes. In fact, good psychological preparation is known to reduce not only the stress but also the amount of sedation needed.[15] Part of this assessment can be performed by a trained nurse.

Consent

Over the years, informed consent has changed from an ethical notion to a legal requirement. Express consent must be obtained from at least one parent or legal guardian, ideally 24 hours before the procedure. Each unit should develop a code of practice suitable to its mode of operation; nevertheless, written should be preferred to oral consent. In some units, a leaflet is distributed containing the main information. It is the physician's duty to provide information about the nature of the procedure, its reason, and its benefits but also the risks, complications, and alternatives to the procedure. Children should be involved in the discussion, encouraging active participation in their own health care at an age-appropriate level of understanding.[16–24] An increasing number of units let adolescents sign their own consent form, although this has no legal value. The endoscopist should countersign to prove that information was provided. In case of intravenous sedation, some units allow one parent to stay during the procedure. When research biopsies are to be collected, a separate consent should be signed after approval by the institutional review board or local ethical committee.[17,25–30]

Preparation

The patient should fast to minimize the risk of pulmonary aspiration. Recognized as a cornerstone in safe practice, the duration of the fasting itself has not reached worldwide consensus, and every institution follows its own protocol.[31] In an attempt to rectify this, guidelines have been established,[32,33] and we recommend that gastroenterologists read those published in the country of practice. However, some general lines can be drawn: the conventional 8-hour fast applied in adult practice is suitable only for the older child. General consensus allows a last feed 4 hours prior to sedation in those 0 to 5 months old. Some institutions are more flexible and allow clear fluid, including breast milk, to be given up to 2 hours prior to sedation. In older children, feeds will be stopped 6 hours before sedation.

Adequate venous access is important not only for administration of medication but also in case quick rever-

sal is needed. It is good practice to check patency just before the start of the procedure.

DURING THE PROCEDURE

Monitoring

At present, pediatric patients undergoing an endoscopy must have some sort of sedation. The use of the different agents available is discussed below; however, all can cause side effects and, at times, unpredictable reactions. Therefore, cardiopulmonary monitoring is essential to provide optimum safety and conditions for both the patient and the endoscopist.[34] The basic equipment includes a pulse oximeter, to monitor oxygen saturation and heart rate, a pressurized oxygen source, and a suction outlet. A ventilation bag with age-appropriate–sized masks and suction tubes should be ready and checked before the start of each procedure. Most equipment also includes a blood pressure monitoring device. In addition, an immediately accessible resuscitation trolley is an essential element. The last important factor for the comfort and safety of the patient is the room temperature, which should be adjustable and appropriate for the age to avoid hypo- or hyperthermia. The safest practice is that one person, a nurse or a physician, trained in pediatric life support is fully dedicated to the vital signs surveillance throughout the procedure.

Sedation

Potential complications of upper endoscopy among children must not be underestimated and are related to both endoscopy methods and the medications used for sedation. The most frequent complication in endoscopy is slight to profound hypoxemia, which is mainly due to sedation, resulting in a dispute between the advocates and opponents of the need for sedation in adult cooperative patients. Unsedated upper endoscopy in children has also been documented and judged to be safe and feasible in motivated children.[35] However, 80% of these patients would choose to have sedation if upper endoscopy was to be repeated.

From a pediatric point of view, there is no doubt that children are unable to express their pain and fear. Consequently, the rationale and aims of sedation are evident in pediatrics and are shown in Table 67.2-1. At present, this attitude is shared by both American[36] and English[37] adult gastroenterologists because only 2% of physicians perform endoscopy without any medication in these countries. Furthermore, sedation must be administered to all pediatric patients, especially in infants and young children,[38] because pain is a real problem in medically and surgically treated pediatric patients, even neonates.[39]

TABLE 67.2-1 AIMS OF SEDATION IN PEDIATRIC GASTROINTESTINAL ENDOSCOPY

To give maximal comfort to the child during endoscopy
To aim for amnesia of endoscopy
To facilitate quick, precise, and safe endoscopy
To use safe and short half-time agents that provide a rapid onset and effective sedation with minimal side effects

Preparatory information and psychological preparation are mandatory before esophagogastroduodenoscopy (EGD). Indeed, they can reduce anxiety and procedural distress in both adults and children.[40] Furthermore, less sedation seems to be necessary after good psychological preparation.[41] In addition, a calm and relaxed atmosphere in the examination room is recommended. In our experience, hypnosis can also be of great help to patients who need repeated procedures.

Local anesthetic agents are widely used by adult[37] and pediatric gastroenterologists. Local anesthesia can be a good complement to intravenous sedation. Indeed, the amount of medication necessary seems to be reduced owing to the lack of pharyngeal stimulation. In our experience, this positive effect is correlated to the level of sedation depth, being more effective in the "lighter" sedation regimen. When used, local anesthesia with lidocaine, without exceeding a dose of 5 mg/kg body weight, can be recommended.

As regards pharmacologic sedation, the most important goals are maximal comfort, endoscopy amnesia, and a short-term effect drug, leading to short time of onset of sedation, length of procedure, and recovery time. The widespread use of sedation in EGD in children imposes the need for resuscitation and monitoring equipment in the endoscopy room. Standard monitoring must include an electrocardiogram, a blood pressure monitor, and a pulse oximeter. Pediatric anesthesiologists are becoming increasingly involved in this process because the risk of disaster is great when a child is sedated and scoped by the same person.

The types of sedation can be described as follows:

- Intramuscular "lytic or so-called Toronto cocktail"
- Intravenous diazepam or midazolam alone
- Intravenous diazepam or midazolam with meperidine, fentanyl, or alfentanil (± atropine)
- Intravenous propofol
- Miscellaneous drugs
- Endotracheal anesthesia

Half-life time and bolus dose of drugs used for sedation are shown in Table 67.2-2. It is important to note that half-life times are often different between children and adults.

Historically, the combination of intramuscular meperidine, promethazine, and chlorpromazine, the so-called "lytic or Toronto cocktail,"[42] was used for a large variety of pedi-

TABLE 67.2-2 HALF-LIFE TIME AND BOLUS DOSE OF DRUGS USED FOR SEDATION DURING ESOPHAGOGASTRODUODENOSCOPY

DRUG	$T^1/_2$ (H) CHILD 3–10 YR	$T^1/_2$ (H) ADULT	IV BOLUS DOSE
Diazepam	7–18	20–50	0.1–0.2 mg/kg BW
Midazolam	1.2	2–3	0.1 mg/kg BW
Meperidine	1.5–4	2–5	1–2 mg/kg BW
Fentanyl	1.1	2.5–5	1–3 µg/kg BW
Alfentanil	1.0	1.6	7.5–10 µg/kg BW
Ketamine	2.2	2.5–2.8	2 mg/kg BW
Propofol	0.5	4–7	2–3.5 mg/kg BW

BW = body weight; IV = intravenous.

atric procedures. Nevertheless, the efficacy of its sedative effect is limited in EGD, and an intravenous sedation must be favored. Benzodiazepines were the most routinely used method of sedation because of their anxiolytic, sedative, amnesic, and muscle-relaxing properties. Among them, diazepam was the usual choice for many years; however, its half-life time is long, discouraging its use in newborn and young children. Moreover, diazepam has respiratory-depressing effects, especially when combined with narcotics.[43] At present, midazolam replaces diazepam because it has more sedative potency, more anterograde amnesic activity, and fewer local effects at the injection site.[44] In addition, midazolam has a short time of uptake and elimination. The effective bolus dose is of 0.1 to 0.2 mg/kg body weight. It also has a potent respiratory negative effect, which is dose dependent and enhanced by concomitant use of opioids. To shorten the total time of EGD, some adult gastroenterologists recommend reversing the effect of midazolam. However, this would not seem necessary in children because they recover rapidly after correct medication.

Opioids have also been used for many years and are synergistic with benzodiazepines.[45] They are useful in providing analgesia and sedation, as well as in decreasing the need for other drug requirement and allowing smooth recovery. Their disadvantages include nausea, vomiting, respiratory depression, hypotension, dysphoria, hallucinations, and bile duct spasm. Meperidine is a long-acting narcotic analgesic that is commonly used in pediatric EGD even if it does not represent a modern anesthesiologist approach.[46] This is explained by its large safety margin in the inexpert hands of endoscopists. Doses for intravenous sedation are a bolus of 1 to 2 mg/kg body weight and should then be titrated. Meperidine is generally used with benzodiazepine,[44,47] and 80% of patients have total amnesia after the procedure.[48] As a combination of drugs was advocated to improve the quality of sedation, other drugs were tested alone or in combination. Among them, fentanyl (1–3 µg/kg body weight) and alfentanil (7.5–10 µg/kg body weight) were studied because of their quicker effect. In a study, we compared a combination of alfentanil-midazolam with meperidine-midazolam.[38] Alfentanil-midazolam had a shorter sedation time and recovery period, with a lower risk of hypoxemia.

The recent development of propofol, a sedative-hypnotic agent, has led to an improved quality of sedation during pediatric EGD.[49] The main advantages of this drug are rapid recovery, less prolonged sedation, and a lower incidence of nausea. The dose is 1 to 3.5 mg/kg body weight as a bolus, followed by a titrated infusion of 0.1 to 0.3 mg/kg body weight/min. Propofol can be used in association with benzodiazepine.

Other drugs have been proposed: intramuscular ketamine, especially in children older than 7 years,[50] or inhaled anesthetic gas. Chloral hydrate and atropine are frequently used in addition to a standard regimen of sedation.

Finally, endotracheal anesthesia is rarely performed because effective deep intravenous sedation is satisfactory in most cases. It can be used for young children (< 2 years), for patients who undergo upper and lower endoscopy in the same session, or for interventional procedures.

Antibiotic Prophylaxis

A transient bacteremia seldom occurs during a procedure and is usually cleared spontaneously by healthy patients.[51] The reported incidence ranges from 0.5 to 4.2% for diagnostic EGD.[51–55] It is important to distinguish diagnostic endoscopy from therapeutic endoscopy, which carries a higher incidence: 8.9% for variceal ligation, 11% for endoscopic retrograde cholangiopancreatography, 15.4% for variceal sclerotherapy, and 22.8% for esophageal dilation.[53,56]

The incidence might even be lower in the pediatric population as shown by the two prospective studies carried out to determine the incidence of bacteremia that identified 1 episode in 75[54] and 4 probable contaminations in 108 children.[57] Neither mucosal biopsy nor polypectomy seems to be a risk factor.[58,59] These episodes of bacteremia are asymptomatic and short-lived. The most feared complication, endocarditis, is reported in a few patients, [60,61] mainly those with risk factors.[52,54,56,62–65] Antibiotic prophylaxis has been demonstrated to be efficient and to diminish incidence by 49%.[66] Over the years, the number of patients with a cardiac implant has steadily increased, yet no evidence of any increase in the incidence of endocarditis has been reported. To answer the question of which patient should benefit from prophylaxis, guidelines have been established by several organizations.[67–74] On average, about 1 to 3% of patients undergoing endoscopy will need prophylaxis.[75] Despite these publications, inappropriate use of antibiotics reaches up to 90% of cases, with a tendency to overtreat patients, [76,77] although failure to treat is also reported.[78] The institution of a mechanism for continuous quality improvement, as simple as monthly meetings, could significantly reduce overprescription by half.[76]

Most pediatric centers use protocols following the guidelines issued by the American Heart Association (AHA) and the American Society of Gastroenterological Endoscopy (ASGE)[68,79] recommending administration of prophylactic antibiotics only in selective situations.[74] Specific clinical conditions recognized as such are cardiac lesions with high or moderate risk of bacterial endocarditis.[80–83] Durack has established a list of high, moderate, and low risks, which are summarized in Table 67.2-3.[51] Available recommendations are not standardized, with the ASGE proposing prophylaxis only in high-risk patients, whereas, recently, the AHA proposed a simplified regimen for moderate-risk patients. It consists of dropping the gentamicin and the second dose of antibiotics. The latter dose is thought to be unnecessary because prolonged serum levels above the minimal inhibitory concentration are obtained with amoxicillin.[84,85] Whereas the parenteral route is recommended in the first group, oral administration is proposed as a choice in the latter. Mitral valve prolapse is addressed specifically by Dajani, and physicians treating such a patient should refer to his publication.[70]

Other special conditions are immunocompromised, either neutropenic or immunosuppressed, children. Neutropenia, depending on the level of cells, is seen as either a high (neutrophils < 100×10^9/L) or a moderate (neutrophils 100–500×10^9/L) risk in Europe[73,86] but not in America and Canada.[68] An American-Canadian survey of

TABLE 67.2-3 CARDIAC INFECTION RISK LEVEL

HIGH RISK	MODERATE RISK	NEGLIGIBLE RISK
Prosthetic valve	Congenital malformations	Isolated ASD
Surgically constructed pulmonary shunt or conduit	Acquired valvular dysfunction	Surgical repair ASD, ventricular septal defect, patent ductus arteriosus
Previous endocarditis	Hypertrophic cardiomyopathy	Pacemakers
Complex congenital cyanotic heart disease	Mitral valve prolapse with regurgitation and/or thickened leaflets	Physiologic or functional heart murmurs
		Previous Kawasaki disease or rheumatic fever without valvular dysfunction

ASD = atrial septal defect.

current practice in 15 pediatric gastroenterology centers showed that only 20% give antibiotics for upper endoscopy and 33% for lower endoscopy, yet no data on the rate of infectious complications were reported.[74] Patients carrying a central line, a ventriculoperitoneal shunt, or an orthopedic implant are also problematic patients, for which no recommendations are published. In the same study, current practice indicates that 20% will give antibiotics for upper endoscopy and 40% for lower endoscopy.[74] The final decision should be made case by case, leaving room for clinical judgment and experience.

Gram-positive bacteria are the most commonly implicated germs. Therefore, in case of cardiac risk, the recommended regimen is as follows[73,74]:

- A combination of ampicillin (50 mg/kg, maximum 2 g) and gentamicin (2 mg/kg, maximum 120 mg) intravenously or intramuscularly 30 minutes prior to the procedure. Some centers will give an additional oral dose of amoxicillin (25 mg/kg) 6 hours later or ampicillin (25 mg/kg, maximum 1 g) intravenously.
- In patients allergic to penicillin, amoxicillin is replaced by vancomycin (20 mg/kg, maximum 1g) or teicoplanin (6 mg/kg).

In addition to cardiac risks, several conditions need special attention:

1. In neutropenic patients, infections can be caused by anaerobes; as a result, metronidazole (7.5 mg/kg) is added to the above regimen.
2. In patients with central lines, prosthetic material (especially orthopedic), ventriculoperitoneal shunt, or ascites,[87] some centers recommend the use of prophylactic antibiotics.
3. During interventional procedures (stricture dilation, sclerotherapy, percutaneous endoscopic gastrostomy [PEG]), a prophylaxis is mandatory because the risk of complication following infection is higher. For example, the rate of skin infection is higher in PEG without antibiotic prophylaxis.[88]

AFTER THE PROCEDURE

Monitoring

The monitoring set during the procedure will be continued, particularly for small infants for the next 15 to 30 minutes, either in the recovery room or on the ward. If asleep, the patient is placed in the lateral position. If midazolam was used, the child stays on the ward for a minimum of 2 hours. Parents are informed that amnesia is a common effect of the drug and can last up to 8 hours. Drinks are allowed 1 hour after the end of the endoscopy; special attention is brought to the deglutition if anesthetic spray was used to avoid choking.

Cleaning the Scopes

Contaminated equipment can transmit infection either from one patient to another or, less commonly, to staff. Implicated germs can be either pathogenic or opportunistic in the case of the immunocompromised patient. Proper and adequate cleaning is a major concern for the patient's and staff's safety. Thus, guidelines have been formulated and modifications made in recent years by several societies.[67,89–92]

Legal Rules. Endoscopes, accessories, disinfectants, sterilizing devices, and washing machines are submitted to local laws. For example, in Europe, they must be in conformity with the European rules.

Levels of Disinfection. Three categories to which medical devices can be assigned are critical, semicritical, and noncritical. Noncritical items (eg, floors, walls, blood pressure cuffs, and furniture) come into contact with intact skin but not with mucous membranes. Semicritical items are devices that come into contact with intact mucous membranes or skin that is not intact. In general, these items should be free of all microorganisms, with the exception of small numbers of bacterial spores. The flexible scopes (gastroscope, duodenoscope, and colonoscope) are referred to as semicritical or class II. They need a high level of disinfection with mycobactericidal, viricidal, and sporicidal activity, the necessary exposure time being determined by the time it takes to inactivate 10^6 resistant non–spore-forming test microorganisms, including, for example, hepatitis B virus, human immunodeficiency virus (HIV), and *Mycobacterium tuberculosis* var. bovi. Items assigned to the critical category present a high risk of infection because they penetrate skin or mucosa or come into contact with normally sterile tissues or the vascular system. They need sterilization when possible; if not achievable, liquid chemical germicides can be used with long exposure. Accessories are defined as such; therefore, grasping forceps, polypectomy snares, injection needles, and cytology brushes should be sterilized or disposed of.

Disinfectants. Two percent glutaraldehyde is the most widely used product. At room temperature, it inactivates most bacteria in 1 minute, HIV in 2 minutes, and hepatitis B virus in less than 5 minutes.[93–98] For hepatitis C virus, the risk persists because ribonucleic acid can be present in

the operating channel and on the biopsy forceps, and a longer washing time (20 minutes) is necessary.[95,96,99] The same washing time will eliminate microbacteria, in particular *M. tuberculosis*. Although this risk has not been described in digestive endoscopy, it is important to remember that some equipment can be shared with bronchoscopes, a device that can be in contact with such germs.

Glutaraldehyde creates frequent adverse reactions that can be severe. Reports of allergy, dermatitis,[100] conjunctivitis,[101] and rhinitis and asthma [102–104] in staff and colitis in patients, owing to improperly cleaned scopes, have been made.[105,106] To limit these disadvantages, procedures take place in a dedicated room, well ventilated with a minimum of 12 volumes/hour.

A suitable alternative is peracetic acid (0.2–0.35%), which has the advantage of being less of an irritant; however, it is more expensive and less stable than glutaraldehyde. It acts by releasing free oxygen and hydroxyl radicals. It has rapid activity against vegetative bacteria, mycobacteria, fungi, and viruses.[107–109] It can cause cosmetic damage to the scopes, but without functional alterations. It can be used in association with hydrogen peroxide (7.5%). It has bactericidal and viricidal activity after 5 minutes and kills spores and mycobacteria in 10 minutes.

Chloride acid and superoxide water are highly microbicidal and are used in some units. The ASGE recommends a final rinse with 70% alcohol for its drying effect on the channels.

Tap water is usable for manual washing of semicritical scopes, but sterile water is necessary for the cleaning of critical ones. For the washer or disinfectors, the water has to be of level II (< 10 opportunistic microorganisms/100 mL at 22°C and 37°C, without *Pseudomonas aeruginosa*). This quality of water is obtained by using filters (0.2–5 µm).

Biologic Controls. Hospital tap water should be controlled on a regular basis, in particular for the presence of *P. aeruginosa*. Final rinse of the scopes requires a microbiologic quality of level I: < 100 opportunistic microorganisms/100 mL at 22°C and 37°C, without *P. aeruginosa* per 100 mL.

Regular checking (once per trimester) of the scopes is necessary by swabs at the level of the canals, exits, outer sheath, and biopsy cap. This allows the identification of poor technique and the modification of clinical practice.[110] In case of contamination after glutaraldehyde sterilization, 50 mL of a sterile mixture containing Tween 80–lecithin is injected in each canal or 200 mL from a common irrigator. If the bottles of liquid lavage, the cleaning water, and the knobs are autoclaved, the vehicle responsible for the contamination is often the biopsy channel. A new washing cycle needs to be performed, including an alkaline enzymatic washing product for 15 minutes and a phase of sporicidal disinfection for 60 minutes. If the problem persists, the scope should be sent to the manufacturer, which will decide if the internal sheaths need to be changed. The remaining water in the washer or disinfectors, along with the incoming water, needs to be checked monthly.

Flexible endoscopes cannot be autoclaved. For the material that tolerates it, water vapor at a temperature of 134°C for 18 minutes should be used after a wash without glutaraldehyde.

Recently, there has been concern about Creutzfeldt-Jakcob disease (CJD) and neurodegenerative diseases in general. Their causative agent, the prion, is resistant to most physical and chemical inactivation methods; therefore, endoscopy should be reconsidered and avoided whenever possible. In pediatric endoscopic procedures with no potential risk, the above disinfection rules are estimated as sufficient by most sanitary authorities. On the contrary, the recommendation from the Gastroenterologists Working Party of the British Society of Gastroenterology Endoscopy Committee is to destroy and incinerate material used in a patient with definite disease because no safe way to guarantee prion eradication exists.[111–113] This point of view is more severe than the recommendation from infectious disease specialists suggesting that standard cleaning and high-level disinfection protocols would be adequate for reprocessing.[114] In patients suspected of CJD, no endoscopy should be scheduled before confirmation of the disease. Any patient presenting with neurologic signs or recent progressive dementia should be suspected of CJD. In patients at risk of CJD (having received extracted growth hormone or gonadotropin, a family member of a potential or confirmed case, neurosurgical intervention before 1994), the washing of the scope includes two cycles with alkaline without aldehyde for a minimum of 15 minutes before a rinse with tap water and then disinfection with glutaraldehyde. The infectivity of the variant CJD seems to affect the peripheral tissues before the brain. The protein prion (PrPsc) has been found in lymphoid tissues (tonsil, rectum,[115,116] appendix[117]). Although declared cases are rare (less than 150 people globally),[118] the endoscopy remains a risk for infection, and a supplementary chemical inactivation after the two added washes is recommended. Its efficacy on prion has not been proven. Sodium hypochlorite at 6°C or bleach can be used if it does not damage the scopes.

Cleaning of the Scopes. M*anual*. Because nonimmersible endoscopes have practically disappeared from practice, only the steps of cleaning and disinfection of immersible ones are addressed in this section. Disinfection is carried out at the start of the session, between cases, and at the end of the day. Scope attribution is decided before the start of the list to allow alternation and diminish waiting time and stress.

Pretreatment takes place in the endoscopy room. Still connected to the light source, air and water channels are flushed with water for at least 15 seconds to expel all organic material using a special valve supplied by the manufacturer for this purpose. The aim is to thoroughly remove all organic material and blood prior to any contact with disinfectant. Premature contact with the latter will fix the proteins, creating debris in which organisms can become imbedded and keeping them out of reach. All channels are flushed with a detergent solution, without aldehyde. The instrument is then disconnected, and caps are fitted when required (videoendoscopes). A cleaning

brush is passed through the suction or biopsy channel until it comes back visually clean.

The instrument is tested for leaks and checked for faults or damage before being fully immersed in neutral or enzymatic detergent. All removable parts (water or air, suction valves, biopsy cap) are removed, the scope is washed, and the distal end, knobs, and valves are individually scrubbed with a soft toothbrush. An all-channel irrigator is put in place, and the channels are irrigated with the solution (minimum of 150 mL) for at least 5 minutes. This step is crucial because it markedly reduces microbial contamination load[119,120] and contamination of multiple-use forceps.[121] The scope and accessories are rinsed under fresh tap water and the channels are irrigated (minimum 300 mL) before air is insufflated to remove fluid residue. All of the equipment is immersed in the disinfectant for the correct contact time, depending on the level of disinfection that needs to be achieved: with 2% glutaraldehyde, according to the ASGE and the French Society of Digestive Endoscopy (FSDE), 10 to 20 minutes for intermediate level and 60 minutes for high level, and, according to the ASGE, FSDE, and the Asia-Pacific Congress of Digestive Endoscopy, 10 to 20 minutes before/after storage[92,122–125]; with peracetic acid, 5 minutes for intermediate level and 10 minutes for high (sporicidal) level.[113,124] The dilution needs to be respected and not fall below 1 to 1.5%.[114] Endoscopes and valves are rinsed with a large volume of water, 300 mL passed through the channels to avoid toxic reactions (colitis). Tap water, preferably with filter, is used when an intermediate level of disinfection is necessary and sterile water if a high level is wanted. Once more, the agent is eliminated by air insufflation. Endoscopes are connected to the light source and forced air-dried. They are then stored hanging vertically in a designated ventilated cupboard. Valves and the biopsy cap are removed and lubricated with silicone oil.

Disinfectant solution needs to be changed depending on the physicochemical stability of the product itself and the number of procedures carried out. Any cloudy solution should be renewed.

Automated Washer and Disinfectors. These machines are being used increasingly in the endoscopy suite. They need regular maintenance and control. These machines have the advantage of providing standardized disinfection and rinse, heat to optimize the process, filtered tap water, automatic record of the washing parameters, and a closed system diminishing exposure to fumes. It should be stressed that some crucial cleaning steps still need to be performed manually: the cleaning itself, the check for leaks, the alcohol rinse, and forced air drying.

Accessories. Reprocessing of these medical devices is a subject of discussion. The complexity of the material (crevices, wire coils, retractable components) and the fact that they breach mucosa classify them as critical. Therefore, they need to be sterilized or disposable. The standardized protocol for reprocessing has been shown to be reliable.[126] Recommendations are that single-use accessories are encouraged when available. Reprocessing and reuse of medical equipment intended for single use has clinical and legal implications.[127] Some working groups propose that consent should be obtained if reuse is intended.[123] However, the cost implications also raise some concerns.[128]

Some accessories that are difficult to clean can be ultrasonically (> 30 kHz) cleaned prior to disinfection or autoclaving. Sterilization should be achieved with water vapor at 134°C for a minimum of 18 minutes, the so-called "prion cycle."

Environment. Owing to common adverse reactions to glutaraldehyde, specific criteria relating to exposure levels have been established. They are defined in terms of average exposure standard and maximum exposure level, which are issued in most countries. The maximum exposure level is 0.05 ppm of glutaraldehyde in the United Kingdom and 0.2 ppm in France.

Staff. Endoscopy staff should be trained not only in the correct use of the equipment but also in case of spillage. All staff coming in contact with glutaraldehyde need to complete a health questionnaire and perform yearly lung function tests. Gloves and disposable aprons should be worn and changed regularly because they absorb the substance. Staff are also strongly advised to be vaccinated against hepatitis B.

INDICATIONS

The indications vary with age because newborn babies do not present with the same pathologies as adolescents. Table 67.2-4 shows the indications divided into three age groups.

Gastroesophageal Reflux

One of the major indications for EGD is gastroesophageal reflux (GER) and its complications. GER is defined by the involuntary passage of gastric content into the esophagus. The main pathophysiologic cause of GER is transient relaxation of the lower esophageal sphincter. Patients can present with dysphagia, odynophagia, hematemesis, anemia, weight loss, failure to thrive, epigastric or retrosternal pain, unexplained vomiting, spitting, or irritability depending on their age. Furthermore, abnormal anatomic conditions, such as hiatal hernia, can lead to GER. The diagnosis of hiatal hernia is made when the ascension of

TABLE 67.2-4 INDICATIONS OF ESOPHAGOGASTRODUODENOSCOPY

NEONATES AND INFANTS	TODDLERS	TEENAGERS
Vomiting	Abdominal pain	Abdominal pain
Hematemesis, melena, hematochezia	Hematemesis, melena	Dyspepsia
Apnea	Vomiting	Hematemesis, hematochezia, melena
Failure to thrive	Dysphagia, odynophagia	Weight loss
Diarrhea	Foreign body	Chronic reflux symptoms
Irritability, Sandifer syndrome	Caustic ingestion	Chronic diarrhea
	Chronic diarrhea	Iron deficiency anemia
	Chronic constipation	Caustic ingestion
	Suspected polyp	Cancer surveillance

the Z line is observed more than 2 cm above the esophageal hiatus. Although in newborns, the junction between the pale pink esophageal epithelium and the darker red gastric one takes place at the level of the diaphragmatic hiatus (pinch), with age, it has a tendency to move upward. The Z line is slightly irregular or undulating. The disappearance of multiple small linear vessels at this level helps to identify it. In moderate to severe hiatal hernia, the mucosal gastric folds slide above the diaphragmatic pinch, giving an aspect of pouch (Figures 67.2-1 and 67.2-2). With a deep breath, this becomes even more apparent as the folds move over the hiatus, particularly in retrovision.

The diagnosis of peptic esophagitis is made endoscopically. The typical lesion is a round or linear erosion with focal erythema, exudate, or ulceration usually perpendicular to the longitudinal axis. It is always localized in the lower third of the esophagus. When multiple lesions are present, they can converge and give a stellar shape. The response to treatment and prognosis depends on the gravity of the lesions seen. Thus, it is crucial to evaluate the severity of the esophagitis, which is graded from 0 to 5 (Table 67.2-5). Grade 0 corresponds to a normal esophagus; grades 1A, 1B, and 1C to reflux lesions; and above grade 2 to different severity of esophagitis. It is important to avoid confusion of grade 2 with the normal circumferential cardial redness seen in the newborn (Figures 67.2-3 and 67.2-4). This characteristic aspect is due to specific distribution of intramural veins running in the lamina propria at the level of the upper esophageal sphincter. These vessels can be seen only with the new generation of video-endoscopes.

The exact localization of the lesion is noted. The importance of biopsies remains a subject of controversy because good-quality material is difficult to obtain.[129,130] The percentage of uninterpretable biopsies can be as high as 59% for grasp and 23% for suction. With an adequate technique, good mucosal biopsies can be obtained with grasp forceps and evaluated under a microscope. Erosions and ulcerations associated with an inflammatory infiltrate are seen only in patients suffering from severe esophagitis. In fact, it is useful to take biopsies in a patient suspected of GER with a macroscopically normal esophagus. Two biopsies will be taken 2 or 3 cm above the Z line and fixed in formol.[131] The ESPGHAN has established criteria to help the correlation of histologic findings with diagnosis (Table 67.2-6). The presence of basal zone hyperplasia of the epithelium, elongated stromal papillae, and vascular ingrowth reflects reflux, whereas inflammatory infiltrate, ulceration, or modification of the epithelium confirms a microscopic esophagitis.[129,131–134]

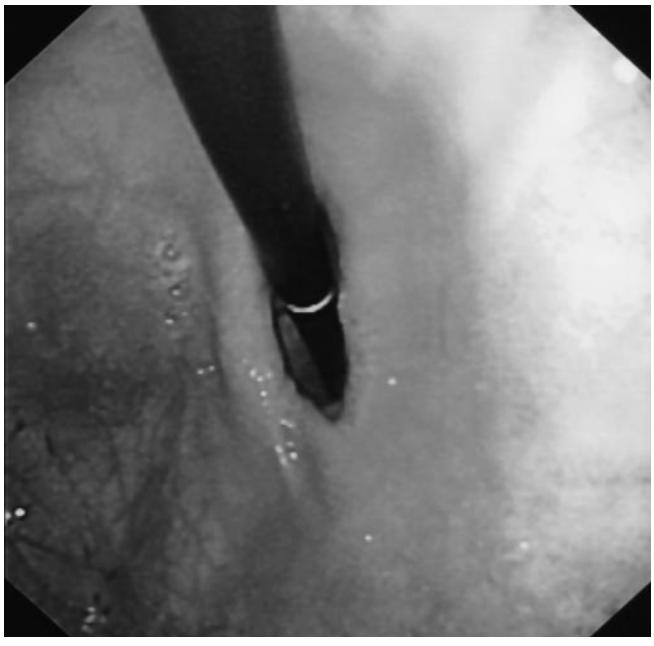

FIGURE 67.2-1 Hiatal hernia in retroversion.

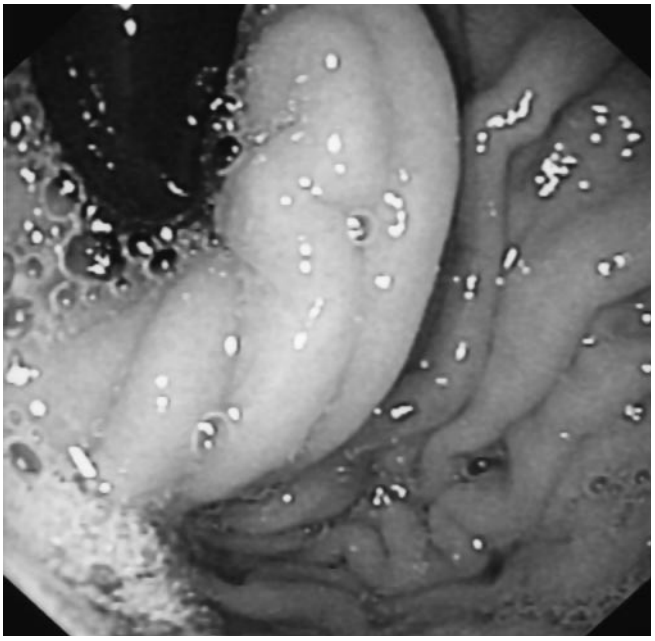

FIGURE 67.2-2 Nissen fundoplication in retroversion.

Endoscopic examination is the only way to diagnose and establish the extent of Barrett esophagus. The typical lesion is an orange-pink spot standing out on the gray-white squamous mucosa (Figure 67.2-5).[135] Because it is frequently described in published pediatric series, it should be suspected in cases of peptic stenosis. Its existence is possible when the Z line is more than 3 cm above the superior limit of the gastric folds, with or without the presence of hiatal hernia. The use of vital colorants, such as Lugol or

TABLE 67.2-5 ESOPHAGITIS GRADE

GRADE	ASPECT
0	Normal mucosa
1	Unique or multiple, nonconfluent erythema, loss of vascular pattern or exudate appearing as red patches or stiae
2	Longitudinal noncircumferential erosions with a hemorrhagic tendency of the mucosa, with friability (bleeding to light touch: 2A) or spontaneous bleeding (2B)
3	Identical lesions with circumferential tendency
4	Ulcers, metaplasia (4A) or stricture (4B)
5	Endobrachyesophagus associated with any of the above lesions

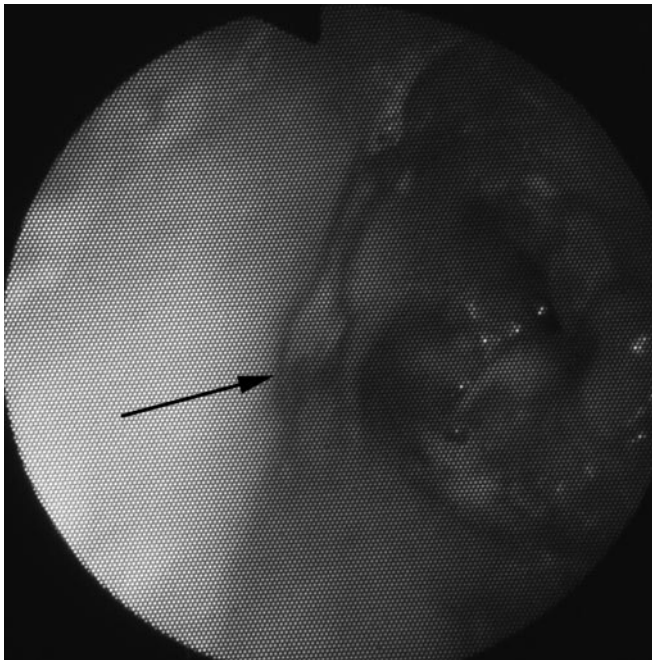

FIGURE 67.2-3 Grade II esophagitis with stellar ulcer (*arrow*).

TABLE 67.2-6 HISTOLOGIC AND DIAGNOSTIC CORRELATION

GRADE	HISTOLOGY	DIAGNOSIS
0	Normal mucosa	Normal esophagus
1A	Basal zone hyperplasia	Reflux
1B	Elongated stromal papillae	
1C	Vascular ingrowth	
2	Inflammatory infiltrate of the lamina propria	Esophagitis
3	Inflammatory infiltrate of the epithelium	
4	Ulceration	
5	Aberrant columnar epithelium	

methylene blue,[136–141] has been proposed. These methods do not help in the most important difficulty: defining the exact position of the esogastric junction. As a time-consuming method, it is not recommended. In conclusion, the diagnosis of Barrett esophagus is made with certainty only when multiple, large biopsies are taken under direct vision and a detailed histologic map is established.[142] The evaluation of the severity helps in predicting healing rate with medication.[143,144] When stenosis is present, biopsies are made only after dilatation. The risk of adenocarcinoma exists (one case was reported in an 11-year-old child[142]) and imposes a surveillance with an EGD and multiple biopsies every 2 years after 10 years of age. As a matter of fact, upper endoscopy is a crucial tool in the diagnosis and follow-up of peptic esophagitis and Barrett esophagus.[145]

Upper Digestive Hemorrhage

There is little doubt that EGD has a superior yield to barium meal in localizing an upper digestive hemorrhage,[146–148] identifying the lesion in more than 80% of cases.[148,149] Ideally, endoscopy is performed in the 6 to 12 hours after stabilization of the patient. In the case of severe hemorrhage, surgeons should be informed and ready to intervene. In young individuals without signs of active bleeding, it can be delayed up to 12 hours, to a maximum of 24 hours.[148] If the patient shows an altered state of consciousness, the procedure is performed under general endotracheal anesthesia to protect the lungs. An endoscope with axial vision adapted to the age of the patient must be employed. The operating channel is often blocked by blood clots; therefore, an endoscope with an operating channel of a diameter of 2.8 mm at least should be chosen

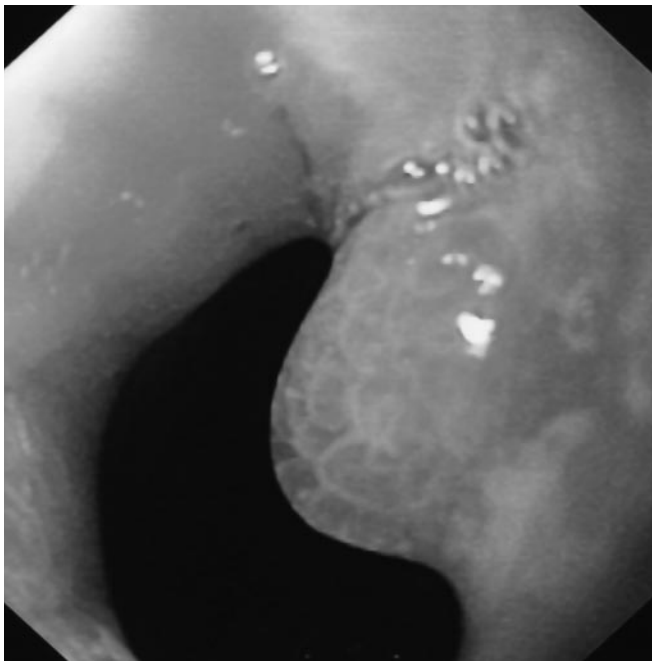

FIGURE 67.2-4 Inflammatory polyp of lower esophagus.

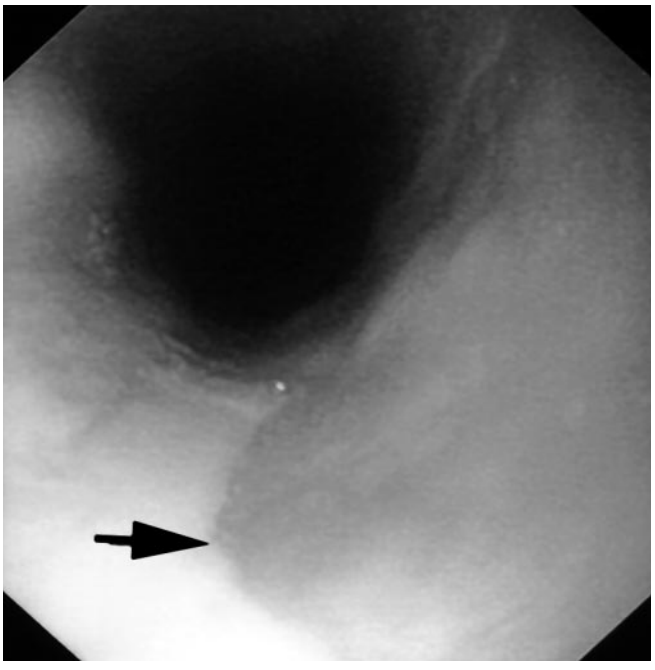

FIGURE 67.2-5 Esophageal gastric heterotopy (*arrow*).

when possible. The exploration is made with slow progression to find the origin of the blood. The instrument is advanced at least up to the genu inferius (Figures 67.2-6 through 67.2-9). Most lesions are to be found in the antrum or in the bulb. The exploration by retrovision of the fundus and the cardial regions is, however, necessary to examine these areas correctly. It might be necessary to change the patient's position to shift the blood from the great curvature. Unless the EGD takes place straight after the event, no active bleeding is normally seen. When the bleeding is still active, its cause is evident at endoscopy unless profuse bleeding obliterates the vision. When the hemorrhage has stopped, it is possible to see a stigma, generally a vessel or a clot adherent to it. In most cases, the clot only partly covers the erosion. In a few hours, the clot disappears, leaving behind a few brown spots. A hemorrhagic base indicates active bleeding within the last 48 hours. In the absence of such signs with a single lesion, it is assumed to be the cause; if multiple erosions are present, no certainty can be reached. Although common in adults, this presentation is rare in children.

At the end of the emergency EGD, three situations can be encountered: in about 70% of cases, the origin of the hemorrhage is found[148,150] or the diagnosis remains uncertain because multiple lesions have been found; finally, no mucosal lesion is seen; therefore, no diagnosis can be reached. This eventuality decreases with the delay of the endoscopy from 82% before 24 hours to 48% after 72 hours.[150] In the case of a negative endoscopy, a second procedure can be performed within 24 hours if bleeding recommences. The origin of the hemorrhage can be found in the esophagus, stomach, or duodenum depending on the age. The most frequent etiologies are shown in Table 67.2-7. Etiologies differ from one center to another and include esophagitis (Figure 67.2-10), Mallory-Weiss tears, esophageal varices (Figure 67.2-11), gastritis (Figure 67.2-12), gastric ulcers (Figure 67.2-13), duodenitis, and duodenal ulcers (Figure 67.2-14). In addition, some specific causes are seen in newborns and infants, such as swallowed maternal blood, neonatal esogastritis, coagulopathy, vascular anomaly, and gastrointestinal duplication.[151] Some of these causes are related to *Helicobacter pylori*.[152] A nodular aspect of the antrum is highly suggestive of the presence of *H. pylori* (Figure 67.2-15).

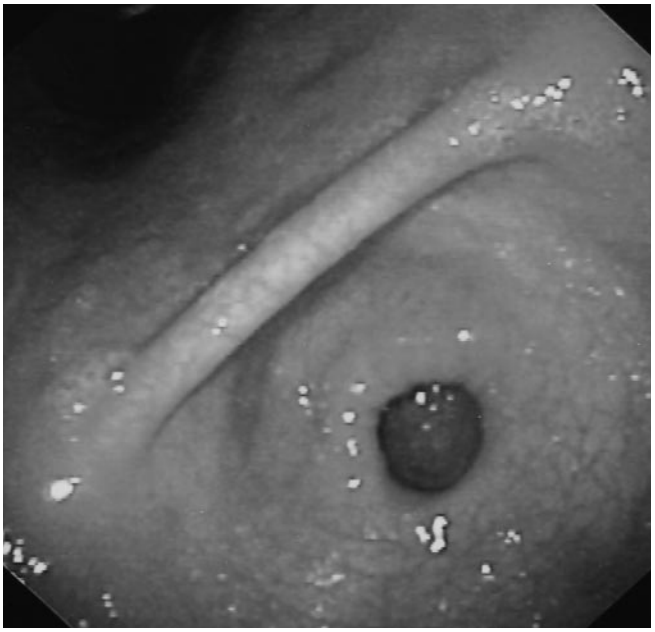

FIGURE 67.2-7 Normal aspect of cardia, angle fold, and fundus in the same view.

Caustic Ingestion

The ingestion of caustic substances is frequent in the pediatric age group.[153] It justifies an EGD in the 12 to 24 hours after the intake, with the exception of bleach. A thorough

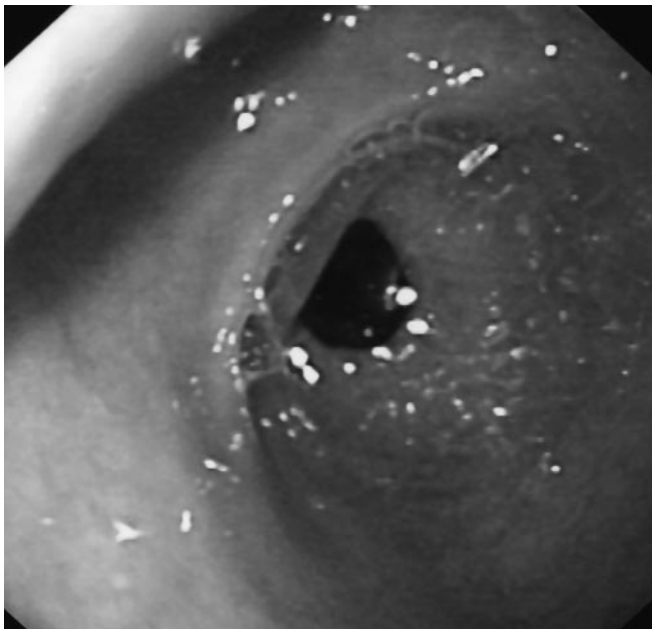

FIGURE 67.2-6 Normal aspect of lesser curvature and open pylorus.

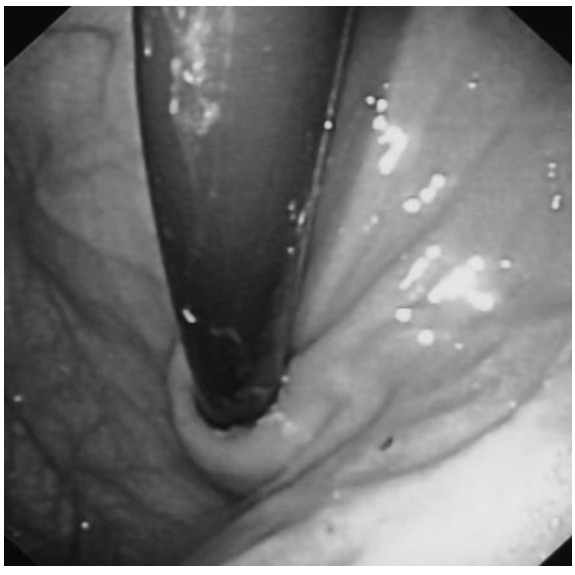

FIGURE 67.2-8 Normal retroversion of fundus of stomach.

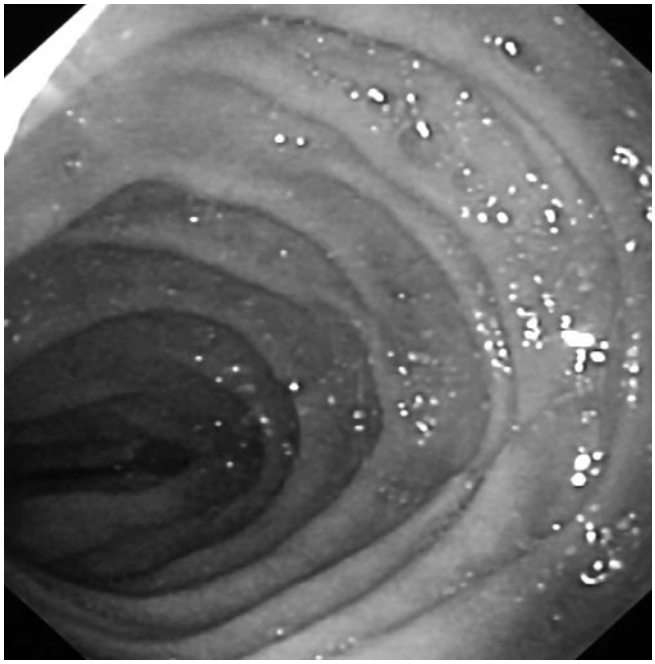

FIGURE 67.2-9 Kerckring fold in normal descending duodenum.

examination of the mouth and pharynx is not sufficient because the presence or not of lesions at this level cannot predict the gastroesophageal integrity. Half of the patients with oropharyngeal lesions have none in the esophagus, whereas around 15% of those without lesions in the top part will have an esophageal injury (J. F. Mougenot, unpublished data, 1997). Alkalis cause a deep liquefaction necrosis extending up to the muscles of the stomach and the esophagus. Acids are responsible for 15% of ingestions, causing a coagulation necrosis that will affect preferentially the stomach, the esophagus being exempt of lesions in 80% of cases (J. F. Mougenot, unpublished data, 1997).

Rigid scopes have been used in the past, but the risk of perforation is high because the instrument becomes blocked at the first proximal lesion. Flexible fibroscopes and videoendoscopes make the endoscopy safer. They can be passed down into the stomach even in the most severe cases. Fibroscopy is used to assess the extent and severity of the lesion, remembering that early examination can underestimate the injury. Early and late complications can be anticipated by the initial extent of the tissue injury. However, it has been suggested by several studies that asymptomatic children following alkali ingestion are not at risk of complications and thus do not need endoscopic examination.[154–159] In summary, the real impact of early endoscopy remains to be proven. A grading system can be used to assess mucosal injury: grade 0, normal mucosa; grade 1, hyperemia and edema; grade 2a, erosion, superficial ulceration, hemorrhage, and white membranes; grade 2b, deep or circumferential 2a lesions; grade 3, multiple ulcerations and areas of necrosis; 3b, extensive necrosis.[160]

Abdominal Pain

Subacute epigastric abdominal pain (present for more than a day and less than 3 months), with anorexia, dyspepsia, weight loss, anemia, and tiredness, that keeps the child awake or away from school needs to be investigated with an upper endoscopy. In our study of 695 consecutive endoscopies in pediatric patients, the association of an epigastric, nocturnal pain related to food intake had a predictive value on the presence of peptic disease with a sensitivity of 90% and a specificity of 37%.[161] The pathologies are shown in Figure 67.2-16. In our opinion, endoscopic examination must be reserved for abdominal pains with organic characteristics.

Recurrent chronic abdominal pains (at least 12 weeks within the preceding 12 months) are present in 10 to 15% of school-age children.[162] They represent one of the most frequent causes of specialized consultation (around 40%). In these cases, a careful clinical examination is crucial before EGD is considered because in only 20%, the investigation will be positive.[162,163] The Rome II criteria can be helpful to individualize functional abdominal pain as recurrent, periumbilical, and diurnal.[164]

Diarrhea and Malabsorption

The EGD with multiple biopsies has succeeded the suction capsule (Crosby, Kugler, Watson, Carey) introduced orally,[165] especially when investigating a malabsorption or a protein-losing enteropathy.[166] Some rules must be respected: three biopsies are taken at the genu inferius to make sure that at least one is well oriented. Some are fixed in formol, whereas others, if needed, are fixed in glutaraldehyde for electronic microscopy or frozen to allow the dosage of the brush border enzymes.

TABLE 67.2-7 ETIOLOGY OF UPPER GASTROINTESTINAL HEMORRHAGE IN CHILDREN (%)

	MOUGENOT AND BALQUET[279] (1978) (*N* = 62)	COX AND AMENT[149] (1979) (*N* = 68)	CHANG ET AL[280] (1983) (*N* = 27)	QUAK ET AL[281] (1990) (*N* = 29)	MOUTERDE ET AL[148] (1996) (*N* = 231)	AYOOLA ET AL[282] (1999) (*N* = 17)
Unknown cause	30.6	16.2	14.8	27.6	19.8	29.4
Esophagitis	4.8	14.7	11.1	17.2	30.7	0.0
Neonatal esogastritis					9.1	
Mallory-Weiss tear				3.5	9.1	
Esophagogastric varices	9.7	10.3		13.8	3.0	11.8
Gastric ulcer	16.1	17.6	33.3		5.6	
Erosive gastritis	14.6	13.2	14.8	27.6	16.2	17.6
Duodenal ulcer	24.2	20.6	29.6	10.3	6.5	5.9
Duodenitis						29.4

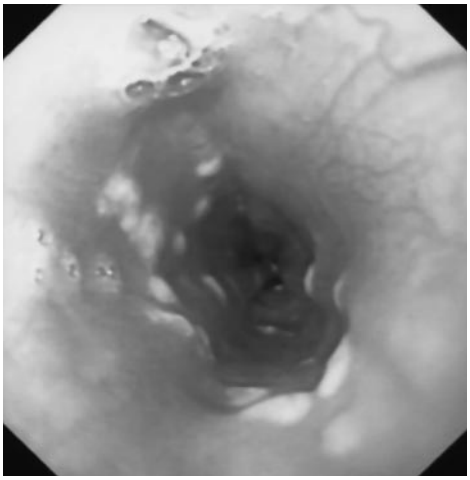

FIGURE 67.2-10 *Candida* esophagitis.

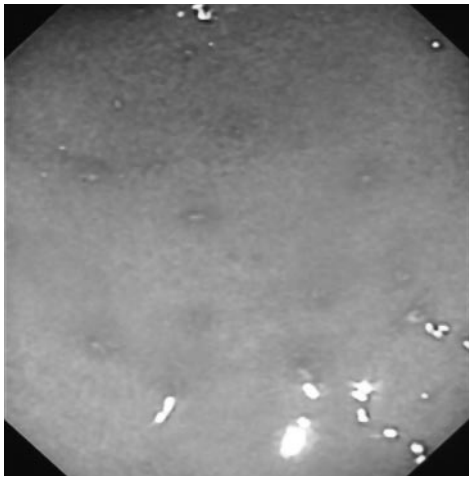

FIGURE 67.2-12 Erosive gastritis.

Some diseases can give a special aspect to duodenal mucosa and should be recognized by the pediatric gastroenterologist: in lipoprotein deficiencies (abetalipoproteinemia, hypobetalipoproteinemia, Andersen disease), a white discoloration of the duodenal mucosa and lipid droplets within the intestinal absorptive cells at biopsy are characteristic owing to the inability of the intestinal cells to export lipids as chylomicrons into the lymphatics[167,168]; a diagnosis of intestinal lymphangiectasia is highly suspect when white villae and/or spots, associated with white nodules and submucosal elevations, are seen.[169,170] Although severe intestinal villae atrophy can be diagnosed with the naked eye when duodenal folds have disappeared, biopsies are compulsory.[166]

Small Bowel Transplant

Control of rejection remains the most difficult problem after intestinal transplant because the intestine seems more susceptible to rejection than any other organ. Because clinical diagnosis of graft-versus-host disease is difficult, endoscopy has become an essential tool in assessing its presence in the allograft.[171–173] An ileostomy is created at the time of the transplant to facilitate regular small bowel biopsies and histologic examination of the intestinal mucosa. The endoscopic appearance of a denuded, hemorrhagic mucosa usually correlates closely with the histologic diagnosis of severe rejection. Early changes can be too subtle to be detected with a standard endoscope, and zoom videoscope is used nowadays to look for edema, blunting of the villae, and expansion of crypt areas by a mononuclear cell infiltrate.[174]

Other Indications

The barium meal has a higher diagnostic yield than EGD for the diagnosis of vessel anomalies and of the esophagotracheal fistula, in which the esophageal orifice is often missed in endoscopy.

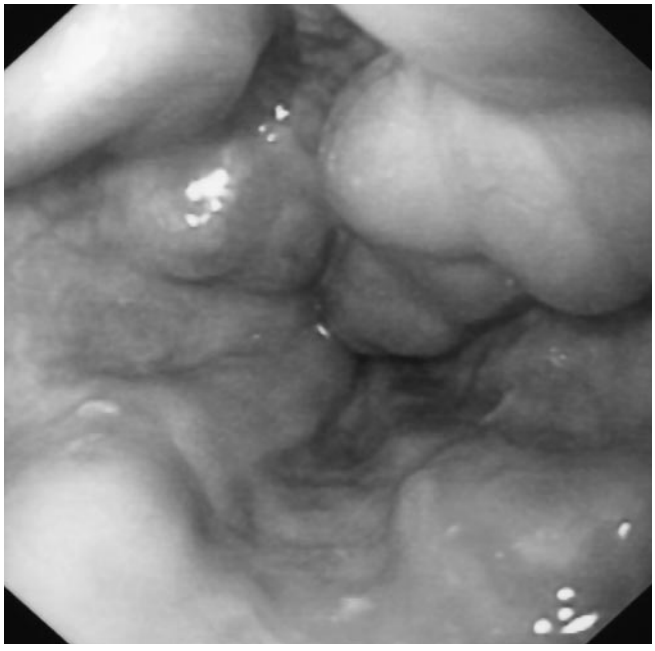

FIGURE 67.2-11 Grade II–III esophageal varices.

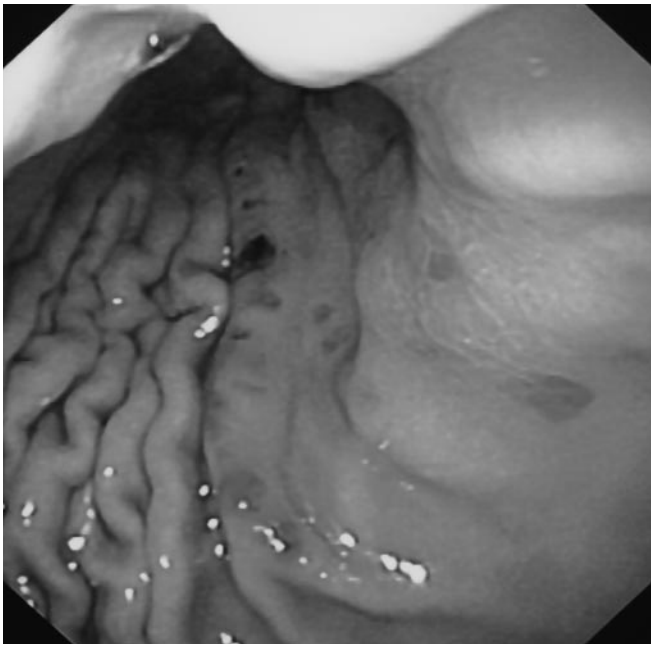

FIGURE 67.2-13 Gastric ulcer with multiple large erosions.

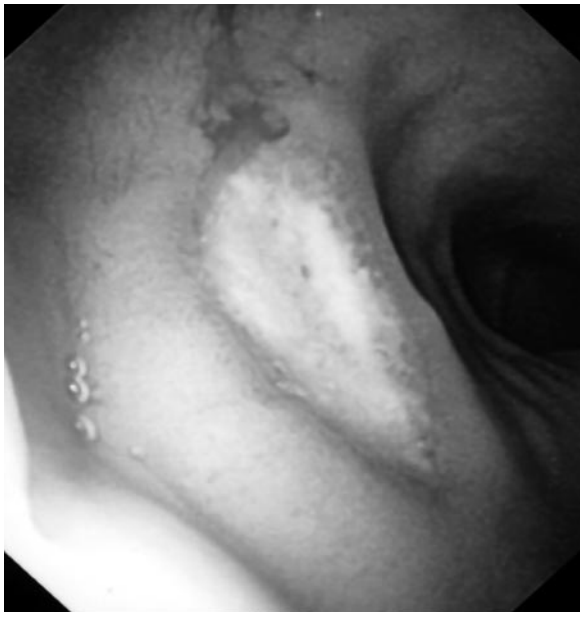

FIGURE 67.2-14 Ulcer of the bulb.

In pyloric stenosis, the first evaluation is made by ultrasonography. In case of doubt, upper endoscopy or barium meal should be performed. Indeed, small endoscopes such as GIF-N-30 (Olympus, Volketswil, Switzerland) and FG-16 X (Pentax, Walliselen, Switzerland) can be passed through a stenosis. Some authors have also proposed balloon dilation of pyloric stenosis.[175]

Despite the fact that primary gastrointestinal cancer is rare in children, EGD can be necessary in cancer surveillance. Endoscopic surveillance is warranted for polyposes syndromes with a higher risk of cancer development. Another indication for EGD can be Munchausen syndrome by proxy, which can present with a broad spectrum of manifestations, such as vomiting, abdominal pain, diarrhea, and failure to thrive.[176] Diagnosis is often delayed, and as there is a risk of mortality due to Munchausen syndrome by proxy, invasive investigation can be mandatory. EGD in this condition has both diagnostic and legal implications.

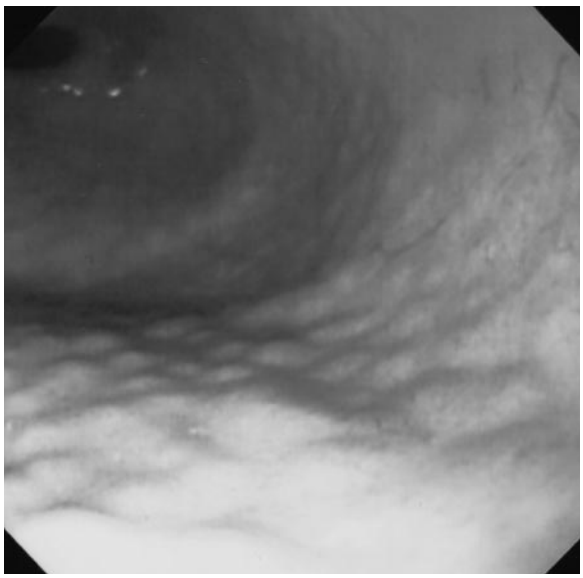

FIGURE 67.2-15 Nodular gastritis due to *Helicobacter pylori* infection.

EGD is an efficient method for follow-up of postsurgical treatment of peptic lesions.

Finally, an upper endoscopy is part of the initial evaluation in suspected inflammatory bowel diseases, and its role is also diagnostic because up to 28% of the granulomas are found only in the upper intestinal tract.[177,178] The integrity of the upper intestinal tract should no longer exclude ulcerative colitis,[177,179] and focally enhanced gastritis does not signify Crohn disease.[180]

CONTRAINDICATIONS

Absolute medical contraindications of upper endoscopy are few and include cardiovascular collapse, an unstable airway, intestinal perforation, peritonitis, and cervical traumas. On top of these pathologies, absences of consent or of competent medical personnel are also situations in which the endoscopy should be either delayed or cancelled.

Relative contraindications are the following: recent digestive surgery, bowel obstruction, coagulopathies, severe thrombocytopenia, and recent food intake. The latter needs to be corrected prior to the procedure.

The major risk with insulin-dependent diabetes is a hypoglycemic episode. The patient should be first on the endoscopy list and advised to reduce the amount of morning insulin.

In a child with hematemesis, an abdominal radiograph must be obtained to eliminate an intestinal obstruction or perforation.

INSTRUMENTATION

ENDOSCOPES

Two types of endoscopes are available that differ in the method of transmitting the image. Both systems require a fiberoptic light guide and a complex objective lens at the distal end to focus the image. The fiberoptic endoscopes use optical fibers. Imaging transmission of light is made by a bundle of glass fibers contained in a cylinder made of a material of inferior refraction index. Each fiber is made of coated glass. The coating acts as a mirror that reflects light through the fiber into the eyepiece. When the light entering the extremity of the optic fiber hits the interface between the glass with a high reflexion index and the material with a low index, it is transmitted by a series of internal reflexions up to the opposite extremity of the flexible fiber glass. A fiber alone could not do the job, but thousands put together make the transmission of an image possible. For the same image to be reproduced at the extremity as the one seen at the other extremity, the individual fibers need to occupy exactly the same position at the two ends of the scope.[181]

Videoendoscopes use advanced charge-coupled device (CCD) technology. The photo-sensible surface of the CCD is made up of a huge number of tiny image elements or pixels that gather the light. The electric charge of these pixels

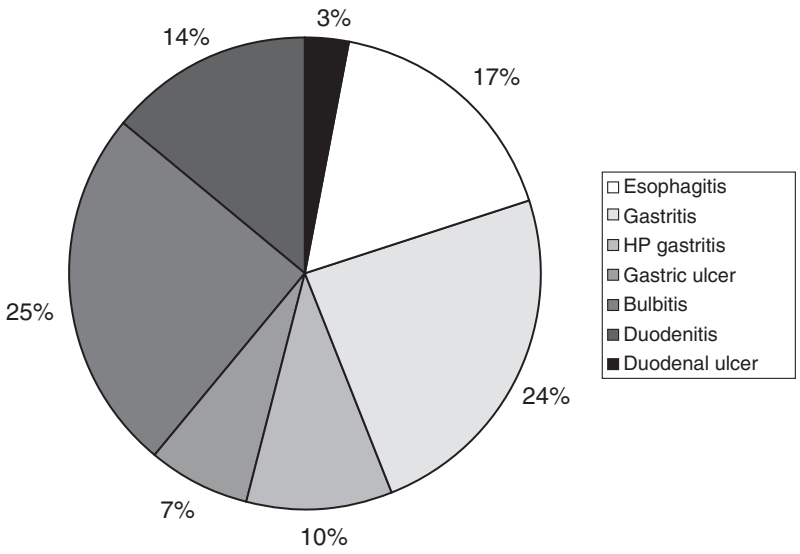

FIGURE 67.2-16 Peptic pathologies found in 695 consecutive endoscopies in a pediatric gastroenterology unit in Geneva, Switzerland. HP = *Helicobacter pylori*.

depends on the intensity and not on the color of the light. Therefore, a CCD is a black and white captor. The color image is created either by the single-plate simultaneous-color CCD chip method or the single-plate red, green, and blue (RGB) surface scanning method. In the former method, a color CCD chip built into the distal tip of the endoscope contains multicolor pixels and can simultaneously capture different color wavelengths of light: red, green, and then blue. Subsequently, the three monochrome images, specific in the intensity and position of the three colors, memorized in the microprocessor are visualized simultaneously, thus reproducing the original picture on the video monitor. In the latter method, the tip of the endoscope incorporates a monochrome CCD chip that can only provide black and white signals, with a multichrome filter added. The last generation of this type of endoscope can have a polychrome chip. This image from the filter (either RGB or a mosaic yellow-cyan-white) is passed through the video processor to modify it in the three primary colors. From a four-pixel block (one yellow, one cyan, two white), in this case, three pixels receive information about the red, four about the green, and three about the blue. The CCD converts the light into an electronic signal, which is then sent to the processing unit, where it is converted for output as a video signal for the monitor to display or to other peripherals (printer, personal computer, monitor, light source) in either RGB mode or Y-C (luminance-chrominance). With the image being directly transmitted to a color video monitor, the inspected areas can be examined fatigue free and simultaneously by several experts when required. However, the cost of the videoendoscope and accessories limits its acquisition.

All fibroscopes and videoendoscopes are waterproof, permitting appropriate washing, decontamination, and disinfection. They house an instrument channel to pass instruments (grasping forceps, polypectomy snares, injection needles, cytology brushes, extractors, retrievers, laser, guidewires, dilating balloons, bougies, diathermic snares) and two accessory channels to insufflate air and inject and aspirate water. Through the smallest diameter of operating channel, 2 mm, fit 5 French instruments, facilitating therapeutic endoscopy in small children (injections, polypectomy). The midsize endoscopes have a 2.8 mm diameter, allowing the passage of 7 French wires. Apart from the duodenoscopes used for endoscopic retrograde cholangiopancreatography, all scopes have an axial vision.

The light source is composed of a generator of cold light (150–300 W halogen or xenon lamps), a light transmission system with automatic light adjustment to ensure sufficient brightness that maintains optimum brightness, and a high-speed rotation filter (20–30 tours/s), which is needed for the sequential RGB videoscope.

Gastroscopes with axial vision, owing to their flexibility and the amplitude of quadridimensional bending capacity (up, down, right, and left), give a full view of the upper intestinal tract from the esophagus to part 3 of the duodenum, including the cardia. An important part of the procedure is to choose the appropriate scope, depending on the size of the patient. The following rules can help in this choice: up to 15 kg, a 9 mm tip diameter can be used; from 5 to 15 kg, 8 to 9 mm; in newborns 2.5 kg to 4 kg, 5 to 8 mm; and in newborns less than 2.5 kg, only 5 mm is possible. In other words, the GIF-N30 (Olympus), the EG-1870K, and the FG-15W (both Pentax) are the only scopes usable in newborns. The GIF-XP20, GIF-XP160 (Olympus), FG-6W (Pentax), and FG-100PE (Fujinon, Wayne, NJ) are usable in term infants up to 4 years of age. Above that age, it is better to use the GIF-P30, P140, or P230. The EG-450PE5 can be used from 1 year of age. In patients older than 10 years, the GIF-XQ40, GIF-160, EG-450WR5 (Fujinon), and EG-2570K (Pentax) are preferable. The scopes incorporating an instrument channel size of 2.8 mm are better for therapeutic use. Under general anesthesia, they can be used in patients as young as 12 months old. Table 67.2-8 resumes the technical characteristics of gastroscopes that can be used in pediatrics.

TABLE 67.2-8 TECHNICAL CHARACTERISTICS OF GASTROSCOPES USED IN PEDIATRICS

ENDOSCOPES	FIELD OF VIEW (DEGREES)	OBSERVATION RANGE (MM)	WORKING LENGTH (M)	DISTAL TIP DIAMETER (MM)	BENDING CAPACITY (DEGREES)	CHANNEL DIAMETER (MM)
Fujinon						
FG series (fiberoptic)						
FG-100PE	105	5–100	1.030	9.5	U/D 210-90 R/L 100-100	2.2
EVE-400 Series (video)						
Gastroscopes						
G5-CCD 410000.0 pixels						
EG-470N5	120	2–100	1.100	5.9	U/D 210-90 R/L 100-100	2.0
EG-450P5E	120	4–100	1.100	8.2	Idem	2.2
EG-450WR5	120	4–100	1.100	9.4	Idem	2.8
EG-450WR5 optical magnification	140	6–100	1.100	9.8	Idem	2.8
Super CCD 1.2000000.0 pixels						
EG-490WR5	140	6–100	1.100	9.8	U/D 210-90 R/L 100-100	2.8
EG-490ZWR5 optical magnification	140	6–100	1.100	10.8	Idem	2.8
Duodenoscope						
ED-450XL8	120	100/15	1.250	12.5	U/D 130-90 R/L 90-110	3.2
Olympus						
EOS						
GIF N30	120	3–50	0.93	5.3	U/D 180-180 R/L 160-160	2.0
GIF-XP20	100	3–100	1.030	7.9	U/D 210-90 R/L 100-100	2.0
GIF-P30	120	3–100	1.025	9.0	Idem	2.2
GIF-XQ40	120	3–100	1.030	9.8	Idem	2.8
EVIS EXERA (video)						
Gastroscope						
GIF-XP160	120	3–100	1.030	5.9	U/D 180-90 R/L 100-100	2.0
GIF-160	140	3–100	1.030	8.6	U/D 180-90 R/L 100-100	2.8
GIF-160	140 retro	3–100	1.0300	10.9	U/D 210-90 R/L 100-100	2.8
GIF-Q160Z	140/75(télé)	8–100	1.030	10.9	U/D 210-90 R/L 100-100	2.8
Duodenoscope						
PJF-160	100/5	5–60	1.235	7.5	U/D 120-90 R/L 90-90	2.0
EVIS (video) 230 and 240						
GIF-N230	120	3–50	0.96	6.0	U/D 180-180 R/L 160-160	2.0
GIF-XP240	120	3–100	1.03	7.7	U/D 210 -90 R/L 100-100	2.2
GIF-XQ240	140	3–100	1.03	9.0	Idem	2.8
JF-240	100	5–60	1.235	12.6	U/D120-90 R/L 110-90	3.2
Pentax						
W Series						
Gastroscope						
FG-15W	95	3–50	0.925	5.3	U/D 180-180 R/L 160-160	2.0
FG-24W	105	3–100	1.050	7.8	U/D 210-120 R/L 120-120	2.4
FG-29W	100	3–100	1.050	9.8	Idem	2.8
70K Series						
EG-1870K	140	5–100	1.050	6.0	U/D 210-120 R/L 120-120	2.0
EG-2570K	140	5–100	1.050	8.7	U/D 210-120 R/L 120-120	2.4
Duodenoscope						
ED-3270K	140	5–100	1.050	13.0	R/L 110-90	3.5

Forceps

A range of biopsy forceps exists apart from standard models: the "crocodiles" have teeth on the edge to help in grasping the mucosa. Others have a spike in the center to facilitate the grip on the mucosa and avoid sliding.

Technique

Endoscopy can be performed either single or two handed. In the first method, the endoscopist keeps the right hand on the shaft throughout the procedure, while the left hand is on the controls. The middle finger helps with the up/down wheel and the thumb reaches the right/left wheel. This method needs considerable practice but has the advantage of leaving the driver independent, needing less personnel. In the second method, the two hands are used to maneuver the controls, the shaft being pushed or withdrawn by a second person. The right hand is then used to move the right/left control. Easier from the start, this method can be performed more rapidly.

The help of experienced personnel is crucial. A minimum of two people, in addition to the operator, are present in the room. It is preferable that they could have met the parents and child before the endoscopy to answer questions. One will be responsible for the patient, making sure that the patient is safe and comfortable at all times. The second will assist the operator and operate the forceps when necessary.

At the start of the procedure, the patient is usually positioned on the left side, although some gastroenterologists will put the patient flat on the back. A small pillow under the head, but not the shoulder, will straighten the neck. The head is slightly flexed forward. In children with teeth, the use of a mouthguard with or without straps is recommended because bites to instruments can damage the fibers. It also helps keep the instrument on the midline. The scope is checked (suction, insufflation, and light on; zoom and brakes off) once more before lubrication is applied. Lastly, up and down movements are rehearsed to make sure that they are made in the axis of the pharynx. The room light is dimmed to allow good vision for the endoscopist. The introduction of the scope is a delicate moment owing to the extreme sensibility of the oropharynx. Two crucial rules are to be respected throughout the procedure if complications are to be avoided: never advance blindly and, if lost, come back. Four landmarks should be recognized during the procedure: cardia, angulus, pylorus, and superior duodenal angle (see Figures 67.2-6 through 67.2-8).

Three basic methods exist to intubate the esophagus:

- The safest way is under direct vision (Figure 67.2-17). The shaft is held at 30 cm and slowly introduced with the tip deflected downward up to the cricopharyngeal sphincter. In passing, the tongue, arytenoids, and vocal cords are localized to avoid impaction on the piriform fossae. If, in advancing, the teeth are seen, the shaft is twisted to put it back on the midline. Whenever landmarks are lost, withdrawal and starting again are the simplest and safest solution. The cricopharyngeal sphincter closes the entrance to the esophagus, making it difficult to locate. After a short waiting time, it will relax, allowing the intubation of the upper posterior esophagus. Insufflation of air or gentle pressure can help to relax it. In a cooperative child, swallowing will relax the upper esophageal sphincter, but it is often not obtainable in pediatrics.
- Another method uses blind manipulation. Holding the scope as before, the shaft is passed up to the back of the mouth. At this level, the tip is deflected upward and gently pushed forward. This will stretch the mucosa and allow the passage of the cricopharyngeal sphincter. Constant reorientation is necessary because the shaft easily comes out of the midline.
- An alternative method is with finger guidance. Probably the easiest for the inexperienced practitioner, it does, however, carry the risk of bites to either the scope or the fingers. The shaft is held with the right hand at about 5 cm, the mouthguard slipped over it. The shaft is pushed while the second and third fingers of the left hand are put over the tongue to help guidance. The cooperation of the patient is required as the sphincter is passed by active swallowing.

Under vision, the scope is progressively pushed forward to the lower sphincter. The Z line, the junction between the esophageal squamous mucosa and the gastric columnar, is located, usually 1 cm above the diaphragmatic hiatus. Its position in centimeters is recorded, remembering that it might be difficult to spot in newborns and infants. The diaphragmatic hiatus is situated between 12 and 40 cm from the incisors, depending on the age and size of the patient. The lower sphincter is often passed blindly owing to its contraction. The tip of the shaft is bent gently downward and to the left because the esophagus angles to the left side to avoid impaction in the fundus wall. To improve vision, air is insufflated, the lesser

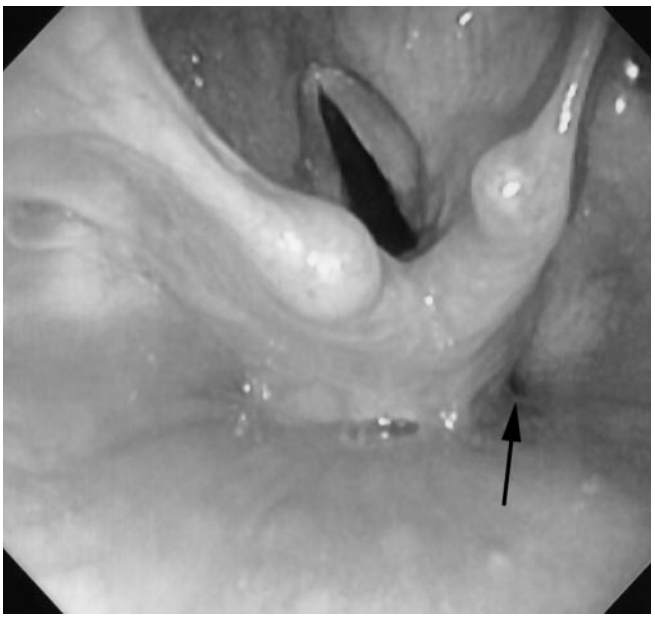

FIGURE 67.2-17 Normal laryngeal and pharyngeal structures. Upper esophageal sphincter (*arrow*).

curvature being on the right, the angulus in the distance. When liquid is present, suction is used to eliminate any risk of aspiration. At this point, most endoscopists will-progress rapidly to the duodenum, careful examination of the mucosa being achieved on the way back. This method implies experience to avoid traumatic lesions (suction or rubbing) that could cause confusion afterward. Yet it has the advantage of avoiding the overinflation required for retrovision that causes discomfort. Progression is made with a clockwise (left) rotation of 90°, bending the tip upward. This double maneuver brings the pylorus into view. The bending can be quite extreme in a young child or infant because the angle is acute. To put the pylorus into the antrum axis, the tip is angled down. At this point, it helps to reduce the amount of air present in the stomach. The shaft is then advanced toward the pylorus, which will open, either spontaneously or with stimulation, by air insufflation. The intubation of the pylorus is achieved with the tip slightly bent down and right. Past the pinch, the tip tends to get impacted because the gastric loop straightens up. Withdrawal and air insufflation confer a view of the pale mucosa of the bulb, the anterior wall placed to the left and the posterior wall to the right. The superior duodenal angle is visualized before passage to the second part of the duodenum is attempted. This progression, usually carried out blindly because of the sharp angle, needs to be made with care. Pushing will bring the tip in front of the duodenal angle; it is then bent to the right and finally rotated simultaneously at 180° to the right and up (150°). Finally, to obtain an optimal view, withdrawal is normally necessary because of the paradoxical progression of the endoscope owing to the straightening of the gastric loop. Sometimes rectification of the last maneuver is needed with deflection of the tip upward and to the left. Often the Vater papilla is seen. Biopsies are taken before withdrawal. Most endoscopists will use this moment to carefully examine the duodenal, bulbic, gastric, and esophageal mucosa. Circumferential movements with air insufflation will provide well-distended mucosa in order not to miss small lesions. A retroversion maneuver in the stomach (J maneuver) is the only way to fully visualize the fundus, the lesser curvature, and the cardia. While back in the proximal part of the antrum, a 180 to 210° angulation is imposed to bring into view the angulus and the lesser curvature. Keeping the angulation, a 180° rotation around the shaft's axis will allow visualization of the greater curvature and the fundus. The withdrawal of the instrument will bring the cardia into view.

Taking biopsies is one of the main reasons for performing an endoscopy, along with visualization of the mucosa. Most gastroenterologists agree that they should be obtained even in the case of a normal mucosa.[10] The primary sites to biopsy are the distal duodenum, antrum, and esophagus. To achieve good-size specimens, some rules have to be followed. The forceps is approached at a 90° angle to the mucosa and not tangentially. The forceps protrudes no more than 3 cm from the tip of the scope to allow good control over it. This is important not only for the quality of the specimens but also to reduce the risk of perforation and hemorrhage. A fold biopsy is more effective because a "double" biopsy is obtained. In a limited space such as the esophagus, a good trick is to sharply bend the tip of the shaft. Firm pressure needs to be applied to the mucosa before slowly closing the forceps. Multiple specimens are taken, especially in the duodenum, so as not to miss focal villae atrophy in case of lesions. Biopsies are transferred with the help of the needle onto a piece of paper and flattened. To avoid crushing, stretching, or puncture injuries to the tissue, the back of the needle is used. The mode of fixation depends on which pathology is suspected. Clo-test (Astra Zeneca AG, Zug, Switzerland) will typically require a fresh antral biopsy, to test for the presence of *H. pylori*, by its production of urea. When cultures are needed, a small drop of saline is placed on top, and they are sent to the appropriate laboratory immediately. If special immunocytochemistry or disaccharidase levels are wanted, frozen specimens are needed. Finally, for electron microscopy, glutaraldehyde should be ready.

The whole procedure with collection of biopsies takes around 15 minutes in experienced hands.

COMPLICATIONS

Complications are uncommon during endoscopy when performed by well-trained pediatric gastroenterologists—probably less than 1%.[182] They are mainly due to the anesthesia or to the procedure itself. Endoscopy in children tends to be performed under deep sedation, making a hypoxic episode possible. On the introduction of the tube, the saturation usually drops by 5% from the basal level before quickly recuperating. However, in Bendig's series, desaturation (< 90%) was observed in 7 of 60 children.[183] Nasal oxygen is usually sufficient to correct the hypoxia. In the rare cases in which no amelioration is obtained, withdrawal of the scope is necessary. Difficulties mainly arise from inefficiency of the sedation. Aspiration is always possible, although respected fasting time reduces the risk. Allergic patients can react to the medications or to the latex. Finally, rare complications are hypotension, arrhythmia, and malignant hyperthermia.

If we now look at the complications arising from the actual procedure, they mainly consist of perforation, hematoma, air embolism and infections:

- The complications described in children owing to the actual procedure are the following: Perforation can occur, usually owing to excessive force or air insufflation on an already damaged mucosa. This mostly involves the esophagus. The majority of reported cases are due to therapeutic endoscopy and are discussed in other chapters. Mucosal tears without perforation have been described in children,[184] yet this seems to be a complication seen more frequently in the elderly presenting with hiatal hernia.[185] To minimize the risk, it is crucial to never push forward without vision. At times, it is impossible to avoid having the scope against the mucosa. In these circumstances, as long as the vessels are seen passing by in front of the camera, this means

that the scope is sliding on the mucosa and that there is no danger. In contrast, if the mucosa turns white, too much pressure is being applied, and the scope needs to be withdrawn. In case of perforation, surgical referral is urgent to choose between a conservative and surgical treatment. Nevertheless, signs of perforation can appear with some delay.

- Intramural duodenal hematoma has been reported after endoscopic biopsies.[186–195] Although it is a rare complication, it seems to occur more often in children than in adults.[189] The clinical presentation mimics abdominal occlusion with severe abdominal pain and vomiting, usually starting in the 12 hours postendoscopy. Often it is associated with pancreatitis.[189,190,192,195] Spontaneous resolution is always obtained between 4 days and 2 weeks, with fasting, nasogastric suction, and fluid replacement. Total parenteral nutrition is sometimes necessary. Surgical drainage, although sometimes performed, is unnecessary and therefore contraindicated.
- Fatal massive air embolism has been reported in a 10-year-old and a 4-month-old child who had been subjected to a Kasai procedure[196,197] because of potential vessel leakage.
- Infectious complications can result from the patient's own microbial flora (autologous), from patient to patient by way of the endoscope (exogenous), or between the patient and the staff. The most frequent microorganisms depending on the mode of transmission are *Salmonella* spp, *P. aeruginosa*, *Mycobacterium* spp, and *H. pylori*.[182,198] These can be seen in cardiac-risk patients (see the antibiotic prophylaxis section); only one case of sepsis has been reported in a child without increased risk.[199] Bacteremia seems rare[52,54,57]; therefore, prophylactic antibiotics are given only to selected patients.

CAPSULE

The invention and development of more sophisticated endoscopes have allowed gastroenterologists to see further into the gut. Whereas in adults, push enteroscopy has enabled the further enhancement of possibilities and visualization of up to 90 to 120 cm beyond the ligament of Treitz,[200,201] experience in children is limited.[11] Another technique, sonde enteroscopy, in expert hands, allows us to see approximately 50 to 70% of the small bowel, the ileocecal valve being reached in only 10%.[202] Nevertheless, 30% of the small intestine could not be visualized, lying beyond our reach. Twenty years ago, Dr. Iddan had the idea of developing a video camera that would fit inside a pill.[203] Technology was not ready, and the idea was put on hold. With the turn of the millennium, engineering breakthroughs, particularly in the area of complementary metal oxide silicon, application-specific integrated circuit, and white light-emitted diode (LED) technologies, allowed all of the components of the camera to be put on a single chip, reducing both its size and power consumption. The wireless capsule endoscopy was born,[204] giving access to the so-called gut "black box."

The advantage compared with conventional upper endoscopy is that neither anesthesia nor air insufflation is necessary, making the whole procedure much more pleasant for the patient. Yet it cannot replace endoscopy for the following reasons. First, although the capsule often passes into the colon while the videotaping continues, its battery runs out before the journey through the colon is complete, not allowing a full view. However, a bowel preparation would be necessary. Furthermore, an important limitation is the inability of the device to take biopsies, although the manufacturers anticipate that improvements may permit this within the years to come. Therefore, the capsule does not replace traditional endoscopy in disorders in which a histologic diagnosis is necessary, for example, celiac and Crohn disease. Another drawback is that it may be difficult to determine the exact location of an abnormality seen. Because of these limitations, the capsule does not replace traditional endoscopy.

Indications

The main indication is obscure gastrointestinal bleeding[205] or iron deficiency anemia after a negative panendoscopy. However, it has been used to diagnose specific disorders that were otherwise difficult to establish, such as vascular abnormalities, polyposis,[206] and suspected Crohn disease.[207,208]

After animal studies demonstrated effectiveness, capsule endoscopy was used in small human prospective studies and proved to better define bleeding sites (55–76%) compared with conventional diagnostic procedures (21–30%).[209–211] When compared with barium studies, the capsule was considered diagnostic in 45% of patients versus 27%, with 31% versus 5% of the causes of obscure bleeding being found.[206] Reported diagnostic yield reaches up to 62.9%.[212] The amount of literature demonstrating its superiority in diagnosing the cause of blood loss is growing, but pediatric trials are needed.

Contraindications and Complications

A small risk exists that the capsule may become lodged in the intestinal strictures or diverticulae. The incidence, estimated around 1%, increases if the patient has undergone major abdominal surgery or suffers from a stenotic disorder.[213] We advise practitioners to perform a barium and follow-through prior to using the capsule to exclude stricture. Abdominal radiography should be considered in patients who do not observe passage of the capsule and whose imaging result does not indicate passage of the device into the cecum. The manufacturers themselves say that the capsule should not be used in patients with symptoms of a bowel obstruction, including nausea and vomiting and abdominal distention.

Instrumentation

The only wireless capsule endoscope available is produced by Given Imaging Ltd, Yoqneam, Israel, the M2A Capsule Endoscope, and is currently available in 33 countries worldwide. The Food and Drug Administration in the United States[214] and the Health Protection Branch of Health and Welfare in Canada recently approved its use in

adults only. The capsule, measuring 11 × 26 mm, has a weight of about 4 g and contains a magnifying (1:8) color camera, four light sources, a radio transmitter, and batteries. With a 140-degree viewing angle, the capsule allows relatively complete viewing of the small intestine, but neither the velocity nor the direction can yet be controlled. Insufflation of air is unnecessary because the focal point of the lens is 1 mm. Images taken twice per second are continuously transmitted to wires placed on the body and captured on a recording device much like a Holter monitor, worn on a belt. The battery power is able to take 50,000 to 60,000 color images and lasts about 6 to 8 hours. These images are downloaded on a computer and viewed by the gastroenterologist as a video; analysis can take up to 2 hours. There is a learning curve in recognizing abnormalities even for experienced gastroenterologists because the aspect of the images is different to that acquired in a conventional way. A blood-sensing algorithm, using color pattern recognition, has recently been added to the software to help detection of intraluminal blood.

Technique

The capsule is swallowed with a glass of water by the patient in the morning and then travels down the gut propelled by peristalsis. Neither bowel preparation nor sedation is needed, the patient having fasted 6 hours prior to the study. Although the minimal age mentioned by the manufacturer is 11 years, it has been used in younger children. The actual limitation is due to the capacity of the patient to swallow it and not the size of the capsule itself. To overcome this problem, some centers insert the capsule endoscopically in children as young as 4 years old. Patients may then perform their regular daily activities while wearing the recording device for the next 8 hours. The capsule has a mean gastric transit time of 80 minutes and a mean small intestinal transit time of 90 minutes.[204] The disposable capsule is usually passed, in the absence of any marked motility disorder, in the next 24 to 48 hours.

INTERVENTIONAL PROCEDURES

Foreign Bodies

Foreign body ingestion is a relatively common reason for consultation in the pediatric emergency room. More than 50% of cases are children aged 5 years or less, and most are boys. The ingestion of the foreign body is not generally voluntary, except for neurologically impaired or psychiatric patients and youngest siblings. A large variety of small objects and toys can be ingested, although coins are the most frequent (Figure 67.2-18). The vast majority of foreign bodies pass through the entire gastrointestinal tract without any problem. Indeed, only 10 to 20% will become impacted. The most frequent localization of impaction is the cervical esophagus. However, it can also occur in the medial and distal parts of the esophagus. When foreign bodies have passed through the esophagus, 95% will be spontaneously eliminated in 4 to 6 days, sometimes longer (3–4 weeks). If they pass through the esophagus, they can become lodged in the pylorus, genu inferius of the duodenum, or ileocecal valve or on acquired or congenital stenosis. As to size, foreign bodies of a diameter of more than 20 mm or a length more than 50 mm (30 mm for infants) are particularly prone to impaction.[153] In more than 80% of cases, the foreign body is opaque, most frequently a coin.[215] The nonopaque foreign body is mainly retained food, often associated with a known history of previous repair for esophageal atresia.

Whenever a foreign body is suspected, thoracic and abdominal radiography must be performed. Every esophageal foreign body that is blocked for more than 24 hours must be removed immediately by endoscopy. Some studies have compared endoscopy, Foley catheter, and bougienage technique, especially for coin removal.[215,216] They favor Foley catheter or bougienage on a cost-effective basis.

For intragastric foreign bodies, interventional endoscopy is performed immediately only in cases of risk factors (a large foreign body with high risk of incarceration, a sharp foreign body with risk of perforation or hemorrhage, and all symptomatic ingestions). In other cases, a more conservative approach can be chosen, with an endoscopy only after 3 days to 4 weeks of nonprogression of the foreign body, depending on the experience of the center.

The special case of battery ingestion should be mentioned.[217] All types of batteries contain potassium or sodium hydrochloride and are not completely watertight. Consequently, their ingestion can lead to esophageal, gastric, or intestinal lesions such as burns or perforation, which can be lethal. Consequently, ingested batteries must be removed within 24 hours.

The endoscopic removal of foreign bodies must be performed under endotracheal anesthesia for all localizations to ensure airway protection, especially in case of an inadvertently released object.

Several devices are available: rat tooth and alligator forceps, polyp snares, retrieval nets, helical baskets, and hooded sheaths. Their specific use is related to the type of

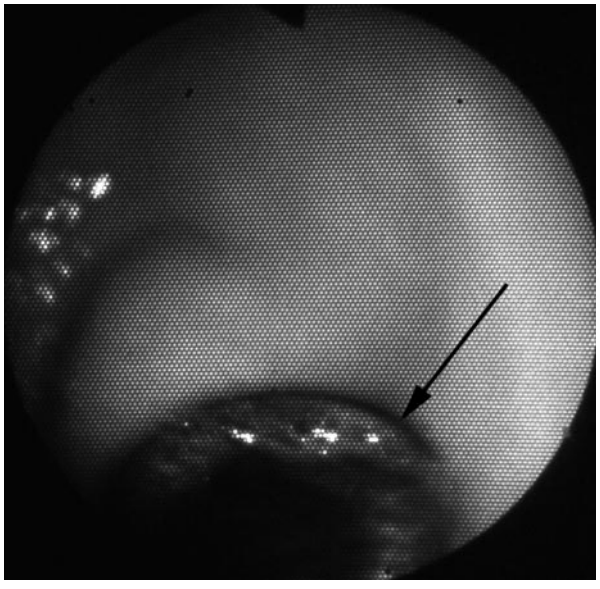

FIGURE 67.2-18 Antral foreign body (*arrow*: coin).

foreign body. It is mandatory that the removal should be carried out under view control and with optimal positioning (pointed end trailing and object close to the tip of the endoscope or in a protective device covering the end of the endoscope) to avoid unsafe removal, especially for sharp objects.

A postremoval surveillance of 12 hours is recommended.

Stricture Dilation

Esophageal stenosis dilatation is indicated in case of esophageal strictures, which can be secondary to several origins: congenital, postoperative, peptic, caustic, infectious, postvariceal sclerotherapy and postradiotherapy. Postoperative strictures are common complications in up to 50% of patients after surgery for esophageal atresia.[218] Peptic stenosis can also be secondary to severe GER with esophagitis, especially in neurologically handicapped children.

Since Trendelenburg in 1883, esophageal stenosis has been dilated either by antegrade or retrograde bougienage, when a gastrostomy can be used. Several types of bougie dilators are available. They develop an axial force along the esophagus, with a high risk of perforation. They are used both blindly following introduction through the mouth or on a guidewire.

An effective and safer alternative to bougienage is the balloon catheter dilation (Figure 67.2-19). Its major advantage is that it dilates uniformly the esophageal stenosis with local stationary radial forces owing to balloon inflation.[219] Indeed, a Gruentzig balloon was developed in 1981 and has been used successfully since.[220] At present, several balloon catheters are available. They can be used under radiologic control with an angiographic guidewire, with a rate of resolution of symptoms of 100% and 70% for congenital and acquired anomalies, respectively.[221]

On the other hand, other balloon devices can be used directly in the operating channel and are called the TTS system ("through the scope" system). However, the maximal dilation diameter is of 8 mm for a 2.8 mm channel and of 12 mm for a 3.5 mm channel. With this technique, the balloon can be placed across the stricture under view control. Then the balloon is inflated with a constant pressure between 3,000 and 4,500 mm Hg for 1 to 3 minutes. The benefit of this procedure is due to the fact that endoscopists can immediately visualize the effect of the dilation on the stricture. In our unit, we use larger balloon catheters (maximum 20 mm diameter), which are introduced by mouth in an antegrade approach. Simultaneously, a flexible endoscope of small size (5 mm) is introduced to control the proper passage of the structure, as well as the positioning of the balloon. Using this method, we have not experienced perforation during the last decade.[222]

This procedure can be performed under antibiotic prophylaxis, and a thoracic radiograph is obtained in case of suspicion of perforation. The reintroduction of nutrition is recommended after 24 hours if the patient remains asymptomatic. The number of dilations and their frequency depend on the type and size of the stricture. Consequently, an exact agenda cannot be recommended but will be modulated by the efficiency of the first dilation.

Note that an antisecretory medication (eg, omeprazole) can be useful after dilation to avoid relapse.

Achalasia is a special indication for balloon dilation. The treatment of this condition necessitates the use of a 35 to 40 mm balloon to reach a pressure of 500 mm Hg, to allow the rupture of the muscular layer gradually over at least two or three sessions. The results of this technique compared with conventional surgery are contradictory but are comparable to the latter in some studies.[223] The rate of esophageal perforation for achalasia is between 1.4 and 4%, with a rate of mortality around 0.5%. Recently, the injection of botulinum toxin in the lower esophageal sphincter was described as a potential alternative to balloon dilation or Heller surgery.[224,225]

Bleeding Control

Nonvariceal hemorrhage can be endoscopically treated either by thermal or nonthermal methods. Thermal coagulation includes laser, heater, monopolar, or multipolar probes. Nonthermal coagulation consists of injection of agents that can be either sclerosing or vasoconstrictive. Both seem to have the same efficacy, yet the injections are easier and cheaper and can be done with smaller endoscopes.

The two main clinical applications are active bleeding of an ulcer and a visible vessel. In active bleeding, injection of epinephrine (5–10 mL of 1:10,000) in the four angles of the lesion is the method of choice. Bleeding stops in 80 to 85% of cases.[226] Addition of a sclerosing agent, such as 1% polidocanol, is recommended in cases of visible vessels.[227] Some factors are responsible for failure: the ulcer's size (> 2 cm), hypovolemic shock, localization on the lesser curvature, and the presence of active bleeding.[228] Therapeutic endoscopy for active bleeding needs a visual control 24 hours later.

In case of severe hemorrhage, with an unsuccessful therapeutical endoscopy (5 to 8% of the adults, exceptional in children), surgery might be needed.[226]

Endoscopic Therapy of Variceal Hemorrhage

Variceal bleeding is the most common cause of severe gastrointestinal bleeding among children and accounts for one-third of all deaths related to cirrhosis in adults. However, the hemorrhage can be due to intra- or extrahepatic causes in childhood. Indeed, extrahepatic portal venous

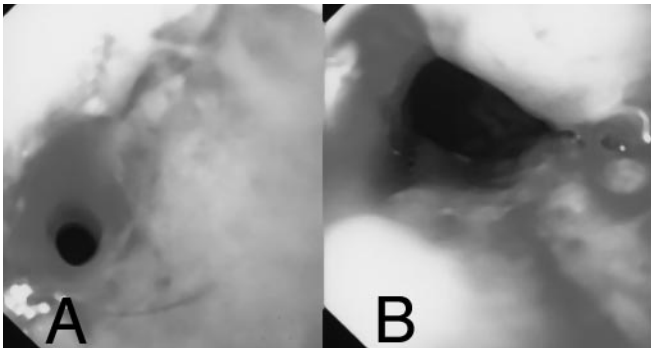

FIGURE 67.2-19 Severe esophageal stricture due to gastroesophageal reflux: *A*, before, *B*, after balloon dilation.

obstruction is a more frequent cause of bleeding in children when compared with adults. Unlike patients with cirrhosis, mainly owing to biliary atresia, those with extrahepatic portal hypertension have a better prognosis because of their liver function preservation.[229] The causes of death attributable to variceal bleeding include recurrent variceal hemorrhage, liver failure, hepatic encephalopathy, and infections. Portal hypertension is characterized by a pressure above 15 mm Hg, which leads to an impaired blood flow into the portal system. In portal hypertension, the mediastinal veins are organized in esophageal varices and in paraesophageal mediastinal veins. Esophageal varices can develop under the effect of pressure higher than 10 to 12 mm Hg.[230] However, their rupture does not seem to be due to a direct relationship between bleeding risk and exact level of portal pressure but to an imbalance between portal hypertension and resistance of the vessel wall.[231]

Endoscopic diagnosis of esophageal varices must be precise. Esophageal varices can be white or blue and are classified in four grades.[232] Grade I corresponds to varices that disappear with air insufflation, grade II corresponds to nonconfluent varices that remain identical with air insufflation, and grade III corresponds to confluent varices that cause an obstruction of the esophageal lumen (see Figure 67.2-11); the addition of red signs (cherry red spots, red wale markings, hematocystic spots, telangiectasia, varices overlying varices) leads to grade IV, with a higher and more precocious risk of bleeding. The classification of gastric varices is simpler: grade I in case of potential presence and grade II when the varices are overt, with red signs, which are easy to differentiate from gastric folds (Figure 67.2-20). Finally, the hypertensive gastropathy is secondary to the hyperkinetic syndrome owing to portal hypertension (Figure 67.2-21). It can be moderate or severe with purpuric spots, vessel ectasia, or erosions. Gastrointestinal bleeding secondary to portal hypertension is mainly due to rupture of esophageal varices. Among children, this situation represents only 15% of the causes of upper gastrointestinal hemorrhage, but it is often severe and life threatening. In specialized centers devoted to liver diseases and transplant, this percentage can be higher.

Adult guidelines for the diagnosis and treatment of gastrointestinal bleeding secondary to portal hypertension were published by the American College of Gastroenterology Practice Parameters Committee.[233] Some good reviews are available for children,[234,235] but no guidelines exist. Consequently, the optimal approach and treatment of children are still controversial, often depending on each center's competences and experience.

The diagnosis of variceal rupture is possible in up to 50% of cases.[236] The endoscopy can reveal a diffuse or localized deposit on a varice. The main localization of the variceal rupture is in the last 3 to 4 cm of the esophagus. Some endoscopic signs have a good positive predictive value for future bleeding, such as large-sized varices, cherry red spots, and varices of the cardia. Seventy-five percent of children with bleeding present with all of these signs.

To date, many modalities for treating variceal bleeding have been recommended, including pharmacologic therapy, endoscopic treatment, surgical approach, and radiologic shunting. Unfortunately, none of the existing methods of treatment are optimal because the association of different therapies can be helpful.

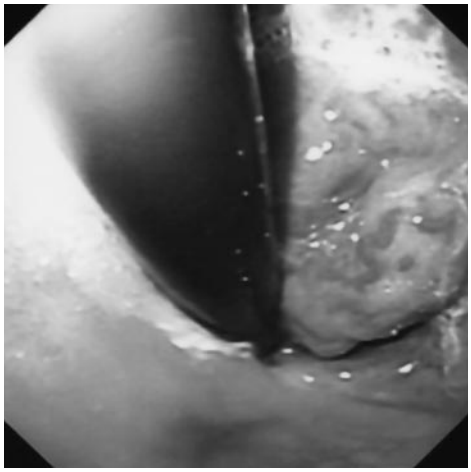

FIGURE 67.2-20 Cardial varices with red signs.

Endoscopic Sclerotherapy. Endoscopic sclerotherapy (EST) was the first efficient endoscopic approach in both adults[237] and infants[238] to treat both acute bleeding and risk of relapse, with less morbidity and mortality when compared with surgery (Figure 67.2-22). Ideally, EST is realized after a short delay (eg, 24 hours) and under endotracheal anesthesia. The timing of EST is weekly for 3 weeks and then monthly until the total eradication of the esophageal varices. Three techniques have been used: paravariceal, intravariceal, and both para- and intravariceal injections. At present, the most common procedure is the intravariceal injection. The most popular sclerosing drug in Europe is polidocanol 1 to 2%. Note that sodium morrhuate, ethanolamine, and absolute alcohol are also used. With a mean dose of 2.5 mL of polidocanol for each injection and a total amount of 10 to 12 mL, the eradication of

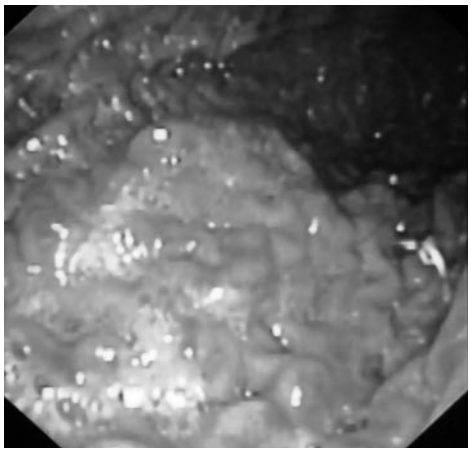

FIGURE 67.2-21 Congestive gastropathy due to severe portal hypertension in patient with cystic fibrosis.

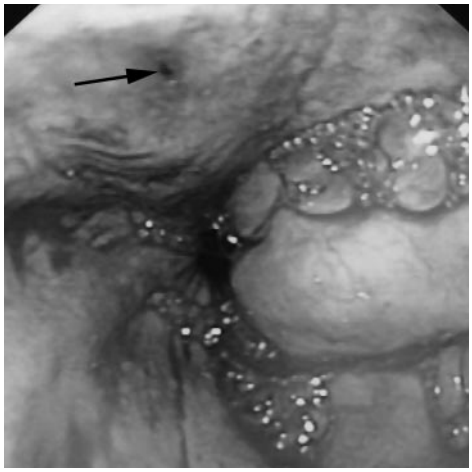

FIGURE 67.2-22 Esophageal varice after sclerotherapy with polidocanol and methylene blue. *Arrow* indicates injection point.

the varices is obtained after four to five sessions of EST. In a recent pediatric study, 95% of the patients had their varices successfully eradicated after a mean of 4.5 EST sessions.[239] The risk of a recurrence of bleeding is different among the studies depending on the realization of a liver transplant or a surgical shunt, ranging from 2[239] to 26%.[240] A rate of 10% must be considered realistic even if the rate of recurrence of varices is higher.

EST causes numerous side effects. Up to 50% of patients complain of dysphagia lasting for 2 days. Owing to the chemical properties of the sclerosing products, ulcers can appear in 17% of cases.[239] They can be responsible for precocious, severe rebleeding. Sucralfate, anti–histamine$_2$, or proton pump inhibitor drugs can be used to accelerate ulcer healing. Strictures are also potential complications of EST and were encountered in around 20% of cases.[239] The most serious complication of EST is perforation, which was described in up to 4% of the series[241] and in 1.4% of this recent study.[239] Its mortality is high. Perforation can also lead to esopleural or esobronchial fistulae. Infrequent complications such as sepsis,[182] bacterial peritonitis,[242] bacterial meningitis,[243–246] cerebral abscess,[245,246] and permanent paraplegia[247] have also been reported. Consequently, prophylactic antibiotics are prescribed because they have been shown to reduce bacteremia and bacterial peritonitis. The use of *N*-butyl-2-cyanoacrylate (Histoacryl), which coagulates instantaneously on contact with blood, is rare among pediatric patients and only related to bleeding of fundus varices. The risks of permanent obstruction of the channels of the endoscope are high. For other localization of bleeding varices, such as the duodenum or rectum, the procedure must be evaluated in each case by the operator.

Endoscopic Variceal Ligation. Endoscopic variceal ligation (EVL) was reported more recently and seen as a safe and quick procedure in adults.[248] EVL was introduced for pediatric patients only recently.[249,250] This procedure is limited in children by the size of the ligation device and by the fact that fewer bands can be applied per session owing to the small esophageal lumen. Apart from these restrictions, EVL is easy to realize, with a low complication rate. The latter is related to the mechanical strangulation of varices with little tissue inflammation and injury. The only serious complications are related to the use of an overtube: laceration, perforation, and punching injury of the esophagus. These lesions can provoke retrosternal pain or dysphagia.

Comparison of Different Modalities of Endoscopic and Medical Treatment. Most studies reported that EVL is superior to EST in terms of speediness, safety, rebleeding, and complications in adults.[237] EVL was also compared with EST in pediatric patients in a recent study.[251] No significant differences were found between EVL and EST in stopping bleeding and achieving variceal eradication. EVL eradicated varices in fewer endoscopic sessions and had significantly lower rebleeding and complication rates. However, recurrence of esophageal varices was similar in both EVL and EST groups after eradication. Consequently, both adult and pediatric data seem to favor EVL rather than EST to prevent risk of rebleeding.

Both EVL and EST are more effective when associated with vasoactive agents than alone. On the contrary, the combination of EVL and EST in the same session does not seem to be recommended because the complications increase more than the benefits.

Finally, endoscopic therapy is less effective than radiologic transjugular intrahepatic portosystemic shunt (TIPS) in terms of variceal rebleeding. However, TIPS must be considered as a rescue procedure before a liver transplant because the risk of occlusion in TIPS is high, as well as the development of encephalopathy.

PERCUTANEOUS ENDOSCOPIC GASTROSTOMY

In the early 1980s, Ponsky and Gauderer developed a PEG,[252] drastically changing the pediatric approach to enteral feeding, in particular in patients with swallowing difficulties. This minimally invasive, sutureless method is nowadays used worldwide to help provide appropriate nutritional intake

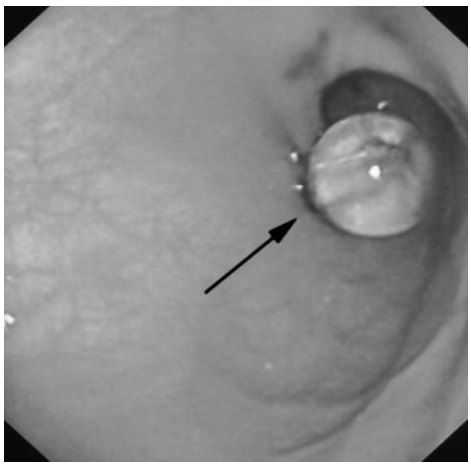

FIGURE 67.2-23 Gastrostomy button MYCKEY type (*arrow*).

with comfort and easy care. Ethical and moral concerns were raised with the increasing number of procedures, and guidelines for indications have been drawn.[73,74]

In the pediatric population, the intervention takes place under general anesthesia on a fasted patient. The patient is at high risk of infection, and antibiotic cover is recommended by gastroenterologist societies[73,74] because it reduces the rate of both peristomal and systemic infections to less than one-quarter.[252–257] At even higher risk are patients suffering from diabetes mellitus.[258] The most commonly recommended regimen is amoxicillin or intravenous cephalosporin.

Although the risks and potential benefits of enteral access catheter placement must be weighed in each patient, certain anatomic and pathologic conditions may increase the likelihood of complications. The absolute contraindications to percutaneous feeding tube placement are uncorrectable coagulopathy and unfavorable anatomy, with inability to bring the anterior gastric wall in apposition to the abdominal one. Relative contraindications are massive ascites, peritoneal dialysis, active gastritis or peptic ulcer disease, and gastric varices. Examples of unfavorable anatomy include malrotation; interposition of the colon, spleen, or liver between the stomach and anterior abdominal wall; intrathoracic stomach; and previous gastrectomy. Hepatomegaly, severe scoliosis, and obesity are not contraindications but may impede gastric transillumination and subsequent placement of the PEG.

A portion of patients needing gastrostomy suffer from conditions associated with reflux, such as neurologic impairment, myopathies, cystic fibrosis, and chronic respiratory failure. The indication of performing a simultaneous fundoplication is still a subject of controversy[88] because it seems to bear a higher complication incidence.[259] In addition, contradictory data result from the studies performed, some showing that the PEG procedure tends to aggravate the reflux, at times antireflux treatment being necessary,[260–263] whereas some show no effect.[259,264–266] Careful evaluation of the clinic and the severity of reflux will provide the elements to decide for a prophylactic fundoplication in a case by case approach, systematic antireflux surgery being increasingly abandoned.

Three techniques have been described to insert the stoma: the "pull-through," in 1980, by its inventor[261]; the "push-pull" technique, in 1981, by Sacks and Vine[267]; and, finally, the "introducer" technique by Russell and colleagues 3 years later.[268] Only the techniques most commonly used in pediatrics are described here. Patients lie flat on their back. The abdomen is disinfected with an antiseptic solution. An endoscope is passed down to the stomach and air is insufflated to approximate the stomach to the abdominal wall. The tip of the endoscope is then applied against the anterior wall, with transillumination helping to choose the exact place for the gastrostomy. The site of choice is located at the junction of the external two-thirds of a line drawn between the umbilicus and the midportion of the left inferior costal margin. Gastric indentation is created by digital pressure on the abdominal wall to check the final stoma position. After a small incision of the skin, the needle is passed into the gastric cavity. At this point, it is important that the endoscopist keep the stomach well inflated to avoid interposition of either the spleen, the liver, or, especially, the transverse colon. A string is then passed through the needle, to be seized by endoscopic snare or forceps and brought out through the mouth of the patient together with the gastroscope. The feeding catheter is then fixed to the oral end of the wire and is gently pulled back through the patient's mouth into the stomach and pulled out through the abdominal wall. The feeding tube ends with a stabilizer or "bumper," shaped either as a crossbar, an X, a T, or a cup, allowing it to be retained in the stomach. The maintenance of apposition of the peritoneal and serosal surfaces will create adherence. One should make sure that no excessive tension is applied to the wall by viewing it directly with the endoscope. An external retaining crossbar helps to maintain the right pressure. Daily rotation to 90° will avoid not only skin lesions but the proliferation of the gastric mucosa. Feeding through the device can start 6 hours postintervention. When the feeding tube is no longer necessary, the device is cut at the skin level. Good practice recommends performing it with endoscopic assistance to retrieve the bumper. Bowel obstruction and perforation caused by the internal part, left within the stomach to pass spontaneously, have indeed been reported in the literature.[269–271]

The feeding tube can be replaced in a second time by a button (Bard [Bard Medica, Volketswil, Switzerland], with a mushroom, or MYC-KEY [Cosanum, Schlieren, Switzerland], with a balloon as a bumper [Figure 67.2-23]). These devices, more esthetic and easier to conceal under the clothes, contain an antireflux valve that helps to avoid skin lesions. Once the gastrostomy tube or the button has been removed, the orifice will close itself in 48 hours. Delay of gastrostomy closure has rarely been observed up to 2 years later.[272]

The rates of adult morbidity and mortality are lower than those for surgical gastrostomy: 3 to 12% versus 30% for morbidity and 1 to 12% versus 16% for mortality.[273–276] Therefore, it should be preferred to surgical gastrostomy if no contraindications are present. In children, severe complications are observed in 3% of the PEG cases, including aspiration bronchopneumonopathies and migration of the bumper at the tip of the gastrostomy tube inside the abdominal wall, also called the "buried bumper syndrome." The condition resulting from excessive traction on the tube, sometimes secondary to growth of the patient, is the most common complication encountered. The button seems more likely to cause it than PEG.[272] Endoscopically, the bumper is partially or not seen, buried in the mucosa. Direct viewing will distinguish it from newly described pseudotumoral proliferative gastric mucosa, revealing an ulcerate mass or polyps. Peritonitis occurs immediately after insertion of the PEG or at the time of button change. It is due to stomal separation, tube malposition, or leakage. Rare cases of gastrocolic fistula have been reported in children, probably owing to misplacement of the colon at the time of insertion.[272,277,278] Risk factors are abdominal surgery that took place prior to insertion of the PEG and

insufficient transillumination. Head supraelevation might reduce this risk by displacing the colon to the lower abdomen. Necrotizing fasciitis starting around the site and ulcers are rare complications. Two cases of hemoperitonitis have been personally encounted by one of the authors. The bleeding is caused by the lesion of parietal gastric vessel and can be fatal if the diagnosis is not made.

Less severe complications happen in 7 to 13% of the interventions, mainly represented by peristomal infections and pneumoperitonium, proving air leakage around the hole. When minor complications, such as granulation, are counted, the rate goes up to 44%.[272] When it takes place immediately after PEG insertion, simple traction on the feeding tube will abolish it by reducing the space between the stomach and the abdominal walls. Feeding through the tube should be delayed. If still present at 48 to 72 hours, laparoscopic exploration needs to be discussed. Local infections need to be taken seriously because they can lead to sepsis. Complication can also occur during replacement, the tube being not well introduced in the stomach. Endoscopic control will avoid missing such a problem. Secondary displacement of the button end has been seen. Granulation tissue sometimes grows around the site, occasionally causing oozing of blood. Treatment consists of chemically burning the tissue by topical application with silver nitrate ($AgNO_3$) sticks.

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3. *Ileocolonoscopy and* Enteroscopy

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This chapter focuses on technique and clinical application of ileocolonoscopy and enteroscopy in childhood. The impact of endoscopic investigations and therapies on specific disease processes and comparison with alternative methods are also discussed. Topics to be covered include the following:

- Patient preparation
- Bowel preparation
- Endoscopy facilities
- Monitoring and sedation or anesthetic
- Equipment
- Basic and advanced techniques in diagnostic and therapeutic ileocolonoscopy
- Indications
- Complications
- Follow-up and surveillance ileocolonoscopy
- New diagnoses
- Diagnostic comparison with noninvasive investigations
- Enteroscopy: indications, techniques, and complications

ILEOCOLONOSCOPY

Safe, informative, and effective ileocolonoscopy performed in a child-friendly environment with the minimum of distress to child and parent alike is a sine qua non for best-practice care of children and adolescents with conditions affecting the ileum and colon, such as inflammatory bowel disease, allergic colitis, and polyposis syndromes.

The care of children and adolescents differs in important ways from that of adults. This is reflected in the emphasis placed on various aspects of ileocolonoscopy, such as the frequent use of general anesthesia, the number and location of mucosal biopsies, and the routine inclusion of ileal intubation during a complete examination. The question of who should conduct the procedure continues to receive attention among pediatric gastroenterologists. It is generally accepted that a pediatrician, preferably with experience in pediatric gastroenterology, should be involved in the care of the child or adolescent and, ideally, should carry out the procedure. There can be few more satisfying experiences in medicine than making a clinical judgment and diagnosis in a child, confirming the nature and extent of the disease oneself by endoscopy, treating appropriately, and then visually demonstrating the success of such endeavours to child and parent by a follow-up procedure.

The practice of pediatric ileocolonoscopy has evolved dramatically over the past 15 to 20 years. Improvements in skill and technique have followed the advances in technology, from the advent of fiberoptic endoscopes used in pediatrics during the late 1970s[1] to the latest small-diameter, electronic videoendoscopes designed specifically for younger children.[2–8]

TRAINING

Much debate has surrounded the issue of the minimum procedure number required during training to achieve competence because a paucity of data exists to support one view or another. Guidelines issued by the North American Society of Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN) in 1997 recommended a minimum of 50 colonoscopies. The guidelines were revised in 1999, increasing the threshold number to 100 and including numbers for therapeutic techniques such as snare polypectomy (20), stricture balloon dilation (15), and injection therapy or electrocautery (20).[9–11] It is apparent that some ongoing skill assessment is needed during training. A logbook of procedures undertaken with a trainer's assessment of skill level will undoubtedly become common practice in recognized training centers. This will shift the emphasis from "number counting" to skill assessment, which is sensible given that trainees learn at different rates. However, some minimum numbers are likely to remain.

It has previously been suggested that training on endoscopy stimulators may reduce the time needed to reach competency in endoscopy. Ferlitsch and colleagues randomly allocated beginners in adult ileocolonoscopy to either receive training on the GI-Mentor (Symbionix Ltd, Lod, Israel 71520) or no training for 3 weeks after baseline assessment.[12] They were subsequently reassessed on the endoscopy simulator, and a statistically significant difference between the two groups was seen. The Royal Free Centre for Paediatric Gastroenterology has a Symbionix GI-Mentor (Figures 67.3-1 and 67.3-2; <www.symbionix.com>), to which trainees now have access. Looking at the ileocolonoscopy skill graphs (Figure 67.3-3), it is clear that three individuals (numbered 8, 9, and 10 in the figures) exhibited much steeper learning curves and seemed more competent having completed a smaller number of procedures than the other seven. These three had had a 6-week program of training on the ileocolonoscopy simulator prior to their first "live" ileocolonoscopy. The trainees log their performance by saving it under their own identifiable code, and the trainer can assess this at any time by logging into the simulator in

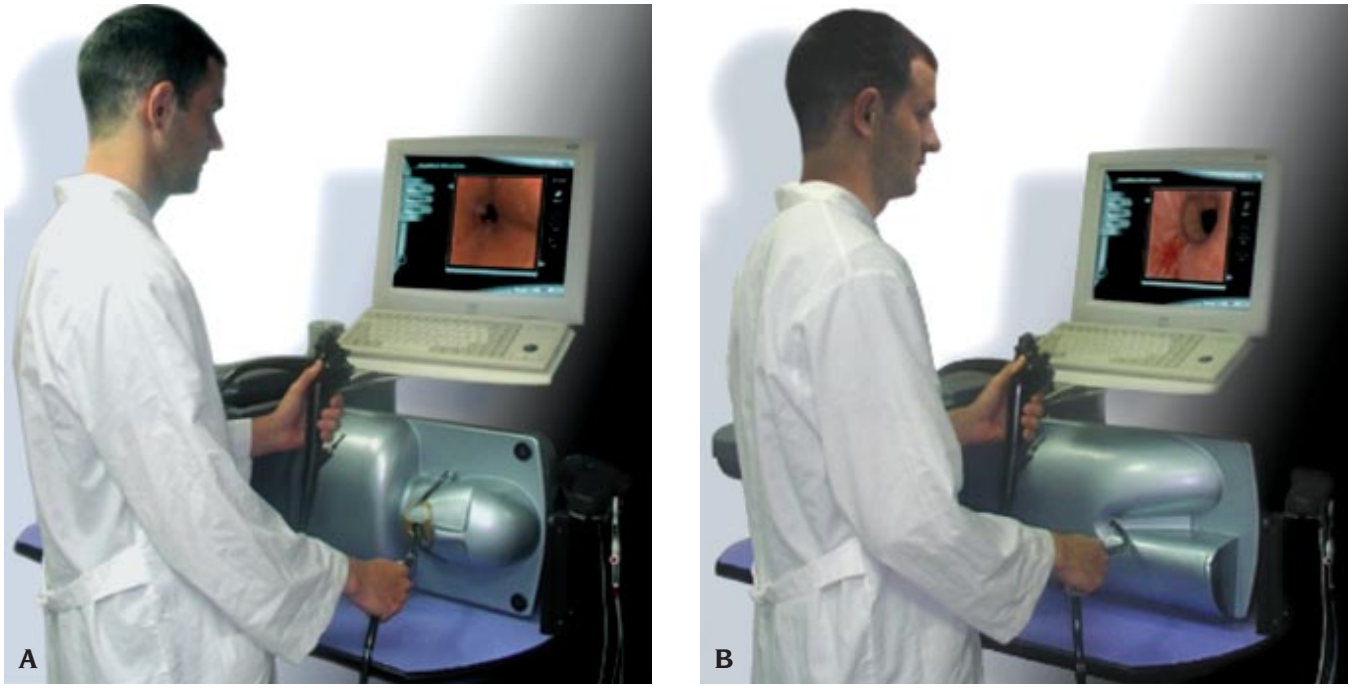

FIGURE 67.3-1 Symbionix GI-Mentor simulator for *A,* endoscopy and *B,* colonoscopy training.

trainer mode and so assess and feed back to the trainees their performance. This interactive approach is flexible and accelerates the learning process.

It is clearly preferable and more effective to learn the basic techniques on a model rather than a patient, and this approach does seem to work, at least on the basis of this preliminary experience. As these tools become more widely available, they are likely to become the first step and would seem to be the ideal introduction to training in pediatric endoscopy and ileocolonoscopy, before actual patient procedures are undertaken.

To collate the views of all pediatric gastroenterologists, the recent World Congress on Pediatric Gastroenterology convened a working group on endoscopy, and one of its remits was to examine the issue of training.[13] This has recently been updated to coincide with the 2004 World Congress meeting in Paris but is not yet published. Three main areas requiring attention were identified: the estab-

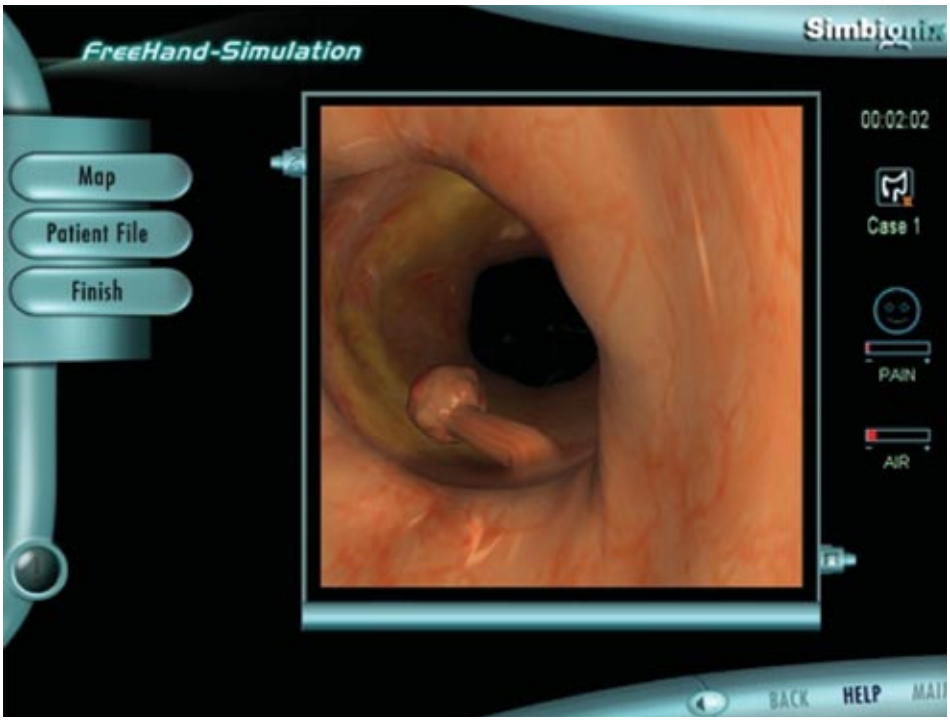

FIGURE 67.3-2 Symbionix GI-Mentor simulator for endoscopy and colonoscopy training: polyp on screen image.

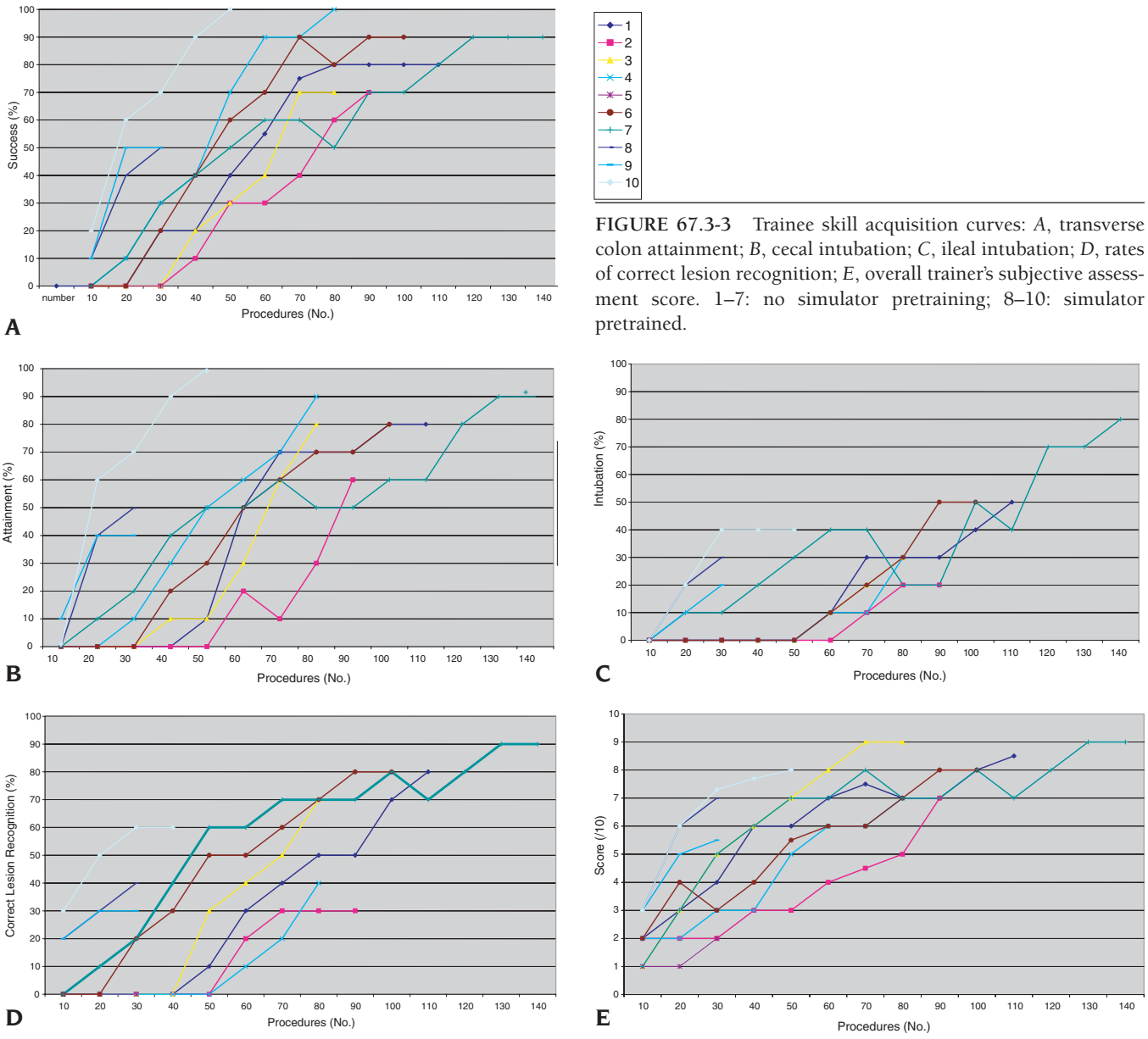

FIGURE 67.3-3 Trainee skill acquisition curves: *A*, transverse colon attainment; *B*, cecal intubation; *C*, ileal intubation; *D*, rates of correct lesion recognition; *E*, overall trainer's subjective assessment score. 1–7: no simulator pretraining; 8–10: simulator pretrained.

lishment and recognition of "Centers of Excellence in Pediatric Endoscopy," the use of training videotapes, and attendance at live and "hands-on" courses in pediatric endoscopy. The recommended goals for training were similar to those of the NASPGHAN position paper of 1999:

1. The ability to recommend endoscopic procedures based on the findings of a personal consultation and recognize indications, contraindications, and diagnostic or therapeutic alternatives for endoscopic procedures
2. To perform procedures safely, completely, and expeditiously
3. To correctly identify most endoscopic findings and perform endoscopic intervention as needed
4. To integrate endoscopic findings into a management plan
5. To understand risk factors and to recognize and manage complications
6. To recognize personal and procedural limitations and know when to request help
7. To be familiar with national organizational guidelines regarding sedation and monitoring for pediatric gastrointestinal procedures
8. To identify age- and indication-appropriate endoscopic equipment
9. The proper cleaning and maintenance of endoscopic equipment with special attention to infection control measures

Quality assurance has received little attention to date and is an issue that may begin to assume more importance, for instance, in the United Kingdom, where Clinical Governance and increased accountability are taking place. A multidiscipline body has been set up in the United Kingdom to address training issues, the Joint Advisory Group on gastrointestinal endoscopy (<www.thejag.org.uk>), and it oversees all training issues; ongoing assessment, revalidation, trainer and training center accreditation, and certification in endoscopic training are just some of the difficult issues now being dealt with effectively by this body, the

view being that only through proper training can standards be improved in general. Striking differences in technical standards and appropriateness of ileocolonoscopy between centers emerged in one study in adult practice in Italy.[14] Quality improvement has been addressed by the American Society for Gastrointestinal Endoscopy.[15]

ENDOSCOPY FACILITY AND PATIENT PREPARATION

Ideally, both the child and the parents should be offered a preparatory visit to the endoscopy unit to answer questions and defuse any potential concerns and anxieties regarding the procedure and admission. Younger children undoubtedly benefit from preadmission visits and the involvement of a play therapist to enable some understanding of what is to take place and why.[16–18] Diagrams may help in explanations to older children. Preparatory videotapes are also useful for informing the patient and parent regarding what to expect. Units can benefit from devising a sample videotape specific to their own facility. A reduction in anticipatory anxiety may even reduce the amount of intravenous sedation required.[19] A child-friendly decorated endoscopy room with age-appropriate videotapes and familiar faces is important at this time of high stress. Parents may stay to watch the procedure in some units when intravenous sedation is provided. Most anesthesiologists would object to having parents present during administration of a general anesthetic, beyond the initial induction. Improved medical compliance and belief in the treatment are potential advantageous consequences of allowing parents to directly view the initial disease and its remission at follow-up ileocolonoscopy.[20] Young children often request photographs or a videotape of the ileocolonoscopy, and even older adolescents may view the procedure themselves.

A full screening is important to identify potential sedation or anesthetic risks. Although there is little correlation of mildly deranged peripheral coagulation indices with hemorrhage after mucosal biopsies, more pronounced bleeding diatheses may require forethought and appropriate blood product backup.[21] Properly informed consent should be obtained with an information sheet detailing potential complications and their incidence, and a separate consent should be signed in the event of research biopsies being requested.

Guidelines concerning antibiotic prophylaxis in children with lesions susceptible to endocarditis or in the immunocompromised child are available in the literature.[22] A low rate of bacteremia owing to bacterial translocation across the bowel wall has been demonstrated following pediatric ileocolonoscopy.[23] A combination of intravenous or intramuscular ampicillin (50 mg/kg, maximum 2 g) and gentamicin (2 mg/kg, maximum 120 mg) 30 minutes before and 6 hours after the ileocolonoscopy is generally recommended. Vancomycin (20 mg/kg slow intravenous infusion 1 hour before) can be substituted for ampicillin in those with documented penicillin allergy. It would seem reasonable to administer antibiotic prophylaxis in the rare case of percutaneous cecostomy or sigmoidostomy as for percutaneous gastrostomy insertion.

BOWEL PREPARATION

Poor bowel preparation is a major factor that may prevent or impede successful ileocolonoscopy. Although administration of regimens is not always easy, modern protocols can be remarkably effective in clearing the colon and ileum. Until 5 or 6 years ago, large volumes of oral electrolyte lavage solutions were used with variable success, coupled with the significant disadvantages of nasogastric administration and potential for fluid-electrolyte shifts in smaller children and infants. In one study, 40 mL/kg/h resulted in clear fecal effluent after a mean of 2.6 hours.[24] Subsequently, more favorable results and compliance were reported with low-volume oral agents and enemas, along with decreased oral intake.[25–28] Use of sodium phosphate preparations was associated with a transient rise in mean serum sodium and phosphate but with no change in serum calcium.[26,27] Refinements were made to these oral and enema regimens as newer preparations, which were more acceptable to children, became available; low-volume nonabsorbable polyethylene glycol preparations are becoming increasingly popular in pediatric units and are well tolerated, with no observable electrolytic disturbance.[29,30] Table 67.3-1 outlines several low-volume regimens that have been used successfully in children. The regimen employed in our unit, shown in Table 67.3-2, combines the beneficial effects of oral low-volume administration with the backup of an enema 1 to 2 hours beforehand if no clear fecal effluent is observed.[31] No clinically significant fluid shifts or electrolyte imbalances have been observed in over 2,000 colonoscopies over a 5-year period in our unit.

The benefit of an intravenous antispasmodic agent administered directly before the ileocolonoscopy has recently been demonstrated.[32] Hyoscyamine 0.5 mg was

TABLE 67.3-1 SUCCESSFUL RECENT LOW-VOLUME REGIMENS FOR THE PREPARATION OF THE BOWEL FOR ILEOCOLONOSCOPY

STUDY	REGIMEN	DIET	SUCCESS RATE
Gremse et al, 1996[27]	Oral sodium phosphate (45 mL/1.7m^2) 6 pm and 6 am for am procedure	Clear liquid 24 h	18/19
da Silva et al, 1997[26]	Oral sodium phosphate (22.5 mL if < 30 kg, 45 mL if > 30 kg) pm and 5 am for am procedure	Clear liquid after first dose	10/14
Pinfield et al, 1999[30]	Sodium picosulfate with magnesium citrate (2.5 g < 2 yr, 5 g 2–5 yr, 10 g > 5 yr per dose) 24 and 18 hr preprocedure	Clear liquid for 24 h	32/32 (3 vomited)
Dahshan et al,1999[29]	Magnesium citrate and X-prep	Clear liquid for 48 h	

TABLE 67.3-2 BOWEL PREPARATION FOR CHILDREN UNDERGOING ILEOCOLONOSCOPY

Clear fluids for preceding 24 h (12 h for infants receiving no solid intake)		
5 pm	Senokot	1–2 mg/kg (max 30 mg)
	Sodium picosulfate	2.5 g if < 1yr 5 g if 1–4 yr 10 g if > 4 yr
1 h preprocedure if no clear fecal effluent		
6 am	Repeat sodium picosulfate dose 1 h before procedure Phosphate enema (1/2 if < 1 yr)	

given in this study of adults. An alternative could be hyoscine 20 mg adminstered intravenously. The use of such an agent given just prior to colonoscopy is determined by personal preference. Their use may facilitate ease of luminal visualization, but it also may increase the compliance of the colon, theoretically allowing a greater chance of loop formation. They are certainly of benefit in spastic colonic situations. It should be remembered that they work only for a short period of time, however—perhaps as short as 5 minutes—and they may be readministered in certain situations, such as when one needs to relax a haustral fold if a polyp is just beyond and obscured by it, or occasionally when one needs to relax a spastic ileocecal valve.

MONITORING AND SEDATION

Debate has surrounded the relative merits and safety of sedation and general anesthesia for upper gastrointestinal endoscopy and ileocolonoscopy in children for the past 5 to 10 years.[33–35] The use of sedation for endoscopy has been comprehensively reviewed recently.[35] The proponents of general anesthesia maintain that sedation is merely a financial or logistic expedient when the services of an anesthesiologist are less accessible.[33,34,36] In one US study, the cost of an endoscopy under general anesthesia was twice that under sedation.[37] It is generally agreed that children should receive, at a minimum, an analgesic in combination with a hypnotic for potentially painful or frightening procedures.[38]

It is also acknowledged that the concept of "conscious" (or "safe") sedation is not the prevailing clinical scenario in sedation in gastrointestinal endoscopy in pediatrics, and to get the requisite cooperation, and therefore a properly conducted procedure with mimimum distress to the child, deep sedation is usually necessary. It is further recognized that there are attendant safety issues of airway maintenance in this situation, and at the very least, a specific individual with appropriate advanced pediatric life support skills should be responsible for the child's cardiorespiratory welfare during such a procedure. The vast majority of pediatric gastrointestinal endoscopy under the age of 8 years in the United Kingdom, for instance, now occurs under general anesthesia.

When a child is sedated, resuscitation equipment should be easily accessible, and one or more people trained in pediatric advanced life support should be responsible for maintaining the airway and monitoring respiration, heart rate, blood pressure, and oxygen saturation.[39,40] Older children can sometimes benefit from conscious sedation such that they can participate in observing their ileocolonoscopy.[20] Sedation of younger children can be aided by environmental comforts such as a soothing voice or dimmed lights.[41] In all age groups, it is often necessary to use deep sedation because of the pain that can be associated with this procedure.[42] With deep sedation, it is clear that the risks are significant, including hypotension, respiratory compromise, and even respiratory arrest.[43] Combinations of benzodiazepines (midazolam in preference to diazepam) and opioids (pethidine or meperidine and fentanyl) are reported, with the occasional report of ketamine.[35,44] Table 67.3-3 lists some of the commonly used sedation regimens with the reversal agents.

Recent studies examining the safety of general anesthesia for day-case ileocolonoscopy in children refute claims that there may be more risk of perforation because the operator cannot judge the degree of discomfort as a marker of impending traction injury.[45,46] There is indeed a lack of evidence to support the contention that there is a higher complication rate with a general anesthetic than with sedation.[47] In fact, the airway is protected in a more effective and safer manner than with sedation, especially in upper endoscopy, with an improved operator satisfaction.[48]

EQUIPMENT

Most modern units employ adult and pediatric videocolonoscopes, and the general technical specifications for the pediatric instruments differ little between manufacturers (Table 67.3-4). When and in whom to use a pediatric colonoscope is mainly a matter of personal preference. We use personal judgment based on age and/or body weight. In general terms, the lower limit for the adult colonoscope is 3 to 4 years of age and/or 12 to 15 kg. The extra stiffness of the adult versions diminishes the likelihood of forming sigmoid loops, but extra care must then be taken, especially in younger children and with general anesthesia, not to advance against undue resistance, to avoid the unlikely complication of colonic perforation. The larger diameter of the adult colonoscopes can also lead to problems of

TABLE 67.3-3 SEDATION AND REVERSAL MEDICATIONS COMMONLY EMPLOYED IN PEDIATRIC ILEOCOLONOSCOPY

Midazolam: IV initial dose 0.05–0.1 mg/kg then titrate to maximum 0.3 mg/kg or 10 mg, whichever is lower[3]
Oral dose 0.75 mg/kg or 15 mg, whichever is lower[34]
Fentanyl: IV initial dose 0.5–1.0 μg/kg, then titrate to maximum 5 μg/kg[3]
Meperidine/pethidine: IV initial dose 0.5 mg/kg, then titrate to maximum 2 mg/kg or 75 mg, whichever is lower[34]
Flumazenil: IV dose 0.02 mg/kg (maximum 0.2 mg) and repeat every minute to maximum of 0.05 mg/kg or maximum 1 mg[3]
Naloxone: IV dose 0.1 mg/kg (maximum 2 mg) and repeat every 2–3 min to maximum 10 mg[3]

TABLE 67.3-4 TECHNICAL SPECIFICATIONS OF VARIOUS PEDIATRIC COLONOSCOPES

PARAMETER	FUJINON (EC-410 MP15)	OLYMPUS (PCF 240L/I)	OLYMPUS VARIABLE STIFFNESS (CF 240AL/I)	PENTAX (EC-3440PK)
Angle of vision (deg)	140	140	140	140
Depth of field (mm)	6–100	4–100	3–100	6–100
Distal end (mm)	11	11.3	12.2	11.5
Insertion tube (mm)	11.1	11.3	12.0	11.4
Channel (mm)	2.8	3.2	3.2	3.8
Angle up/down (deg)	180/180	180/180	180/180	180/180
Angle right/left (deg)	160/160	160/160	160/160	160/160
Working length (mm)	1,520	1,330 1,680	1,330 1,680	1,500

maneuverability within the smaller colonic lumen of a young child. The variable stiffness colonoscope (see Table 67.3-4) may negotiate some of these problems. A control dial on the upper shaft of this small-diameter colonoscope (Olympus XCF-240AL/I, Olympus Corporation, Tokyo, Japan) allows an increase in the stiffness of the insertion tube when passing through the sigmoid and transverse colon to avoid looping.[49]

More recently, magnifying colonoscopes have been developed, and their value in combination with dye spray or chromoscopy in various gastrointestinal diseases has been described.[50] For instance, the decrease in the number of cryptal openings in ulcerative colitis can be observed and correlated to disease activity,[51] but this does not substitute for histologic assessment.

For insufflation, there may be some advantage awarded by the use of carbon dioxide in place of air because it is more rapidly absorbed, leading to less patient discomfort and, theoretically, less risk of perforation.[52,53]

ILEOCOLONOSCOPY BASIC TECHNIQUE

Getting Started and Patient Positioning

The patient is usually positioned in the left lateral knee to chest position, although some operators prefer the right lateral position, citing easier sigmoid negotiation. Certainly, if the procedure is not subsequently allowing easy access to the splenic flexure, then patient repositioning from one side to the supine and then to the other side may be advantageous. In general, frequent turning of the patient is conducive to easier ileocolonoscopy as a whole and is to be advocated. An assistant stands on the operator's left to administer any abdominal pressure that may subsequently be deemed necessary to control, or try to prevent, loop formation in the sigmoid or transverse colon.

Practical Tips in Ileocolonoscopy

One important "trick" in learning ileocolonoscopy is to grasp the concept of the lumen and the positions of a clock face. For instance, if the lumen is at 9 o'clock, then to enter this requires anticlockwise rotation combined with upward deflection of the scope tip from the "neutral" position of 12 o'clock. Similarly, a combination of upward deflection of the tip with clockwise rotation of the colonoscope will allow entry of the lumen, suggested by a dark crescent, if seen at anywhere clockwise from 12 o'clock to 6 o'clock. Obviously, one may equally use downward tip deflection combined with the opposite rotatory control to that with upward tip deflection, and the execution and teaching of this concept are at personal discretion. With either approach, this is the most important maneuver that can be learned to assist in three-dimensional spatial orientation in the colon.

Prolonged "side viewing" of the bowel wall as it slides by should be avoided. Generally, the only place where, very temporarily, the lumen should be out of view is the occasional difficult negotiation of the splenic flexure. The patient's position may be changed throughout the procedure to facilitate removal of loops and to allow a better view of the lumen because the gravity-dependent material in the colonic lumen changes position. Relatively minimal insufflation of air is desirable in the sigmoid colon because excess air may increase the chance of sigmoid loop formation (carbon dioxide, provided by a specific commercially available delivery system attached to the colonoscope, because the insufflatory gas of choice may be preferable because it is absorbed much more quickly, decreasing pain and the very unlikely chance of perforation; see "Complications").

In handling the colonoscope, it is good practice to have a flat unimpeded surface on which to place the remainder of the colonoscope that is not yet inserted; this is particularly important because then any resistance encountered by the operator to forward advancement of the colonoscope can be attributed to colonic obstruction or loop formation within the child's colon. Hence, relatively quickly, the trainee can acquire a realization of the normal expected resistance to scope advancement. This, in turn, allows understanding of the likelihood of loop formation, without any external resistance to scope advancement, causing confusion with regard to the behavior of the colonoscope within the patient.

Generally, in ileocolonoscopy, gentle scope advancement with clear lumen visualization is desirable, and, usually, only the forefinger and thumb will be required to advance the colonoscope. If greater pressure is required, then the operator is not performing an optimum procedure, and loop formation is likely to have occurred.

Rectal Intubation

Prior to any colonoscopy, it is considered good practice to perform an anal and then a rectal digital examination, the latter to avoid missing, by colonoscopy, very low-lying rectal polyps (although, where possible, retroflexion of the colonoscope in the rectum should occur prior to removal of the instrument to avoid missing lesions close to the anal

margin). Adequate water-soluble lubrication, avoiding the tip of the instrument, allows easy passage into the rectum, which can occur with or without digital guidance from the operator's index finger. Posterior positioning of the tip and air insufflation will allow visualization of the rectal mucosa and the three semilunar folds, or valves of Houston, occurring on alternating sides of the lumen. Subsequently, direct visualization of the bowel lumen is mandatory, except in some circumstances at the splenic flexure. If, at any point, a maneuver results in loss of visualization of the lumen, then reversal of what the operator has just done will often return the lumen to view; if not, the gentle scope retraction combined with minor tip deflections using the wheels and minor rotation of the scope in both directions will usually result in reorientation in the lumen. Obviously, if luminal contents are blocking the view, then lens cleaning will help.

Sigmoid and Descending Colon

Gentle torquing of the shaft clockwise and anticlockwise combined with upward or downward tip deflection and scope advancement is ideal for negotiating the sigmoid colon—the so-called "torque-steering" technique. The initial sigmoid fold or valve can usually be passed by 90 to 120° of anticlockwise torsion. The different loops encountered in the sigmoid are demonstrated in Figure 67.3-4. A so-called N loop may be overcome by transabdominal pressure by an assistant on the apex of the loop pushing toward the feet (see Figure 67.3-4A). This often allows a so-called α loop to form, which can usually be tolerated as the instrument advances toward the splenic flexure (see Figure 67.3-4B). Reducing an α loop is accomplished by initial clockwise rotation and then slow removal of the colonoscope, keeping the lumen in the center of the field of vision. This may not be possible until the transverse (or even ascending) colon has been entered, in which case, it may be assisted by hooking the tip of the scope over the splenic flexure. Paradoxical movement of the tip forward may be observed as the instrument is withdrawn and the bowel "concertinas" over the colonoscope. Abdominal pressure in the left iliac fossa may be helpful. The sigmoid and descending colon are relatively featureless, with less haustral folds than more proximally in the colon.

Splenic Flexure and Transverse Colon

Nonlooped colonoscope length used at this point might be 40 cm in older children and even 20 to 25 cm in those

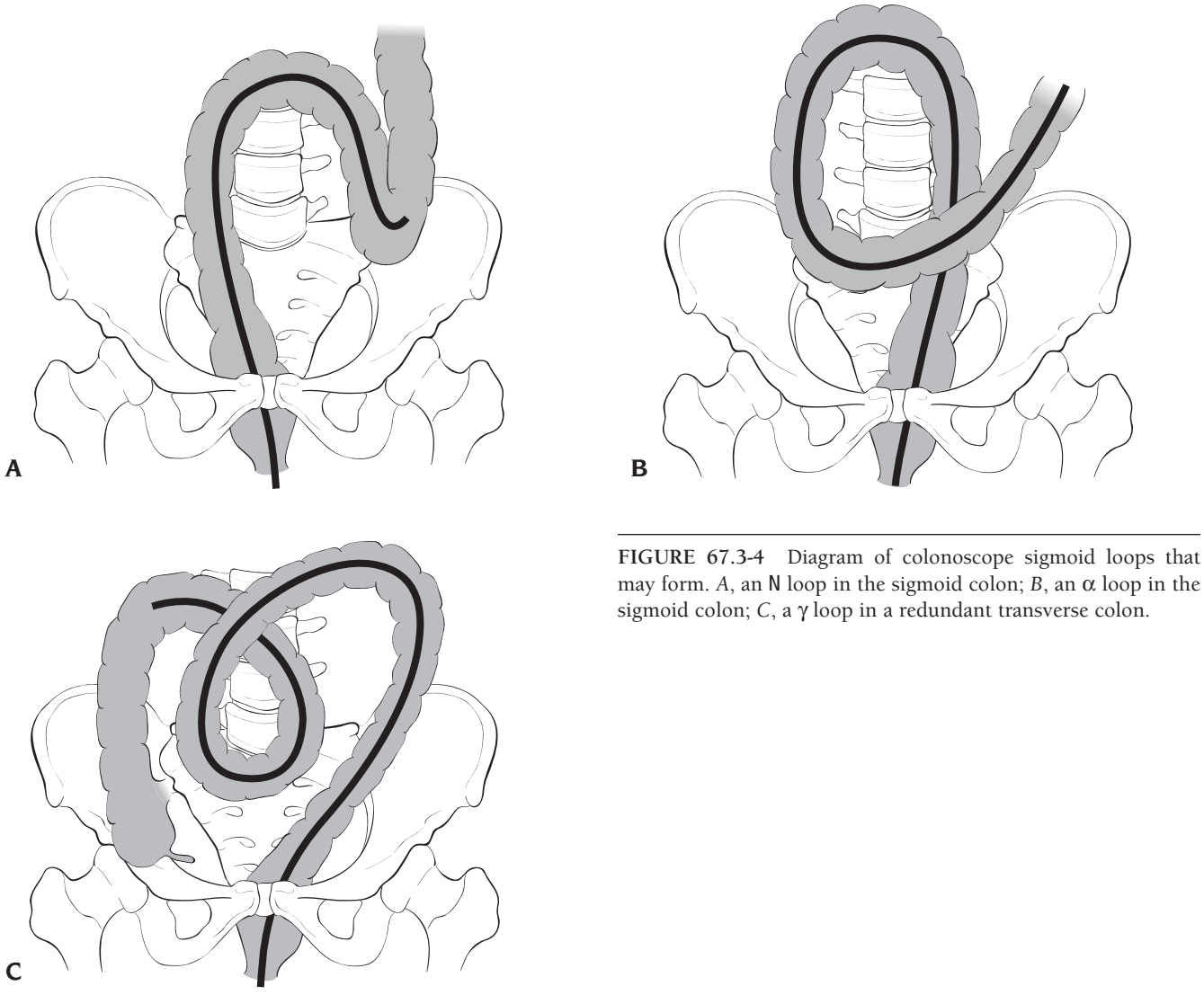

FIGURE 67.3-4 Diagram of colonoscope sigmoid loops that may form. *A*, an N loop in the sigmoid colon; *B*, an α loop in the sigmoid colon; *C*, a γ loop in a redundant transverse colon.

under the age of 3 to 4 years. This is valuable in determining whether a loop is present. At the splenic flexure, the spleen may then be seen as a dark blue transmural discoloration (Figure 67.3-5). When negotiating the splenic flexure, the most successful combination of tip maneuvers is that of clockwise, right, and up followed by anticlockwise after passing the flexure. Occasionally, placing the patient in the right decubitus position may assist. The transverse colon is recognized by the triangular haustral folds and is usually easily passed. Supine or right decubitus positioning may ease this. A loop in the shape of a U may occur in a dependent transverse colon, which is supported by abdominal pressure. The more difficult γ loop may occur in a redundant transverse colon (see Figure 67.3-4C). In addition, a good bit of advice is to apply gentle suction as the tip is advanced again in an attempt to concertina a potentially long dependent transverse colon over the colonoscope, thus maintaining a relatively short colonoscope and, hence, good control and maneuverability.

Hepatic Flexure and Ascending Colon

Nonlooped colonoscope length used at this point might be 60 cm in older children and even 40 cm in those under the age of 3 to 4 years. This is valuable in determining whether a loop is present. The hepatic flexure is also recognized by the dark, usually blue, discoloration seen through the bowel wall, and positional change to the supine or right decubitus may again facilitate identification of the lumen. The combination of right, up, and clockwise followed by anticlockwise rotation and suction down into the ascending colon once around the sharply angled hepatic flexure is usually the most effective maneuver, but various combinations, including position change and scope withdrawal, may be required. Another tip is to remember that it is easy to be too far advanced into the vault of the hepatic flexure, leading to advance into a blind end, and often slight withdrawal of the instrument may reveal the fact that one is trying to negotiate this blind-ended area. The two or three sharp folds then observed may then be most successfully negotiated by tip deflection using both up/down and left/right wheels with minimal advancement of the scope. This is most easily performed in the supine patient position, however.

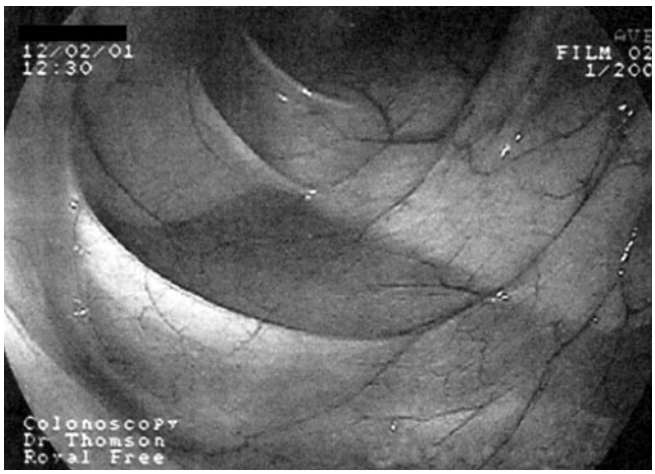

FIGURE 67.3-5 Dark shadow of spleen seen through colon at splenic flexure.

Once the hepatic flexure is negotiated, the transverse colonic γ loop may be reduced with anticlockwise or clockwise rotation followed by withdrawal of the colonoscope and suction. Loop withdrawal is essentially informed guess work initially. Studies with the colon Map guider developed by Dr. Christopher Williams and Olympus, based on using a colonoscope with an inbuilt electromagnetic loop that allows accurate real-time colonoscope three-dimensional positioning by detection using an external positioning device and displayed on a screen next to the patient, have shown that even expert colonoscopists get the type of loop present wrong in half of the cases.[54–56] Once one starts to remove the loop, using rotation only initially, a tip is to gently start to remove the colonoscope and try to determine whether within-patient resistance is increasing or whether the colonoscope is trying to push your hand away from the patient as the loop unfolds. Usually, trying clockwise or anticlockwise combined with instrument withdrawal will, with experience, allow early determination of which rotation direction is likely to be successful in "delooping" the colonoscope. It is best to try to maintain good luminal vision during this procedure, but, not infrequently, the lumen is lost; however, if this loop removal technique is effective, it is then not unusual to find oneself then looking at the appendiceal orifice and hence the cecum because the scope will have naturally traveled down the ascending colon. It is important to remember that the ascending colon, which in children is of variable length, may be as short as 5 cm in some younger cases.

Cecum

Three useful ways to ensure that one has reached the cecum are as follows:

- Observing the colonoscopic illumination in the right iliac fossa (using the specific high-intensity light transillumination application available with some colonoscopes is not usually necessary in children, excepting with some obese adolescents, for whom it can be helpful when applied in a dark environment)
- Digitally indenting the abdominal wall over the right iliac fossa and observing the corresponding effect on the colonic wall with the colonoscope
- Identifying the triradiate fold, appendicele orifice, and (especially if gas bubbles or ileal effluent are being excreted from it) the typical two lips–like appearance of the ileocecal valve (Figure 67.3-6A)

A good maxim is that if there is any doubt in the operator's mind about having reached the cecum, then one is usually at the hepatic or even splenic flexure. Only about 80 cm of scope from the anus is needed when all loops are removed in an adult, and in smaller children, only 40 to 60 cm may be needed. This assumes normal anatomy of the ascending colon and cecum. Obviously, cecal strictures can confuse the picture.

Ileal Intubation and Its Importance

The ileocecal valve is present approximately 1 to 4 cm distal to the appendicele orifice opening into a smooth asymmetric fold and opens perpendicular to the axis of the colon. Figure 67.3-6 shows the steps of the easiest technique for ileal intubation. Removal of any colonic loops is important to allow for a responsive scope with no paradoxical movement. Figures 67.3-6B and 67.3-7 show the valve maneuvered to the 6 o'clock position, usually after clockwise rotation of the scope and wheel-tip deflection to maintain a centered cecal view. Anticlockwise rotation can also be used but is less efficient. If too much gas is present, then the cecum may be "tented," and this should be suctioned prior to an ileal intubation attempt. Figures 67.3-6C and 67.3-8 show the insertion of the biopsy forceps such that just the tip or the first few millimeters are visibly exposed beyond the end of the scope. The scope is then inserted just beyond the fold (using the downward deflecting wheel with the scope as above already in the 6 o'clock position), and the tip is inclined downward so that the forceps gently press into the wall. Slight left inclination may be required at this point to open the valve like a pair of lips on slight withdrawal of the scope (see Figure 67.3-6C). Once the valve is opened, the scope may be passed into the ileum with further downward deflection. Often this is facilitated by small right and left deflections with an assistant pressing on the abdomen over the transverse colon to support a dependent transverse and also prevent loop formation. In the absence of ileocecal valve strictures, and with practice, this technique will allow an ileal intubation rate of 100%. Perforation of the cecum or ileum with this technique is a theoretical concern raised by some observers unfamiliar with this technique, but this has not occurred in our experience of over 5,000 ileocolonoscopies and is extremely unlikely.

An alternative technique is "blind" intubation of the ileocecal valve. This involves the same positioning of the valve at 6 or 9 o'clock and then slowly withdrawing the scope back from just beyond the valve's fold while insufflating with air and deflecting the scope tip downward. The disadvantage of this technique is that it is not under direct vision.

Ileum

The ileal mucosa will have the typical velvet-like appearance of small bowel, with the presence of smoother raised areas, which are Peyer patches, and, occasionally, lymphonodular hyperplasia of varying degrees. Villi are more easily seen if the lumen is flooded with water. The ileal surface is shown in greater relief with a spray of standard blue or black ink (methylene blue in a 1:20 dilution may also be used); this is also useful in showing the detail of sessile polyps in the colon. Deeper intubation of the ileum by either technique is similar to duodenal negotiation during upper gastrointestinal endoscopy, and up to 40 cm of ileum can be observed.

It is pertinent here to discuss the diagnostic need for entering the ileum in children suspected of inflammatory bowel disease. Williams and colleagues, in 1982,[7] reported

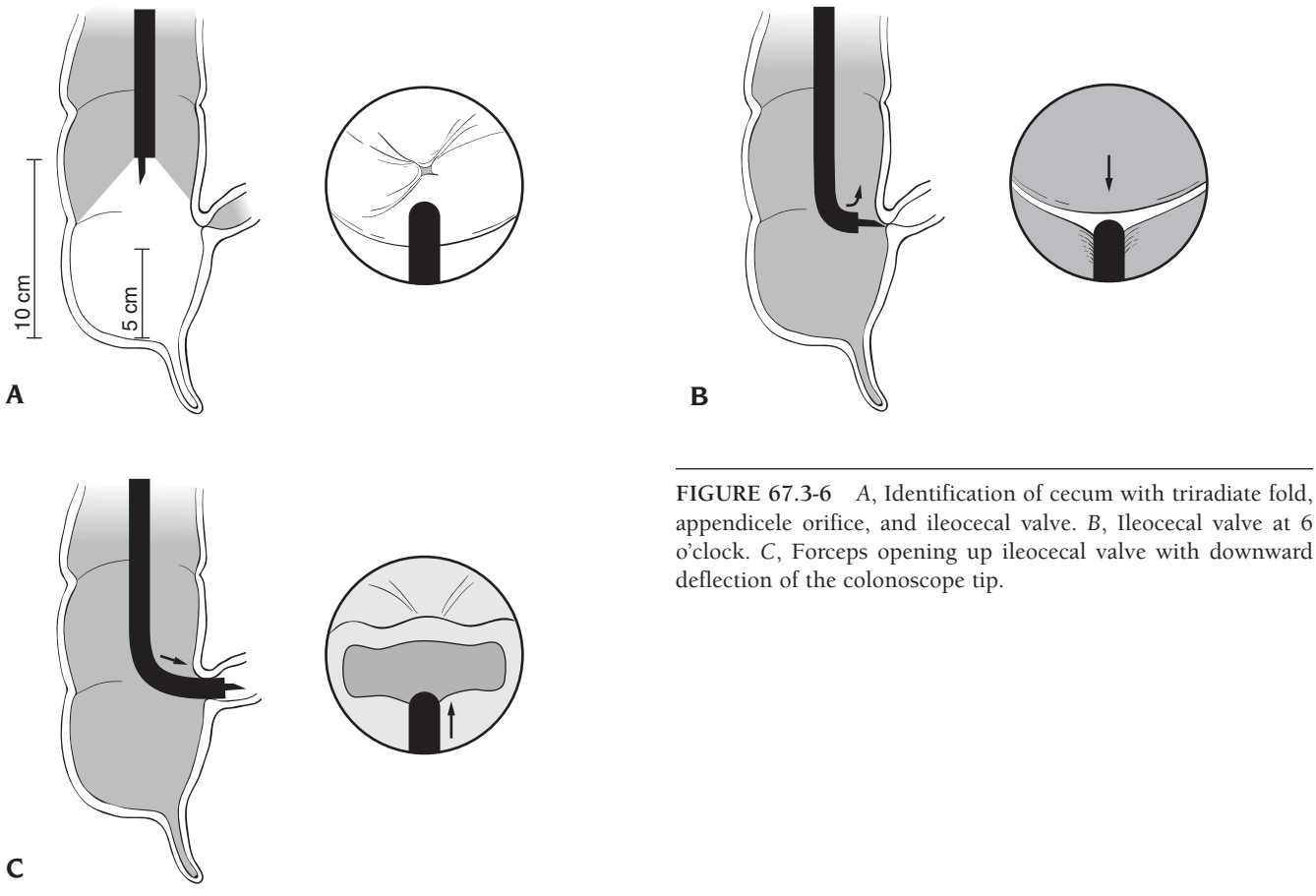

FIGURE 67.3-6 *A*, Identification of cecum with triradiate fold, appendicele orifice, and ileocecal valve. *B*, Ileocecal valve at 6 o'clock. *C*, Forceps opening up ileocecal valve with downward deflection of the colonoscope tip.

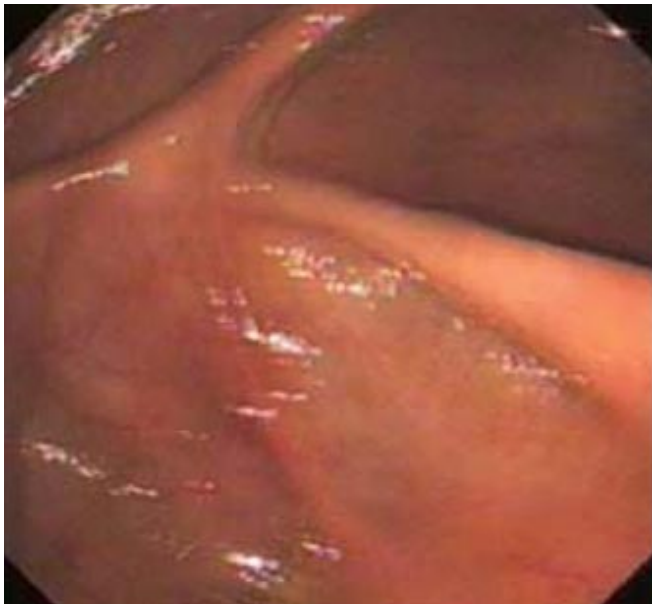

FIGURE 67.3-7 Ileocecal valve at 6 o'clock.

their experience of total ileocolonoscopy in children in which the terminal ileum was examined in 63 patients. In 6 children, ileitis detected by ileocolonoscopy was the sole finding of Crohn disease, which was previously unrecognized by radiologic contrast studies. Lipson and colleagues compared ileoscopy and barium studies, with an endoscopy specificity of 0.96 for diagnosis of Crohn disease in the terminal ileum.[57] In 14 of 46 children, ileoscopy revealed diagnosis, which would otherwise have been missed. This study also made clear that the endoscopic appearances could be completely normal, yet the diagnosis of Crohn disease could be made histologically by the presence of granulomata. Also, a pronounced lymphoid hyperplasia pattern was present radiologically in 24% of children and would have been a source of error in two cases had contrast radiographs been relied on to make the diagnosis without ileoscopy. More recently, Deere and colleagues showed that sigmoid, colonic, and rectal biopsies confirm the diagnosis of inflammatory bowel disease in only 60% of cases, and diagnosis based on morphologic criteria was possible in only 85% of cases when the cecum was reached without ileal intubation.[58] Geboes and colleagues assessed 300 patients, including adolescents and children, and found endoscopic and histologic ileal lesions in 123 and 125, respectively, of whom no colonic disease was present in 44.[59] Ileal biopsies were essential for the diagnosis in 15 patients and contributory in 53. There are, of course, other reasons apart from the principal one, that is, diagnosis of chronic inflammatory bowel disease, for entering the ileum in children. For instance, ileoscopy will facilitate diagnoses of other causes of ileitis such as infection with tuberculosis or *Yersinia*.[60] In addition, therapeutic dilation of short terminal ileal strictures by per endoscopic balloon catheter may be attempted.

Scope Withdrawal

A more careful inspection of the colon is necessary on withdrawal of the scope, especially for the presence of polyps, which may have remained hidden behind a haustral fold during the initial insertion of the scope. Biopsies should be taken from all areas, including normal-looking mucosa to allow for accurate histologic diagnosis. Biopsy technique is similar to esophagogastroduodenoscopy, with the exception that many colonoscopic biopsy forceps have a central barb, allowing more than one biopsy to be taken each time the forceps are passed.

Lastly, before removing the scope from the anus, a retroflexion maneuver obtained by maximum upward and right or left tip deflection and slight advancement of the scope into the rectal vault, followed by rotation clockwise and anticlockwise through 180°, completes the examination. This is necessary to observe the anorectal junction and distal rectum. Distal ulcers, inflammation, or even polyps can be missed if this is not done.

ADVANCED TECHNIQUES

Polypectomy

This is the most common therapeutic intervention, and the requirements for safe and effective polyp identification and removal include the following:

- Adequate sedation or general anesthetic to prevent patient movement
- Adequate bowel preparation
- Careful observation on insertion and withdrawal, changing the patient's position as needed
- Familiarity with the cauterization technique and power settings
- Availability of hemostasis techniques
- Normal or corrected coagulation indices

If bowel clearance is not good, then small or medium polyps may be missed, and feces may hamper their

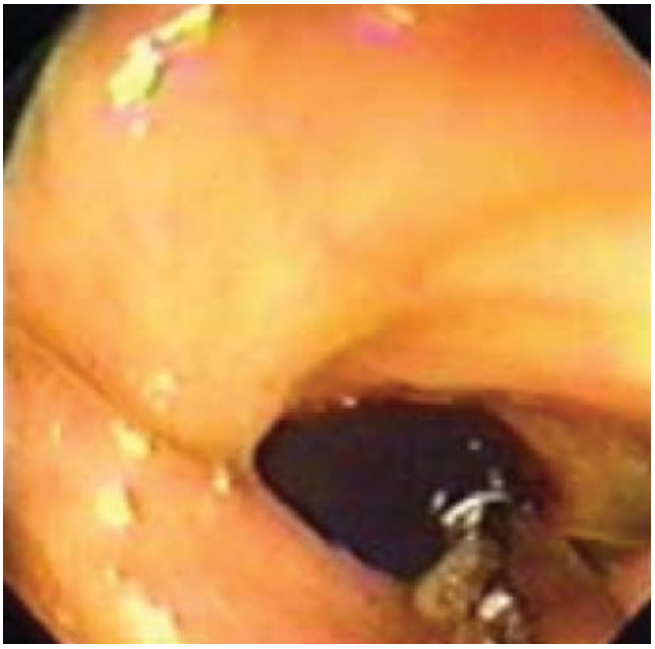

FIGURE 67.3-8 Forceps opening up ileocecal valve with downward deflection of the colonoscope tip.

removal. Sudden movement of the patient may cause premature removal of an ensnared polyp without electrocautery and lead to hemorrhage. Ensnaring the polyps may also be impeded. Delayed postpolypectomy hemorrhage is rare, but parents should be advised as to this possibility, although this is not a reason for routine overnight observation in the hospital following the procedure.

Polyps should be removed when first encountered because they may be difficult to subsequently locate. The size of a polyp and whether it is pedunculated or sessile determines the mode of removal. Those less than 5 mm in diameter may be removed by normal "cold" or monopolar or bipolar "hot" biopsy forceps (Figure 67.3-9). Monopolar forceps provide tissue for histology and cauterize surrounding tissue, whereas bipolar forceps ablate most of the tissue, making histologic interpretation more difficult.[61,62] Minisnares (15–20 mm long) may also be used for removal of small polyps by cold technique or cautery.[63] Generally, polyps greater than 5 mm require an electrocautery snare, which are available in varying sizes and shapes (eg, hexagonal, oval, crescent) (Figure 67.3-10). Most are monopolar, and the advantages of bipolar snares are not compelling.[64]

Polyps with stalks are ensnared around their neck, and if a monopolar current is employed, the colonoscope is used to angle the polyp away from the colonic wall. Otherwise, current is transmitted from the polyp to contact areas on the colonic wall with the potential for perforation (Figure 67.3-11). "Pulsed cut" (also called "blended cut and coagulation") and pure "coagulation" set at 15 to 30 watts are the favored types of current rather than pure "cut," which will not adequately cauterize the stump. The assistant slowly tightens the snare while current is applied to prevent garrotting of the polyp. Excess current may occasionally lead to transmural damage, with the potential for perforation. For larger polyps with attendant larger stalks, other methods may prevent excessive hemorrhage, for example, attachment of a metal clip to the base of the stalk,[65] prior injection of the stalk with 1:10,000 epinephrine,[46] attachment of detachable loop snares to the stalk prior to polyp removal,[66] and piecemeal removal with specially designed "endo-scissors" and "endo-forceps." This latter technique is particularly useful for large sessile polyps, in which submucosal injection of saline may divide the tissue planes and allow for endoscopic mucosal resection of the polyp (Figure 67.3-12). Rubber bands may be applied to elevate a sessile polyp prior to resection[67] or to the bleeding stalk postpolypectomy.[68] Techniques of endomucosal resection and polyp removal can also be aided by prior endosonographic assessment.

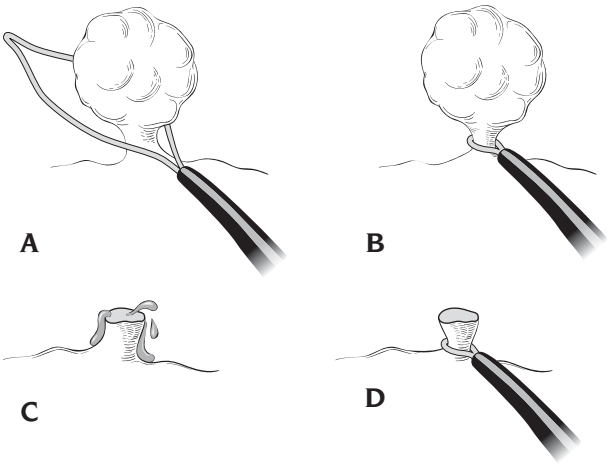

FIGURE 67.3-10 Technique of snaring larger pedunculated polyps. *A*, Polyp lassoed by snare; *B*, snare closed; care not to amputate without coagulation current; *C*, if stalk bleeds, inject base with 1:10,000 epinephrine; *D*, snare assisted coagulation of stump.

Retrieval also depends on size. Small polyps may be sucked up through the suction channel and caught in a suction trap. The snare itself may be used to retrieve larger polyps, or specific retrieval devices such as baskets, grasping forceps, or nets may be helpful. Without a net to gather the polyps, one may need to repeatedly remove and reinsert the scope to collect all of the polyps. Alternatively, when many large polyps are encountered and removed, it may be easier to sift the feces subsequently.

Dilation of Strictures

Through-the-scope balloon dilators are appropriate for ileocolonic dilation, employing the same concept and method as for upper gastrointestinal strictures, employing radiologic screening control. Long-term symptomatic relief can be afforded in some carefully selected patients, including adolescents in reported studies.[69,70] Pressures of 35 to 50 psi in balloons of 12 to 18 mm are available. Theoretically, as for neoplastic or diverticulitis-associated strictures in adults, stent placement could be used as a last resort in inflammatory bowel disease–type strictures, but there are no reported cases of this occurring in childhood as yet.

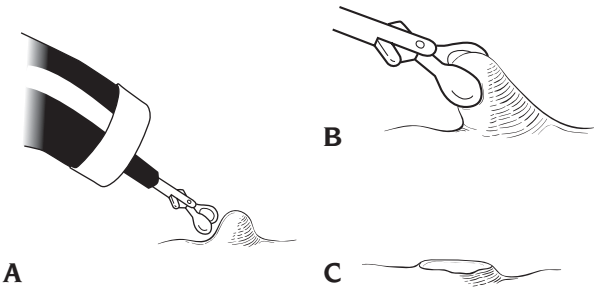

FIGURE 67.3-9 Technique for removal of small polyps using "cold" or "hot" forceps. *A*, Forceps exit scope; *B*, grasp and "tent" polyp; *C*, polyp removed, "hot" biopsy forceps with current.

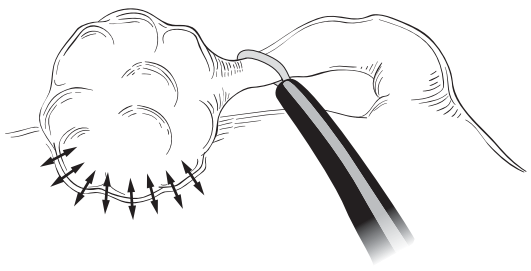

FIGURE 67.3-11 Complication of current dissipating through the colonic wall with the potential for perforation unless the polyp is drawn away from the wall.

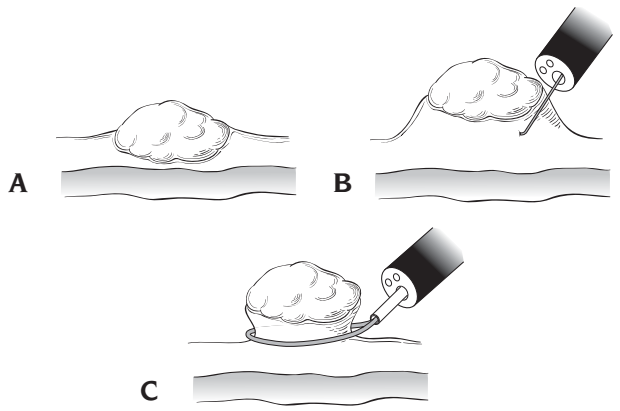

FIGURE 67.3-12 Technique of lifting a sessile polyp (*A*) away from underlying stroma prior to snare polypectomy. *B*, Saline or 1:10,000 epinephrine injected under polyp, creating a tissue plane; *C*, snare and polypectomy. Endomucosal resection may be needed.

Percutaneous Endoscopic Cecostomy

Percutaneous endoscopic cecostomies have been successfully placed in a number of children with intractable constipation using the same technique as for gastrostomy insertion using the pull-through technique. This approach followed reports of effective treatment of obstipation and fecal impaction in children by appendicostomy and intestinal button implantation using antegrade continence enemas, the so-called ACE procedure.[71] Intraoperative ultrasonography was used to confirm the position of the cecum just below the layers of the abdominal wall. No complications were reported in one series.[72]

A newer technique of percutaneous sigmoidostomy has recently been reported for the treatment of low left-sided refractory constipation, again employing a pull-through technique, with some success.[73]

Therapy of Lower Gastrointestinal Bleeding

Interventions used to prevent bleeding during polypectomy and mentioned earlier may also be used to manage post-polypectomy bleeding. Apart from colitis and polyps, other causes of bleeding are angiodyplasias and arteriovenous malformations. Angiodysplasias are rarely reported in childhood (mean age 2–3 years) and are more easily diagnosed by selective regional angiography than by ileocolonoscopy despite occurring in the left hemicolon. In children, control of bleeding has required surgical excision.[74] Arteriovenous malformations must be subject to angiographic assessment before any endoscopic therapy is contemplated because many of these apparently innocuous-looking luminal lesions may have large vascular beds. Examples of equipment that may be used to stop a localized lesion from bleeding include heater probes,[75] bipolar cautery probe, hot biopsy forceps, neodymium:yttrium-aluminum-garnet laser set for thermal coagulation,[76] and argon plasma coagulation.

ENDOSONOGRAPHY

Endoluminal ultrasonography of the rectum has been an established technique for years; however, more recently, an echocolonoscope has allowed combined examination of the mucosa and the bowel wall. This is a forward-viewing colonoscope with the transducer (7.5 MHz) situated in the rigid tip of the scope.[77] Alternatively, an ultrasound miniprobe can be introduced via the biopsy channel (7.5 or 12.5 MHz). A fluid interface is necessary for all endosonography, and this can be achieved either with a fluid-filled balloon or filling the relevant colonic segment with water. Because this may be time-consuming, it is easier to concentrate on the region of interest rather than attempt to examine the entire colon. In adult practice, staging of cancers is the major indication for endosonography. In children and adolescents, indications for this technique might include suspicion of early invasive cancer arising from an adenoma, assessment of the extent and depth of sessile polyps to guide reception technique, assessment of colonic strictures/fistulae/anastomoses, assessment of the extent and depth of inflammatory bowel disease, assessment of the extent and depth of vascular lesions, examination of rectal and colonic portal hypertension with varices, and suspicion of lymphoma.

Inflammatory bowel disease appears as wall thickening and subsequent loss of the normal layer structure of the colon with progressive inflammation. Although theoretical differentiation between ulcerative colitis and Crohn disease is possible owing to the transmural nature of Crohn disease, it has been shown recently that active ulcerative colitis can have echotexture changes extending into the submucosa and that these changes correlate with disease activity.[78] Surgical decisions were made in one study of patients with Crohn disease[79] in which endoscopic ultrasonography was used to differentiate between superficial and transmural involvement. An ileoanal pouch was undesirable when transmural disease was identified. Perirectal and pericolonic fistulae and abscesses have been seen using the rigid rectal ultrasound probe, and this is a potential application for endoscopic ultrasonography.[80] Catheter probe–assisted endoscopic ultrasonography in inflammatory bowel disease has advantages over an echocolonoscope, which may be technically difficult to use. One study recently showed that wall thickness was twice as great in active inflammatory bowel disease, but ulcerative colitis could not be differentiated from Crohn disease.[81] Loss of wall structure correlated with disease activity score in the Crohn disease group, and wall thickness correlated with disease activity in the ulcerative colitis group. Other parameters, such as superior mesenteric artery maximum flow velocity and increased Doppler ultrasonography–demonstrated mural blood flow, are being examined as viable noninvasive substitutes for determination of post-treatment ileocecal Crohn disease activity, thus potentially avoiding the need for follow-up ileocolonoscopy, as some units advocate.

FOREIGN BODY REMOVAL

Foreign bodies in the lower gastrointestinal tract are rare in childhood. Occasionally, objects such as percutaneous endoscopic gastrostomy flanges or other ingested foreign bodies may become impacted at the ileocecal valve, necessitating endoscopic removal.[82]

REDUCTION OF INTUSSUSCEPTION AND SIGMOID VOLVULUS

Pneumatic reduction of intussusception has been described with simultaneous identification of the intussuscipiens, in one case a polyp, which was removed, and in another case, terminal ileal lymphonodular hyperplasia.[83,84] I have used air insufflation to reduce an ileocecal intussusception caused by terminal ileal Crohn disease. Reduction of sigmoid volvulus is a more common indication, particularly among patients with chronic constipation.

PER ENDOSCOPIC MANOMETRY

Per endoscopic manometry has limited application but has been studied in adults specifically identifying abnormal motor patterns and hypertension of the ileocecal valve.[85]

COMPLICATIONS OF ILEOCOLONOSCOPY

Complications, excluding those due to sedation, are summarized in Table 67.3-5. The literature to date reveals over 3,000 colonoscopies under 20 years of age reported, with 5 perforations—4 postpolypectomy and 1 in a patient with severe ulcerative colitis. Ten procedure-related minor complications are noted, including four small postpolypectomy hemorrhages, three cases of postprocedure abdominal pain with spontaneous resolution, one common peroneal nerve palsy secondary to periprocedure positioning, and two with a postprocedure fever for more than 24 hours.[1,4,5,45,46,75,86,87] This equates to a complication rate owing to the procedure itself of approximately 0.3% and, without polypectomy, of about 0.05%. This is in keeping with the British definition of "minimal" risk and the American definition of "minor risk over minimal."[88]

Perforation itself may well be relatively operator independent, occurring often in those with underlying connective tissue disease. Even enema treatment for constipation can cause perforation in patients with Ehlers-Danlos syndrome.[89] A single case of a child with serosal surface tears owing to a rigid colonoscope and a large sigmoid loop was reported in 1974.[90] Flexible pediatric colonoscopes or the new variable-stiffness colonoscopes may prevent this nowadays. Following electrocautery removal of a polyp, the stalk and the contralateral wall should be examined closely for evidence of mucosal perforation. The merits of conservative therapy of selected cases of colonic perforation have been discussed,[91] and it would seem reasonable to adopt conservative management, for instance, in the case of silent asymptomatic perforations and those with localized peritonitis without signs of sepsis who continue to improve clinically without intervention.[92] In one study in adults, only 3 of 21 patients were managed nonsurgically, and there was no difference in the morbidity or mortality between primary repair and resection and anastomosis.[93] In another, conservative management was successful in 13 of 48 patients, and 12 of the 13 were postpolypectomy perforations.[94]

In contradistinction to adults, bacteremia has not been detected in children after ileocolonoscopy.[23] In addition, modern cleaning machines seem to largely prevent the glutaraldehyde-associated colitis reported in the past.[95]

Polypectomy is the main cause of hemorrhage, and, as noted above, there are very few reported cases of this occurring in pediatrics. Hassall and colleagues noted minor bleeding, which stopped spontaneously in 2 children from 38 polypectomies in 136 children,[4] and there are no cases reported that required transfusion in children. Resnaring a bleeding stalk and maintaining pressure for 10 to 15 minutes are the first steps and, if unsuccessful, can be followed by injection of 1:10,000 epinephrine or thrombin or further electrocoagulation. Later bleeding has been reported in adults with a median delay of 37 hours in about half of one series.[96]

Splenic rupture is rarely seen and will present with hypovolemia and shoulder tip or abdominal pain within 24 hours of the ileocolonoscopy.[97] Similarly, direct trauma to the tail of the pancreas is the proposed mechanism of injury in the rare case of pancreatitis reported.[98]

Because of the rarity of complications in pediatrics, most pediatric endoscopists, when presented with such a clinical situation, will be unfamiliar with the etiology of the symptoms, and colleagues' opinions should often be sought.[99]

TABLE 67.3-5 PROCEDURE-RELATED AND POSTPROCEDURE COMPLICATIONS IN PEDIATRIC ILEOCOLONOSCOPY

DIAGNOSTIC PROCEDURE RELATED
Vasovagal reactions
Hemorrhage
Perforation: traction serosal, direct transmural
Pancreatitis
Splenic trauma
THERAPEUTIC PROCEDURE RELATED
Perforation
Hemorrhage
Thermal injury: transmural
POSTPROCEDURE
Distention and discomfort (less if CO_2 insufflation used)
Delayed evidence of perforation or hemorrage

INDICATIONS FOR ILEOCOLONOSCOPY

The main indications, both diagnostic and therapeutic, for ileocolonoscopy in childhood and adolescence are set out in Table 67.3-6. The most common indication in the pediatric age group is, of course, suspicion of inflammatory bowel disease. As part of first-line investigation, an upper endoscopy is also indicated because up to 20% of inflammatory bowel disease cases can be differentiated into either Crohn disease or indeterminate colitis by upper gastrointestinal endoscopy.[100]

The mucosal appearances of ulcerative colitis and Crohn disease are well described and are not reiterated here.[3,101] Ileocolonoscopy of acute colitis is generally held to be a safe and reliable tool in expert hands and can help to differentiate ulcerative colitis from other pathologies and also allow prognostication.[102,103] Per rectal bleeding is also an important indication for ileocolonoscopy. With col-

TABLE 67.3-6 DIAGNOSTIC AND THERAPEUTIC INDICATIONS FOR ILEOCOLONOSCOPY IN CHILDREN AND ADOLESCENTS

DIAGNOSTIC
Suspected inflammatory bowel disease
Follow-up for assessment of efficacy of inflammatory bowel disease treatment
Suspected allergic colitis
Suspected colitis owing to other causes, eg, chronic granulomatous disease
Lower gastrointestinal hemorrhage
Chronic diarrhea
Cancer surveillance
Graft-versus-host disease
Endosonography
Manometry
THERAPEUTIC
Polypectomy
Foreign body removal
Percutaneous cecostomy
Stricture dilation
Reduction of intussusception

itis, the blood will usually be mixed in with the stool and may be associated with pain, whereas polyps are the most common cause of painless rectal bleeding.[104] Follow-up ileocolonoscopy allows assessment of treatment efficacy and can demonstrate to older children and parents these beneficial effects, promoting continued compliance.

Allergic enterocolitis usually presents in the first year to 2 years of life and may present even in the exclusively breastfed infant whose mother is ingesting antigens such as cow's milk protein.[105] A case can be made for removing the potentially offending antigen from the diet of the child or, if breastfed alone, from the mother. Ileocolonoscopy is not usually needed in such a circumstance, although it has been advocated as a diagnostic tool—the so-called "colonoscopic allergen provocation," in which, in one study in adults, 77% had a positive local visible mucosal reaction with histologic evidence of recruitment of mast cells and activation of eosinophils.[106] Simple dietary manipulation is usually sufficient to make the diagnosis in infants. Ulcerative colitis and Crohn disease are extremely rare under the age of 2 years. If appearances at ileocolonoscopy or on histology of biopsies are in keeping with such diagnoses, then it is important to think of other underlying reasons for these findings in this age group. This may include diagnoses such as enterocolitis of infancy,[107] glycogen storage disorders, chronic granulomatous disease, neutrophil chemotactic defects, and infectious enterocolitides, among others. Rare enteropathies such as tufting enteropathy may also involve the colon.

It must not be forgotten that infections such as amebic dysentery, *Campylobacter*, *Salmonella*, *Shigella*, and other common enteritides can mimic idiopathic colitis, and *Yersinia* and tuberculosis can cause ileitis that mimics Crohn disease.[51,60,108]

Enterobiasis has also been reported to cause ileal and colonic ulceration.[109] Thicker 5 μm sections stained with Ziehl-Neelsen and analysis of tissue samples by polymerase chain reaction may improve detection for tuberculosis.[60,110]

Vascular anomalies may be a rare cause of lower gastrointestinal bleeding. These lesions may have minor mucosal involvement but an extensive intra- and extramural vascular supply. Endoscopic ultrasonography with or without Doppler may help to delineate the extent of such lesions before angiography is considered or treatment is attempted. Because of the large size of some vascular lesions, simple mucosal injection, sclerosis, or heat coagulation may lead to greater bleeding. Blue rubber bleb nevus syndrome is another unusual example of vascular malformation, as is angiodysplasia, which can easily be missed if the colon is not very clean after bowel preparation.[74] Vasculitis, masquerading as inflammatory bowel disease, may have similar ileocolonic mucosal appearances. Clues to this diagnosis are the involvement of other organs and systems such as loss of beat to beat variation on electrocardiography representing atrioventricular node ischemia owing to vasculitis, perinuclear antineutrophil cytoplasmic antibody positivity, and skin lesions. Celiac axis, renal, and superior or inferior mesenteric angiograms may reveal multiple microaneurysms.

Polyps are found in 80% of instances within the rectosigmoid colon alone, reach peak incidence around 5 to 6 years, and are not usually seen in infancy.[111] Hamartomas make up the vast majority and are termed juvenile, inflammatory, or retention polyps. Approximately one-third of juvenile polyps cause anemia and often are lost by autoamputation. Even in expert hands, 10% or so of polyps can be missed at ileocolonoscopy, and the technique affording the best chance of detection is noted above. One in five rectosigmoid polyps will be accompanied by additional polyps in the proximal colon—hence the need for a full examination even if a polyp has been removed from a distal site. The recurrence rate may be 25%, but this is not well documented. Juvenile polyposis coli is often defined as the presence of more than 5 polyps, although there may be as many as 100 or more, and they may not be confined to the large bowel.[111,112] Dysplastic change with a carcinoma risk of 15% by age 35 years and 68% by 60 years is reported.[113] Adenomas can occur rarely and macroscopically are difficult to distinguish from hamartomas, dictating the need for histologic confirmation, and mixed polyps have been reported.[114,115] Familial adenomatous polyposis, an autosomal dominant disorder associated with the *APC* gene,[111] typically has hundreds or thousands of adenomas in the colon; screening in both of these conditions is dealt with in the following section. Several other syndromes may manifest with polyps and include Peutz-Jeghers, Turcot, Cronkhite-Canada, and hereditary flat adenoma syndromes.[116]

There are less than 200 cases of colonic carcinoma reported in the literature in children, and these represent less than 1% of all childhood tumors. Carcinoma has been reported in the absence of familial adenomatous polyposis or hereditary nonpolyposis colon carcinoma germline mutations.[43] Abdominal pain is the most common presenting symptom, occurring in 95% of patients,[117] and an abdominal mass is the most common finding, occurring in more than 50% of cases. Rectal bleeding is less common than in adult studies. Carcinoembryonic antigen had a

specificity of 77% and a sensitivity of 68% in those under 18 years in a recent study.[118] Because this is too low to be clinically reliable, the requirement is for full ileocolonoscopy.[119]

Graft-versus-host disease (GVHD) following bone marrow, kidney, or liver transplant can have colonopathy in both acute and chronic forms. Focal apoptosis of the glandular epithelium is typical in acute GVHD,[120] and mucosal changes reminiscent of chronic idiopathic inflammatory bowel disease occur in chronic GVHD.[121]

FOLLOW-UP AND SURVEILLANCE ILEOCOLONOSCOPY

It is the practice in many units to perform a follow-up ileocolonoscopy 2 to 3 months after the start of treatment in a newly diagnosed case of inflammatory bowel disease. This practice is based on a number of premises, including evidence from studies such as those by Modigliani and colleagues, which showed that only 29% of adults with Crohn disease in clinical and biochemical remission actually achieved endoscopic remission.[122] This has a number of advantages. It allows the physician to observe the mucosal efficacy of the therapy because, in many instances, such as steroid use in colitis, the clinical improvement of the patient may not be mirrored by the mucosal improvement, which is regarded by most as the most important meter of a successful treatment regimen.[20] Ileocecal transcutaneous Doppler ultrasonography may be of benefit as a noninvasive alternative to repeat ileocolonoscopy in this situation, as noted above. In addition, the activity of mucosal inflammation may determine the long-term risk for carcinogenesis in the bowel.

NEW DIAGNOSES

Appreciation of more subtle lesions and pathology has been possible more recently as a consequence of combining high-definition videoendoscopy with sophisticated diagnostic techniques of mucosal immunology such as immunohistochemistry and gated flow cytometry. One such example is that of the histologically nonspecific colitis and ileal lymphonodular hyperplasia (Figure 67.3-13), which has been recognized recently in children with regressive autism or pervasive developmental disorder, and it is not considered to be due to any exogenous trigger, as has widely and wrongly been reported with respect to the measles, mumps, and rubella vaccine, but to be entirely independent of this or other triggers and may well be of autoimmune origin, but this is yet to be fully explored.[123,124] Nonspecific colitis appears macroscopically as granularity, loss of vascular pattern, pronounced lymphoid follicles surrounded by erythema described as the "red halo" sign (Figure 67.3-14), and aphthoid ulcers in a small proportion of cases (Figure 67.3-15). Diffuse infiltration of the lamina propria with macrophages and lymphocytes and enlarged germinal centers of lymphoid follicles containing tingible body macrophages are noted in the colon. Lymphoid follicular hyperplasia with reactive and expanded germinal centers is found in the terminal ileum.[123] Further work on this observation has revealed a specific lymphocytic colitis less severe than classic inflammatory bowel disease. When compared, however, with cohorts with no histologic or clinical disease, developmentally normal children with ileal lymphonodular hyperplasia, Crohn disease, and ulcerative colitis, this group had significantly increased basement membrane thickness, mucosal $\gamma\delta$ cell density, $CD8^+$ density, and higher intraepithelial lymphocyte numbers. There were also higher CD3 and plasma cell density and greater crypt proliferation, and the disruption of epithelial but not lamina propria glycosoaminoglycans with a negative human leukocyte antigen (HLA)-DR in the

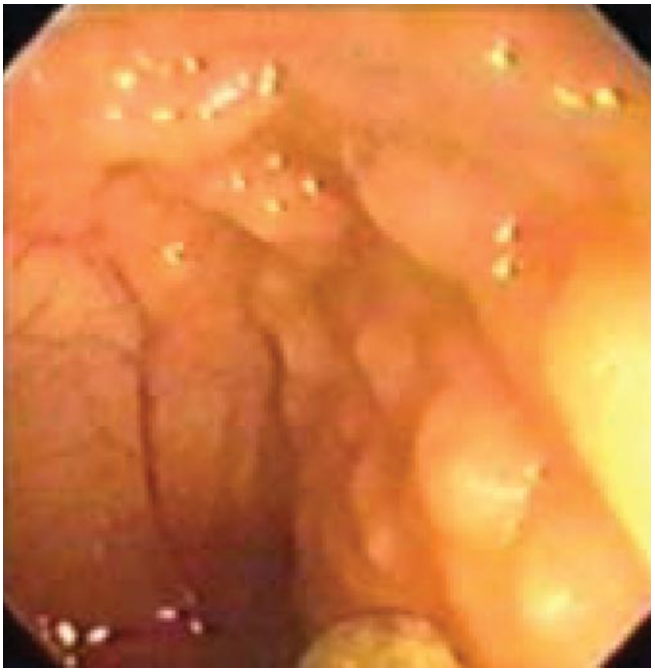

FIGURE 67.3-13 Terminal ileal lymphonodular hyperplasia.

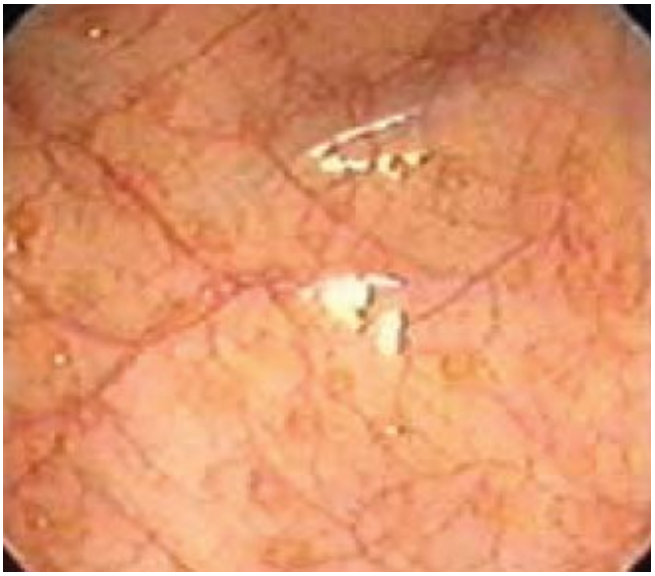

FIGURE 67.3-14 Multiple prominent lymphoid follicles in the colon surrounded by intense erythema. The so-called "halo" sign.

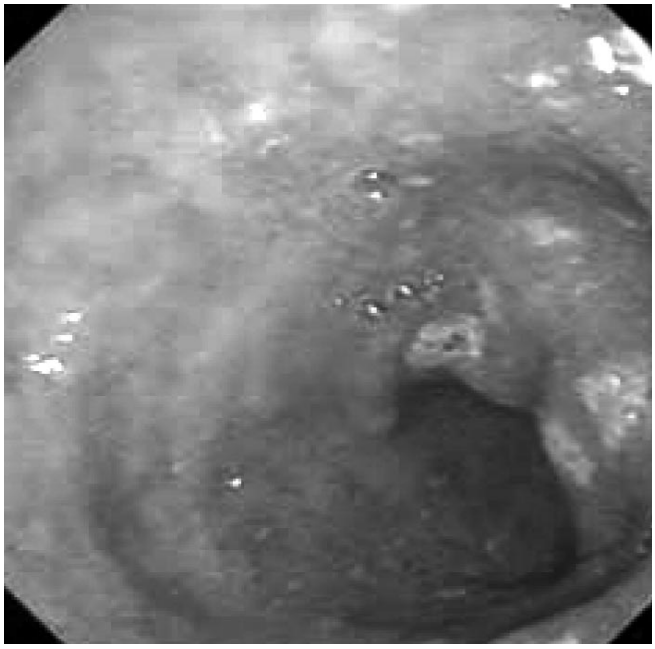

FIGURE 67.3-15 Evolution of inflamed lymphoid follicle into aphthoid ulcer.

epithelium suggested a T helper 2 response.[125] Other groups have reported similar ileocolonic lesions in patients with autism, pervasive developmental disorder of childhood, and attention-deficit/hyperactivity disorder.[126] Upper gastrointestinal pathology such as reflux esophagitis and nonspecific gastritis or duodenitis in this group of patients has also been noted recently.[127,128]

Fibrosing colonopathy has been identified in patients receiving excessive pancreatic enzyme supplements for treatment of both cystic fibrosis[129] and non–cystic fibrosis–related illness.[130]

Collagenous colitis presents with watery diarrhea, crampy abdominal pain, and a distinctive histology of the colon that includes a subepithelial collagen band and prominent chronic lamina propria inflammation with an increase in intraepithelial lymphocytes.[131] There have been only two reports of this in children, and this remains controversial.[132] Most cases occur in women over 50 years of age.

Lymphocytic (also called microscopic) colitis presents in a similar manner and is also idiopathic in origin but occurs equally between the sexes.[96] The histologic appearances are similar but without the collagen band. It is not seen commonly in children.

Diversion (also known as disuse) colitis arises in the colon, which is excluded from the fecal stream. It is time dependent, occurring with increasing frequency the longer the segment of bowel is not used, ranging from 3 to 36 months postsurgery.[133] Histology is typical of nonspecific colitis. The absence of luminal short-chain fatty acids is the most popular theory of pathogenesis because colonocytes require these for energy. Bacterial flora normally produce short-chain fatty acids from undigested carbohydrate and fiber.[134] Surgical reanastomosis is the treatment of choice, but topical fatty acids have been applied successfully when surgery is not possible.[135]

Other, more recently described disorders include autoimmune colitis, associated with autoimmune enteropathy and antienterocyte antibodies, and tufting enteropathy involving the colon.[136]

ILEOCOLONOSCOPY VERSUS OTHER DIAGNOSTIC TECHNIQUES

Although ileocolonoscopy is the gold standard for the diagnosis of pediatric inflammatory bowel disease, other noninvasive modalities may play a role in the diagnosis and assessment of treatment efficacy. Technetium hexamethylpropyleneamine oxime (Tc-HMPAO) labeling of granulocytes does not involve as much radiation burden as indium 111–tagged white cells and has been compared with ileocolonoscopy.[137] Jobling and colleagues showed that a Tc-HMPAO scan had 90% sensitivity and 75% specificity compared with biopsies at ileocolonoscopy, but this study had information from the ileum in only 12 of 39 cases—hence potentially overestimating the sensitivity and specificity of the technique.[138] The main disadvantages of the technique were lack of histologic confirmation of the disease process and lack of localization of the inflammation. More recently, the value of white cell–labeled scanning has been further questioned owing to the lack of accuracy in disease localization and sensitivity levels of approximately 75%.[139] Steroids decrease the white blood cell uptake of Tc-HMPAO by up to 50% and hence may decrease further the sensitivity of this technique. However, disease in the terminal ileum may be identified where it might otherwise have been missed because of unsuccessful endoscopic intubation of the terminal ileum.[140] Charron and colleagues showed that 6 of 106 cases of inflammatory bowel disease would have been mislabeled as ulcerative colitis or Crohn disease by technetium scan alone.[141]

Lipson and colleagues found a barium meal and follow-through to the terminal ileum to be a highly sensitive technique, especially for small bowel disease.[57] Tribl and colleagues reported a lower sensitivity of 91% but a specificity of 100%; hence, the radiologist's experience seems to be important.[142] The disadvantage of lack of histologic confirmation of the type of inflammation remains, and the diagnostic confusion owing to terminal ileal lymphonodular hyperplasia is well documented.[137] The radiation burden is also significant.

In experienced hands, ultrasonography has a sensitivity of 88% and a specificity of 93% for inflammatory bowel disease in children. Transabdominal ultrasonography assesses the bowel wall thickness in different areas, especially focusing on the terminal ileum.[143]

Spiral computed tomography (CT) may be useful in determining the nature of pericolic masses or in fistulating disease. Some authors suggest that Crohn disease can be differentiated from ulcerative colitis on the basis of finding greater colonic mural thickness in Crohn disease and greater submucosal fat deposition in ulcerative colitis, but many CT findings are nonspecific.[144] Three-dimensional CT, used to screen for carcinoma in adults, has potential application for identifying polyps in children, although the disadvantages of

lacking histologic confirmation or therapeutic intervention remain. Current magnetic resonance imaging does not adequately recognize pediatric Crohn disease.[145]

The application of the novel technique of wireless capsule endoscopy (WCE) for visualization of the small bowel for occult pathology such as undiagnosed bleeding sources, mid- to small bowel polyps, and mid- to small bowel inflammatory pathologies is now well documented.[146] The use of this technique for diagnosis by direct vision of mucosal pathology in the ileum is useful (although one shortcoming is its relative contraindication in the presence of strictures) and has also been used to identify lesions in the colon; however, in the presence of good ileocolonoscopic technique and with the inability of WCE to obtain biopsies for histologic confirmation of pathology (although the next generation of capsules may have a limited biopsy facility), its application to colonic investigation remains somewhat limited to date. External steering of the capsule has recently been reported, opening up possibilities of retropulsion in the colon rather than just reliance on normal peristalsis.[147]

ENTEROSCOPY

Enteroscopy, now a standard endoscopic procedure in adult medicine and recently reviewed,[148,149] came of age because of the realization that the small bowel did indeed have specific pathology requiring not only diagnostic but also therapeutic expertise. It is of particular use in those with unexplained gastrointestinal bleeding. Pediatric enteroscopy literature is limited to Sonde-type and intraoperative-assisted push enteroscopy.[150–152] Sonde enteroscopy has largely been abandoned in favor of push enteroscopy,[153,154] given the desire for therapeutic capability. The techniques of per oral push enteroscopy and laparoscopy-assisted enteroscopy continue to evolve. In addition, newer methods of examining the small bowel are being developed and include virtual enteroscopy and WCE small bowel examination (as discussed above).[155] As noted, WCE does not allow therapeutic intervention as yet, but its diagnostic yield is certainly as good as if not better than enteroscopy for pathologies such as occult or obscure small bowel bleeding sources.[147,156]

Instruments and Technique

Although a pediatric colonoscope can be used for enteroscopy, specifically designed enteroscopes up to 230 cm in length are now available. The Olympus SIF Q140 has a diameter of 10.5 mm and is 250 cm long. A push enteroscope, like a colonoscope, allows four-way tip deflection to 160 to 180 degrees. There are no enteroscopes or overtubes specifically designed for pediatric application, reflecting the lack of widespread use of this technique in children. An overtube, typically 60 to 100 cm in length with a soft Goretex tapered tip, stiffens the enteroscope within the stomach and upper duodenum, limiting looping, thereby allowing deeper advancement into the small bowel.[153] An enteroscope can be introduced 120 to 180 cm beyond the ligament of Treitz, and with laparoscopic assistance, even the terminal ileum can be reached, allowing lesions such as a Meckel diverticulum to be found.[152]

Preparation for enteroscopy is the same as for upper gastrointestinal endoscopy, although the procedure may be substantially longer and more uncomfortable. Therefore, it is the practice at my unit to use general anesthesia even in adolescents. Patients are positioned left lateral or semiprone. After normal examination of the esophagus and stomach, air is removed, and minimal insufflation of the stomach allows deeper penetration into the small bowel when not using an overtube. At 60 to 80 cm in older children and adolescents, the ligament of Treitz is found, and extreme tip deflection is needed to find the lumen. The first jejunal loop is more readily identified because it is straighter and travels down to the pelvis. If using an overtube, which has been threaded over the enteroscope prior to oral insertion, this is deployed down the esophagus and into the second part of the duodenum; prepyloric deployment will not aid in deeper small bowel penetration. Some exponents use fluoroscopy to aid in overtube tip positioning.[148] When advancing the overtube, the enteroscope needs to be pulled back with clockwise rotation to straighten it, similar to the maneuver used to achieve the shortened scope position during endoscopic retrograde cholangiopancreatography. Variable-stiffness enteroscopes may soon be developed, which may remove the need for an overtube.

Indications

Table 67.3-7 details potential pediatric indications for push enteroscopy. One of few studies in children investigated the possibility of Crohn disease in children with growth retardation.[157] A number of reports demonstrate the utility of push enteroscopy in adults. In one recent series, a bleeding source was identified in 64% using push enteroscopy and an overtube.[158] Transfusion requirements can be significantly decreased in cases of angiodysplasia in the jejunum when cauterized at enteroscopy.[159,160] Nonsteroidal drugs can cause acute and chronic small bowel hemorrhage, perforation or obstruction, and an enteropathy, all diagnosed by push enteroscopy.[149,161,162]

TABLE 63.7-7 INDICATIONS FOR ENTEROSCOPY IN CHILDREN AND ADOLESCENTS

DIAGNOSTIC
Obscure gastrointestinal bleeding (after endoscopy and ileocolonoscopy)
Iron deficiency anemia (especially if history of nonsteroidal drug use)
Extent of Crohn disease
Polyposis syndrome surveillance
Lymphoma (suspicion or follow-up post-treatment)
Lymphangiectasia
Intestinal obstruction
Graft assessment after small bowel transplant
Enteroclysis
THERAPEUTIC
Therapy of hemorrhagic lesions (eg, cauterizing angiodysplasia)
Polypectomy
Stricture dilation
Nasojejunal tube placement
Percutaneous jejunostomy tube placement

There are no data in children regarding nonsteroidal anti-inflammatory drug small bowel disease. The assessment of graft survival following small bowel transplant can be assessed by push enteroscopy. However, most often a jejunostomy is fashioned through which it is much easier to advance an enteroscope or simply use a standard endoscope. Biopsies taken for disaccharidase activity and histologic assessment are also helpful in early detection of small bowel allograft rejection.[163,164] Indeed, a zoom endoscope has recently been used to detect the early structural changes associated with graft rejection.[165]

Direct percutaneous jejunostomies can be sited using the same pull-through technique employed in percutaneous gastrostomy insertion.[166] Nasojejunal tubes can be placed accurately using a guidewire-assisted technique.[167] Finally, polypectomies can be successfully completed with a normal snare technique.

The indications for Sonde enteroscopy in children are very limited and might include a situation in which initial surgical exploration for a site of suspected small bowel bleeding is deemed undesirable.

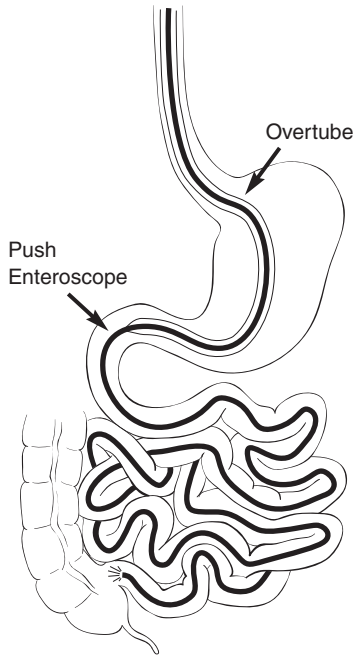

FIGURE 67.3-16 Extent of laparoscopy-assisted enteroscopy.

Intraoperative or Laparoscopy-Assisted Enteroscopy

Intraoperative or laparoscopy-assisted enteroscopy starts with conventional enteroscopic jejunal intubation followed by surgical assistance. The endoscopist's role is relatively passive, deflecting the tip of the instrument while the surgeon, either with hands or with laparoscopic instruments, concertinas examined parts of the small bowel over the enteroscope. Both the mucosal and serosal surfaces can be examined. Very little air is insufflated into the bowel to avoid hindering the surgeon. Dimmed lights in the operating field also help to identify the position of the tip of the instrument. In experienced hands, all of the small bowel is examined in 60% of cases, taking up to 2 to 3 hours (Figure 67.3-16).[168] An enterotomy may be used to insert a sterilized enteroscope in some situations. Lesions can be marked by injection of ink or placement of a suture. Intraoperative or laparoscopy-assisted enteroscopy is the most successful technique for identifying sites of obscure gastrointestinal bleeding with diagnostic yields of between 83 and 100%.[169] Laser or bipolar coagulation can be used, and resection of lesions is recommended if intraoperative.[170] This technique has demonstrable advantages in the assessment of the extent of polyposis syndromes. "On-table" enteroscopy has a better pick-up rate for polyps at laparotomy than external transillumination and palpation.[111] When attempted in Crohn disease, up to 65% of patients have had lesions not previously identified in the small bowel by other investigations, including direct vision of the serosal surface of the bowel.[171] As previously mentioned, occult Crohn disease can be identified in children using enteroscopy.[157] Partial intestinal obstruction and Meckel diverticulum have also been identified at intraoperative enteroscopy.[150,152] Small bowel neoplasia must not be forgotten as the second most common cause of obscure gastrointestinal bleeding in younger patients, accounting for 5 to 10% of cases in young adults.[168] Exploratory laparoscopy and enteroscopy are important in preventing missed diagnoses.

Complications

Complications are not often encountered with simple push enteroscopy, but when the overtube is employed, significant patient discomfort has been described.[153] Other, rare complications of the overtube include pharyngeal tear, Mallory-Weiss tear, gastric mucosal stripping, pancreatitis, and duodenal perforation.[158,172–174] Intraoperative enteroscopy has a 5% incidence of perforation and, in one series, a 50% incidence of mucosal laceration.[152] Prolonged ileus has been occasionally described.[173] None of these rare complications have been reported in the limited studies investigating children.

SUMMARY

Pediatric ileocolonoscopy and enteroscopy differ significantly from their adult parallels in nearly every aspect, including patient and parent management and preparation, selection criteria for sedation and general anesthetic, bowel preparation, expected diagnoses, instrument selection, imperative for terminal ileal intubation, and requirement for biopsies from macroscopically normal mucosa. The basic technique of ileocolonoscopy in children is illustrated and accompanied by discussion of advanced techniques such as endosonography, cecostomy, and therapy of lower gastrointestinal bleeding. The advantages and disadvantages of other noninvasive investigations are compared with those of ileocolonoscopy, and the former are generally held to be second best. The main aim of this chapter is to highlight those differences and to provide a workable guide for those involved or training in the discipline of pediatric ileocolonoscopy or enteroscopy.

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4. Gastrointestinal Endosonography

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Gastrointestinal endosonography or endoscopic ultrasonography (EUS) is a relatively new endoscopic procedure in which a high-frequency ultrasound transducer is located on the tip of a flexible endoscope. It is unique in that it is able to obtain ultrasonographic images of the gastrointestinal tract and its surrounding structures. The arrival of EUS in the United States began in March 1980 with publication of a report by DiMagno and colleagues of a gastroscope equipped with an ultrasound probe.[1] Since the inception of EUS, it has been a novel modality looking for a purpose. Through the more than two decades since the first report, the indications for its use have increased but have not reached overall widespread use, especially in pediatrics.

EUS is not currently part of standard training programs in the United States, and few endoscopists are expert in the interpretation and performance of this modality. With further access to and improved technology of the equipment, pediatric endoscopists may develop more indications for this imaging technique.

Current indications for EUS in the adult population include evaluation of gastrointestinal submucosal lesions, cystic and solid pancreatic masses, gastrointestinal malignancy staging, and EUS-guided fine-needle aspiration (FNA).[2] In pediatrics, there has been a paucity of reports in the literature. Many of the adult indications can be applied to our patients, but, recently, there have been some small reports of other indications pertaining specifically to children, which will be outlined in this chapter.

FUNDAMENTALS OF ULTRASOUND IMAGING

Sound is a physical force that is transmitted through solid or liquid as a wave. The frequency of sound waves is measured in cycles formed per second. Their unit of measurement is a hertz. Medical imaging commonly uses frequency of waves in the 3.5 to 20 million Hz or megahertz (MHz).

Sound waves are formed by special ceramic crystals that have a piezoelectric property. The crystals vibrate to form sound waves, which are sent to tissues and reflected from the tissues back to the crystals. The incoming waves are then translated to an electrical signal, creating the ultrasound image. The transducer uses multiple crystals designed in an array to transmit and collect a series of waves to and from the tissue. Because of the multiple crystals, a two-dimensional image is created by multiple reference points that are reflections of the depth and span of the tissue being imaged.

Because sound waves travel at different speeds through different types of tissues, structures can be identified by their differences in hypo- or hyperechogenicity. Sound waves travel easily through fluid but not through air or bone. The speed of transmission is determined by the stiffness of the imaged tissue. Fat and collagen are the most reflective and therefore the brightest layers. Muscle or lean solid mass is less reflective and lighter. Interface between two tissues also creates an image owing to differing acoustic properties. Typically, lower frequencies (5–7.5 MHz) are used to image objects far away from the transducer, whereas higher frequencies (12–30 MHz) give greater detail closer to the transducer (eg, the gastrointestinal wall).

Ultrasound waves are better transmitted through a liquid medium than through an air medium. This phenomenon causes difficulties in transcutaneous ultrasonography because of areas filled with air, such as lungs and bowel. This problem is overcome within the gastrointestinal tract either by distending the gastrointestinal lumen with water or using a water-filled balloon attached to the end of the echoendoscope to improve the transmission of ultrasound waves.

EQUIPMENT AND TECHNIQUE

Current equipment available for EUS includes radial (Figure 67.4-1) and linear array (Figure 67.4-2) echoendoscopes. Both forms of echoendoscope employ regular endoscopy to guide the instrument into position before ultrasound imaging commences. More recently, catheter-based ultrasound probes (Figure 67.4-3) have been developed for use through standard endoscopes.

The original radial array echoendoscope displays a 360° cross-sectional view with gut wall layers and surrounding structures (Figure 67.4-4). The gastrointestinal wall (including the esophagus, stomach, small intestine, and rectum) has a five-layer pattern that correlates to the various histologic layers (superficial mucosa, deep mucosa, submucosa, muscularis propria, and serosa) (Figure 67.4-5). Advantages to the images in this view include the ability to compare similar images in orientation with computed tomographic (CT) cross-sectional imaging. Recent developments of this technology have included the addition of Doppler technology to detect blood flow. The smallest radial array endoscope has a 12.7 mm outer diameter (GF-UM130, Olympus America Inc., Melville, NY, USA). The disadvantages of this modality are the inability to view and guide a needle biopsy device and the inability

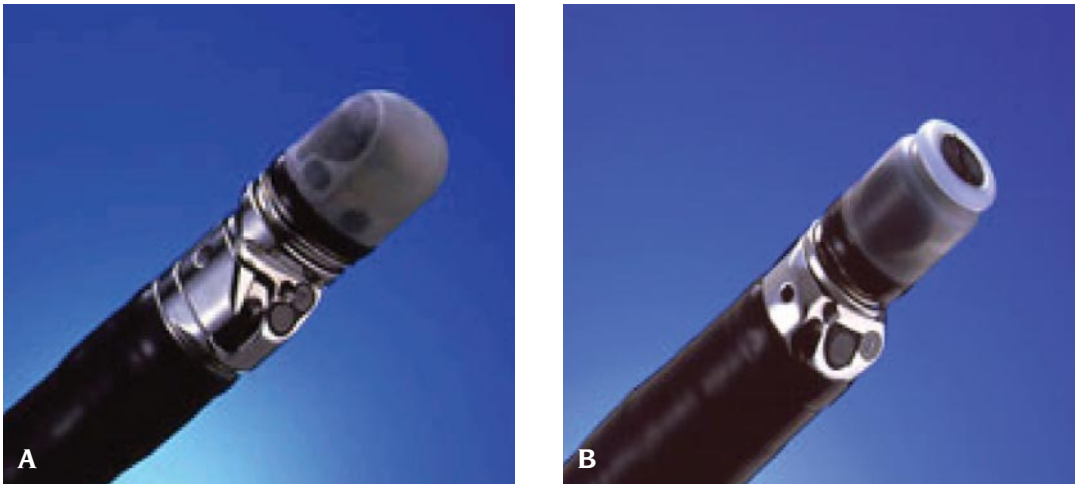

FIGURE 67.4-1 *A*, Radial ultrasound endoscope (GF-UM30P). *B*, Radial ultrasound endoscope (GF-UM130). Courtesy of Olympus Corporation (Tokyo, Japan).

to use the endoscope in small, young patients because of its large outer diameter.

The linear array echoendoscope provides a field of view in a configuration parallel to the endoscope (Figure 67.4-6). This instrument was initially developed for use with needle biopsy devices. The depth of the needle can be viewed when obtaining fluid or tissue. Along with the ability to use needle biopsy devices, the linear array echoendoscope can also facilitate use of Doppler imaging of blood vessels. A disadvantage to this type of EUS is its "forward oblique" view. For endoscopists familiar with side-viewing endoscopes, this may not be problematic, but for the average endoscopist, it can be disorienting. Another disadvantage similar to the radial array endoscope is its large outer diameter. The smallest linear array endoscope has an outer diameter of 12.1 mm (Pentax EG-3620U, Pentax Precision Instrument Corporation, Orangeburg, NY, USA).

Catheter-based ultrasound probes consist of a small-diameter transducer within a catheter sheath that can be introduced through a standard endoscope that has at least a 2.8 mm channel diameter. Catheter-based ultrasound probes using a variety of frequencies are available (5, 7.5, 12, 20, and 30 MHz). Typically, these probes are introduced into water-filled gut lumens to generate images. Probes are also available with a balloon sheath that can be filled with water to improve the image quality. Again, the disadvantage for the small pediatric patient is the need to use an endoscope that has at least a 2.8 mm channel diameter.

INDICATIONS

Because EUS is a fairly new modality and is just receiving acceptance in the adult patient population, use in the pediatric population is slow but steady. In the adult population, accepted indications for use include differential diagnosis of submucosal lesions, gastrointestinal malignancy staging, evaluation of the biliary tree, diagnosis of cystic or solid pancreatic masses, and FNA.[2] Recent case reports and retrospective publications with inclusion of some pediatric patients up to age 18 years have revealed some newer investigational indications for use of EUS in the pediatric population.

ESOPHAGUS

EUS has been used to differentiate eosinophilic esophagitis with normal controls.[3,4] Although histopathology is the gold standard method of diagnosis for this entity, findings

FIGURE 67.4-2 Linear array endoscope (EG3630-U). Courtesy of Pentax Precision Instrument Corporation (Orangeburg, NY, USA).

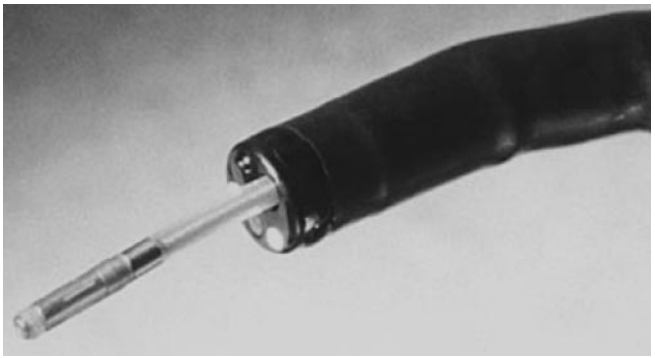

FIGURE 67.4-3 Catheter-based ultrasound probe (UM-3R). Courtesy of Olympus Corporation (Tokyo, Japan).

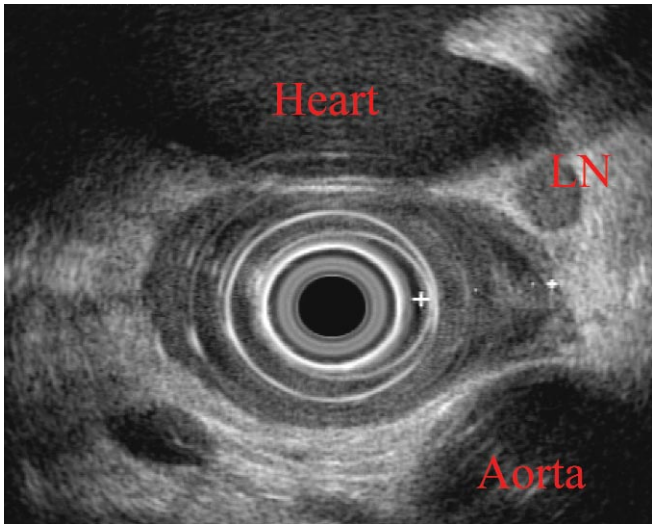

FIGURE 67.4-4 Esophageal wall layers and surrounding structures. LN = lymph node. Courtesy of J. Farrell, MD.

of esophageal wall thickening with significant expansion of the esophageal wall were noted in the subjects with eosinophilic esophagitis versus controls. Similar findings of esophageal wall thickening have been seen in subjects with gastroesophageal reflux disease[5,6]; thus, EUS only complements endoscopy and histology for the diagnosis of reflux esophagitis. Because EUS was used as an adjuvant for diagnosis of eosinophilic esophagitis and gives similar findings in patients with gastroesophageal reflux disease, this modality may be more useful as a follow-up tool in patients who are being treated for their eosinophilic esophagitis as opposed to a primary diagnostic tool.

The use of EUS in patients with congenital esophageal stenosis (CES) has been successful in a few reports for determination of the optimal treatment approach.[7–9] There are three histopathologic types of CES: tracheobronchial remnants, membranous diaphragm, and fibromuscular hypertrophy. The type of CES must be determined before deciding on dilatation versus surgical resection. Dilatation of tracheobronchial remnants carries a higher risk of perforation and a poor success rate, with the eventual necessity of surgical resection. EUS is able to visualize cartilage associated with tracheobronchial remnants and thereby eliminates attempts at dilatation.

Many studies have used EUS in the clinical setting of portal hypertension. A specific study was undertaken to determine if variceal wall thickness correlates with the risk of variceal bleeding.[10] Using these data, along with variceal pressures, wall tension was determined to estimate the risk of bleeding. However, the researchers were unable to provide similar findings in patients with multiple varices.

An established indication for use of EUS in adults is staging of esophageal cancers and the diagnosis of mediastinal lympadenopathy. Pediatric patients may have other etiologies for mediastinal lymphadenopathy, including infectious or granulomatous disease (eg, sarcoidosis). EUS was shown to assist in the diagnosis of histoplasmosis among patients with symptoms of dysphagia and a midesophageal submucosal mass.[11] Savides and colleagues also tried to determine the cost-effectiveness of EUS versus CT scan of the chest.[11] In their institution, the cost was comparable, but the advantage of EUS was the availability to obtain tissue via FNA. They also found that EUS provided greater resolution of periesophageal lesions and a greater sensitivity of calcified lymph nodes. The real benefit of the role of EUS-guided FNA in the evaluation of pediatric mediastinal processes may be in the avoidance of more invasive and costly diagnostic procedures (eg, mediastinoscopy and thoracoscopy).

EUS has also been used for post-treatment assessment of patients with achalasia.[12] Patients with achalasia who were treated with dilation versus botulinum toxin injection underwent EUS to assess esophageal wall damage post-treatment. Transient esophageal mucosa-submucosa diameter was increased postdilation but not post–botulinum toxin injection. Also of note, the thickening normalized within 24 hours of the procedure.

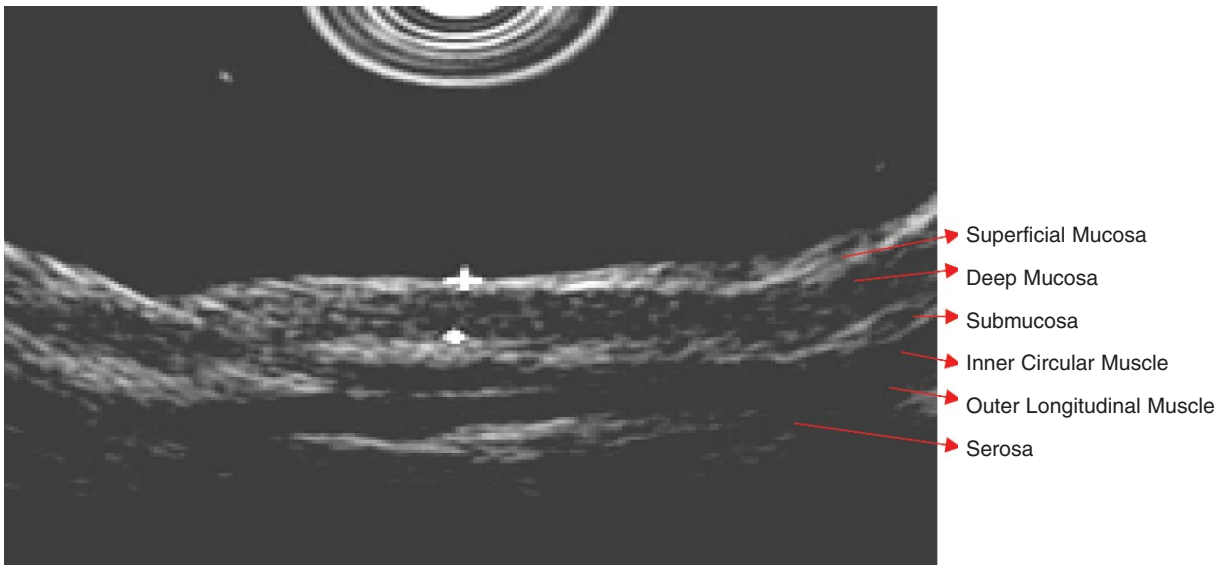

FIGURE 67.4-5 Endoscopic ultrasonography tissue layer correlation. Courtesy of J. Farrell, MD.

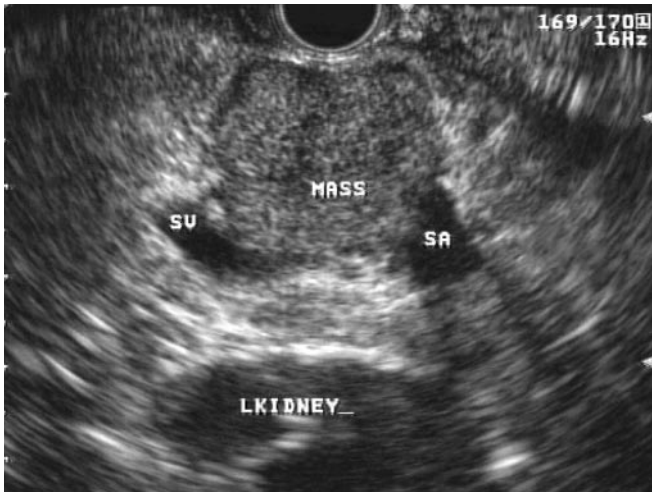

FIGURE 67.4-6 Linear array image of pancreas tail mass. Courtesy of J. Farrell, MD. SA = splenic artery; SV = splenic vein.

Stomach

Dieulafoy lesion can occasionally be seen in the pediatric patient population. Fockens and colleagues used EUS to determine the location of this abnormal, large submucosal vessel.[13] On finding such a vessel, they performed sclerotherapy. Unfortunately, they used a rotating sector scanner that did not have Doppler capability, and with the perpendicular scanning view, needle-guided injections were difficult. Use of the linear array would most likely alleviate this problem.

Foregut duplications are a rare abnormality, but generally two-thirds are found within the first year of life. An investigational indication for EUS is defining the relationship of these duplications to their intraluminal tract.[14] Because EUS can differentiate between cystic and solid structures and is in greater proximity to the lesion, it can be a reliable tool to diagnose this entity. Also, endosonographic aspiration of the cyst can be performed to identify cells consistent with duplication. The wall layers can be identified in duplications to match the gastrointestinal tract (Figure 67.4-7).

Pancreas/Biliary Tree

There are a significant number of established indications for EUS use in adults in the pancreas and biliary tract. In children, reports of EUS use include diagnosis of solid pseudopapillary tumors of the pancreas (Figure 67.4-8),[15] blunt pancreatic trauma,[16] drainage of pancreatic pseudocysts (Figure 67.4-9),[17] preoperative localization of insulinomas and gastrinomas,[18] and as a screening tool prior to sphincterotomy for acute biliary pancreatitis.[19,20]

EUS-guided FNA has also been used in children for the diagnosis of solid pseudopapillary tumors of the pancreas.[15] Owing to the malignant potential of the tumor, radical resection is the mainstay of treatment in adults. The investigators postulated that surgeons reluctant to perform radical resection in children when the diagnosis is in question might be reassured by a definitive diagnosis with the assistance of EUS. EUS may also be the best modality to obtain the FNA because of the location of the mass.

Pancreatic trauma can occasionally be seen in the pediatric population. EUS has been compared with CT for diagnosis and follow-up of pancreatic trauma.[16] Several advantages were established, including mobility of the EUS equipment, lack of radiation, ability to do multiple serial examinations, and no requirement of contrast material with sensitivity equal to that of CT. Disadvantages include increased time consumption for the EUS examination and diagnosis dependent on operator expertise.

Drainage of pancreatic pseudocysts with the use of conventional endoscopy has been reported since 1981.[21] EUS-guided drainage was first described in 1996.[22] EUS plays an important role in the evaluation of pancreatic pseudocysts prior to endoscopic drainage. More recently, an EUS-guided one-step drainage procedure was used in three patients, including one pediatric patient.[17] A curved linear

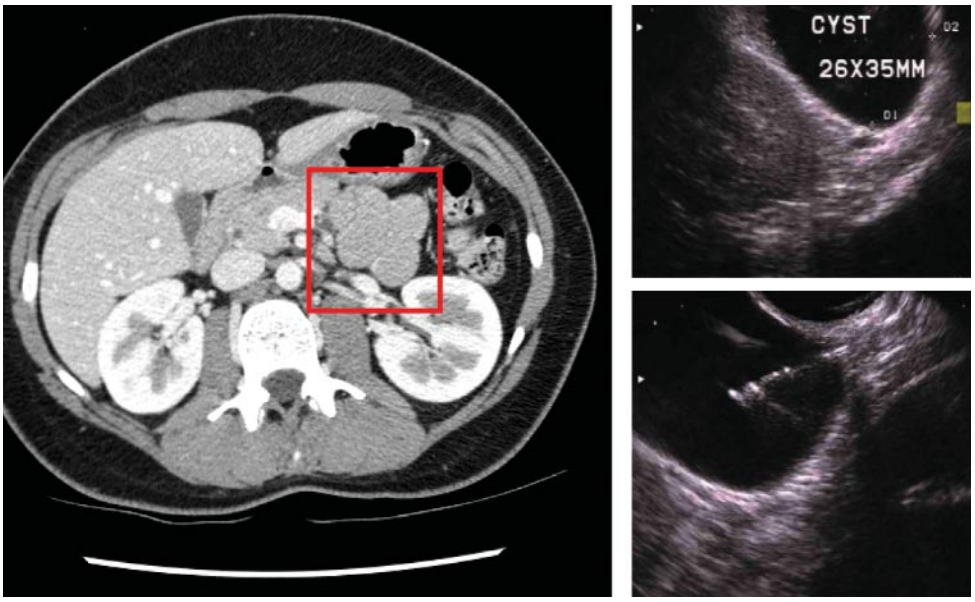

FIGURE 67.4-7 Gastric duplication cyst (computed tomographic scan and endoscopic ultrasonographic images with fine-needle aspiration). Courtesy of J. Farrell, MD.

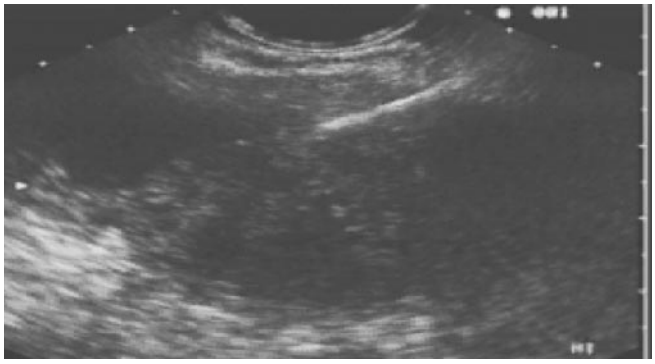

FIGURE 67.4-8 Endoscopic ultrasonography–guided fine-needle aspiration of a pancreatic mass. Courtesy of J. Farrell, MD.

array EUS with a 22-gauge needle and a 7 French drainage tube were used in this procedure. Although there were a small number of patients in this report, EUS-guided one-step drainage, with its ability to identify and avoid intramural vasculature, may be an advantageous procedure over a radiologic or a surgical approach.

EUS was compared with somatostatin receptor scintigraphy (SRS), transabdominal ultrasonography, CT, and magnetic resonance imaging (MRI) for the preoperative localization of insulinomas and gastrinomas.[18] The authors found that EUS and SRS had comparable sensitivity and significantly greater sensitivity for localization of gastrinomas than ultrasonography, CT, or MRI. EUS was the most sensitive modality for localization of insulinomas than all of the others.

Endoscopic sphincterotomy is beneficial for patients with biliary pancreatitis, but it can have serious complications. The use of EUS for prescreening patients prior to sphincterotomy can reduce the complications by avoiding unnecessary cannulation of the papilla.[19]

Colon

Established indications in adults for using EUS in the lower gastrointestinal tract are primarily for staging of rectal cancer, but EUS investigational use in children has included evaluation of the rectal venous system in patients with portal hypertension,[23,24] differential diagnosis in suspected cases of inflammatory bowel disease,[25,26] and EUS evaluation of perirectal and perianal complications of Crohn disease.[27,28]

Yachha and colleagues were able to use EUS to compare children with extrahepatic portal venous obstruction and controls to determine the size, number, and location of varices.[23] They determined that EUS was able to detect the presence of rectal varices better than endoscopy. They also suggested that EUS may be useful in differentiating between portal rectopathy and inflammatory colitis.

An attempt to use EUS for differentiating Crohn disease and ulcerative colitis has been inconclusive.[27] Many studies have tried to compare wall thickness and discrimination between mucosal and transmural inflammation. No consistent conclusions are seen among these studies. However, EUS has been used to correctly identify perirectal and perianal complications of Crohn disease better than fistulacontrast studies and CT.[28]

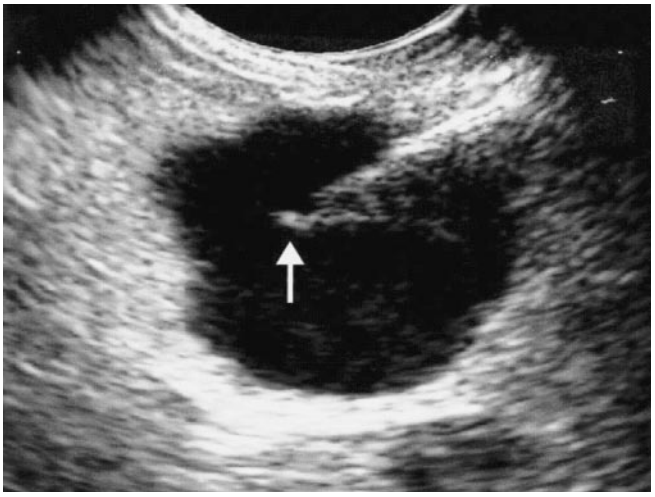

FIGURE 67.4-9 Endoscopic ultrasonography–guided fine-needle aspiration of a pancreatic cyst (*arrow*, tip of needle).

CONCLUSIONS

EUS, including EUS-guided FNA, is a new modality that has many established indications in the adult patient population. Its pediatric indications have increased somewhat since its inception, but owing to the lack of awareness among general physicians, it may be underused. As clinical indications increase, training in this technique will also need to increase.

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5. Endoscopic Retrograde Cholangiopancreatography

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> Tell me and I forget. Teach me and I remember. Involve me and I learn.
>
> —Benjamin Franklin (1706–1790)

Over the past 30 years, endoscopic retrograde cholangiopancreatography (ERCP) has become an integral element in the diagnostic and therapeutic tools of the gastroenterologist. It has grown from a limited procedure, performed by a few, to a mainstream modality for the diagnosis and treatment of a wide variety of benign and malignant hepatobiliary and pancreatic disorders. Since the report of the first successful cannulation of the ampulla of Vater by McCune and colleagues in 1968,[1] the role of ERCP for diagnosing and treating these diseases has evolved, providing patients with a minimally invasive method to treat problems that previously required open surgery.

ERCP is currently used for the management of bile duct stones; benign and malignant biliary obstruction; benign and malignant pancreatic neoplasms and diseases of the major and minor papilla; acute and chronic pancreatitis; bile duct injuries; pancreatic duct disruption and pseudocysts; pain syndromes considered to be of pancreatic, biliary, or sphincter of Oddi origin; certain congenital and acquired hepatic conditions affecting the biliary tract; and bleeding or infection suspected of being of hepatic, biliary, or pancreatic origin.[2–4]

Although ERCP has been widely applied in adult patients for a long time, only recently has it become an accepted modality in pediatrics. The limited use of this technique in children might be caused by multiple factors, including (1) a relatively low incidence of pancreatic and biliary diseases in childhood and a low index of clinical suspicion; (2) limited availability of pediatric instruments; (3) a lack of expertise among pediatric gastroenterologists to perform ERCP; (4) reluctance of adult gastroenterologists to study small children; (5) the impression that ERCP in children is technically more difficult to accomplish; (6) difficulty in the effective evaluation of a therapeutic result; and (7) a lack of well-defined indications and safety of ERCP in children.[5–7]

Since the first report of a successful ERCP in a 3.5-month-old child in 1976,[8] the field of pediatric ERCP has expanded dramatically, driven by the growing subspecialty of pediatric gastroenterology, refinements in design, and worldwide distribution of small-diameter duodenoscopes.[9,10]

TECHNICAL CONSIDERATIONS

ERCP is a relatively complex combined endoscopic or radiographic procedure that requires multiple skilled personnel. Our goal is to provide state-of-the-art patient care, and success depends on the combined efforts of members from the departments of surgery, medicine, pediatrics, and radiology. Hence, one weak link in this multidisciplinary venture might limit success.[11–13]

Preprocedure Preparation

According to the Standards of Practice Committee from the American Society for Gastrointestinal Endoscopy (ASGE), "preparation for endoscopy in pediatric age patients requires attention to physiologic issues as well as emotional and psychosocial issues in both the patient and the parent or guardian."[12] The anticipation of potentially painful or noxious procedures provokes intense anxiety in children. Parental anxiety is heightened by protective feelings, wanting to shield the child against harm and unnecessary discomfort. Much of this anxiety may be relieved by providing optimal age-appropriate information and counseling to the patients and parents, with a detailed description of all aspects of the procedure.[9,12] With psychological preparation, children are less anxious and more cooperative during the procedure, requiring less sedation.[14]

Dietary Restrictions

Dietary restrictions are necessary to minimize the risks of pulmonary aspiration of gastric contents. In pediatric patients presumed to have normal gastric emptying, the fasting interval before endoscopy should be a minimum of 2 hours for clear liquids. Guidelines for fasting after ingestion of milk and solids are diverse and related to age. Fasting from milk or solids should be for 4 hours in infants under 5 months, 6 hours in those 6 to 36 months, and 8 hours in those over 36 months of age.[12,15]

Informed Consent

Written informed consent must be obtained from the appropriately designated parent or guardian after careful explanation of the risks and benefits of the entire procedure. Adolescents might be offered the option to review and sign the consent form to acknowledge their active participation in the process.[9]

Sedation

Premedication is not routinely used for pediatric endoscopy.[16] When necessary, oral or rectal administration of benzodiazepines might be used prior to intravenous sedation or anesthesia, improving the ease of separation from parents and of intravenous insertion.[12]

Most ERCPs are performed with the benefit of conscious sedation, deep sedation, or general anesthesia, although some experts give no sedation to selected patients.[17] The proper approach to sedation remains a major controversy among pediatric endoscopists.[9,18]

The relatively large diameter of standard duodenoscopes (eg, 11–12 mm) may cause discomfort and compress the soft-walled trachea in young children. The prone position during ERCP also compromises chest and lung excursion and may result in hypoventilation and hypoxia in a sedated child. With these considerations in mind, general anesthesia with endotracheal intubation might be appropriate for some children. Insufficient outcome data exist to make firm recommendations about the comparative adequacy, safety, and cost of sedation and general anesthesia in children undergoing ERCP (Table 67.5-1).[19]

An individual trained in pediatric monitoring and at least basic pediatric life support should be present in addition to the endoscopist for the entire duration of the procedure. Pulse oximetry and hemodynamic monitoring should be routinely used.[12]

Equipment

Fluoroscopic equipment should be suitable for children of all ages, minimizing radiation exposure.

In neonates and infants younger than 12 months, ERCP is performed with a pediatric duodenoscope, which has an insertion tube diameter of 7.5 mm, a channel of 2 mm, and an elevator (Olympus PJF–160 – Olympus, Hamburg, Germany). This endoscope, which is also preferred for children less than 2 years of age, accepts cannulas and other accessories that are 5 French or smaller. A standard diagnostic duodenoscope (insertion tube diameter approximately 11–12 mm) with a 3.2 mm operating channel may be used in most children older than 2 to 3 years. Therapeutic large-diameter (> 13 mm) endoscopes with a 4.2 mm channel are infrequently required to place 10 French stents in larger adolescents.[5,19]

Antimicrobial Prophylaxis

The frequency of bacteremia associated with upper or lower gastrointestinal endoscopy with or without biopsy is low, ranging from about 2 to 5%.[20,21] In immunocompetent children, clinically relevant bacteremia is very infrequent following routine endoscopic procedures.[22] The reported risk of bacteremia with ERCP (5–6%) is similar to that for upper and lower endoscopy unless an obstructed biliary ductal system is present.[20]

Infections associated with gastrointestinal endoscopic procedures are rare, including those performed in children.[22] In a meta-analysis of antibiotic prophylaxis in ERCP, Harris and colleagues concluded that antibiotic prophylaxis prior to ERCP may reduce the incidence of bacteremia, but this has little clinical relevance. Prophylaxis does not substantially reduce the incidence of sepsis or cholangitis; thus, the routine use of antibiotic prophylaxis cannot be recommended.[23]

The American Heart Association and the ASGE have developed criteria for the use of prophylactic antibiotics for endoscopic procedures based on the associated risk of developing endocarditis. ERCP in the absence of ductal obstruction is considered to be of low risk, and routine antibiotic prophylaxis is not recommended. Evaluation should be performed on a case by case basis.[20,24,25] For endoscopic procedures associated with increased rates of transient bacteremia (ERCP with known or suspected bile duct obstruction), prophylaxis is recommended for patients at "high risk" for the development of infective endocarditis (eg, prosthetic heart valves, a previous history of endocarditis, complex cyanotic congenital heart disease, and surgically constructed systemic-pulmonary shunts). No prophylaxis is recommended for patients with those cardiac lesions and conditions at no increased risk for infective endocarditis over the general population (eg, previous coronary artery bypass, graft surgery, cardiac pacemakers and implanted defibrillators, mitral valve prolapse or previous rheumatic fever without valvular dysfunction or regurgitation). There are insufficient data to recommend routine prophylaxis for patients with cardiac lesions or conditions at intermediate risk for the development of infective endocarditis (eg, most congenital cardiac malformations, rheumatic and other acquired valvular dysfunction, even after valvular surgery, hypertrophic cardiomyopathy, and mitral valve prolapse with valvular regurgitation). The endoscopist may consider prophylaxis on a case by case basis.[24,25]

Snyder and Bratton surveyed 14 academic pediatric centers in the United States and Canada to determine the current practices of these groups with regard to antimicrobial prophylaxis in ERCP. In patients with cardiac disease at high risk for endocarditis, all centers used prophylaxis. In patients with a moderate risk, 64% of the centers used prophylaxis.[20]

The Working Party for the British Society of Gastroenterology Endoscopy Committee recommends antibiotic prophylaxis for all patients undergoing ERCP with evidence of biliary stasis or pancreatic pseudocyst. Oral ciprofloxacin or parenteral gentamicin (or parenteral quinolone, cephalosporin, or ureidopenicillin) is recommended.[26] There are no pediatric data to guide antibiotic prophylaxis for ERCP.[5,20]

Procedure Length

The time for total examination (from introduction of duodenoscope to withdrawal of the instrument) varies considerably according to expertise and the difficulty of the

TABLE 67.5-1 PEDIATRIC ENDOSCOPIC RETROGRADE CHOLANGIOPANCREATOGRAPHY SERIES

AUTHOR, YEAR (REF)	PATIENT	PROCEDURE (N)	SUCCESS (%)	AGE		GA (%)	INDICATIONS (%)			THERAPY (%)	COMPLICATIONS (%)			
				RANGE	MEAN		BILIARY	PANCREATIC	PAIN		PANCREATIC	HEMORRHAGE	PERFORATION	INFECTION
Riemann and Koch, 1978[145]	18	18	100	6–17 yr	NR	11	17	83	0	0	0	0	0	1 (death)
Cotton and Laage, 1982[29]	20	25	96	7–16 yr	NR	65	20	50	30	5	0	0	0	0
Guelrud et al, 1987[146]	23	23	96	19–150 d	67d	26	100	0	0	0	0	0	0	0
Allendorph et al, 1987[144]	39	39	92	6 mo–18 yr	12.5 yr	46	28	54	18	10	10	0	0	0
Heyman et al, 1988[28]	12	12	33	3–58 wk	11.6 wk	100	100	0	0	0	0	0	8	0
Buckley and Connon, 1990[72]	42	42	97	1–19 yr	10.5 yr	38	36	43	21	12	5	2	0	0
Guelrud et al, 1991[17]	32	32	94	16–150 d	49d	19	100	0	0	0	0	0	0	0
Putnam et al, 1991[147]	38	42	93	14 mo–19 yr	12.3 yr	32	36	64	0	0	8	0	0	0
Dite et al, 1992[148]	19	19	100	4–16 yr	11.9 yr	68	5	68	26	0	0	0	0	0
Brown et al, 1993[7]	92	121	94	4 mo–19 yr	10.9 yr	54	20	77	3	16.5	3	0	0	2.5
Brown and Goldschmiedt, 1994[76]	25	42	100	22 mo–19 yr	NR	NR	64	36	0	68	8	0	0	0
Richieri et al, 1994[149]	19	19	100	1–18 yr	9.6 yr	58	58	42	0	5.3	5.3	0	0	0
Guelrud et al, 1994[105]	51	NR	98*	1–18 yr	NR	0	0	100	0	35	8	0	0	0
Derkx et al, 1994[150]	20	22	90	4–19 wk	12 wk	100	100	0	0	0	0	0	0	0
Mitchell and Wilkinson, 1994[151]	40	40	90	6–80 wk	12 wk	100	100	0	0	0	0	0	0	0
Abu-Khalaf, 1995[100]	16	16	100	2 mo–18 yr	10.5 yr	31	56	25	19	25	0	0	0	0
Ohnuma et al, 1997[152]	73	75	88	8–300 d	71 d	100	100	0	0	0	0	0	0	0
Tagge et al, 1997[13]	26	26	96†	6 mo–19 yr	10 yr	58	92	8	0	61.5	4	0	0	0
Tarnasky et al, 1998[73]	10	10	100	0.5–16.9 yr	8.8 yr	80	100	0	0	100	0	0	0	0
Graham et al, 1998[107]	17	17	94	3–16 yr	11.2 yr	88	0	100	0	53	13	0	0	0
Hsu et al, 2000[122]	22	34	100	1.5–17 yr	10.7 yr	68	0	100	0	67.6	6	0	0	0
Teng et al, 2000[68]	42	50	100	57 d–15 yr	NR	80	64	36	0	12	0	0	0	2
Poddar et al, 2001[6]	72	84	97	11 mo–14 yr	9 yr	0	61	28	11	30.6	7	0	0	1.4
Pfau et al, 2002[153]	43	53	94.3	1–18 yr	13.5 yr	60.4	47	53	0	45.2	3.8	1.9	0	0
Liguory et al, 2001 (unpublished)	51	51	92	6 mo–15 yr	8 yr	100	29.4	54.9	15.7	43.1	2	0	0	0

Adapted from Etzkorn KP et al.[18]

GA = general anesthesia; NR = not reported.

*83% success in therapeutic examinations.

†87% success in therapeutic examinations.

examination. The duration of a diagnostic examination ranges from 2 minutes to 1 hour.[17,27] In most series, the mean time is between 10 and 30 minutes.[6,17,28,29] For therapeutic ERCPs, it is difficult to define the "normal" mean time, considering the myriad of interventions available. In one study, the average time for ERCP, including both diagnostic and therapeutic studies, was 58 minutes.[12]

DIAGNOSTIC AND THERAPEUTIC INDICATIONS

Indications for diagnostic and therapeutic ERCP in pediatrics (see Table 67.5-1) are, for the most part, similar to those established for the adult population. However, the relative frequency of each indication differs because children are much more prone to congenital abnormalities and trauma than malignancy.

Diagnostic ERCP in the Era of Magnetic Resonance Cholangiopancreatography

Over the last two decades, several new diagnostic modalities have been developed and refined, such as ultrasonography (transabdominal and endoscopic), computed tomography (CT) (single and multislice helical), magnetic resonance imaging (MRI) and magnetic resonance cholangiopancreatography (MRCP), CT-virtual cholangiography, and laparoscopic surgery with intraoperative cholangiography. These techniques have proven useful in the diagnosis and staging of pancreatic and hepatobiliary diseases and may obviate the need for diagnostic ERCP in some cases.

MRCP is a noninvasive test for imaging the biliary and pancreatic ducts, which provides high-quality images without administration of exogenous contrast material or use of ionizing radiation.[30–36]

MRI relies on radiofrequency pulse-induced excitation of protons within a magnetic field to generate an image. Conventional MRI uses a combination of T_1- and T_2-weighted sequences to image abdominal organs (eg, liver, pancreas biliary tract, and duodenum). MRCP relies on heavily T_2-weighted sequences because fluid-containing structures have a much longer T_2 than solid tissue. Thus, stationary fluid in the biliary and pancreatic ducts serves as an intrinsic contrast medium, resulting in images similar to those obtained through ERCP.[30] Intraluminal filling defects and the nature of ductal stenoses and/or dilatation can be readily demonstrated. With current magnetic resonance scanners, images are acquired in a few seconds, virtually eliminating image degradation from motion artefacts. The entire biliary tract can be imaged in a single breath-hold of 20 seconds or less with high spatial resolution so that structures such as fourth-order intrahepatic bile ducts and small stones are detected.[35] No special preparation is required, although fasting for 2 to 4 hours is recommended to reduce gastric and duodenal fluids.[31]

Different MRCP pulse sequences have been used: steady-state free precession, two-dimensional and three-dimensional fast spin echo techniques, rapid acquisition with relaxation enhancement (RARE), and half-Fourier RARE. MRCP image sequencing differs from center to center because of rapidly evolving technology, equipment and software differences, and radiologist preferences and expertise. Currently, most centers are not equipped to perform state-of-the-art MRCP images.[30]

MRCP is a useful noninvasive alternative to diagnostic ERCP, especially when ERCP study is difficult or technically inadequate (eg, Billroth II surgical bypass, biliary-enteric anastomoses, periampullary diverticula, duodenal obstruction). It can also be used for planning surgical, endoscopic, and radiologic interventions.

The accuracy of MRCP in comparison with ERCP is very high. Adequate images can be obtained in 95 to 98% of patients.[37–39]

Currently, a limitation of MRCP is that its resolution is inferior to that of ERCP. Although MRCP detects stones as small as 2 mm, the spatial resolution of MRCP is not sufficient to detect small stones and crystals in a consistent fashion. It may not be able to detect small ampullary or distal bile duct tumors either.[35]

Absolute contraindications include the presence of a cardiac pacemaker, cerebral aneurysm clips, ocular or cochlear implants, and ocular foreign bodies. Relative contraindications include the presence of a cardiac prosthetic valve, neurostimulators, and metal prostheses. Claustrophobia accounts for 1 to 4% of failed or inadequate studies.[30,40]

In pediatrics, MRCP has been used to diagnose biliary atresia (BA) and other congenital or acquired pancreaticobiliary disorders (eg, choledochal cysts).[41–43] However, further studies are needed to support routine use of MRCP in pediatrics.[31]

Defining Normal Duct Size in Pediatrics

The size of a normal common bile duct (CBD) was measured in children between 7 and 16 years and varies from 2.1 to 4.9 mm, just below the entry of the cystic duct. The diameter of the pancreatic duct in the head ranges from 1.4 to 2.1 mm, and in the body from 1.1 to 1.9 mm (all measures are corrected for radiographic magnification).[29]

BILIARY INDICATIONS FOR ERCP

Indications for ERCP in children are listed in Table 67.5-2. Investigation of neonatal cholestasis is unique to pediatrics, although the role of ERCP remains controversial.[5,19] Structural causes of neonatal cholestasis include BA, choledochal cysts, choledocholithiasis, intrahepatic bile duct paucity or hypoplasia, neonatal sclerosing cholangitis, and congenital bile duct stricture.[19]

In children older than 1 year and adolescents with biliary disorders, the most frequent indications are obstructive jaundice, known or suspected choledocholithiasis, abnormal liver enzymes in children with inflammatory bowel diseases, abnormal biliary findings in abdominal ultrasonography, and therapeutic ERCP.[5]

Biliary Atresia

In a child with neonatal cholestasis, symptoms and signs of BA overlap those of idiopathic neonatal hepatitis and other rare causes of cholestasis.[17,44] Early identification

TABLE 67.5-2 BILIARY INDICATIONS FOR ENDOSCOPIC RETROGRADE CHOLANGIOPANCREATOGRAPHY

DIAGNOSTIC	THERAPEUTIC
Investigation of neonatal cholestasis	Sphincterotomy
Biliary atresia	Sphincteroplasty (balloon dilation)
Choledochal cyst	Stone extraction
Choledocholithiasis	Stricture dilation
Biliary obstruction owing to parasitic infestation	Stent placement
Dilated intrahepatic bile duct	Nasobiliary drainage
Benign and malignant biliary strictures	
Primary sclerosing cholangitis	
Biliary obstruction or leaks after liver transplant	
Preoperative and postoperative evaluation (laparoscopic cholecystectomy)	
Bile plug syndrome	
Abnormal findings in other examinations	
Manometric evaluation of the sphincter of Oddi	

of BA before 8 weeks of age may be associated with better surgical results (Kasai procedure) and improved clinical outcome.[17,27,44]

BA is a localized, progressive obliteration of the extrahepatic and hilar bile ducts that uniquely presents in the first months of life. The disease occurs in approximately 1 in 8,000 to 1 in 15,000 live births, with a 1.4:1 female-to-male predominance. It accounts for 30% of all cases of cholestasis in young infants. If untreated, it leads to progressive fibrosis and, ultimately, cirrhosis and death.[44,45]

No single test or combination of tests is consistently reliable in differentiating BA from other forms of cholestasis.[27] Imaging techniques such as ultrasonography and hepatobiliary scan are useful procedures but are often inconclusive. The sensitivity and specificity of scintigraphy, with concomitant administration of phenobarbital, are about 95% and 93%, respectively. However, failure of excretion may be seen in neonatal hepatitis as well. Currently, the most reliable test, aside from exploratory laparotomy, is percutaneous liver biopsy (up to 93% accuracy).[44] However, the histologic features of giant cell transformation and bile duct proliferation can be seen in both neonatal hepatitis and early stages of BA.[17]

Duodenal intubation has been reported to exclude BA if bilirubin or bile is found in the fluid. However, 10% of infants in whom bile is not found will not have BA.[17]

The combination of several tests may improve the accuracy of the diagnosis. Discriminant analysis using clinical criteria, scintigraphic excretion scan, and liver biopsy permit accurate diagnosis of either BA or neonatal hepatitis in 80 to 94% of young infants.[17] Thus, 10 to 20% of infants might need surgical exploration to establish a diagnosis. For these children, ERCP may be of diagnostic value.[5,17,27,28,44]

ERCP is the procedure of choice for investigation of structural abnormalities or obstructive lesions of the extrahepatic bile ducts and gallbladder when the intrahepatic ducts are not dilated.[28] Three types of anatomic findings have been described in patients with BA[5,17]:

- Type 1: no visualization of the biliary tree. This is observed in about 35% of the cases. No bile is seen in the duodenum. A normal pancreatogram is obtained.
- Type 2: visualization of the distal CBD and gallbladder without visualization of the main hepatic or intrahepatic ducts (35% of the cases). No bile is seen in the duodenum, and a narrow and irregular distal CBD might be present. The pancreatic duct is normal.
- Type 3: type 3a includes visualization of the gallbladder and the complete CBD with biliary lakes at the porta hepatis; in type 3b, both hepatic ducts are seen with biliary lakes. It is present in 30% of cases. Bile can be seen in the duodenum. The pancreatic duct is normal, and a narrow distal CBD is present.

When the biliary tree is partially visualized (types 2 and 3), the diagnosis of BA is made and confirmed by surgery. When the biliary tree is not opacified and only the pancreatic duct is seen (type 1), the diagnosis is suspected, and exploratory laparotomy is indicated.

It is important to emphasize that absence of CBD opacification alone, despite several maneuvers to opacify the biliary tree, does not establish atresia.[16,27] In most situations, clinical, biochemical, and liver biopsy findings in conjunction with filling of the pancreatic duct without CBD opacification and absence of bile in the duodenum may be sufficient to establish the diagnosis of BA prior to surgical intervention.[16] Of the 310 infants with neonatal cholestasis reported in the literature and reviewed by Guelrud, the diagnosis by ERCP was incorrect in only 5 (1.6%) patients.[5]

MRCP has been used in neonates and infants for the diagnosis of BA, and the results are promising.[40] However, further studies are required.[30,43]

Choledochal Cysts

Choledochal cysts are uncommon anomalies of the bile ducts, with probable congenital origin, associated with anomalous pancreaticobiliary junction in 80 to 90% of cases.[46] The incidence is higher among Japanese, being rare in Western countries, where its prevalence is estimated in 1 in 100,000 to 1 in 150,000 newborns.[47] The female-to-male predominance is 3 to 4:1. These cysts are usually found during infancy or childhood (80% of patients are younger than 10 years of age),[48] although, in some series, 30% are found in adults or among the elderly.[49,50]

The most common classification accepted nowadays was proposed by Todani and colleagues,[51] expanding the original classification of Alonso-Lej and colleagues[52] by including intrahepatic cysts and redividing the extrahepatic disease:

- Type I cysts are present in 80 to 90% of cases and are further classified according to the shape of the affected segment. A type IA cyst involves cystic dilation of the CBD, with marked dilation of part of or the entire extrahepatic biliary tree. The gallbladder commonly arises from the cyst, and the intrahepatic biliary tree is normal. Type IB cyst involves focal, segmental dilation of the CBD, usually of the most distal part of the duct. A normal segment of CBD is present between the cyst and the cystic duct. Type IC cyst involves fusiform dilation of the CBD, along with diffuse, cylindrical dilation of the common hepatic duct. The gallbladder arises from the dilated CBD, and the intrahepatic biliary system is not dilated.
- Type II cysts are present in 2% of cases and are considered true choledochal diverticula.
- Type III cyst, found in 1.5 to 5% of the patients, is a choledochocele that involves only the intraduodenal portion of the CBD. Characteristically, the papilla appears as a hemispherical cystic structure protruding into the duodenal lumen. The terminal end of the CBD is blunt and bulbous, unlike the normal tapered appearance. The cystic structure enlarges further during injection of contrast material into the distal CBD. A rounded, cystic structure filled with contrast material is noted when patients are upright.[53]
- Type IV cysts are subclassified in two groups. Type IVA cyst involves dilation of the intra- and extrahepatic bile ducts and is present in up to 20% of patients. Cholangiography shows gross cystic dilation of the extrahepatic biliary tree, with extension of the cystic dilation into the intrahepatic biliary tree. The intrahepatic dilation may affect multiple segments and be smooth and fusiform or irregular. Type IVB cysts also involve dilation of multiple segments but are confined to the extrahepatic bile duct. They are much less common than type IVA cysts. Cholangiography shows multiple segmental dilation of the CBD. The intrahepatic biliary tree is normal.
- Type V cyst (Caroli disease) involves dilation of one or several segments of the intrahepatic bile duct.[51,54]

The origin and formation of choledochal cysts have been a matter of considerable investigation and debate.[55]

The classic triad of choledochal cyst is characterized by abdominal pain, jaundice, and abdominal mass. Unusual presentations include rupture of the choledochal cyst with bile peritonitis, pancreatitis, and bleeding esophageal varices owing to biliary cirrhosis.[56] Intermittent fever, vomiting, elevation in serum transaminases and amylase, abdominal pain, and jaundice might be related to bouts of cholangitis and pancreatitis.[57]

Many complications have been associated with choledochal cysts, including cholelithiasis, choledocholithiasis, cystolithiasis, pancreatitis, intrahepatic abscesses, biliary cirrhosis, portal hypertension, and biliary carcinoma.[46]

Malignancy related to choledochal cysts is more frequent with advancing age, being reported in 23 to 40% of the cases.[58,59] These rates are extremely high if compared with biliary carcinoma in the general population (0.003–0.004%).[60] The development of cancer seems to be related to bile stasis and prolonged contact with the epithelium. It might be related to pancreatic juice reflux to the biliary tree, leading to chronic irritation and metaplasia. Most cases are detected in advanced stages, with poor prognosis. Although cyst excision eliminates the potential site for neoplasia, it does not exclude the possibility of developing cancer in the intrahepatic ducts. Long-term follow-up is always mandatory.[61]

Abdominal ultrasonography and CT are important in detecting cystic masses in close relation to the pancreatic head and hepatic hilum. CT is more accurate in depicting intrahepatic bile ducts and the distal parts of the CBD. When the cyst is round and markedly dilated, with no evidence of intrahepatic ductal dilation, its biliary origin is difficult to determine. Cystic lesions such as mesenteric, omental, ovarian, renal, adrenal, and hepatic cysts; gastrointestinal duplication; hydronephrotic kidneys; and pancreatic pseudocysts are the main differential diagnoses when a huge choledochal cyst lacks intrahepatic involvement at CT.[54] Hepatobiliary scintigraphy using technetium 99m is useful in this setting.

ERCP is the most sensitive method to define the biliary system anatomy and depict anomalous pancreaticobiliary junction and biliary malignancies.[59,62] It is invasive and operator dependent, requiring a meticulous technique. In this setting, it has been associated with a particularly high risk of pancreatitis, probably related to the fact that, in the presence of a common channel, cyst opacification often requires repetitive injections of the pancreatic duct.[62,63]

Virtual cholangiography using CT with two- or three-dimensional visualization of the biliary tract has also been used in the diagnosis of choledochal cysts. Spinzi and colleagues emphasized its advantages in depicting the entire biliary system and the capability of showing mucosal lesions.[60] However, the need for contrast administration and adequate hepatic metabolism (absorption and excretion) limits its application.

MRCP is a noninvasive method capable of examining the entire biliary tree and the pancreatic duct in two or three dimensions. The development of fast acquisition techniques for MRCP has reduced acquisition time to a few seconds and made possible the performance of dynamic studies after secretin stimulation.[63] MRCP can identify the anomalous pancreaticobiliary junction in up to 82% of the cases. It also shows good correlation with ERCP with regard to choledochal cysts and anomalous pancreaticobiliary junction diagnoses.[63–65] MRCP has established value in the diagnosis of choledocholithiasis and biliary obstruction. However, small stones and gallbladder lesions (carcinoma and mucosal hyperplasia) often associated with anomalous pancreaticobiliary junction are difficult to demonstrate.[65] Endoscopic ultrasonography can be useful in this situation.[66]

Percutaneous drainage of choledochal cysts has been reported and is supposed to help in the decompression of the biliary tree, making the surgical approach easier.[46] Dilation of the sphincter of Oddi through ERCP has also been reported, relieving symptoms.[67] The ease of stent placement provides an alternative to urgent surgery in this group of sick children.[12] However, owing to the high potential of complications, including malignant degeneration, the treatment of choice is always surgery. According to the case, a choledochojejunal anastomosis can be performed, although cyst excision and Roux-en-Y hepaticojejunostomy are preferred.[49,54]

In type III cysts (choledochocele), the incidence of cholangiocarcinoma does not appear to be as high as for type I and IV cysts. Currently, in more than 50 cases of type III cysts, no cases of cholangiocarcinoma have been reported. Thus, resection of a type III cyst may not be mandatory for control of future cancer risk. Type III cysts have been effectively treated with transduodenal sphincterotomy or sphincteroplasty. The goal of therapy is to establish effective drainage of the CBD and pancreatic duct.[45,53,68]

Choledocholithiasis

In infants and children, choledocholithiasis rarely occurs.[69–71] Perforation of the extrahepatic biliary tract with resulting biliary ascites is the more common presentation of CBD stones in infants.[69]

Although the sensitivity of abdominal ultrasonography for cholelithiasis is in excess of 95%, reported sensitivities for choledocholithiasis range from 18 to 74%. No pediatric data are available. Sensitivities of conventional CT scan range from 76 to 90%.[35]

Early studies of MRCP in choledocholithiasis noted sensitivities ranging from 81 to 92% and specificities ranging from 91 to 100%. Recent works using state-of-the-art techniques yield sensitivities of 90 to 100%, specificities of 92 to 100%, and positive predictive values of 93 to 100%. Negative predictive values range from 96 to 100%.[34] Based on these results, MRCP is being used with increasing frequency as a screening examination for the detection and exclusion of CBD stones, especially in patients with a low or moderate probability of having stones. The limitations of MRCP are as follows: (1) inferior resolution to that of ERCP; although MRCP detects stones as small as 2 mm, the spatial resolution of MRCP is not sufficient to detect small stones and crystals in a consistent fashion; (2) impacted calculi (not surrounded by bile); (3) patients with metal clips, pneumobilia, and hemobilia; (4) the latest advances are not uniformly available worldwide; (5) a lack of therapeutic options.[2,30,31,35]

ERCP is very sensitive in detecting CBD stones, although, occasionally, small stones may be missed.[2] However, with the introduction of MRCP, the focus of ERCP has shifted in many institutions from its use both as a diagnostic and therapeutic tool to its use primarily as a therapeutic procedure.[35]

Many reports in the pediatric literature confirm the ability of ERCP to treat CBD stones.[12,70–74] A variety of techniques are used, including endoscopic sphincterotomy (ES), balloon dilation of the papilla (sphincteroplasty), balloon or basket removal, and stent and nasobiliary drain placement (Figure 67.5-1).[12]

ES is a well-established procedure in adult patients with choledocholithiasis. In children, it was first reported in 1982[29] and has been performed in infants as young as 5 months old.[73,75,76] Because the long-term consequences of ES in children are unknown, endoscopic balloon dilation of the sphincter of Oddi seems to be an attractive technique.[12,73] Evidence that sphincter function returns after balloon dilation may prove to be a major advantage, particularly in a younger age group. Further evidence is required to confirm that this is the case. There must be some concern about whether balloon dilation is justifiable in patients with stones more than 8 to 10 mm in diameter because lithotripsy then seems inevitable.[77]

Biliary microlithiasis causing pancreatitis in a 14-year-old boy has also been reported.[78]

Primary Sclerosing Cholangitis

Primary sclerosing cholangitis (PSC) is an inflammatory disease of the biliary tract of uncertain etiology, occurring with a prevalence of 1 to 6 in 100,000.[32] It is characterized by recurrent fever, abdominal pain, and jaundice resulting from fibrosing and inflammatory obstruction of the biliary tree.[79] At presentation, about 70% of patients report slowly progressive symptoms, such as a gradual onset of fatigue and pruritus.[32]

The diagnosis of PSC is based on a combination of the clinical features, cholestatic biochemical profile, and histologic and radiographic abnormalities.

ERCP provides an accurate and sensitive method of diagnosing PSC. The typical cholangiographic features include diffuse multifocal annular strictures, intervening segments of normal or slightly ectatic ducts, diverticular outpouchings, and short band-like strictures involving the intrahepatic and/or extrahepatic biliary tree. Pruning of the peripheral biliary tree and irregularities of the duct walls may be found.[5,32,79]

Recently, MRCP has been evaluated in patients with PSC, with high sensitivity (85%) and specificity (99%) for the diagnosis of PSC.[32]

With regard to therapy, patients with major ductal strictures are candidates for endoscopic treatment with ES and balloon dilation to relieve the obstruction and delay the progression to cirrhosis.[5,80]

Cholangiopathy Associated with HIV Infection

Although pancreatic, hepatic, and biliary complications of human immunodeficiency virus (HIV) infection are well recognized in adults,[81–84] there have been few studies in children. Findings include cytomegalovirus infection, Kaposi sarcoma, giant cell transformation, granulomatous hepatitis, steatosis, portal inflammation, and cholestasis. Sclerosing cholangitis has been reported in children with immune deficiencies other than acquired immune deficiency syndrome (AIDS) and in HIV patients. Pancreatitis has been associated with infections with cytomegalovirus,

Cryptosporidium, *Pneumocystis*, and *Mycobacterium avium-intracellulare*.[85] ERCP can be safely performed on children with HIV infection. ERCP findings can direct treatment; it can also be used as a therapeutic procedure.[85]

Parasitic Infestation

Ascaris lumbricoides is the most common helminth in the human gastrointestinal tract. It is estimated that more than 1 billion people in the world are infected, and the prevalence is inversely related to the level of sanitation, personal hygiene, and agricultural development.[86,87] Children are predominantly affected. Most cases have a benign course and respond well to treatment.

Most intestinal *Ascaris* infections are asymptomatic or mildly symptomatic, but classic biliary symptoms occur if the large-sized *Ascaris* worm migrates into the bile duct. Patients may present with typical biliary colic, acute cholangitis, acalculous cholecystitis, hepatic abscess, or acute pancreatitis. Biliary colic or pancreatitis is often related to a worm impacted at the ampulla.[87]

The treatment should start with conservative measures, including hydration, analgesics, antispasmodics, and antihelminthic therapy. For patients unresponsive to these, ERCP may be performed.

ERCP plays an important role both in the diagnosis of the infestation in the biliary and pancreatic ducts (serpigi-

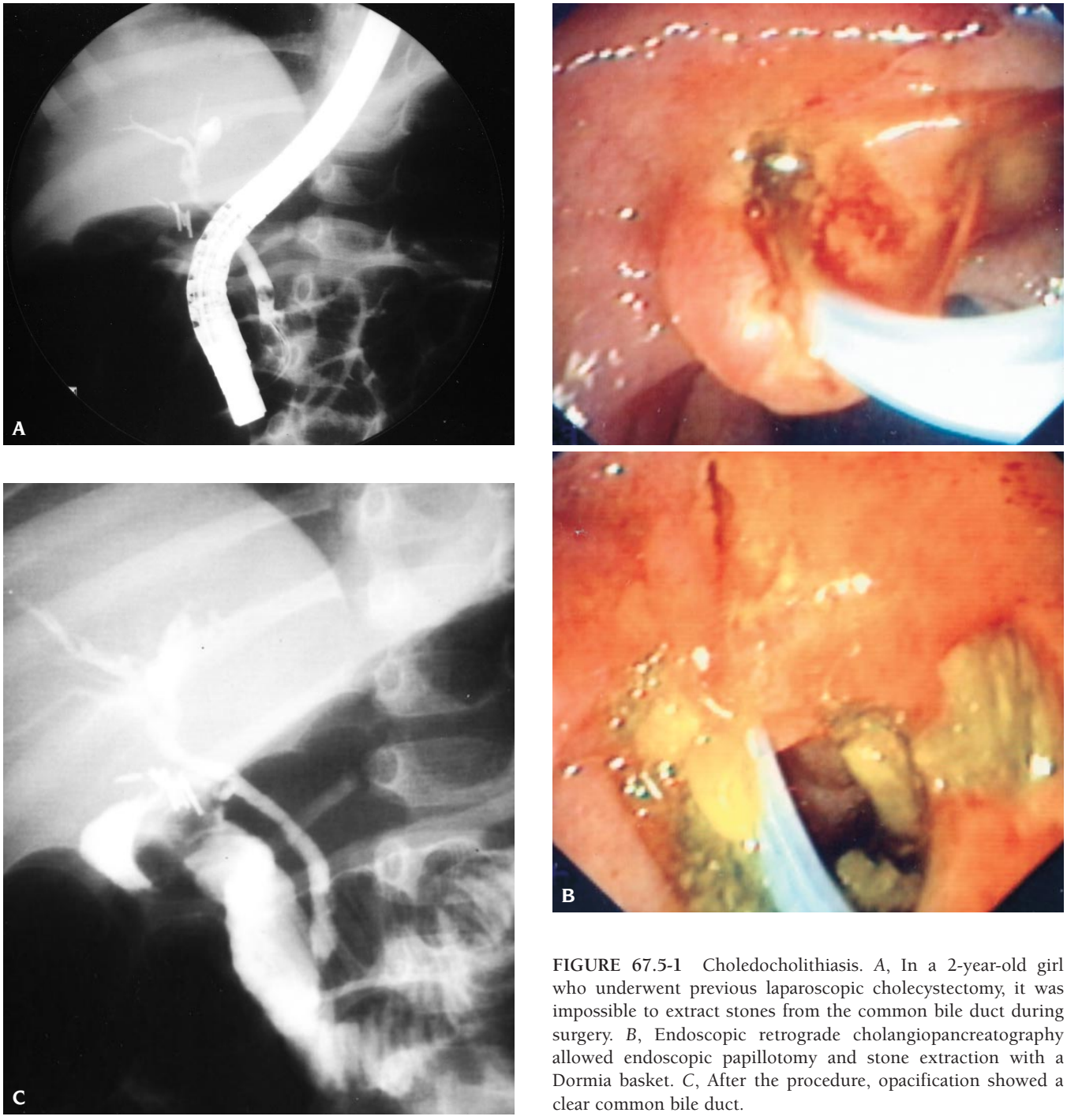

FIGURE 67.5-1 Choledocholithiasis. *A*, In a 2-year-old girl who underwent previous laparoscopic cholecystectomy, it was impossible to extract stones from the common bile duct during surgery. *B*, Endoscopic retrograde cholangiopancreatography allowed endoscopic papillotomy and stone extraction with a Dormia basket. *C*, After the procedure, opacification showed a clear common bile duct.

nous images causing filling defects) and in the treatment of this disease, removing the worms and permitting the decompression of the duct, with or without sphincterotomy. Rapid improvement of patients with pancreatic ascariasis after endoscopic removal is reported.[86, 88–90]

Duodenal Duplication Cyst

Duplication cysts of the gastrointestinal tract are rare congenital anomalies that occur predominantly in males. Duodenal duplications represent only 4 to 12% of all intestinal duplications.[91,92]

Severe vomiting, relapsing pancreatitis, bowel obstruction (mimicking hypertrophic pyloric stenosis), and pain during the first 2 to 4 years of life are possible early presenting symptoms. They rarely present with gastrointestinal bleeding owing to ulceration produced by acid-secreting ectopic gastric mucosa.

Real-time ultrasonography is useful in determining a duodenal duplication cyst. A peristaltic wave is shown passing through the cystic structure.[91] ERCP provides accurate information about the exact location, size, and communication to the biliary and pancreatic tract, which is necessary for guiding the therapeutic endoscopic intervention.[92]

Duodenal duplication cysts cannot be differentiated from a choledochocele by endoscopy. However, the histologic features of a duodenal duplication cyst are quite distinct. These include the presence of a muscular lining with or without ectopic gastric or pancreatic tissue.[53]

In the past, these patients have been managed surgically.[91] In recent years, a few reports of endoscopic drainage have been published with symptomatic improvement.[91,92]

Biliary Tract Complications after Liver Transplant

Biliary tract complications are common after orthotopic liver transplant, occurring in 13 to 35% of individuals.[93–95] Bile leaks and anastomotic strictures are the most common complications.

Leaks can occur with both duct-to-duct anastomoses and Roux-en-Y choledochojejunostomy. These can be divided into cystic duct leaks, incidental intrahepatic injury, T-tube tract leaks, and anastomotic leaks.[93]

Endoscopic treatment for bile leaks includes biliary stent placement with sphincterotomy, biliary stent placement alone, nasobiliary tube placement with sphincterotomy, and sphincterotomy alone. Excellent results are reported (up to 100% of fistula closure) after endoscopic treatment.[94]

Anastomotic strictures or stenosis can occur after choledochocholedochostomy or Roux-en-Y choledochojejunostomy and are thought to be caused by faulty surgical technique.[94] In some patients, a transient narrowing at a duct-to-duct connection appears within the first 30 to 60 days. This type of stenosis is usually caused by postoperative edema and inflammation and responds very well to balloon dilation and temporary stent placement.[93] The second type of stricture appears later, usually after 3 months. Although the narrowing responds well to initial dilation, the response is short-lived, and the relapse is predictable.[93] Dilation can be performed with either graduated bougies or balloons. In all instances, a balloon larger than one of the limbs of the anastomosis should never be used. In practice, balloons between 4 and 8 mm are used. The use of a stent is quite important because it prevents edema or intramural hemorrhage, which can obstruct the anastomosis. Hence, a stent prevents any leakage of bile from the anastomosis in the event of a microscopic perforation or disruption.[93] Stents must be exchanged periodically (usually every 3–4 months) for long periods (until stricture resolution occurs). The response to endoscopic treatment is usually very good.[94]

Another type of stricture is the nonanastomotic stricture (donor duct). The causes of these strictures are less clear and are associated with occlusion of the hepatic artery, prolonged cold ischemia, rejection, and ABO blood group incompatibility.[94] By comparison with anastomotic strictures, the success rate for nonsurgical management of nonanastomotic strictures is low (58% in one series).[94]

Unexplained Abdominal Pain

Abdominal pain is a frequent cause of disability and the most common reason that a patient will seek consultation with a gastroenterologist. The perception of pain is purely subjective and is heavily influenced by psychosocial aspects. For the gastroenterologist, the differential diagnosis of abdominal pain is a common but challenging clinical problem.[96] Whereas the diagnosis of pancreatic or biliary disease in patients with "classic" clinical features is usually straightforward, patients with the sole symptom of abdominal pain might represent a "Pandora's box."

Many of these patients undergo a battery of tests, including ERCP.[97] However, the role of ERCP in patients with pain and absence of obvious obstructive disorders of the pancreatic and bile duct is less clear.[98]

In pediatrics, ERCP has been used in many patients with unexplained abdominal pain (see Table 67.5-1). However, the role of ERCP in these patients is disappointing. This is probably due to the fact that most children with unexplained pain are suffering from a functional disorder in which ERCP is clearly unlikely to demonstrate any organic problem. In such children, if investigation is really envisaged, MRCP may be a more appropriate test.[6,7,72,99,100]

PANCREATIC INDICATIONS

Pancreatic indications for diagnostic and therapeutic ERCP are listed in Table 67.5-3.

Acute Pancreatitis

In adults, the two most common causes of acute pancreatitis are alcohol and gallstones. Both situations are rare in children. Other causes include drugs, infectious agents, hypertriglyceridemia, trauma, biliary tract anomalies, and pancreatic ductal obstruction.[101,102]

In patients who present with the typical findings of acute pancreatitis (elevated pancreatic enzymes, abdominal pain), ERCP has no role except when the diagnosis of acute biliary pancreatitis with concomitant cholangitis is suspected (presence of fever and abnormal liver chemistries). In most noncomplicated cases, noninvasive

TABLE 67.5-3 PANCREATIC INDICATIONS FOR ENDOSCOPIC RETROGRADE CHOLANGIOPANCREATOGRAPHY

DIAGNOSTIC
Unexplained persistent acute pancreatitis
Recurrent pancreatitis
Congenital disorders
Biliary anomalies (choledochal cyst or anomalous pancreaticobiliary union)
Pancreatic anomalies (pancreas divisum, annular pancreas, short pancreas, pancreatocele)
Duodenal anomalies (duodenal or gastric duplication cysts, duodenal diverticulum)
Cystic fibrosis
Hyperlipidemia/hypercalcemia
Acquired disorders
Parasitic infestation (*Ascaris*)
Sphincter of Oddi dysfunction
Pancreatic trauma
Medications
Chronic pancreatitis
Pancreatic mass
Preoperative evaluation
THERAPEUTIC
Pancreatic sphincterotomy
Dilation of pancreatic stricture
Stone removal
Stent placement
Pseudocyst drainage
Nasopancreatic drainage

imaging studies such as abdominal ultrasonography and CT can define the extent of the disease, diagnose and quantify necrosis, and determine whether pseudocysts are present. MRCP is also a promising test.[2]

In patients with severe biliary pancreatitis, trials comparing early ERCP versus delayed ERCP show a benefit of early intervention, at least in a subset of patients.[2,103]

Indications for ERCP in acute biliary pancreatitis should be as follows[103]:

- Before cholecystectomy: in the presence of concomitant cholangitis, obstructive jaundice, or severe disease or in patients who suffer an in-hospital exacerbation
- After cholecystectomy: in patients with unsuccessful laparoscopic or open CBD exploration or patients with smoldering disease (± sphincter dysfunction or ductal disruption)

Anecdotal reports of therapeutic ERCP in acute biliary pancreatitis in children have been published.[104] However, no consistent data exist on the role of ERCP in acute pancreatitis in children.

Recurrent Pancreatitis

Recurrent pancreatitis is characterized by episodes of acute pancreatitis, manifested by unexplained recurrent bouts of abdominal pain, with intervening asymptomatic intervals of varying duration. It is rarely recognized in children because the index of clinical suspicion is low.[105] Diagnosis is often missed for several months or years. Patients are symptomatically treated during an acute attack and discharged.

Recurrent pancreatitis can be divided into two groups, depending on whether the cause is nonobstructive or obstructive.[105] Nonobstructive causes include hereditary factors, cystic fibrosis, hyperlipidemia, trauma, medication, and hypercalcemia. Obstructive causes include choledochal cysts, pancreas divisum, duodenal diverticulum, duodenal duplication, parasitic infestation, and anomalous pancreaticobiliary junction.

MRCP is a promising, noninvasive method of identifying and ruling out structural abnormalities as a cause of acute pancreatitis in children with early-stage pancreatitis.[106] However, currently, it is ERCP that offers the definition of the CBD and pancreatic ductal system that is necessary to make decisions in the management of these children. Whatever the underlying cause of pancreatitis, the possibility that there may be an anatomic abnormality amenable to endoscopic therapy or surgery should always be considered.[102,105,107] ERCP has been found useful in the identification of treatable causes in 40 to 75% of children with recurrent pancreatitis.[5]

Endoscopic therapy in recurrent pancreatitis includes standard biliary sphincterotomy, dual sphincterotomy of the pancreatic duct sphincter and the CBD sphincter, minor papilla sphincterotomy, pancreatic stone extraction, pancreatic endoprosthesis insertion, cystogastrostomy or cystoduodenostomy (Figure 67.5-2).

Pancreas Divisum. Pancreas divisum is a congenital anomaly caused by failure of fusion of the dorsal and ventral endodermal buds during gestation. Each duct drains via its own separate orifice, the major papilla of Vater for the ventral duct of Wirsung, and the minor accessory papilla for the dorsal duct of Santorini.[108] It is the most common congenital variant of pancreatic ductal anatomy and has been found in approximately 5 to 14% of autopsy series and 0.3 to 8% of ERCP studies.[105,108,109] However, when patients with unexplained recurrent pancreatitis are studied, the incidence is 25%.[105]

The exact prevalence of pancreas divisum in children is unknown. In 272 cases of successful ERCP performed in children, pancreas divisum was found in 9 (3.3%).[108] In children with recurrent pancreatitis, the incidence ranges between 7 and 22%.[105] The clinical significance of pancreas divisum is controversial. For many authors, it is related to recurrent pancreatitis, arguing that the minor papilla could be too small to allow adequate drainage.[108,110,111] However, others have considered it to be a coincidental finding by reviewing large series of ERCP.[112]

The treatment of pancreas divisum used to be surgical (minor papilla sphincterotomy). In recent years, endoscopic therapy has been directed to decompress the dorsal duct by insertion of endoprosthesis, dilation of the minor papilla, or sphincterotomy of the minor papilla with or without stent insertion. Overall, improvement is seen in 70 to 90% of the cases.[110,113–116]

Guelrud and colleagues treated three children with pancreas divisum by ES of the minor papilla. In one, a pancreatic stent was placed for 2 weeks. Two were asymptomatic during follow-up of 10 to 17 months, and one had

recurrent episodes of abdominal pain without enzyme elevation. Similar results were seen in three other patients treated surgically in the same series.[105]

Other Congenital Anomalies. Annular pancreas, short pancreas, cystic dilation of the pancreatic duct (pancreatocele), duodenal or gastric duplication, and duodenal diverticulum have been reported to be associated with recurrent pancreatitis. ERCP might be useful in the diagnostic evaluation of these entities, especially when less invasive tests, such as CT and MRCP, are inconclusive.[106]

Anomalous Pancreaticobiliary Junction. Anomalous pancreaticobiliary junction is a congenital malformation defined as a communication of the CBD with the pancreatic duct to form a long common channel outside the duodenal wall and therefore not under the influence of the sphincter of Oddi. During an ERCP, anomalous pancreaticobiliary junction is considered to be present when the common channel measures more than 15 mm or when its extraduodenal portion is more than 6 mm long.[117,118] This anomaly has been implicated as a cause of choledochal cyst, bile duct and gallbladder carcinoma, and recurrent pancreatitis.[117]

According to the classification of Kimura and colleagues,[119] there are two types of anomalous pancreaticobiliary junction:

- Type BP: when the common bile duct appears to join the pancreatic duct
- Type PB: when the pancreatic duct appears to join the CBD

Guelrud and colleagues have proposed a third type, the "long Y," in which there is only a long common channel without CBD dilation.[118] It seems that recurrent pancreatitis is more directly associated with the PB type than the BP type.[118]

One case of balloon dilation as an alternative to surgical biliodigestive anastomosis in a child with a long common channel, choledochal cyst, and multiple biliary stenoses has been reported.[120]

Sphincter of Oddi Dysfunction. Sphincter of Oddi dysfunction is an abnormality in the contractility of this sphincter. It is a benign, noncalculous obstruction to flow of bile or pancreatic juice through the pancreaticobiliary junction. Its pathogenesis is unknown.[98,121]

Sphincter of Oddi dysfunction may be manifested clinically by pancreaticobiliary pain, pancreatitis, or cholestasis. The pain is usually epigastric or in the right upper quadrant, may be disabling, and lasts for 30 minutes to several hours. It may radiate to the back or shoulder and be accompanied by nausea and vomiting. Although typically observed in middle-aged women, it may occur in pediatric or adult patients of any age.[98] It can involve abnormalities of the biliary sphincter, pancreatic sphincter, or both. Three types of suspected biliary SOD are reported:

- Biliary type I: patients with biliary-type pain, abnormal aspartate aminotransferase or alkaline phosphatase (> 2× normal documented on two or more occasions), delayed drainage of ERCP contrast from the biliary tree (> 45 minutes), and dilated CBD (> 12 mm in diameter). The frequency of abnormal manometry ranges between 75% and 95%, and the probability of pain relief by sphincterotomy is between 90% and 95%. Although manometry may be useful in documenting sphincter of Oddi dysfunction, it is not an essential diagnostic study before endoscopic or surgical sphincter ablation.

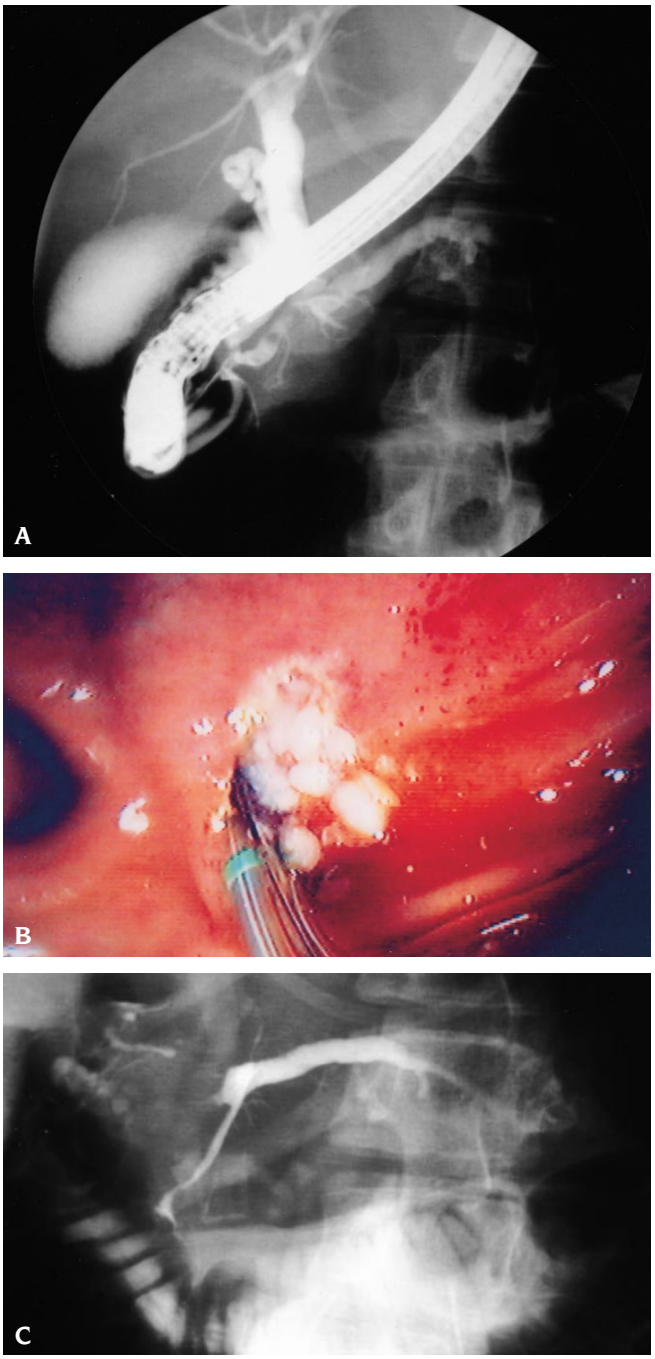

FIGURE 67.5-2 Recurrent pancreatitis. *A*, Recurrent pancreatitis in a 11-year-old girl. Endoscopic retrograde cholangiopancreatography showed a dilated main pancreatic duct with multiple filling defects owing to proteinaceous plugs. The common bile duct was also dilated. *B*, Stones were extracted after pancreatic duct sphincterotomy. *C*, Postoperative opacification showed a Wirsung duct clear of stone.

- Biliary type II: patients with biliary-type pain but only one or two of the above criteria. Manometry is abnormal in 55 to 65% of patients. If manometry is abnormal, about 85% of patients will experience pain relief by sphincterotomy. If manometry is normal, only 35% will benefit from sphincterotomy.
- Biliary Type III: patients with only biliary-type pain and no other abnormality. Manometry is abnormal in 25 to 60% of patients. If abnormal, 55 to 65% will improve with sphincterotomy. If manometry is normal, less than 10% will improve after sphincterotomy.

Manometry is the only available method to measure sphincter of Oddi motor activity directly.[98] ERCP has been used to perform sphincter of Oddi manometry in adults and children with suspected SOD. Because of the difficulties involved in performing manometry on healthy children, values obtained for adults are used as normal for children. Basal sphincter pressure is considered abnormal if it is greater than 35 mm Hg.[117] However, given that there are no normal control data in children, minimal published experience, and inherent technical difficulties with the test, no recommendation can be made regarding the clinical application or reliability of this test in children.[19]

Pediatric patients, like adults, can have pancreatitis secondary to sphincter of Oddi dysfunction. In general, they do not respond as well to endoscopic treatment, for which the complication rate tends to be higher than that for adults.[122]

Chronic Pancreatitis

Chronic pancreatitis is a chronic inflammatory condition characterized by fibrosis, destruction of exocrine tissue, and, eventually, destruction of exocrine and endocrine tissue. Both parenchymal and ductular tissue may be involved. Fibrosis may be accentuated in focal areas, causing sphincter of Oddi or main duct strictures, or it may be diffuse throughout the gland, including small ducts. Three subgroups of chronic pancreatitis are delineated[123–126]:

1. Chronic calcified pancreatitis. This is the largest subgroup, and it is characterized by sporadic parenchymal fibrosis associated with intraductal protein plugs, intraductal stones, and ductal injury.
2. Chronic obstructive pancreatitis. This results from obstruction of the main pancreatic duct and is characterized by uniform ductal dilatation and atrophy, with eventual replacement of acinar cells by fibrous tissue.
3. Chronic inflammatory pancreatitis. This subgroup is characterized by fibrosis, mononuclear cell infiltration, and atrophy. It is associated with autoimmune diseases such as Sjögren syndrome and PSC of the liver.

New etiologies for chronic pancreatitis have been recently defined: autoimmune pancreatitis, eosinophilic pancreatitis, genetic pancreatitis (mutation in the trypsinogen cationic gene responsible for familial pancreatitis, with autosomal dominant inheritance, or recessive mutations in the *SPINKI* gene [serine protease inhibitor Kazal type 1] or in the *CFTR* gene [cystic fibrosis transmembrane conductance regulator], observed with high frequencies in patients with "idiopathic" pancreatitis). Before performing invasive tests such as ERCP, searching for these entities is mandatory if the first bout of pancreatitis occurs before the age of 20 years.[127,128]

Abdominal pain is the most serious clinical problem in chronic pancreatitis and the most common indication for surgery. In an exacerbation of acute inflammation, the characteristics of pain are similar to those experienced during prior episodes of acute pancreatitis. Some patients have evidence of chronic pancreatitis, including intraductal calcification, ductal dilatation, and pseudocyst in the absence of pain.

The later stages of chronic pancreatitis are characterized by spontaneous remission of abdominal pain and the appearance of signs of full-blown insufficiency of both the exocrine and endocrine pancreas. Before the disease reaches this stage, treatment is aimed at producing stable remission of painful symptoms (that respond poorly to medical treatment), prevention of complications (cystic lesions, stenosis of the main bile duct), and halting or slowing the progression of the disease. These objectives are particularly important when the disease appears early in life because it can interfere with the psychophysical development of the child. In a growing child, suspension of oral feedings prescribed during acute attacks or refusal to eat owing to fear of pain can have effects that are much more serious than in adults.[129]

In the treatment of chronic pancreatitis, endoscopy can be proposed in certain circumstances. Endoscopic treatment is useful in treating local complications such as pancreatic pseudocysts and biliary strictures. Endotherapy is also effective in treating postsurgical complications such as pancreatic leakage after pancreaticojejunostomy. However, the main indication is to control painful chronic pancreatitis resistant to medical treatment or recurrent attacks of acute pancreatitis, which frequently appear in the course of chronic pancreatitis. This goal can be achieved by endoscopic drainage procedures in cases of outflow obstruction caused by pancreatic ductal stones, strictures of the main pancreatic duct, or a compressing pseudocyst, all resulting in upstream dilation. These drainage procedures include pancreatic sphincterotomy, stone extraction, balloon dilation of strictures, usually followed by stent insertion, and pseudocyst drainage.[130,131] Most endoscopic studies have concluded that pain diminishes in a majority of patients after endoscopic therapy, particularly in those patients with a dominant duct stricture and upstream dilation.[124,126]

Because of the experience gained in adults and a greater understanding of the mechanisms underlying the pain experienced by children, endoscopic approaches are now being used to treat pediatric patients as well.[129,132] The pain experienced by children with chronic idiopathic pancreatitis appears to be more commonly caused by the transient occlusion of the sphincter of Oddi by protein plugs. In these cases, the most effective approach to restore the flow within the duct is to reduce the resistance to the passage of these plugs by sphincterotomy.[129]

In a study involving 22 children with pancreatitis (6 acute, 6 recurrent, and 10 chronic) treated endoscopi-

cally, Hsu and colleagues observed significant improvement in all 6 outcome parameters analyzed, especially improvement in the frequency and severity of pain, a decrease in health care encounters (emergency department visit, clinic visit, and hospital admission), and improvement in general conditions.[122] In an early stage, patients without diffuse ductal changes in the pancreatogram had the most significant clinical improvement.

Pseudocysts

Pancreatic pseudocysts are nonepithelium-lined fluid collections that result from transient or persistent pancreatic duct disruption. They are common consequences of acute and chronic pancreatitis. Most of the pseudocysts resolve spontaneously. In the setting of chronic pancreatitis, symptomatic pseudocysts are commonly seen in association with pancreatic stones and strictures. Symptomatic, large (> 4 cm), or persistent (beyond 6 weeks) pseudocysts are unlikely to resolve and are at risk of complications. There has been increased interest in nonoperative management of pseudocysts.[5,19,124]

Pseudocysts that connect with the main pancreatic duct and are accessible to guidewire passage and those that abut the stomach or duodenum are addressed by transductal or transmural pseudocyst entry and stent placement.[124]

In adults, successful endoscopic treatment with pseudocyst resolution has been reported in about 80 to 90% of cases within 1 to 2 months. A 15% recurrence rate has been reported. Complication rates are approximately 20%, with a 1% mortality, indicating that this is one of the more dangerous endoscopic therapies.[5,124] Pediatric experience is limited to a few case reports and insufficient to comment on either the efficacy or safety of this procedure in children.[7,19,105,132]

Pancreatic Trauma

Injuries to the pancreas from blunt abdominal trauma in children are rare. Most are minor and are best treated conservatively. The mainstay for treatment of major ductal injuries has been prompt surgical resection.

Diagnostic imaging modalities are the key to the accurate classification of these injuries and planning appropriate treatment. CT has been the major imaging modality in blunt abdominal trauma for children, but it has shortcomings in the diagnosis of pancreatic ductal injury. MRCP is a promising test in these patients.[133]

ERCP has been shown recently to be superior to CT in the diagnosis of pancreatic trauma, allowing the possibility of stent placement (Figure 67.5-3).[134] In reported pediatric cases, clinical improvement is rapid, with complete resolution of clinical and biochemical pancreatitis, resumption of a normal diet, and discharge from hospital.[135]

CONTRAINDICATIONS

Contraindications to ERCP include unstable cardiovascular, pulmonary, or neurologic conditions or suspected bowel perforation. Esophageal stricture is a relative contraindication. Coagulopathies should be corrected before ERCP whenever possible, especially if a therapeutic procedure is envisaged. Aspirin, nonsteroidal anti-inflammatory drugs, and medications that interfere with platelet function should be avoided before and immediately after the procedure.[19]

COMPLICATIONS

ERCP has evolved from a diagnostic test into a primarily therapeutic procedure for a variety of biliary and pancreatic disorders. Many short-term complications are associated with the examination, mainly pancreatitis, hemorrhage, perforation, cholangitis, and cholecystitis, and are related to the anesthesia. Short-term complications are reported to occur after 5 to 10% of ERCPs with or without sphincterotomy (see Table 67.5-1).[136,137]

In children, the major risks and complications of ERCP are the same as in adults. The relative risk for these complications in children is not well established because of the small number of patients in pediatric series.[19]

In a large pediatric ERCP series, the overall complication rate was 11.6%. Pancreatitis occurred in 3.3% (see Table 67.5-1).[7] Hemorrhage and perforation have rarely been reported after ERCP in children.[28,72,138] Rates are likely to be similar to those reported in adults: 0.7 to 2% for hemorrhage and 0.3 to 0.6% for perforation.[139,140] In Guelrud's experience with 184 neonates and young infants, minor complication without clinical significance occurred in 24 (13%) patients. In 220 ERCPs in children older than 1 year, this author observed 1.8% of complications in diagnostic examinations and 10.7% in therapeutic ERCPs.[5]

The long-term complications of ES or use of stents in pediatric patients are unknown.[19]

COMPETENCE IN PEDIATRIC ERCP

Endoscopic competence is difficult to define and almost impossible to quantitate. Strictly defined, "competence" is the ability to carry out a set of tasks or a role adequately or effectively.[141] Competency in diagnostic and therapeutic endoscopy implies a demonstration of appropriate clinical judgment in patient selection and adequate cognitive and technical skills to complete a procedure safely and successfully. Deep knowledge of appropriate indications and contraindications, potential risks, and benefits is also mandatory. The endoscopist must be prepared to manage or to enlist the help of others to manage complications or adverse outcomes expeditiously in an appropriate medical facility.[142]

According to the ASGE, endoscopic competence includes objectives that trained endoscopists will be able to[143]

- Recommend endoscopic procedures based on findings of a personal consultation and in consideration of specific indications, contraindications, and diagnostic or therapeutic alternatives
- Perform specific procedures safely, completely, and expeditiously
- Correctly interpret most endoscopic findings and undertake endoscopic interventions when indicated

- Integrate endoscopic findings or therapy into patient management plans
- Understand risk factors and recognize and manage complications
- Recognize personal and procedural limits and know when to request help

Competency in pediatric endoscopy requires a specialized knowledge of gastrointestinal diseases affecting children. Such knowledge is conventionally gained through formal training in pediatric gastroenterology. However, other physicians experienced in the care of children or adults with gastrointestinal disease may

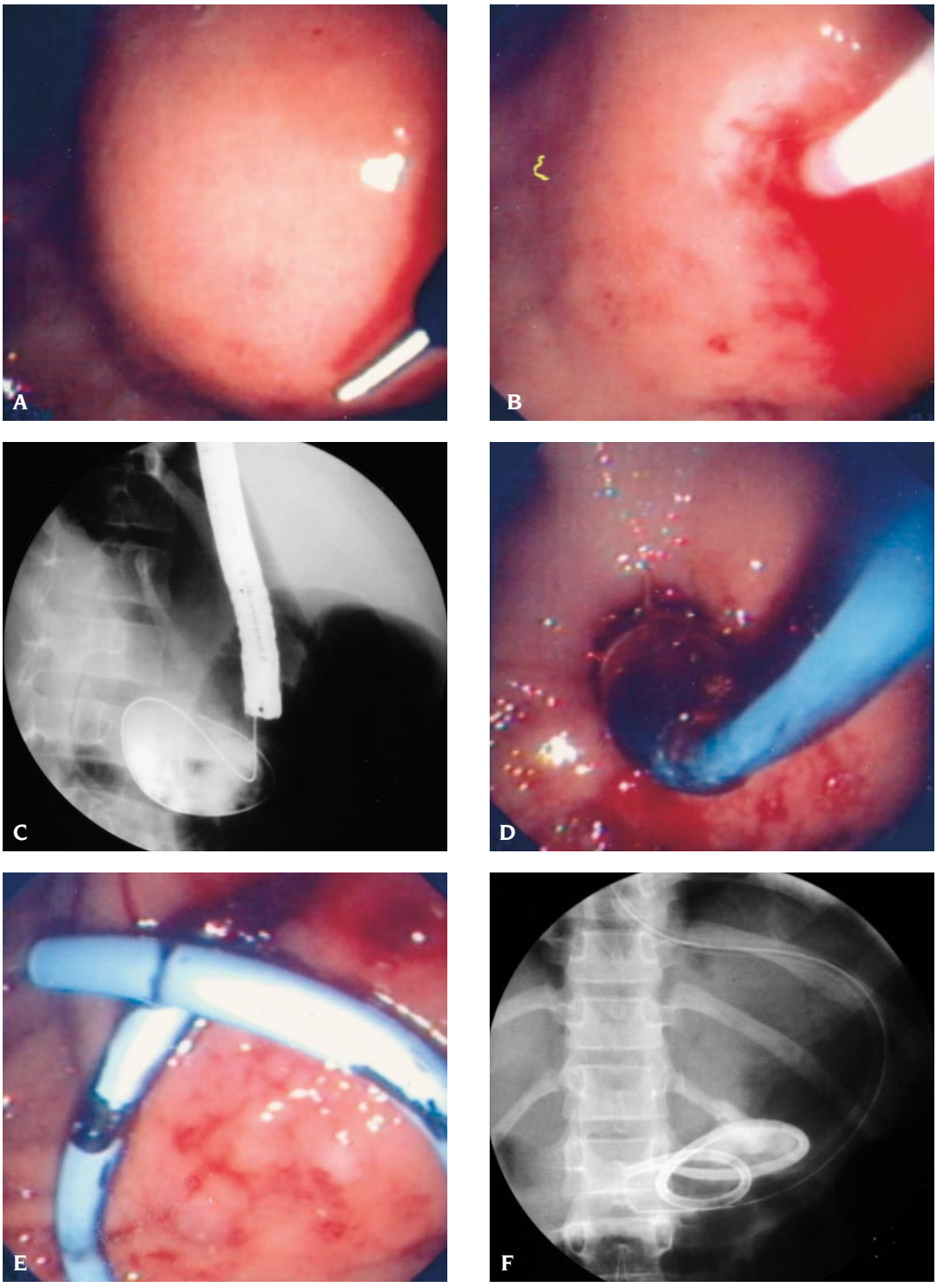

FIGURE 67.5-3 Pancreatic trauma. *A*, Traumatic pseudocyst with obvious bulging into the gastric lumen. *B*, Diathermic needle puncture of the gastric wall toward the cyst's center. *C*, Opacification of the cystic cavity and guidewire placement. *D*, Balloon dilation of the cystic opening. *E*, Insertion of a pigtail stent to maintain the patency of the cystogastrostomy. *F*, Pigtail stent allowing communication between the cystic cavity and the stomach.

acquire suitable cognitive skills to achieve competence in pediatric endoscopy.[142]

CONCLUSIONS

ERCP is a safe and valuable diagnostic and therapeutic procedure in children with presumptive pancreaticobiliary disease. Considerable experience with this endoscopic procedure is a prerequisite before undertaking cannulation in this young age group. It is anticipated that ERCP and its associated endoscopic therapy will find increasing use in the pediatric population, not only for diagnosis and preoperative delineation of the pancreaticobiliary tract but, in many instances, as a substitute for traditional operative therapy.[144] The performance of endoscopy in children assumes an adequate knowledge and understanding of pediatrics. To provide appropriate care for each child, a team approach is often required, including the pediatrician or pediatric gastroenterologist, the surgeon, and the adult endoscopist.[7,12,76]

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CHAPTER 68

LIVER BIOPSY INTERPRETATION

A. S. Knisely, MD

Diagnosis in a liver biopsy specimen resembles diagnosis in a patient. The specimen, or the patient, is interrogated using a standard set of questions, or histochemical techniques. An inventory is made of the findings, or the responses, that differ from expected norms. Both the histopathologist and the clinician conclude their data-taking with a synthesis, an attempt to identify a diagnosis that accounts for any abnormalities recognized. Both the clinician and the histopathologist continue their inquiries when they believe that a better diagnosis is yet to be achieved.

Two points are particularly important in interpreting liver biopsy findings. First, the histopathologist must not work in isolation. Help from clinical colleagues is essential if the histopathologist is best to serve the patient. Second, the histopathologist must not lose information. For that as well, teamwork is essential.

This chapter outlines how to ensure, between the clinician and the histopathologist, that information is not lost as a liver biopsy specimen is obtained and processed. It lists a set of studies likely to permit a first-line diagnosis, while permitting referral, if necessary, of biopsy material in consultation, and gives examples of how each study can prove useful. The question of when biopsy is in order is raised. The chapter ends by discussing several clinical settings frequently encountered in pediatric patients in which biopsy may prove useful, as well as what biopsy may find. It does not pretend, however, to match the comprehensive accounts of liver biopsy interpretation[1] and the pathology of the liver[2] available elsewhere, to which the reader is referred.

MATTERS TO BE SETTLED BEFORE THE BIOPSY PROCEDURE

Much more can now be won from a sample of liver tissue than only morphologic data. For many, even most, purposes, formalin fixation is adequate. But genetic or proteomic information specific to the liver, such as how genomic deoxyribonucleic acid (DNA) is processed into messenger ribonucleic acid (RNA) and beyond, is lost when liver is not snap-frozen immediately on biopsy.

Formalin-fixed liver can be used only for routine microscopy and for a limited range of immunohistochemical and in situ hybridization studies. Snap-frozen liver can be used for quantitative analysis of constituents, microbiologic evaluation, molecular diagnosis, transmission electron microscopy (after thawing in proper fixative), or specialized immunohistochemical studies. It can also be used for all of the studies possible with routinely formalin-fixed liver (again, after thawing in proper fixative). A 2 cm core biopsy specimen provides ample tissue for routine processing and for freezing.

In all but rare settings, liver biopsy is an elective procedure. Referral units that specialize in liver disease routinely ensure that a Dewar flask of liquid nitrogen is brought to the bedside, the ultrasonography suite, or the operating room to receive and to snap-freeze a sample of liver as soon as it is obtained. The tissue can be placed in an embedding compound within a mold, placed inside a vial or capsule, or (perhaps giving easiest access) laid on a labeled piece of aluminum foil, which is then folded to yield a packet. For reasons presented above, immediate snap-freezing of liver tissue must be regarded as the standard of optimal care.

Pediatric gastroenterology and hepatology services have recently extended outside predominantly academic hospitals as specialty trainees take up careers in the wider community. Appropriate support from histopathology services (snap-freezing; storage and shipment at –80°C) should be in place before pediatric liver biopsy is undertaken. If liver biopsy is contemplated where snap-freezing is not available, the possible need for rebiopsy, with attendant risk, must be balanced against any inconvenience of patient transfer to a venue where liver biopsy tissue can be correctly preserved.

HANDLING THE BIOPSY CORE

A biopsy specimen intended for routine histopathologic study should be immersed in formalin as soon as it is obtained. Contact with saline solution or wicking away of fluids into a towel or gauze produces a variety of artifacts and alters immunohistochemical reactivity. Tissue should be processed in mesh cassettes rather than on biopsy sponges, which distort anatomic relationships.

Biopsy specimens often are presented to the histopathologist as “ribbons,” with large numbers of sections closely packed on a single slide. Liver biopsy specimens should be handled differently, with a ribbon divided into individual, serial sections, each mounted on the proper sequentially numbered slide. Even after trimming the block, 12 pairs of such sections at 4 μ or 5 μ extend only some 200 μ into the biopsy core (1,000 μ thick

before fixation and dehydration), conserving tissue against future work. At the Institute of Liver Studies, our practice is that alternate pairs are held on slides as unstained sections, early and late pairs are stained with hematoxylin and eosin (HE), and pairs in the bracketed interval are stained with diastase and periodic acid–Schiff (DPAS) technique, for iron, with orcein, and for reticulin. Abnormalities can be tracked through the core; lesions such as granulomata can be further evaluated where they occur, and with unstained sections on hand, a delay in additional studies is minimal.

Regulators in different venues require retention of glass slides and tissue blocks for different times. The pediatric gastroenterologist or hepatologist working in a facility with care of adults as its primary concern may wish to ensure that pediatric materials are held longer than the minimum time prescribed or even indefinitely.

THE NORMAL LIVER: A REFRESHER

Blood enters the liver at its hilum, through the portal vein and hepatic artery. It passes from portal tracts (Figure 68-1A) along the sinusoids within the lobule into centrilobular venules (Figure 68-1B) and leaves the liver for the inferior vena cava via the hepatic veins. The line of junction and demarcation between portal tract and lobule is known as the limiting plate. Hepatocytes extract various substances from the plasma that bathes their sides and bases. They return others to it; blood passing over hepatocytes is sequentially modified from the portal tract to the centrilobular venule.

Hepatocytes are disposed between sinusoids in cords one cell thick. Their dimensions are similar throughout the lobule. Although they exhibit zonal differences in function, on light microscopy they tend to look alike. They are

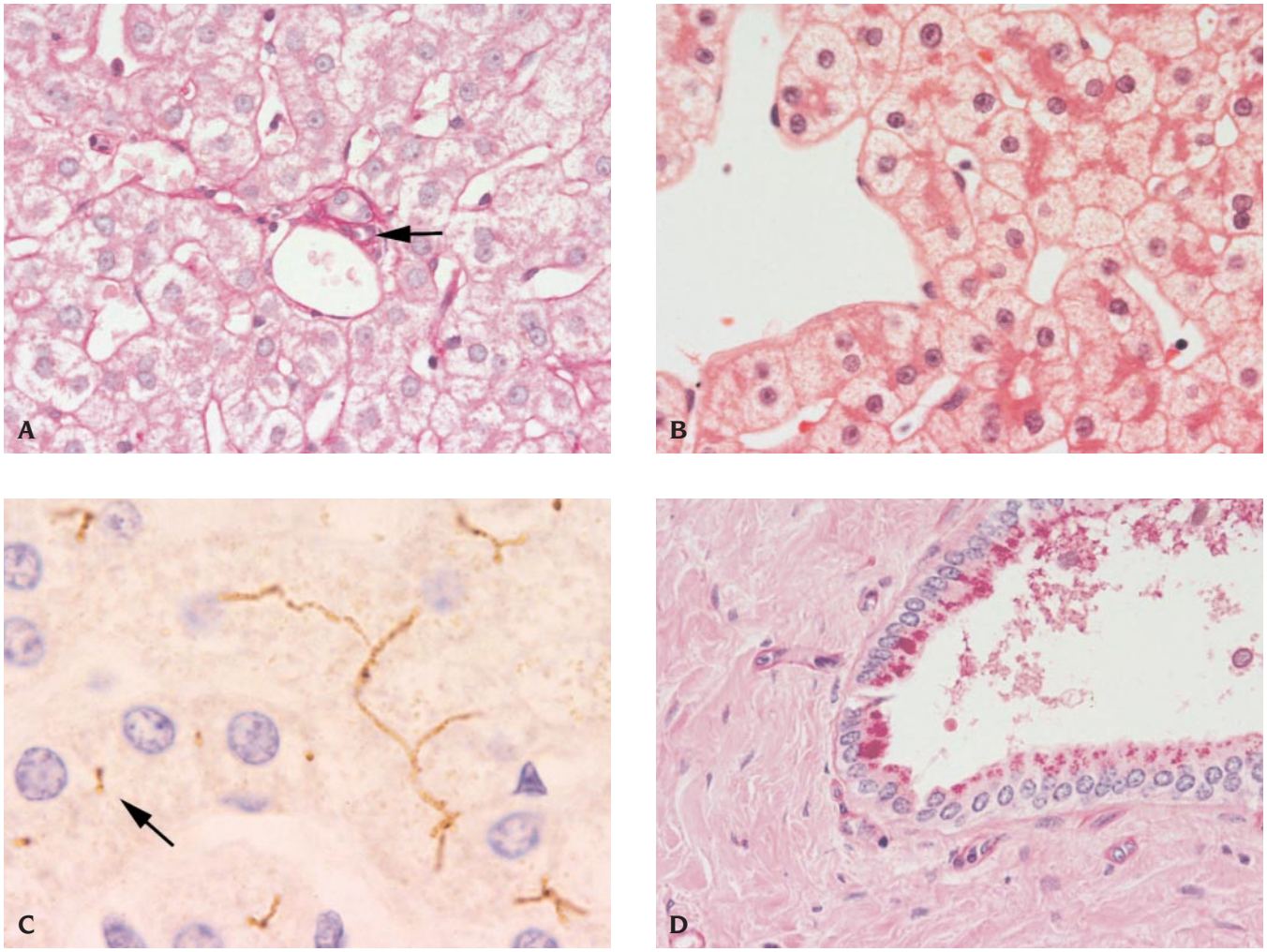


FIGURE 68-1 *A*, An interlobular portal tract, with a large, thin-walled portal venule containing a few erythrocytes; a bile duct circumscribed by basement membrane and lined by cholangiocytes (two nuclei are visible); and a small, thick-walled arteriole (*arrow*). Normal liver. Diastase and periodic acid–Schiff technique with hematoxylin counterstain (DPAS); ×400 original magnification. *B*, A centrilobular venule, with sinusoids and hepatocyte cords of the adjacent lobule. Some of the cords are two cells thick. Lysosomes, whose contents tend to mark with eosin, are clustered at the hepatocytes' canalicular poles. Normal liver (hematoxylin and eosin; ×400 original magnification). *C*, Canaliculi in transverse (*arrow*) and longitudinal section. A hepatocyte may contribute to several canaliculi, as shown. Normal liver. Hematoxylin and antibody against canalicular multispecific organic anion transporter (immunoperoxidase technique); ×1,000 original magnification. *D*, Droplets of mucin are found within columnar cholangiocytes of a septal bile duct, and scant mucus is seen in the ductal lumen. Normal liver (DPAS; ×400 original magnification).

cuboidal or polygonal and uniform in size. They have one or two nuclei, which are generally round in profile and may contain a chromocenter. Hepatocytes may be euploid or polyploid. Their cytoplasm is finely granular and uniform, although pigment may be seen at the aspect most remote from the sinusoid, the canalicular pole.

Portions of two or three hepatocytes may meet at the canaliculus, which is separated by tight junctions from the basolateral aspects of the hepatocytes that form it (Figure 68-1C). An individual hepatocyte participates in forming several canaliculi. Hepatocytes secrete various substances across the canalicular membrane into the bile. This travels from canaliculi into interlobular bile ducts, then into trabecular or septal bile ducts, and finally into segmental or lobar ducts, moving into the hilum in the direction opposite to that taken by the blood. The cholangiocytes of interlobular bile ducts are cuboidal and serous (see Figure 68-1A). Those of larger bile ducts are columnar and mucinous (Figure 68-1D). Large ducts toward the hilum also gradually acquire a mural secretory gland apparatus. Bile passing down canaliculi and ducts is sequentially modified in its transit, and bile at its exit from the liver is a very different fluid from bile within the canaliculus.

Endothelial cells with one set of characteristics line vessels within portal tracts and centrilobular venules. Those that line the sinusoids have different characteristics; they are discontinuous or fenestrated, allowing hepatocytes direct access to plasma. Within the sinusoids are the macrophages known as Kupffer cells. Nerves accompany vessels into the hilum; they enter the lobule in the space of Disse, which lies between sinusoidal basement membrane and hepatocellular basolateral membrane. This space also contains stellate cells, which are contractile and fibrogenic and which store vitamin A. Small numbers of leukocytes, principally small lymphocytes and macrophages, are normally found in portal tracts.

Liver biopsy relies on the axiom that parenchymal disease uniformly involves lobules throughout the liver. This is not true, but as an approximation, it is true enough to be useful. One should bear in mind, however, that neither percutaneous nor transvenous liver biopsy will routinely sample central or hilar structures. Processes that affect them must be studied by specifically directed biopsy.

WHAT ROUTINELY STAINED SECTIONS WILL AND WILL NOT SHOW

Nucleic Acids and Proteins within Nucleus and Cytoplasm: HE

The purple-blue dye, hematoxylin, binds with electronegative substances (ie, nucleic acids or carbonates and phosphates found in mineralized tissues). It marks the DNA of nuclei and the RNA of rough endoplasmic reticulum. The orange-red dye, eosin, binds with electropositive substances (proteins). It marks much of the cytoplasm of most cells, as well as the bulk of interstitial or intercellular material. Shifts in degrees of eosinophilia or hematoxylinophilia signal various metabolic changes (eg, the move from purple-red to orange-red, associated with loss of RNA, that indicates both maturation in erythrocytes and cell death in hepatocytes). HE-stained sections are the mainstay of histopathologic diagnosis.

Glycosylated Substances: DPAS

Diastase digestion removes complex sugars from tissue sections; glycoproteins remain. The aldehyde moieties in these, after oxidation with periodic acid, take up the fuchsin chromophore in Schiff reagent and become magenta. Proteins not coupled with sugars are marked only very palely. DPAS-stained sections, with a hematoxylin (nuclear) counterstain, demonstrate cell outlines and basement membranes excellently. These stand out all the more because the hemoglobin of erythrocytes, which sometimes can obscure tissue details, effectively does not mark. Collections of glycoprotein within cytoplasm (mucin [see Figure 68-1D], the phagocytosed material within Kupffer cells, secondary lysosomes in hepatocytes, and some, but not all, materials accumulated in storage disorders [Figure 68-2A]) also stand out. DPAS staining is valuable in identifying residua of some structures; basement membranes of bile ducts can persist after cholangiocytes are gone. It also permits screening for fungal forms, whose cell walls take the stain.

Iron: Prussian Blue Reaction

Acid pretreatment converts iron complexed as ferritin (or, after partial digestion within lysosomes, as hemosiderin) to its ferric form, which reacts with potassium ferrocyanide to yield bright, crisp Prussian blue. A counterstain such as nuclear fast red is often used to make it easier to identify where in the tissue stainable iron lies (Figure 68-2B). To demonstrate iron may have prognostic implications, as in hepatitis C virus infection, or permit diagnosis of a metabolic disorder, as in hemochromatosis. Because hepatocytes and Kupffer cells or macrophages may contain granular brown material other than hemosiderin (Dubin-Johnson pigment, lipofuscin, malarial pigment), to demonstrate that particular deposits do not contain iron also can be useful.

Materials Rich in Sulfhydryls: Orcein

The purple-black dyestuff, orcein, originally extracted from lichen, complexes with sulfhydryl groups. These abound in elastin (Figure 68-3A), in the surface antigen of hepatitis B virus (Figure 68-3, B and C), and in metallothioneins (see Figure 68-3A). Synthesis of metallothioneins is induced by cytosolic accumulation of several transition metals; the most clinically important is copper. Subclinical cholestasis, without hyperbilirubinemia, is associated with impaired excretion of copper into bile. Metallothioneins bind copper and sequester it within the cytosol. Over time, they accumulate in partly degenerated form in secondary lysosomes—"cuprisomes."[3] These are particularly prominent in periportal hepatocytes. Orcein staining highlights vessels by picking out elastic tissue within their walls. It shows (when elastic tissue is present) that a scar has matured.[4] By demonstrating copper-associated protein,

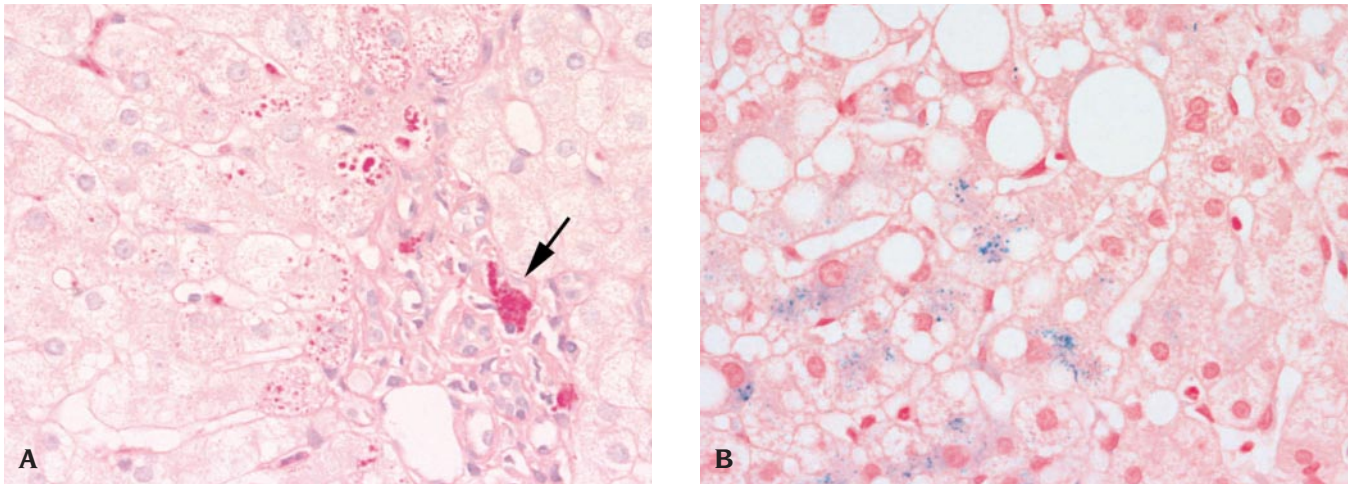


FIGURE 68-2 *A*, Granules and globules of the glycoprotein α_1-antitrypsin have accumulated within cytoplasm of periportal hepatocytes. Portal tract macrophages (*arrow*) contain lipofuscin pigment; the two substances stain alike. α_1-Antitrypsin storage disorder (diastase and periodic acid–Schiff; ×400 original magnification). *B*, Finely granular dark reaction product marks hemosiderin, principally within lysosomes (note a tendency toward pericanalicular accumulation). The large and small optically clear vacuoles represent the lacunae left after elution of neutral lipid during specimen processing (Perls' Prussian blue and nuclear fast red technique; ×400 original magnification).

orcein staining can support an impression, garnered from HE-stained sections, that a cholangiopathy is present even when bilirubinostasis is not apparent, or that cholestasis is likely of long standing.

Type III Collagen: Reticulin

Abnormalities in the architecture of the liver, including the sometimes subtle changes of shrinkage or swelling of hepatocytes associated with perturbed vascular supply, are easily assessed in sections stained for basement membrane collagen ("reticulin," in which collagen type III predominates[5,6]) using a silver impregnation technique. Parenchymal loss with stromal collapse is marked by apposition of reticulin fibers (Figure 68-4A). Hyperplasia of hepatocytes (broadened hepatocyte cords that are two cells rather than one cell thick) is marked by splaying apart of reticulin fibers (Figure 68-4B). Hepatocellular malignancy is marked by a deficiency of reticulin fibers. The fibrosis owing to inflammation or injury is generally accompanied by an increase in reticulin within the fibrous tissue. Assessment of the extent of fibrosis is an important aspect of biopsy diagnosis.

Substances Lost in Processing

The formaldehyde in formalin (an aqueous solution of formaldehyde) stabilizes tissues, as a fixative, by cross-linking them. Formaldehyde cross-links proteins and nucleic acids more efficiently than it cross-links neutral lipids or simple sugars. As a liver biopsy specimen moves from formalin through alcohol into xylene and on to paraffin, the tissue is brought into contact with polar and nonpolar solvents. Most stains are applied in aqueous solution, so to permit staining, sections mounted on slides are again passed through xylene and then through alcohol into water. Once sections are stained, because coverslips are secured with adhesives dissolved in nonpolar media, the sections are exposed to alcohol and then again to xylene. Water, alcohol, and xylene leach many small molecules, such as sugars or bilirubin, and neutral lipid from the tissue (see Figures 68-2B and 68-5, A and B). These then cannot be stained, and statements that they ever were present rely on inference.

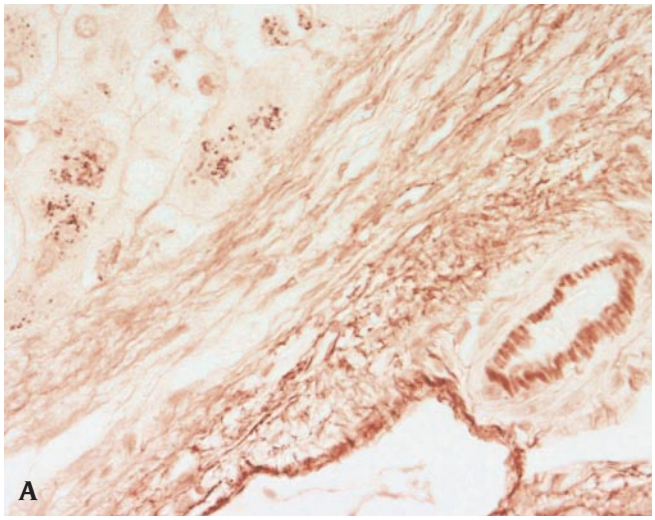
If such substances must be specifically demonstrated, fixation in absolute alcohol or other selected substances rather than in formalin can lessen tissue exposure to polar solvents and can partly conserve small molecules through subsequent handling. Postfixation in osmium tetroxide (osmication) after initial exposure to formalin can cross-link and stabilize neutral lipid against elution, permitting routine processing into paraffin thereafter. Osmication affords histologic detail superior to that available in sections of frozen tissue (from which lipid is not lost) that are stained with oil red O or Sudan black.

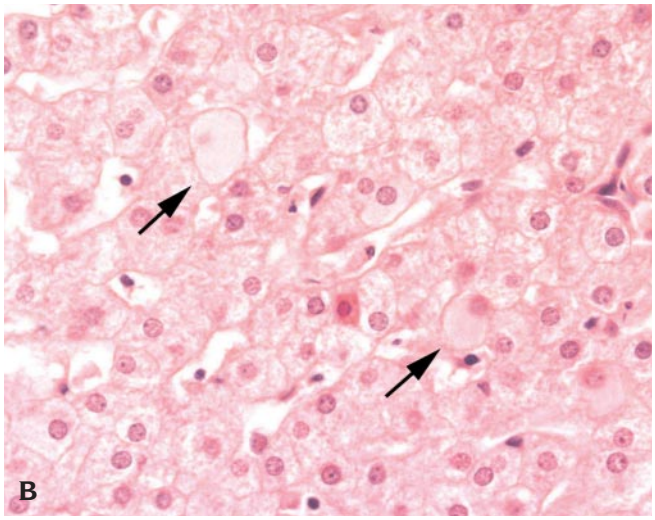
PITFALLS OF ONTOGENY

Postnatal patterns of hepatocellular and bile duct growth in prematurely born infants may differ from those in infants born at term.[7] Standards for the former are not well defined. Caution in interpreting biopsy findings is in order.

The liver is the principal site of fetal hematopoiesis, which can persist into the first postnatal weeks. Foci of erythropoiesis in the lobule and of granulopoiesis in portal tracts should not be confused with inflammation in biopsy specimens from neonates.

Copper-associated protein is normally present in the first several tiers of periportal hepatocytes of infants born at term; it may persist for several weeks or months. The same is true for hemosiderin, which may extend into midzonal or even centrilobular hepatocytes. To see either, which would mark disease in older children, can be disregarded in early infancy.





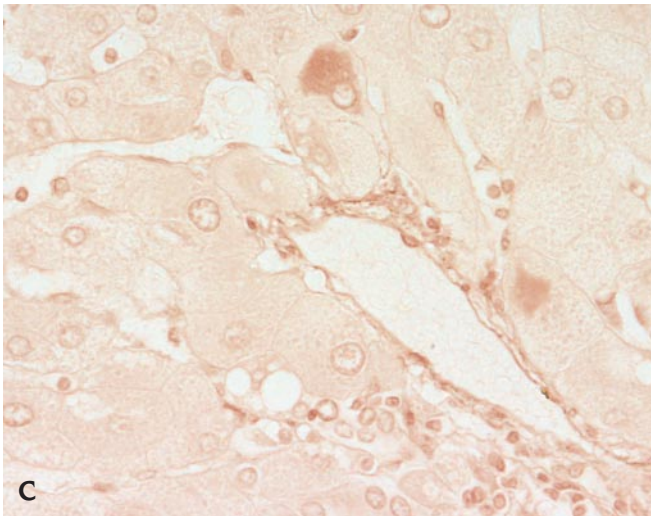
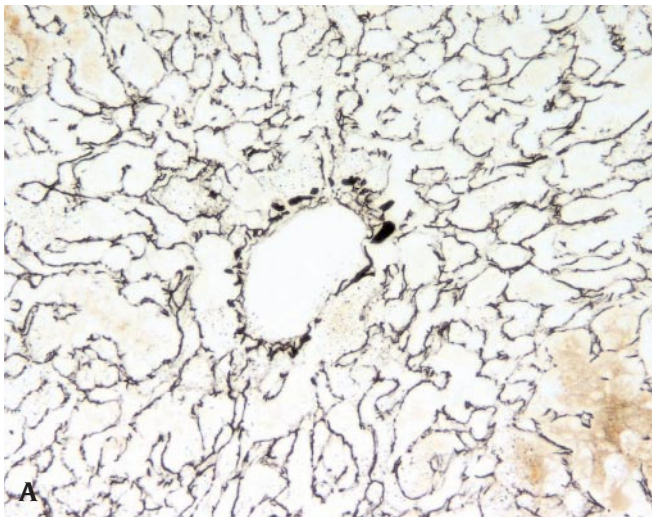


FIGURE 68-3 *A*, Granular dark material, upper left, within hepatocytes represents copper-associated protein. The corrugated, dense elastic lamina of a portal tract artery and individual elastic fibrils within looser portal tract connective tissue also mark. Familial intrahepatic cholestasis 1 at hepatectomy (orcein; ×400 original magnification). *B*, Most hepatocytes have finely granular, eosinophilic cytoplasm; that of several hepatocytes is uniformly pale (*arrows*), resembling frosted glass. A shrunken, densely eosinophilic hepatocyte at the center is undergoing cell death. Chronic hepatitis B virus infection (hematoxylin and eosin; ×400 original magnification). *C*, The cytoplasm of "ground-glass" hepatocytes like those in *B* marks when the change is due to accumulation of hepatitis B surface antigen. Chronic hepatitis B virus infection (orcein; ×400 original magnification).



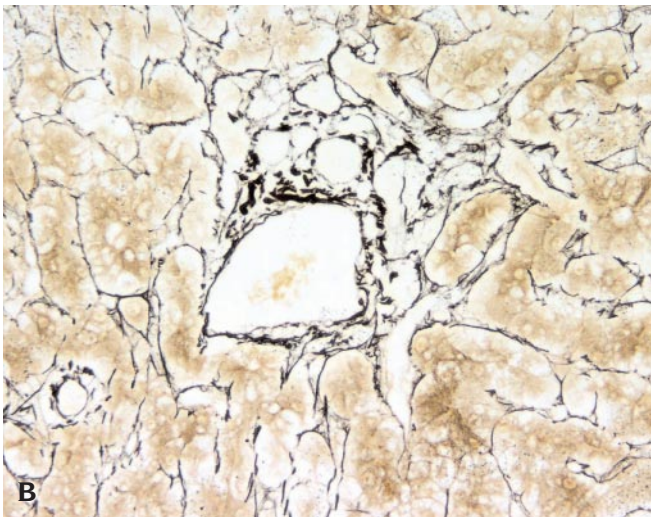
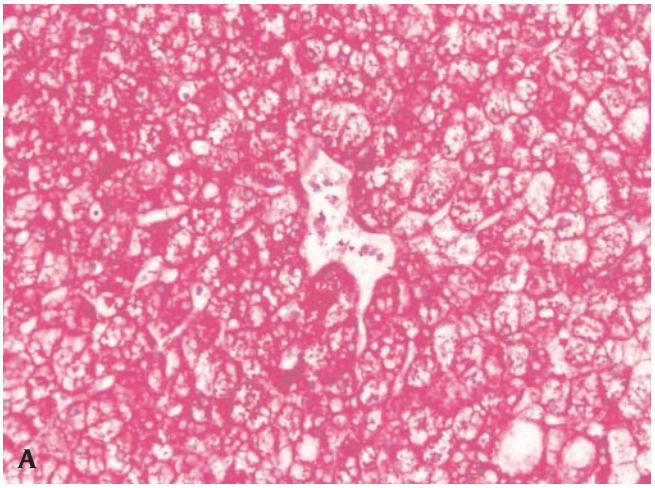
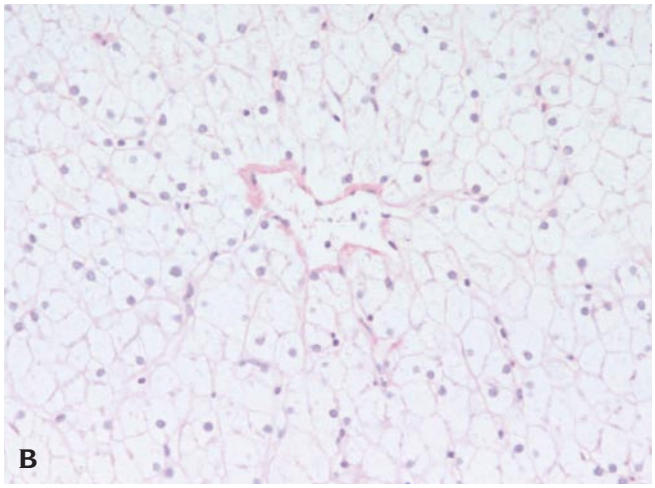


FIGURE 68-4 *A*, Silver impregnation marks reticulin fibers brown-black and defines sinusoidal margins at junctions with hepatocyte cords. They are relatively closely apposed around this centrilobular venule (reticulin; ×400 original magnification). *B*, Same patient as in *A*; acetaminophen (paracetamol) overdose with zonal hepatocyte loss. Hepatocyte cords are wider, and reticulin fibers lie farther apart, in this periportal zone than in centrilobular liver (compare with *A*). Loss of hepatocytes (*A*) and regenerative hyperplasia of hepatocytes (*B*) underlie the differences (reticulin; ×400 original magnification).





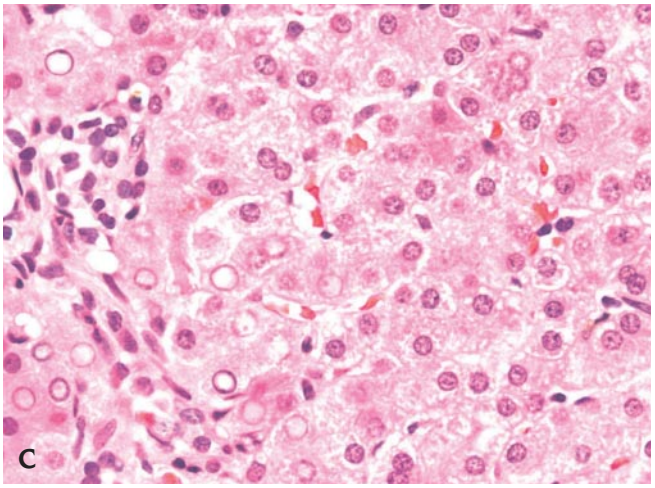


FIGURE 68-5 *A*, Accumulated glycogen within hepatocyte cytoplasm can be demonstrated histochemically. Glycogen storage disease, type I (periodic acid–Schiff technique with hematoxylin counterstain; ×200 original magnification). *B*, Diastase pretreatment of sections eliminates glycogen and, with it, histochemical reactivity (same patient as in *A*). Hepatocellular enlargement is apparent. Glycogen storage disease, type I (diastase and periodic acid–Schiff; ×200 original magnification). *C*, No particular significance inheres in slight enlargement of nuclei, with displacement of chromatin to the periphery by accumulated glycogen, as seen here in periportal hepatocytes (hematoxylin and eosin; ×400 original magnification).

Hepatocyte cords are normally two cells broad in the first several years of childhood. Only at age 4 or 5 years and thereafter should this finding be considered pathologic. In adults, optically clear nuclei in which glycogen has displaced chromatin may signal diabetes. They occur in children without metabolic abnormality (Figure 68-5C).

WHEN IS LIVER BIOPSY IN ORDER?

Leaving aside masses within the liver, most of which continue to require direct examination of tissue for definitive diagnosis, advances in microbiology, clinical biochemistry, clinical immunology, imaging studies, and molecular genetics have made the morphologic study of the liver less and less necessary. This is as it should be; liver biopsy is invasive and carries a small but real risk to the patient.

Specialized metabolic or molecular analysis techniques may be accessible, however, at several centers worldwide at most, with no guarantee that clinically useful results ever will be supplied. Even routinely conducted assays may be seldom done, with uncomfortable delays in receiving results. The techniques used in biopsy diagnosis, in contrast, are very widely employed, with (possibly nondefinitive) results available in only a few hours. Historical experience with evaluation of disease in liver biopsy materials also is extensive.

In addition, even if noninvasive studies strongly suggest a particular diagnosis, how badly a particular disease has damaged the liver often can be determined only at biopsy. This information can be useful in assessing prognosis or, with follow-up biopsy, in evaluating response to therapy.

Biopsy is thus generally used at present to aid in selecting among a small number of likely diagnoses and to guide further work, to corroborate a provisional diagnosis already arrived at on clinical grounds, and to determine the extent of the disease or, over time, the progress of the disease. Satisfactory interpretation often depends on understanding what questions a particular biopsy is hoped to answer.

FREQUENTLY ENCOUNTERED CLINICAL SETTINGS

Conjugated Hyperbilirubinemia in Infancy

Inflammation of the extrahepatic biliary tract with perinatal onset may lead to fibrosis that occludes or obliterates the lumen of the common bile duct (extrahepatic biliary atresia [EHBA]). This process may involve the gallbladder. EHBA is manifest as conjugated hyperbilirubinemia with elevated serum concentrations of γ-glutamyl transpeptidase (GGT) activity (high-GGT cholestasis). EHBA can be palliated by hepatic portoenterostomy. The results of such

surgery are better the earlier it is performed. When imaging study results (failure to identify a normal gallbladder on ultrasonography; in some centers, failure to opacify the biliary tract on percutaneous or endoscopic cholangiography) are ambiguous, the histopathologist thus may be asked to identify changes in the liver that suggest distal biliary tract obstruction.

These changes include edema of portal tracts, fibrosis of portal tracts, an increase in the number of bile duct profiles seen, and the presence of bile plugs in the lumina of principal bile ducts, that is, of bile ducts within portal tracts rather than of recruited ductules at portal tract margins (Figure 68-6). The cholangiocytes of bile duct epithelium are irregularly arrayed, and intraepithelial leukocytes may be found, but this is the nonspecific case in any cholangiopathy. Granulocytes may be seen in portal tracts, but this also is nonspecific and may reflect not inflammation but the postnatal persistence of hematopoiesis. The lobular changes of "neonatal hepatitis" (edema of hepatocytes, with giant cell change and intrahepatocytic bile pigment; rosetting of hepatocytes around dilated canalicular lumina that contain bile plugs; necrosis of occasional hepatocytes; and clusters of hematopoietic elements, usually erythroid [Figure 68-7, A and B]) may be minimal or quite pronounced. Their extent cannot be used definitively to exclude EHBA: florid "neonatal hepatitis" and EHBA may coexist.[8]

High-GGT cholestasis can reflect, beside EHBA, syndromic and nonsyndromic paucity of interlobular bile ducts (PIB); α_1-antitrypsin (A1AT) storage disorder (A1ATSD); neonatal sclerosing cholangitis (NSC); mutation in *ABCB4*, which encodes multiple drug resistance 3 (MDR3), a "flippase" that shifts phospholipid from inner leaflet to outer leaflet of the canalicular membrane (MDR3 disease)[9]; and a long list of rarer entities, as well as infective disorders or defects in intermediary metabolism. These infective disorders or defects in intermediary metabolism are generally identified by microbiologic studies, serologic work, or screening of urine and serum for unusual substances. High-GGT cholestasis also can accompany the nonspecific and highly unsatisfactory diagnosis of "neonatal hepatitis" (see above). Intralobular cholestasis and steatosis tend to mark defects of intermediary metabolism, and the latter is not a feature of EHBA. "Neonatal hepatitis" lacks the portal tract changes of EHBA (see Figure 68-7B).

PIB, A1ATSD, NSC, and MDR3 disease may pass through a phase of bile duct proliferation, with evident injury to cholangiocytes. Features that reliably permit the diagnosis of NSC on histopathologic examination have not yet been identified. NSC must be considered a diagnosis made on cholangiography.[10] MDR3 disease also has no well-defined, specific histopathologic features. To demonstrate a lack of phospholipid in bile, or to identify mutation in *ABCB4*, is necessary for diagnosis.[9]

In only A1ATSD, however, do the degree of portal tract edema and the extent of bile duct proliferation, with bile plugging, sometimes mimic those found in EHBA. Fine granules of A1AT that mark in DPAS-stained sections may be seen in periportal hepatocytes of infants several months old. In infants approximately 6 to 10 weeks old, when the urgency in diagnosing features of distal biliary tract obstruction is greatest, bile pigment and secondary lysosomes, which physiologically contain hemosiderin and metallothioneins, are easily confused on DPAS staining with the minimal quantities of stored A1AT that may be present at that age in A1ATSD. An inconstant but useful feature of A1ATSD is steatosis of periportal hepatocytes.[11] Its presence can shift EHBA down the differential diagnosis rank table.

Issues of definition tend to confound discussions of PIB. The criteria for assigning the diagnosis depend classically on finding fewer than five bile duct profiles in six interlobular portal tracts.[12,13] These criteria, however, were

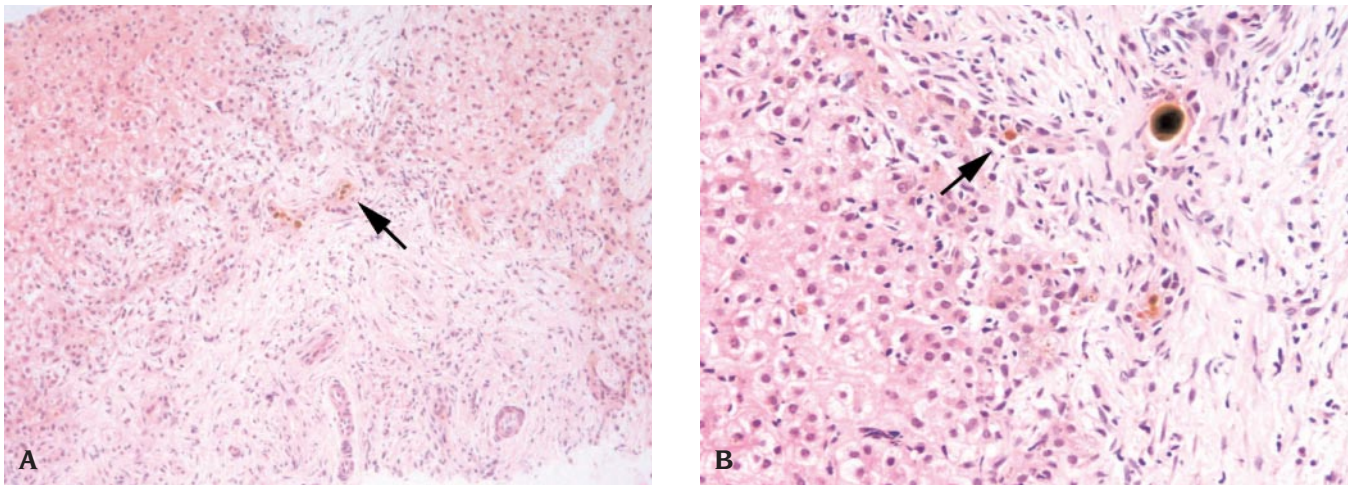


FIGURE 68-6 *A*, In distal biliary tract obstruction, a portal tract is expanded by loose, pale fibrous tissue, suggesting edema. Bile plugs are present in centrally located duct lumina (*arrow*). Extrahepatic biliary atresia (EHBA); biopsy at presentation (hematoxylin and eosin (HE); ×100 original magnification). *B*, Hepatocytes at the limiting plate have adopted a cholangiolar phenotype (the ductular reaction), and bile plugs are seen both in principal bile ducts and a newly recruited ductule (*arrow*). Hepatocyte disarray is generally slight in distal biliary tract obstruction, as seen here, but brisk giant cell change and necrosis also may be found. EHBA; biopsy at presentation (HE; ×200 original magnification).

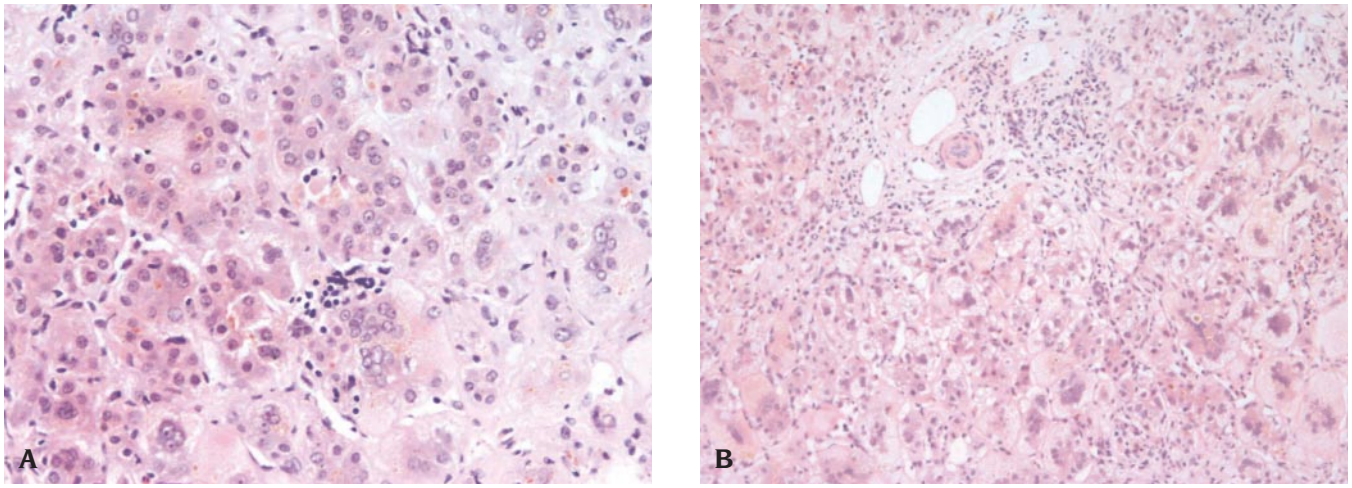


FIGURE 68-7 *A*, Typical "neonatal hepatitis" includes giant cell change and edema, with bile pigment in hepatocytes and canaliculi, as well as lobular hematopoiesis (*arrow*). Compare with *B*, in which such changes are lacking. Biopsy at presentation. Hematoxylin and eosin [HE]; ×200 original magnification. *B*, Portal tracts in usual "neonatal hepatitis" are compact, with, at most, slight ductular reaction. Cholestasis is restricted to the lobule. Compare with *A*, in which the portal tract is expanded and contains bile plugs. Biopsy at presentation (HE; ×100 original magnification).

set before the development of immunohistochemical techniques for marking bile ducts, so that strict use requires examination of HE- or DPAS-stained sections only. Finding six interlobular portal tracts seen in full cross-section can itself be difficult in slender, short needle biopsy specimens of liver. Inflammation or hematopoiesis that blurs structural detail makes identifying bile ducts harder. Personal experience suggests that immunostaining for bile duct cytokeratins often picks out bile ducts that cannot be discerned in routinely stained sections (Figure 68-8A).

Bile ducts identifiable only on immunostaining, however, generally are threadlike, with as few as one or two cholangiocytes seen (Figure 68-8B). They appear hypoplastic—a hypoplasia to be distinguished from hypoplasia of the intrahepatic biliary tree, as PIB is sometimes termed. This appearance is frequent in the setting of intralobular cholestasis, perhaps because stimuli to normal development are lacking when hepatocellular bile secretion lags. But hypoplasia of a bile duct is distinct from absence of a bile duct; hypoplastic ducts may grow and cholestasis resolve. To diagnose PIB, because of its potentially adverse prognosis, seems to require that even minute ducts be absent from more than one of six interlobular portal tracts. Whoever interprets the findings on microscopy when PIB is at issue would be prudent to specify in any report what criteria and techniques were used.

The distal biliary tract is not generally malformed in infants with either syndromic or nonsyndromic PIB. Larger bile ducts are not identifiably lacking. Peripheral larger bile ducts began as interlobular ducts and with growth were

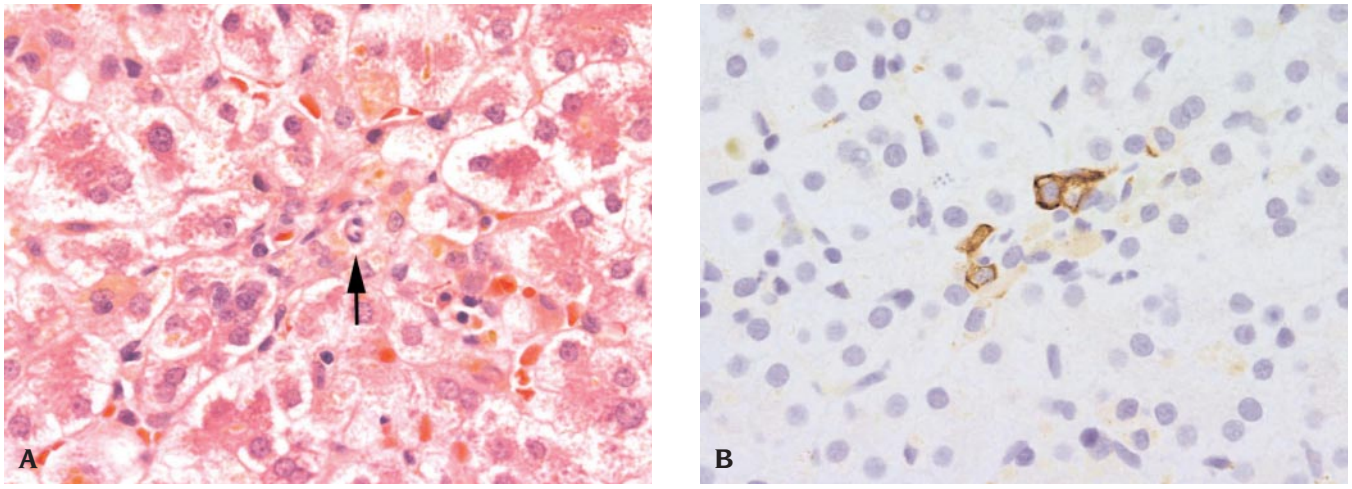


FIGURE 68-8 *A*, An interlobular portal tract in an infant with conjugated hyperbilirubinemia; compare with Figure 68-1A. An arrow indicates the hepatic arteriole. No bile duct can be discerned. Suspected paucity of interlobular bile ducts. Hematoxylin and eosin; ×400 original magnification. *B*, The same portal tract (compare with *A*), several micra distant, proves on immunohistochemical study to contain cells that express cytokeratin 19, which is usually found in cholangiocytes (hematoxylin and antibody against cytokeratin 19 [immunoperoxidase technique]; ×400 original magnification).

remodeled into trabecular or septal ducts. This suggests that bile duct morphogenesis in utero is unremarkable in such infants. Perinatal shifts in bile secretion, with alterations in bile composition,[14] may underlie perinatal development of a cholangiopathy. Septal and interlobular cholangiocytes differ in form (see Figure 68-1). Susceptibility to injury by more caustic bile may also differ between trabecular or septal ducts and interlobular ducts (the smallest, most recently formed, most peripheral ducts).

As mentioned, injured bile ducts may proliferate before they are lost. In EHBA, a vigorous increase in the number of bile ducts is initially the rule, but after several years, end-stage liver disease is characterized by bile duct disappearance.[15] This is generally ascribed to bile duct injury by bile. Such injury seems markedly accelerated in some instances of PIB. Of note is that newly laid down liver in early infancy is at the periphery of both the biliary tree and the liver itself and that newly formed interlobular ducts can be expected to predominate there. In addition, the right lobe of the infant liver grows more briskly after birth than does the left lobe.[16] Routine percutaneous biopsy is most likely to sample liver beneath the capsule in the right lobe, the youngest region. The pace of liver growth slows in later infancy, however (liver weight 75 g at term birth, 150 g at 3 months, 300 g at 1 year[17]). Follow-up biopsy, after duct loss has come to predominate over duct neogenesis, may be useful in assessing whether PIB is present. Histopathologic study cannot distinguish between syndromic PIB (associated with *JAG1* mutation[18]) and nonsyndromic PIB.

Some instances of conjugated hyperbilirubinemia in infancy are marked by a failure of GGT activity to rise. This may be due to severe hepatocellular injury, in which case, low GGT is nonspecific.[19] It may be due to any number of heritable disorders of synthesis of bile acids or their precursor molecules.[20–22] In such a setting, low GGT is likely due to an absence from bile of the bile acids that usually elute canalicular membrane GGT into bile, whence it can reflux into plasma. Finally, it may be due to one of several heritable disorders (arthrogryposis-renal dysfunction-cholestasis syndrome [ARC][23]; mutation in *ATP8B1*, which encodes familial intrahepatic cholestasis 1 [FIC1], a protein of undefined function[24]; or mutation in *ABCB11*, which encodes bile salt export protein [BSEP], the pump that transports bile salts from hepatocytes into bile).[25]

The etiology of ARC is not clear; it seems, however, that deficient expression of GGT at the canaliculus may underlie the failure of GGT activity to rise.[26] The same appears true for FIC1 disease.[27] With BSEP disease, although GGT is normally expressed, a lack of bile acids within bile—as with primary disorders of bile acid synthesis—may be responsible for failure to elute GGT and, accordingly, for the failure of GGT activity to rise.[27] The renal, neuromuscular, and skeletal manifestations of ARC permit its distinction from FIC1 disease and BSEP disease. FIC1 disease and BSEP disease, however, may be difficult to differentiate from one another. Changes of cholate stasis, with hepatocellular injury generally ascribed to retained bile acids, are usually less pronounced in FIC1 disease than in BSEP disease. The former tends to manifest as bland intracanalicular cholestasis (Figure 68-9A) and the latter as giant cell hepatitis (Figure 68-9B). Transmission electron microscopy in FIC1 disease tends to find loose, coarsely granular bile within canaliculi (Figure 68-9C), which is not usually the case in BSEP disease (Figure 68-9D).[28,29] Criteria for histopathologic diagnosis of these two recently distinguished disorders are under study.

Predominantly Hepatocellular Injury (Transaminitis) in Childhood

Damage to hepatocytes and damage to the biliary tract overlap, but two patterns of clinical biochemistry findings can be broadly defined. The first, which is more usual when damage to hepatocytes predominates, is characterized most prominently by elevations in serum concentrations of transaminase activities (the hepatitic pattern). The second, which is more usual when damage to the biliary tract predominates, is characterized most prominently by elevations in serum concentrations of bilirubin and of alkaline phosphatase and GGT activities (the cholestatic pattern). These two patterns also overlap: injury to hepatocytes induces cholestasis, and cholestasis leads to injury to hepatocytes.

On liver biopsy in patients whose clinical biochemistry test results fit the hepatitic pattern, increased numbers of leukocytes are found in portal tracts and in the lobule. Regardless of the speed of clinical onset of disease, whether slow (chronic) or abrupt (acute), most of the leukocytes are the mononuclear forms (lymphocytes, macrophages, and plasma cells) that, rather than granulocytes, characterize chronic inflammation elsewhere in the body. Acute hepatitis and chronic hepatitis are thus differentiated from one another by criteria other than the composition of the inflammatory infiltrate found.

In acute hepatitis, inflammation is predominantly within the lobule; in chronic hepatitis, it is within the portal tracts. Hepatocellular injury is more pronounced in acute hepatitis than in chronic hepatitis, with edema, necrosis of individual hepatocytes (Figure 68-10A), and both hepatocellular and canalicular cholestasis (Figure 68-10B). Macrophages enlarge and become more numerous; their cytoplasm contains ingested debris (see Figure 68-10B). Loss of hepatocytes may be associated with approximation of reticulin fibers to one another (stromal collapse, bridging) (Figure 68-10C). Acute hepatitis may be due to viral infection, toxin or drug ingestion, or autoimmune disease. It may resolve entirely, be so severe that liver transplant is necessary for survival, or evolve into a chronic hepatitis, with varying degrees of scarring and persistent inflammation.

In chronic hepatitis, the extent of inflammation and of injury to hepatocytes is assessed under the general heading of "necroinflammatory activity." Points considered are the distribution of inflammation and the extent of injury to hepatocytes. When inflammation is not restricted to the portal tracts but spills across the limiting plate into the lobule (Figure 68-11), interface hepatitis is present. The proportions of portal tracts involved by interface hepatitis, the extent of their circumference at which the limiting plate is blurred or effaced, and the depth and intensity to which

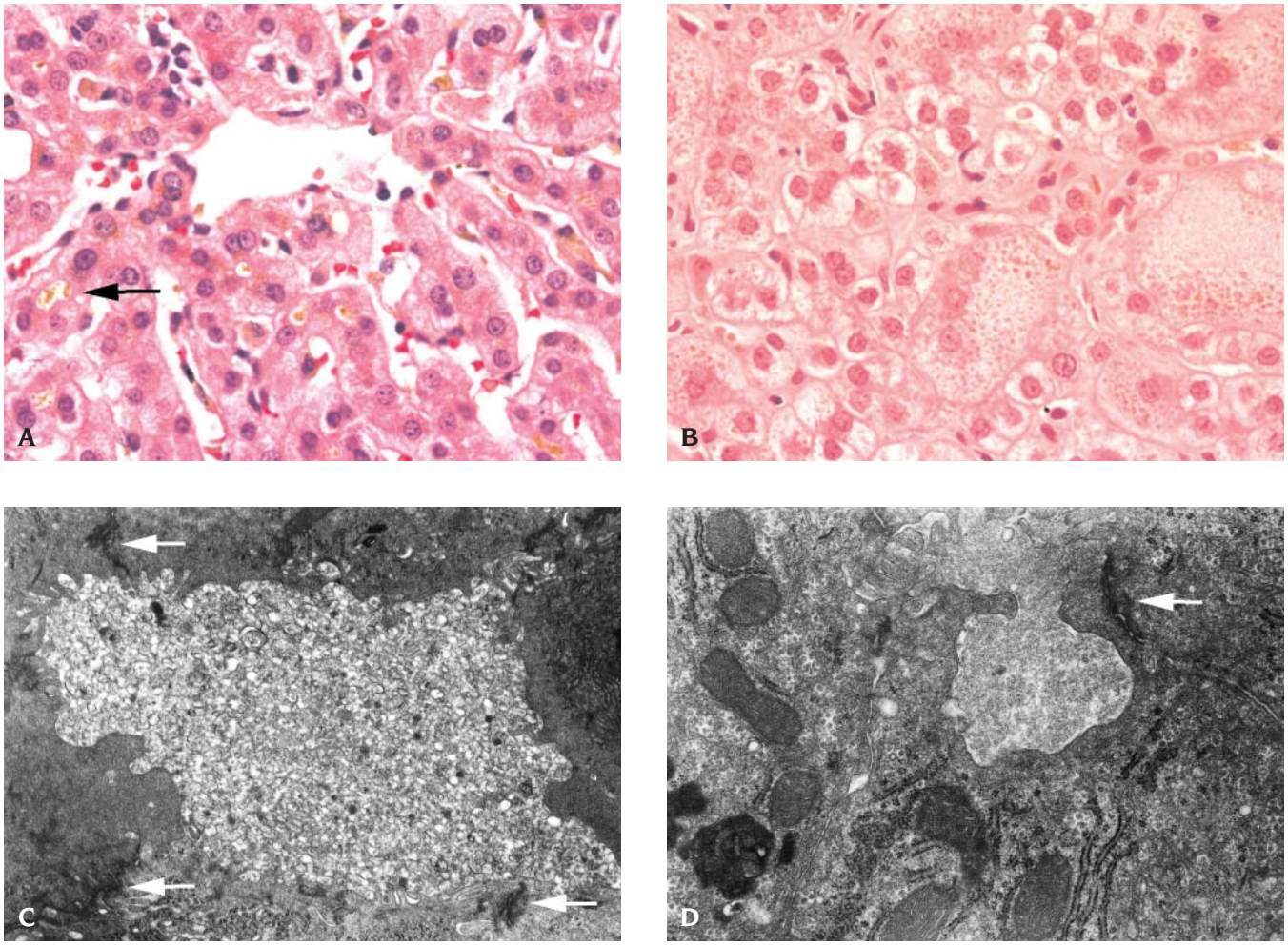


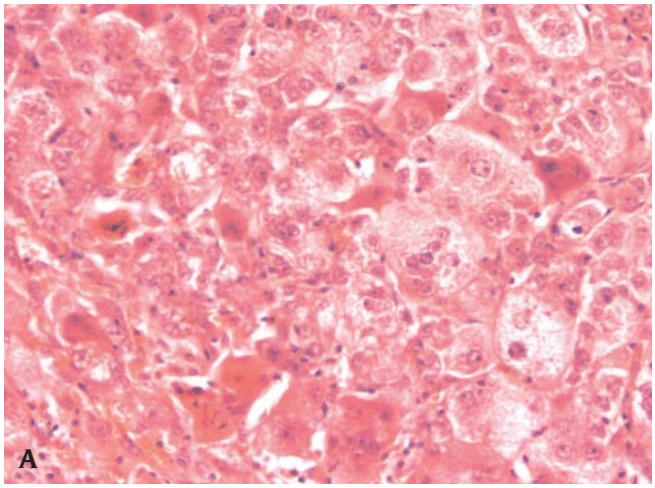
FIGURE 68-9 *A*, Small hepatocytes, varying only slightly in size, with compact cytoplasm border canaliculi containing bile plugs (*arrow*). Kupffer cells also contain bile pigment, but cholestasis within hepatocytes is not apparent. Familial intrahepatic cholestasis 1 (FIC1) disease (hematoxylin and eosin [HE]; ×400 original magnification). *B*, Giant cell change, with bile pigment in cytoplasm, is seen. Edema and variation in hepatocyte size are usual. Bile salt export protein (BSEP) disease (HE; ×400 original magnification). *C*, Circular profiles suggesting coarse granularity occupy the lumen of a canaliculus (tight junctions, *arrows*). FIC1 disease (osmium tetroxide, uranyl acetate, and lead citrate; ×16,500 original magnification). *D*, A canaliculus (tight junction, *arrow*) contains amorphous bile. Microvilli are lacking. BSEP disease (osmium tetroxide, uranyl acetate, and lead citrate; ×20,750 original magnification).

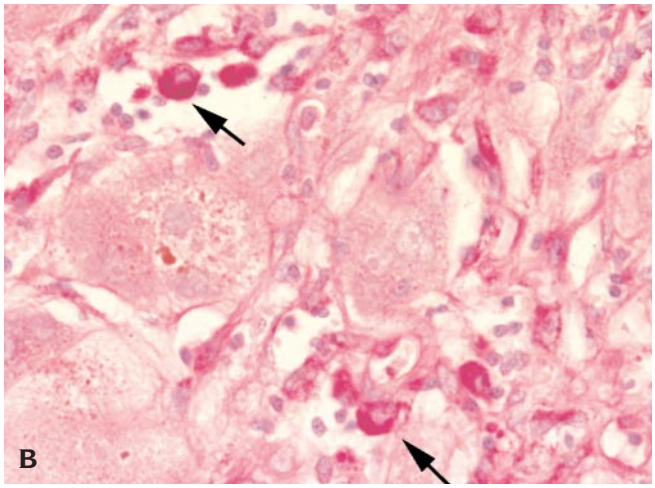
inflammation incurs into the lobule are evaluated. Changes in the appearance of hepatocytes, as listed for acute hepatitis (above), also are weighed. This assessment of injury at the moment of biopsy is called "grading." The damage to the liver caused by hepatitis is manifest as parenchymal loss, scarring, and parenchymal regeneration. If the insult to the liver is not eliminated, cirrhosis ensues. Evaluation of how far along the path to cirrhosis this damage has progressed is called "staging."

Several systems have been developed for numeric grading of the severity of chronic hepatitis and for staging associated scarring.[30–32] They are most useful when comparing instances of a particular disorder with one another and can be misleading when used too rigidly. Even the worst injury owing to hepatitis C virus infection, for example, falls short of what can be found in a florid autoimmune hepatitis. That, in turn, falls short of the devastation seen in non-A, non-B hepatitis with fulminant hepatic failure. Yet each of these varying degrees of necroinflammatory activity warrants designation as "severe"—of its kind.

This deficiency in across the board grading of necroinflammatory activity in chronic hepatitis has been expressly recognized for the changes seen in association with fatty change of hepatocytes (steatohepatitis), for which a specific grading system has been proposed.[33] It has been implicitly recognized in the lack of demand for numeric grading of necroinflammatory activity in neonatal hepatitis. Experience and access to clinical information that will help identify the etiology of a chronic hepatitis are needed for proper flexibility in choosing and applying a grading system for a particular biopsy specimen. Reliance on a numeric score alone is convenient, but the clinical practitioner should ensure, through discussions with colleagues in histopathology and, when possible, through review of findings on microscopy, that as little ambiguity as possible inheres in what, in the setting of a particular etiologic diagnosis, that score is meant to convey.

Morphologically defined chronic hepatitis has causes or associations that include viral infection, autoimmune





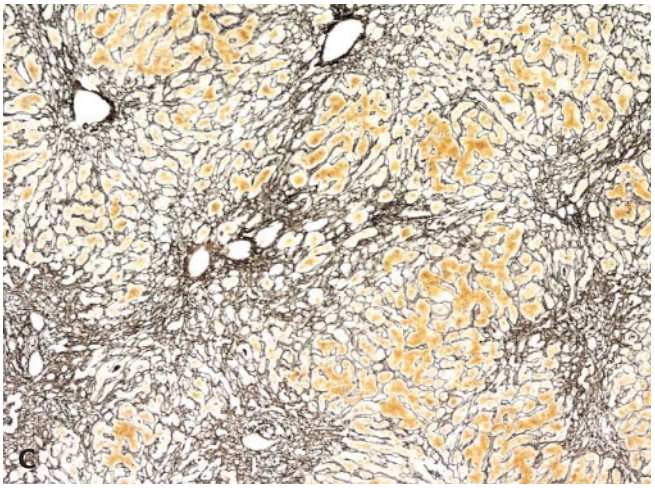


FIGURE 68-10 *A*, Edema, marked anisocytosis, and hepatocyte necrosis (with hypereosinophilia of cytoplasm and pyknosis of nuclei) are found in severe acute hepatitis owing to hepatitis B virus infection and requiring liver transplant for survival (hematoxylin and eosin; ×200 original magnification). *B*, Bile pigment is seen within edematous hepatocytes. Macrophages laden with ingested material (*arrows*) and lymphocytes lie among the hepatocytes. Acute hepatitis B virus infection; same patient as in *A* (diastase and periodic acid–Schiff; ×400 original magnification). *C*, Reticulin fibers are approximated to one another, without intervening hepatocytes, at many sites. The pattern is that of "bridging necrosis." Acute hepatitis B virus infection; same patient as in *A* (reticulin; ×40 original magnification).

disease, toxin exposure, Wilson disease, and fatty change. A wide variety of viruses can infect the liver, with hepatitis B virus and hepatitis C virus being the most frequently encountered at biopsy. Serologic and molecular-biologic studies generally provide diagnosis; as discussed above, biopsy is used to evaluate the extent of injury and for clues to prognosis. Hepatitis B virus infection may be manifest as "ground-glass" inclusions in hepatocyte cytoplasm (see Figure 68-3B); these mark on orcein staining (see Figure 68-3C). Hepatitis C virus infection is associated with expansion of portal tracts by lymphoid aggregates or even follicles, with germinal centers (Figure 68-12A), as well as with steatosis of hepatocytes (Figure 68-12B).

Autoimmune hepatitis is necessarily a serologically assigned diagnosis, but biopsy has a place in evaluating the severity and progression of disease.[34] Some features are more common in autoimmune hepatitis than in other forms of chronic hepatitis. These include plasma cell infiltrates that extend into the lobule (Figure 68-13A) and a tendency to centrilobular injury (Figure 68-13B). Toxin or drug ingestion may trigger an autoimmune hepatitis, and the histopathologic features of toxin- or drug-associated hepatitis overlap somewhat with those of autoimmune hepatitis. Prominence of neutrophils and eosinophils among inflammatory cells may suggest that injury is drug induced.

Wilson disease results from mutation in *ATP7B*, which encodes a hepatic copper-transporting enzyme.[35] It is effectively a pediatric disorder. Wilson disease may come to attention in the second decade with acute hepatic failure superimposed on cirrhosis or be identified earlier during the evaluation of transaminitis. In later stages of disease, copper-associated protein abounds within hepatocytes, but its distribution within the liver is patchy (Figure 68-14A). Cytosolic copper cannot readily be demonstrated histochemically. However, it may be present when cuprisomal copper is not, and to quantitate copper in liver tissue is still important in confirming a clinical diagnosis. Earlier stages are marked by steatosis of hepatocytes, a generally mild chronic hepatitis, and portal tract fibrosis, with relatively scant stainable copper (Figure 68-14B). Steatosis and hepatitis in a child thus should prompt evaluation for Wilson disease.

Steatohepatitis is characterized by steatosis of hepatocytes, portal tract fibrosis with perisinusoidal extension, centrilobular fibrosis, neutrophils within the lobule, hepatocyte ballooning with Mallory hyalin, activation of Kupffer cells, and lipogranulomata formed in response to rupture of fat-laden hepatocytes (Figure 68-15).[33] Not all of these need to be present before the diagnosis can be made. These changes are not distinguishable from those induced by ingestion of alcohol to excess. Steatohepatitis associated with obesity is increasingly reported in children.[36]

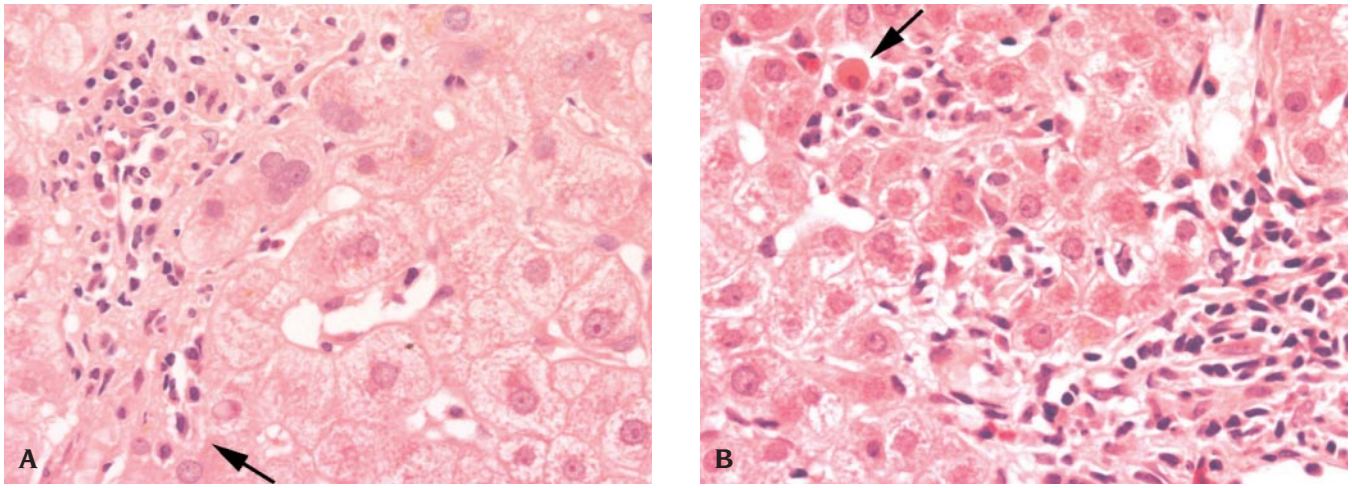


FIGURE 68-11 *A*, A portal tract contains an inflammatory infiltrate. It traverses and blurs the limiting plate, extending a short distance into the lobule. Mononuclear leukocytes are seen within hepatocytes (*arrow*). Hepatocellular anisocytosis is slight. Chronic hepatitis with mild activity; hepatitis C virus infection (hematoxylin and eosin [HE]; ×400 original magnification). *B*, A portal tract contains an inflammatory infiltrate. The present infiltrate extends more deeply into parenchyma and is more dense than that in *A*. It is associated with necrosis of a hepatocyte (*arrow*). Hepatocellular anisocytosis is pronounced. Chronic hepatitis with moderate activity; autoimmune hepatitis (HE; ×400 original magnification).

Predominantly Biliary Tract Injury (Cholestatic Pattern) in Childhood

The changes seen in distal biliary tract obstruction (extrahepatic cholestasis), in several heritable forms of intrahepatic cholestasis, and in hepatitis with secondary cholestasis are touched on above; the changes seen in sepsis are addressed below, with other post-transplant complications. Drugs, including both endogenous and exogenous hormones, can trigger intrahepatic cholestasis that both clinically and at biopsy resembles heritable cholestasis. Such acute-onset cholestasis and its pharmacogenetics are under study. Various spectra of susceptibility to drug-induced cholestasis, with implications for prophylaxis and treatment, may at some point be defined mutationally.

Damage to large and small bile ducts in childhood may itself be a heritable condition, as in NSC[37,38] and cirhin disease (North American Indian childhood cirrhosis).[39] It may be associated with neoplasia, as in Langerhans cell histiocytosis, or with immunodeficiency and its complications.[40] More frequently encountered are the changes seen in association with inflammatory bowel disease, which are generally called primary sclerosing cholangitis. These include an obliterative atrophy of septal or trabecular ducts, which are found rarely in needle biopsy specimens, and proliferative changes in interlobular bile ducts (Figure 68-16A) proximal to sites of large duct scarring. These changes may be associated with mixed or predominantly lymphocytic inflammation and with some degree of interface hepatitis. Frank cholestasis is not generally seen,

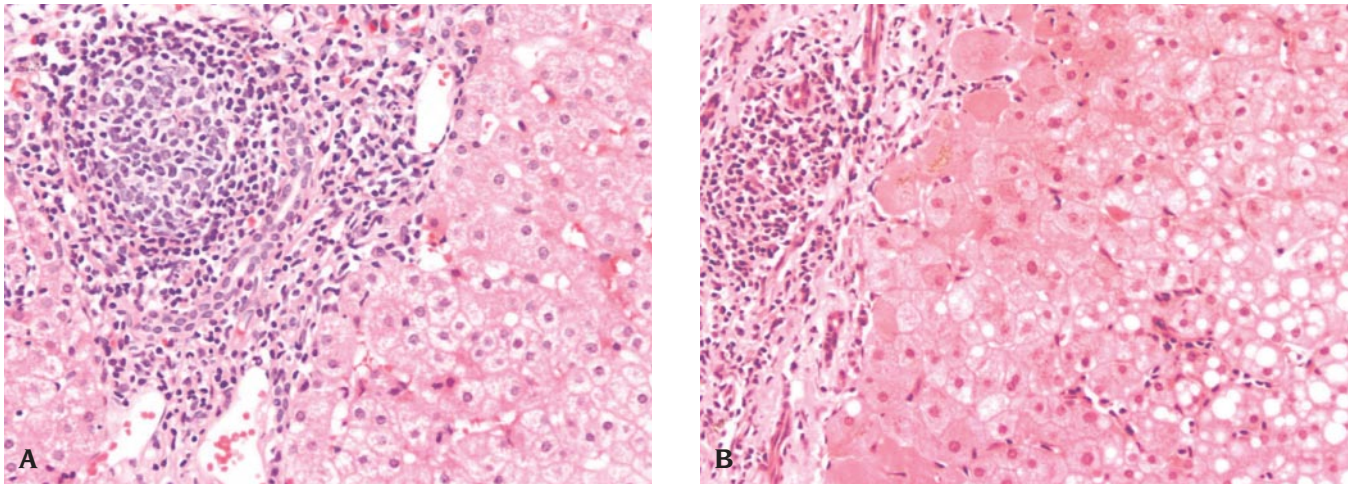


FIGURE 68-12 *A*, A germinal center lies within a portal tract. A bile duct appears draped along the germinal center's border; it is not the focus of inflammation. Slight interface activity is present. Hepatitis C virus infection (hematoxylin and eosin [HE]; ×200 original magnification). *B*, A portal tract contains a lymphoid aggregate. Slight interface activity and lobular inflammation are found. Macrovesicular steatosis of hepatocytes is seen. Hepatitis C virus infection (HE; ×200 original magnification).

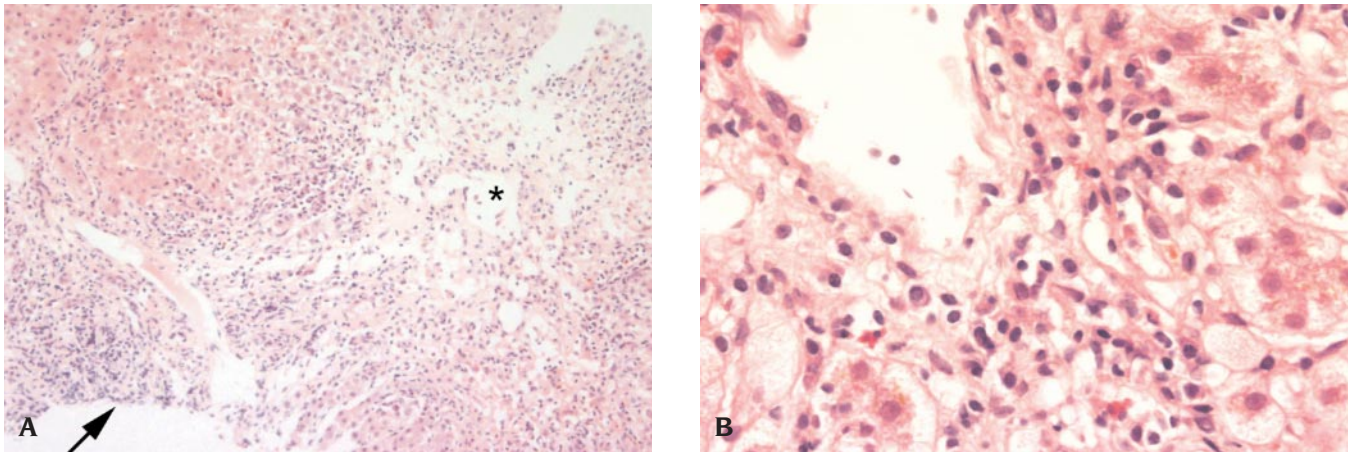


FIGURE 68-13 *A*, Brisk mononuclear leukocyte inflammation extends from the portal tract (*arrow*) to the central vein (*asterisk*), a pattern of injury leading to bridging necrosis and fibrosis. Autoimmune hepatitis (hematoxylin and eosin [HE]; ×100 original magnification). *B*, Plasma cells are conspicuous, with lymphocytes and scattered eosinophils, in a centrilobular infiltrate. Autoimmune hepatitis (HE; ×400 original magnification).

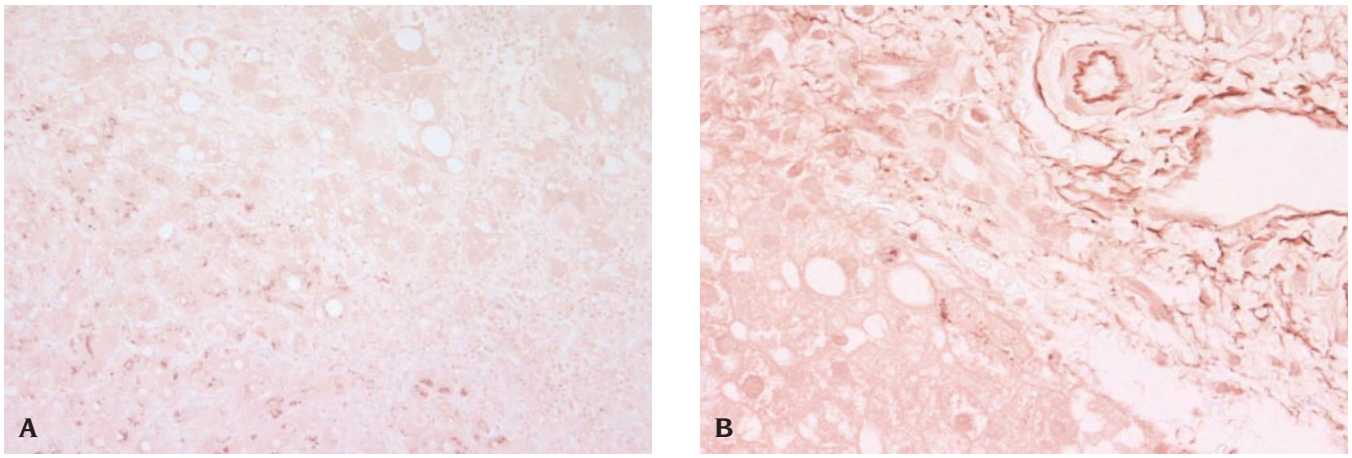


FIGURE 68-14 *A*, A nodule of hepatocytes (note the curved margin) contains substantial deposits of copper-associated protein; elsewhere in the section, none is demonstrated. Steatosis can be identified. Wilson disease at hepatectomy, age 14 years (orcein; ×100 original magnification). *B*, Small quantities of copper-associated protein are seen in hepatocytes at the limiting plate. Steatosis can be identified. Wilson disease at presentation, age 5 years (orcein; ×400 original magnification).

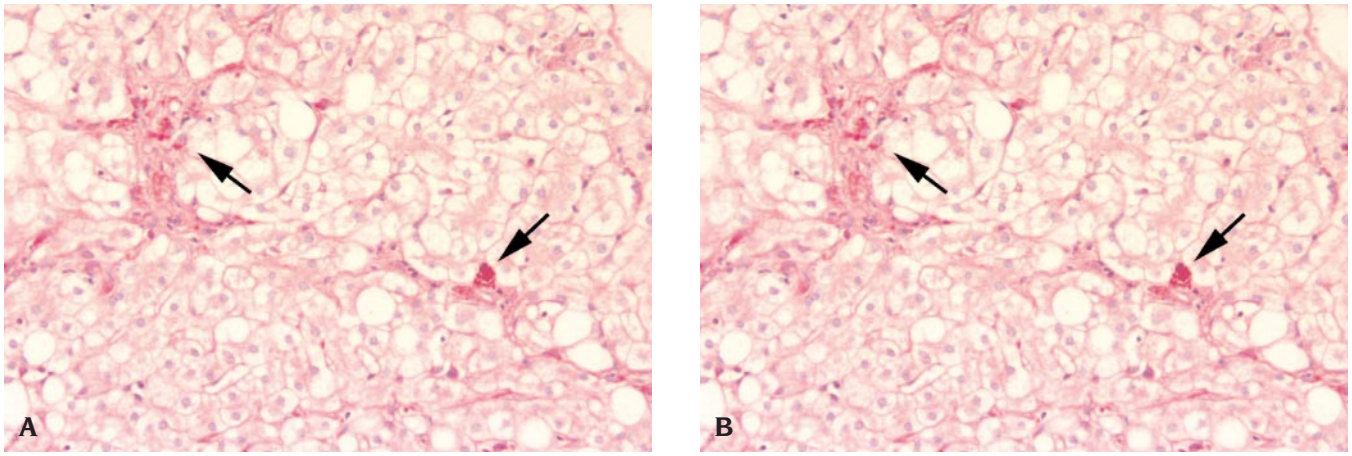


FIGURE 68-15 *A*, Nearly all hepatocytes exhibit steatosis. A small granuloma is present (*arrow*). Nonalcoholic steatohepatitis (hematoxylin and eosin; ×200 original magnification). *B*, "Turnover" pigment arising from necrosis of hepatocytes is seen in Kupffer cells and portal tract macrophages (*arrows*). Fine perisinusoidal fibrosis can be appreciated. Nonalcoholic steatohepatitis; same patient as in *A* (diastase and periodic acid–Schiff; ×200 original magnification).

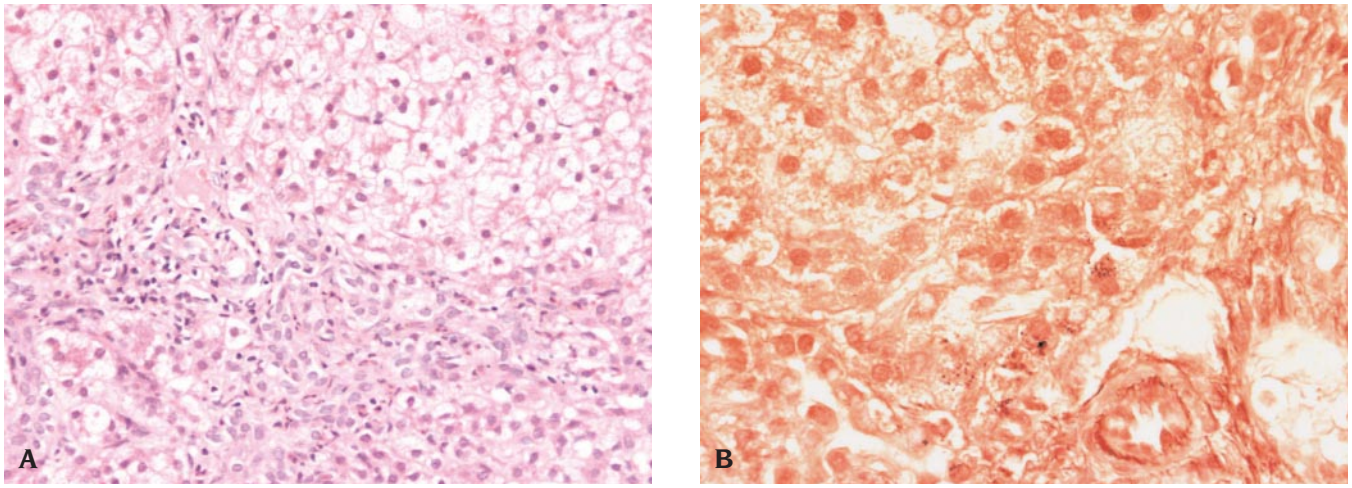


FIGURE 68-16 *A*, A portal tract is expanded by fibrosis and by increased numbers of bile duct profiles. Cholestasis is not seen. A scant acute and chronic pericholangitis is present. Cholangiopathy of inflammatory bowel disease (hematoxylin and eosin; ×200 original magnification). *B*, Copper-associated protein in small quantities is present in periportal hepatocytes. Cholangiopathy of inflammatory bowel disease; same patient as in *A* (orcein; ×400 original magnification).

although the hepatocellular edema of cholate stasis may be found. Copper-associated protein in periportal hepatocytes (Figure 68-16B) may mark the subclinical cholestasis of cholangiopathy and should be sought. If small duct cholangiopathy is found at biopsy and large duct cholangiopathy cannot be documented on imaging study, MDR3 disease[9] should be considered. Some patients in whom features of cholangiopathy clinically dominate features of hepatitis also have autoantibodies.[40,41] These may be considered instances of "overlap" or of an autoimmune sclerosing cholangitis. This diagnosis is made predominantly on serologic grounds.

Portal Hypertension

Some children without apparent metabolic disorder and without clinical biochemistry evidence of hepatic injury are noted to have stigmata of portal hypertension or hepatomegaly. Evaluation of portal hypertension constitutes an indication for liver biopsy. Two conditions identified from time to time in this setting are congenital hepatic fibrosis and portal vein thrombosis. In the former, portal tracts are expanded by fibrosis and contain increased numbers of bile ducts. The profiles and locations of bile ducts are unusual. They appear dilated and often lie at the limiting plate rather than within the collagenous stroma of the portal tract (Figure 68-17A). These changes suggest persistence of a stage in fetal liver development and are one manifestation of the ductal plate malformation.[42] Portal vein branches may appear hypoplastic. Extrahepatic and intrahepatic portal vein thrombosis (hepatoportal sclerosis) sometimes follows umbilical vein catheterization, but it may have no identifiable antecedent. Portal venules of unusually small caliber (Figure 68-17B) or parenchymal atrophy, particularly of centrilobular regions, may suggest that diagnosis.[43]

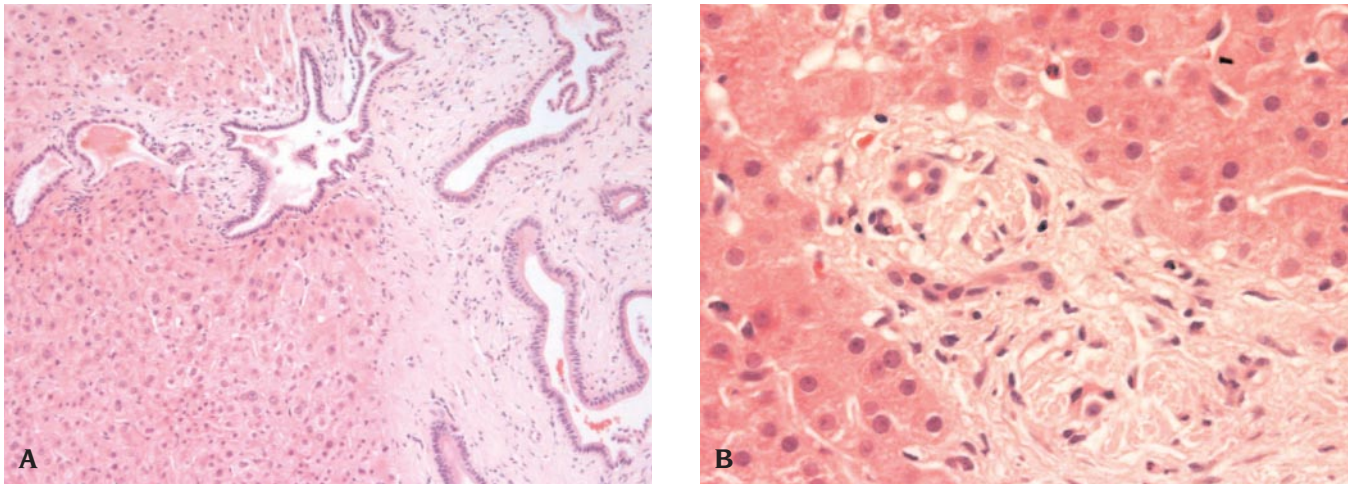


FIGURE 68-17 *A*, Dilated bile ducts in increased numbers are seen in and at the margins of a fibrotic portal tract. The profiles of the ducts are irregular. Congenital hepatic fibrosis (hematoxylin and eosin [HE]; ×100 original magnification). *B*, An interlobular portal tract lacks a portal venule of appropriate size (compare with Figure 68-1A) and exhibits slight fibrosis. History of portal vein thrombosis (HE; ×400 original magnification).

Post-Transplant Complications

Allograft livers are susceptible in adult and pediatric patients alike to rejection; to problems of vascular supply or drainage; to problems of biliary drainage; to infection, sometimes facilitated by immunosuppression; to involvement of the allograft by the original disorder; and to post-transplant de novo "autoimmune" hepatitis. Children who receive hepatic allografts are more likely to experience problems owing to technical aspects of surgery, to infection, and to de novo "autoimmune" hepatitis.

Rejection of allograft livers (leaving aside hyperacute rejection) takes two forms: acute and chronic. In acute rejection,[44] biopsy finds lymphocytic endotheliitis of portal and centrilobular venules, lymphocytic cholangitis, and portal tract inflammation, with lymphocytes and eosinophils (Figure 68-18A). Macrophages and neutrophils may be present as well. Centrilobular injury may extend into the parenchyma, with hepatocyte loss and hemorrhage. Intralobular cholestasis and hepatocellular edema often are seen (Figure 68-18B). Acute rejection is most frequently diagnosed on biopsy in the first weeks after liver transplant, but it can occur at any time if immunosuppression is withdrawn. Intercurrent viral infection may also precipitate acute rejection.[45] A consensus grading scheme for assessing the severity of acute rejection is available.[46] Its utility is probably greater in clinical trials than in day-to-day patient management.

Chronic rejection is characterized by loss of interlobular bile ducts and by a foam cell obliterative arteriopathy affecting larger vessels. Only bile duct injury can generally be assessed in needle biopsy specimens. Bile duct damage, with attenuation and disordered polarity of cholangiocytes (Figure 68-18C), precedes duct loss. A consensus staging scheme for assigning the diagnosis of chronic rejection is available.[47]

Global ischemic injury to the hepatic allograft may result in geographic-pattern infarction, with irregularly distributed regions of sparing and necrosis. Core needle biopsy thus cannot reliably assess the extent of injury. A late sequela of hepatic artery thrombosis can be incomplete biliary tract obstruction owing to scarring and stricture. The ductular reaction (recruitment of periportal hepatocytes to the small cholangiocyte phenotype) is found in both distal biliary tract stricture and acute rejection.[44,48] If edema of portal tracts and substantial numbers of neutrophils are particularly prominent, stricture should be considered.

Bacterial, fungal, and viral infections can damage the allograft liver. When sepsis is among the clinically considered reasons for allograft dysfunction (most commonly soon after transplant), bile plugs in the lumina of newly recruited cholangioles at the margin of the portal tract[49]

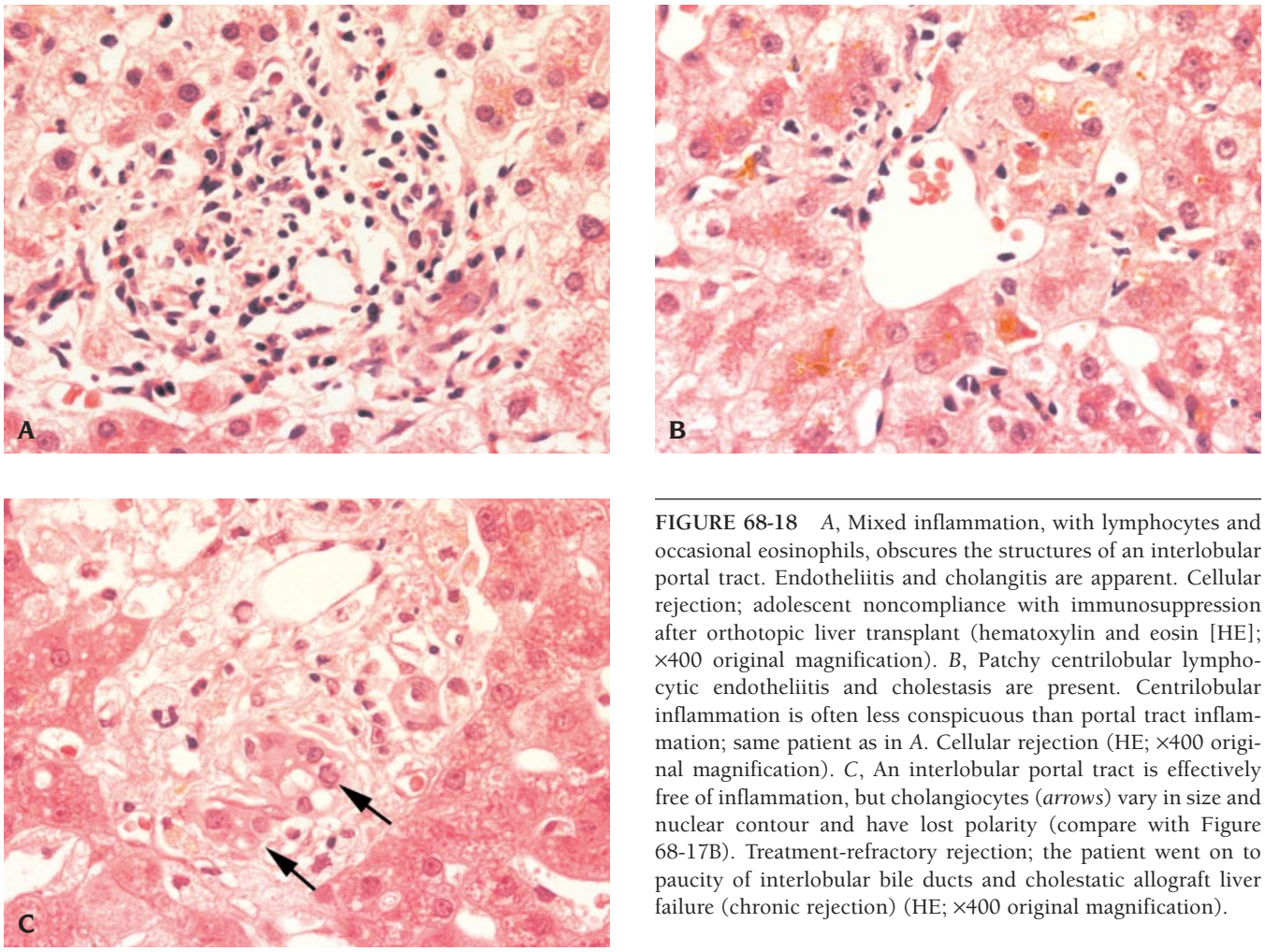


FIGURE 68-18 *A*, Mixed inflammation, with lymphocytes and occasional eosinophils, obscures the structures of an interlobular portal tract. Endotheliitis and cholangitis are apparent. Cellular rejection; adolescent noncompliance with immunosuppression after orthotopic liver transplant (hematoxylin and eosin [HE]; ×400 original magnification). *B*, Patchy centrilobular lymphocytic endotheliitis and cholestasis are present. Centrilobular inflammation is often less conspicuous than portal tract inflammation; same patient as in *A*. Cellular rejection (HE; ×400 original magnification). *C*, An interlobular portal tract is effectively free of inflammation, but cholangiocytes (*arrows*) vary in size and nuclear contour and have lost polarity (compare with Figure 68-17B). Treatment-refractory rejection; the patient went on to paucity of interlobular bile ducts and cholestatic allograft liver failure (chronic rejection) (HE; ×400 original magnification).

may support that diagnosis. Ascending cholangitis is an additional consideration, particularly when, as in EHBA, a limb of bowel has replaced the extrahepatic biliary tract. Fungal infection may complicate ischemic injury to the bile ducts, but it only very rarely involves the liver sampled by core needle biopsy.

Viral pathogens of particular note in pediatric liver transplant recipients include adenovirus, herpes simplex or varicella-zoster virus, cytomegalovirus, and Epstein-Barr virus. Adenovirus, herpes simplex, and varicella-zoster viruses produce pock-like foci of coagulative necrosis randomly distributed within the lobule; adenovirus can also directly involve the biliary tract.[50] Cytomegalovirus causes characteristic inclusions within endothelial cells, cholangiocytes, and hepatocytes, leading in the lobule to microabscess or microgranuloma formation. Epstein-Barr virus infection may lead to post-transplant lymphoproliferative disorder, more frequently so when primary infection occurs at the time of transplant or thereafter.[51] Immunosuppression predisposes the patient to development and progression of Epstein-Barr virus–associated disease. Post-transplant lymphoproliferative disorder may range from a mononucleosis-like syndrome to frank lymphoma. Features distinguishing the portal tract infiltrates of post-transplant lymphoproliferative disorder from those of acute cellular rejection include B-cell rather than T-cell predominance, immunoblastic or plasmacytoid features, and numerous—rather than occasional—cells containing Epstein-Barr virus sequences on in situ hybridization.[52,53]

Some patients who undergo liver transplant for disorders other than an autoimmune hepatitis develop allograft injury characterized by transaminitis, histopathologic findings more characteristic of autoimmune hepatitis than of either acute or chronic rejection, and circulating antinuclear or anti–smooth muscle antibodies. This pattern of disease, called de novo autoimmune hepatitis, appears more frequently in children than in adults and is not generally manifest soon after transplant.[54–56] To see a plasma cell component in an allograft liver biopsy specimen should prompt consideration of this diagnosis.

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CHAPTER 69

INTESTINAL BIOPSY

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SMALL INTESTINAL BIOPSY

Small intestinal mucosal biopsy is a routine procedure in specialty centers in which considerable experience and expertise in the technique itself and in the interpretation of biopsy findings are available. Therefore, safety, minimal disturbance to the child, and reliable results are all appropriately combined. The occasional small intestinal biopsy performed by inexperienced hands may lead to a disturbed child and often yields inconclusive results.

The introduction of the techniques of proximal small intestinal mucosal biopsy to pediatric practice by Sakula and Shiner in 1957[1] was a major advance, and access to tissue samples in children has led to a burgeoning of knowledge of intestinal pathology. It has also led to a better understanding of how the intestine functions normally. Variations on the biopsy capsule format have been produced and include a smaller porthole size to provide safer tissue sampling in children and double portholes[2] to improve sampling of potentially patchy lesions.[3] It is dangerous to use the adult capsule in small children because the size of the tissue biopsy specimen may be too large and may occasionally lead to perforation. Use of the pediatric capsule is safe in the experience of most observers, although a small risk of complications still exists. However, the use of the suction biopsy capsule has been superseded in most centers by endoscopic grab biopsy, which can provide tissue samples of similar quality and size.[4,5] It is possible to combine the two techniques.[6] Endoscopic sampling gives multiple biopsy specimens of adequate quality from one region, and it is possible to use a narrow-bore endoscope to give access to patients of low weight (> 1.8 kg). In addition, endoscopy provides a surface view of the upper gastrointestinal (GI) tract, with the option to biopsy esophageal and gastric regions during the procedure, although it is usually performed under a general anesthetic. Suction biopsy, in contrast, is usually performed in a sedated patient and has no facility for a macroscopic view (thereby requiring screening to position the capsule before taking a sample). Both require sterilization after use, and suction biopsy provides a cheaper route to intestinal sampling. Historically, suction biopsies have been performed toward the duodenojejunal flexure. Small intestinal endoscopic biopsies may be performed more proximally, around the second part of the duodenum, although distal duodenal sampling is perfectly feasible, and it is important to be aware that morphologic features may not be identical in the two regions (eg, in villous height, degree of lamina propria cellularity).

Technique of Small Intestinal Biopsy

Suction Biopsy. The child should fast overnight, although small amounts of water may be given as required. Infants may have a 10 pm and sometimes a 2 am feeding if necessary. On the morning of the biopsy, the child is sedated. The Royal Free Hospital (London, UK) no longer performs suction biopsies, and all intestinal mucosal biopsies are taken via endoscopy. Historically, the following oral regimen for sedation was used: trimeprazine, chloral hydrate, and metoclopramide in an appropriate dose for age. If the child becomes highly restless or distressed during the procedure, intravenous diazepam may be given at a maximum dose of 0.5 mg/kg. This should be done only when there is no risk to the child because of heavy sedation and when resuscitation equipment is immediately available. Grossly enlarged tonsils and any compromise to the upper airways are clear contraindications to its use. Once the child is appropriately sedated, the capsule is passed. This is done in the small child by placing a tongue depressor in the mouth and placing the capsule at the back of the tongue. The depressor is withdrawn, the chin is held up, and the child swallows. The tubing is then gently advanced until the capsule is in the stomach. Resistance is often felt at the cardioesophageal junction. The child is then placed on his right side, and the capsule is further advanced. It should then fall toward the pylorus. The next step depends on whether a flexible or a more rigid tubing is being used and whether there is to be fluoroscopic screening (the preferred technique) or if progress is to be assessed by plain radiography of the abdomen. In addition, if a nonradiopaque tube is used, radiopaque material needs to be injected down the tubing before the position of the capsule can be checked radiologically. Using a flexible tubing and a plain radiograph of the abdomen is a time-consuming procedure, but it does have the virtue of providing an exact record (a radiograph) of the exact site of the biopsy. However, the more rapid technique of using a radiopaque relatively rigid tube and positioning the capsule under fluoroscopic control is preferred. Such a semi-

rigid catheter is the metal, braided angiocardiographic catheter, which successfully transmits torque. It is also helpful to inject some air into the stomach via the capsule. A practical advantage of this technique is its usual speed, which makes the procedure preferable from the child's point of view. Care should be taken to monitor the fluoroscopy time. This should not exceed 2 minutes and usually is far shorter.

Metoclopramide introduced into the tubing in the dose of 2.5 mg for infants younger than 2 years and 5 mg for those older than 2 years usually speeds the passage of the capsule when there is a holdup at the pylorus. Alternatively, cisapride (0.2–0.3 mg/kg) can be given as a single dose via the capsule tubing. Using either procedure, once the capsule is positioned in the fourth part of the duodenum, the duodenojejunal flexure, or the first loop of the jejunum, it is "fired" by suction with a 20 mL syringe and then withdrawn. Ideally, biopsy specimens should be taken from a constant standard site. To ensure that the tube is not blocked (if radiopaque material or metoclopramide syrup has been injected down the tube), it is helpful to inject 2 mL of water followed by 2 mL of air before firing the capsule.

Ideally, some duodenal juice should be obtained either by free drainage before the capsule is fired or at the time of firing. The juice should be examined immediately by light microscopic study, under phase-contrast or high-contrast conditions, for the presence of *Giardia lamblia* and should be sent for culture if bacterial overgrowth or infection (eg, enteropathogenic *Escherichia coli* [EPEC]) is considered a diagnostic possibility.

Endoscopic Biopsy. The majority of centers now perform the small bowel biopsy during upper GI endoscopy. This has led to increased awareness of coexistent gastric and esophageal pathology, such as reflux esophagitis in many infants with cow's milk–sensitive enteropathy (CMSE). Position statements on the role of endoscopy in the investigation of conditions such as esophagitis have been published by both the North American Society for Pediatric Gastroenterology and Nutrition (NASPGN)[7] and the European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN)[8]; however, this chapter concerns only the role of endoscopic small intestinal biopsy in the diagnosis of enteropathy. When pediatric endoscopy was first performed, concerns were expressed about the quality of tissue obtained by endoscopic grab biopsy compared with capsule biopsy. This does not appear to be a problem with modern equipment, and several studies have confirmed that endoscopic biopsies are perfectly satisfactory for diagnostic purposes provided that tissue handling is performed correctly (see below).

The techniques of upper GI endoscopy are considered in detail in Chapter 67, "Gastrointestinal Endoscopy." As for capsule biopsy, it is appropriate to consider sedative premedication for the young child or an apprehensive older child. There has been recent discussion about the relative merits of deep intravenous sedation and general anesthesia in pediatric endoscopy.[9] Although some gastroenterologists use little or no sedation for their adult patients, this is not appropriate in pediatrics. The level of sedation achieved using intravenous benzodiazepines, together with narcotic analgesics such as pethidine, may overlap with general anesthesia, and it is mandatory that an appropriately qualified specialist has continuing responsibility for supervising the safety of the sedated patient throughout and after the procedure. Thus, in addition to continuous monitoring of oxygen saturation, there is an absolute requirement for functional resuscitation equipment and the ready availability of reversal agents such as naloxone and flumazenil. Some centers use a qualified anesthetist in this role, although in other centers, a member of the pediatric gastroenterology team assumes this responsibility. It is thus desirable for pediatric gastroenterologists in training to have undergone certified training in intubation and airway maintenance. Several countries now run registered courses in pediatric advanced lifesaving techniques.

The advent of video endoscopy, which has largely superseded the old fiberoptic instruments, has increased the potential diagnostic efficacy of endoscopy, and recognition of subtle lesions has been enhanced. It is thus easier to detect patchy small bowel lesions, such as CMSE. It is also possible, using very small-diameter "neonatal" endoscopes, to perform examinations in very small infants of less than 2 kg. There is an inevitable trade-off in terms of the quality of biopsy obtained if small biopsy forceps are used, and the choice of forceps is a matter of personal or departmental choice. Diagnostic ability is always enhanced by appropriate tissue handling (see below). Conversely, the largest samples may be rendered diagnostically uninformative by mishandling. Simply dropping a small intestinal biopsy into formalin runs the risk of causing a contraction of the longitudinal muscle layer, making subsequent orientation difficult. Although this may not affect diagnosis of celiac disease, it may make secure diagnosis of subtle enteropathy impossible.

Sample Handling. Once the capsule or endoscopic biopsy forceps has been withdrawn, the biopsy specimens should be rapidly removed from the capsule onto a gloved finger using a blunt seeker. The samples are opened out carefully so that the mucosal surface is facing downward (this can be checked using a dissecting microscope or hand lens if required). A piece of dry black card is then applied gently to the serosal surface for a few seconds, resulting in the sample adhering to the card. The card and sample, with the mucosal surface now facing upward, are placed into cold (4°C) normal saline. The black card optimizes the contrast of the specimen for study and photography. Under the dissecting microscope, the appearance of the mucosa can be assessed, but fixation must be prompt so that tissue autolysis is minimized. If using suction biopsy, it is not easy to repeat the procedure, so samples for which fixation with formalin is to be avoided, for example, electron microscopic study, disaccharidase assay, and immunohistochemical study, should be taken. Using endoscopy, it is a simple process to take additional grasp biopsy samples for any other requirements.

The specimens, still on the black card, are then placed in 10% phosphate-buffered formalin and processed for histologic examination. Routinely, 10 to 20 serial sections are cut, mounted on a single glass slide, and stained with hematoxylin and eosin (HE) and with periodic acid–Schiff (PAS) stain.

Hygiene Precautions. It is good practice when handling the biopsy capsule or endoscopic forceps after firing to wear surgical gloves. It is also advisable to wear safety spectacles or a visor to avoid face splashes with tissue and/or fluids. The endoscope is cleaned in 2% activated glutaraldehyde solution in an automatic, self-contained system. The biopsy capsule should be cleaned thoroughly after use and disinfected for at least 10 minutes in 0.5% chlorhexidine gluconate (weight per volume) in 70% alcohol, followed by a minimum of 1 hour in 2% activated glutaraldehyde solution, rinsed thoroughly in water, and allowed to dry. Great care should be taken when handling glutaraldehyde, and individual exposure must be kept to a minimum by using appropriate personal protection equipment and adequate ventilation.

Morphologic and Other Observations of Small Intestinal Biopsy Specimens

Dissecting Microscopy. The value of initial examination of biopsy samples with the dissecting microscope has been confirmed by many workers, both in adult medicine and pediatrics.[10,11] The following points illustrate the value of this method:

1. It facilitates the orientation of biopsy specimens to optimize histologic sectioning.
2. It allows a study to be made of the three-dimensional arrangements of mucosal architecture.
3. The entire biopsy specimen may be examined, which is particularly important in pediatrics because patchy mucosal lesions often occur (Figure 69-1).
4. Any gross artifactual damage can be recognized along with the adequacy, or otherwise, of the sample, so that a repeat biopsy can be considered while the patient remains sedated or anesthetized.
5. It allows rapid diagnosis of the presence or absence of a flat mucosa.
6. It allows parents to see the mucosa themselves (eg, a flat mucosa [Figure 69-2] and therefore reinforces the need for a gluten-free diet, particularly in postgluten challenges, when symptoms may not arise.
7. It offers the opportunity to take small samples of the specimen for other procedures (eg, electron microscopy, disaccharidase assay), although this is not necessary with endoscopy.

The drawbacks of the method are that if due care is not taken, fixation may be postponed, giving rise to the possibility of autolytic changes, and the severity of an abnormality other than a flat mucosa can be underestimated.

Some authors have considered that this method of examination adds little to histologic diagnosis and that its only value lies in the rapid recognition of a flat mucosa.[12] They instead advocate serial sectioning for histologic study of the whole biopsy specimen. However, such an approach is idealistic because it is not practical in most hospitals, whereas dissecting microscopic study is simple and straightforward and can easily be performed routinely.

In normal, healthy adults, the small intestinal mucosa is characterized principally by finger-like villi, with some leaf-like villi, but in children, the villi tend to be broader. The term "tongue-like" is used to describe such villi, and when they are extremely wide, the term "ridge-like" villi is used. The latter appearance is frequently seen in children in the first 5 years of life (Figure 69-3).[13] The appearance of leaf-like, tongue-like, or thin ridge-like villi on a proximal small

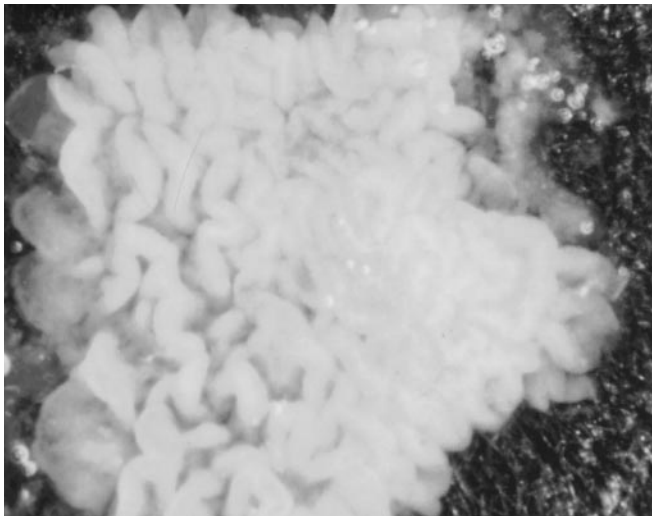

FIGURE 69-1 Dissecting microscopic study: patchy appearance showing ridgelike villi on the left side with low, closely packed ridges on the right (male infant, 13 months, postenteritis syndrome).

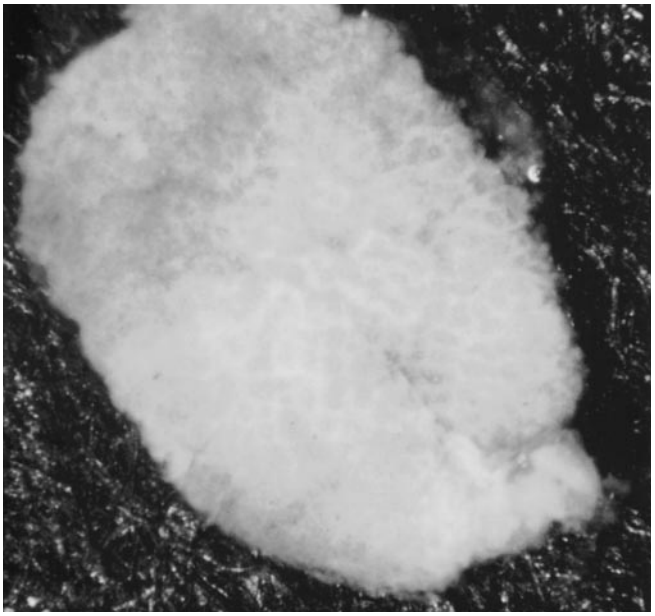

FIGURE 69-2 Dissecting microscopic study: flat mucosa showing visible crypt openings (female infant, 13 months, untreated celiac disease).

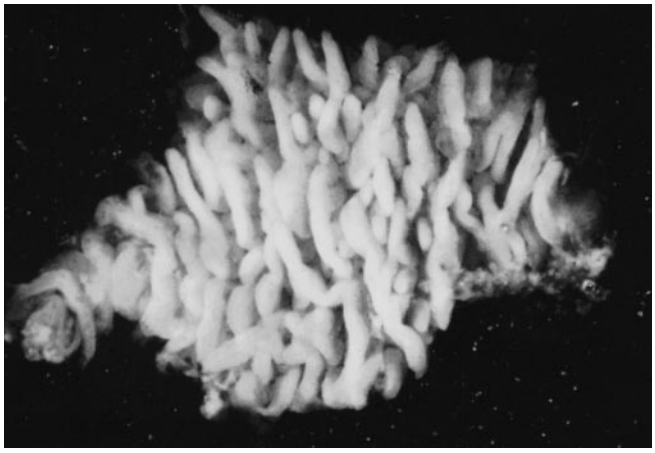

FIGURE 69-3 Dissecting microscopic study: normal appearance of tall, thin, ridgelike villi interspersed with leaflike villi (male infant, 5 months, intermittent diarrhea).

intestinal biopsy is accepted as a normal finding in children. Wright and others found that in childhood controls, the epithelial cell transit time in the crypts was 40% less than in adult controls, and the corrected mitotic index was 20% greater.[14] Thus, in early childhood, the mucosal surface area is reduced, and epithelial cell turnover is greater than in adults. The explanation for these observations is unknown. Because the changes are found in infants of 26 or more weeks gestation who have not been fed,[15] they cannot result from the ingestion of food and bacteria after birth. However, in utero ingestion of amniotic fluid or gastric acid secretions per se may play a role.

Abnormal appearances seen under the dissecting microscope are broadly grouped under two headings: a flat mucosa and a ridged or convoluted mucosa. In both types of mucosa, the normal villous architecture is lost. Mild villous shortening can be difficult to appreciate on dissecting microscopy. Patchy changes in architecture also can be seen with the dissecting microscope.[3]

Proximal intestinal isolated lymphoid follicles can be readily identified by dissecting microscopic study and are more frequent in children between 1 and 2 years old, being uncommon in children older than 6 years of age. Dilated lacteals also may be visible, but they are not indicative of lymphangiectasia in the absence of other clinical features. Endoscopy allows the clear visualization of ileal Peyer patches, which can therefore be selectively biopsied.[16]

Light Microscopy. *Terminology.* Small intestinal biopsy sections are routinely examined with the light microscope after the sections have been stained with HE. Current histologic terminology is unsatisfactory. The earliest reports divided pathologic small intestinal mucosa into subtotal and partial villous atrophy. The former category was characterized by a flat mucosa with thickening of the glandular layer beneath an atrophic epithelium, and the latter was characterized by a less abnormal mucosa. Some authors have further qualified partial villous atrophy with the terms "mild" and "severe." Others have used the term total villous atrophy to describe a flat mucosa. In fact, the mucosa described as either total or subtotal villous atrophy is not a truly atrophic mucosa in that it is not thinner than normal. Currently, most pathologists avoid the terms referred to above and use the term crypt hyperplastic villous atrophy to describe lesions in which villi are shortened and crypts are lengthened. This is the most frequently observed abnormality of the small intestinal mucosa (Figure 69-4). When villous height and crypt depth are approximately equal, this is termed a mild or minor abnormality (Figure 69-4B); a moderate abnormality involves crypt depth greater than villous height (Figure 69-4C), and a flat mucosa (Figure 69-4D) is a severe abnormality. Just as observed under the dissecting microscope, a patchy histologic abnormality may be seen (ie, a patchy enteropathy; Figure 69-5).[3]

It should be recognized that it is possible to find villous atrophy without marked crypt hyperplasia.[17] This may be referred to as crypt hypoplastic villous atrophy; however, crypt dimensions are normal. Thus, a more accurate description is crypt normoplastic villous atrophy. This produces a thin mucosa, and lesions of variable severity are seen (Figure 69-6). It is also possible to see villous hyperplasia with crypt hyperplasia.[18]

Parameters of Analysis. Many parameters of mucosal structure may be appreciated on light microscopic examination. However, classification of the appearance has mainly centered on villous height and crypt depth.

Histologic Normality. A clear idea of what is "normal" is required to evaluate the appearance of the small intestinal mucosa. This is not necessarily easy to determine in childhood because it is not ethical to perform biopsies on healthy children. However, observation of the morphologic features of biopsy specimens in the following situations expands the knowledge of the morphologic features of normal small intestinal mucosa: (1) biopsy specimens from children thought to have GI disease but whose specimens turn out to be normal, (2) control biopsy specimens from children on a gluten-free diet with celiac disease in remission, and (3) postmortem studies of the small intestine from children dying without evidence of GI disease (see Figure 69-4).

It is important to study other aspects of the mucosa along with villous height and crypt depth because these can give specific diagnoses. Aspects to be studied include the following:

- *Organisms*. Look for the presence of luminal, surface-attached, and intramucosal organisms (eg, *G. lamblia*, *Cryptosporidium*, enteropathogenic *E. coli,* and microsporidiosis).
- *Epithelial cells*. Assess the state of the epithelium, in particular the enterocyte, for cell height and degree of vacuolation. The latter finding can indicate abetalipoproteinemia and hypobetalipoproteinemia if extensive vacuolation of villous epithelium is seen in an otherwise normal mucosa. However, this should not be confused with the lesser degree of vacuolation seen in the enteropathies of celiac disease and the postenteritis syndrome. This is usually confined to the villous tip or

to a small number of surface exposed cells in severe enteropathies.[19] Crypt epithelium should be studied for the number of mitotic figures to give an indication of cell production, and the number of goblet cells should be assessed. In some cases of autoimmune enteropathy, a complete absence of goblet cells has been noted.[20] Paneth and endocrine cells also form part of the epithelium, but these are not useful for routine diagnostic purposes. Paneth cell dysplasia can be seen in conditions of increased epithelial cell turnover, and the number of endocrine cells is increased in celiac disease. This can be appreciated by studying histologic sections under fluorescence microscopy because these cells autofluoresce.

- *Intraepithelial cells*. Note the presence and number of migratory, intraepithelial cells, such as lymphocytes, eosinophils, neutrophils, and mast cells. Quantifying

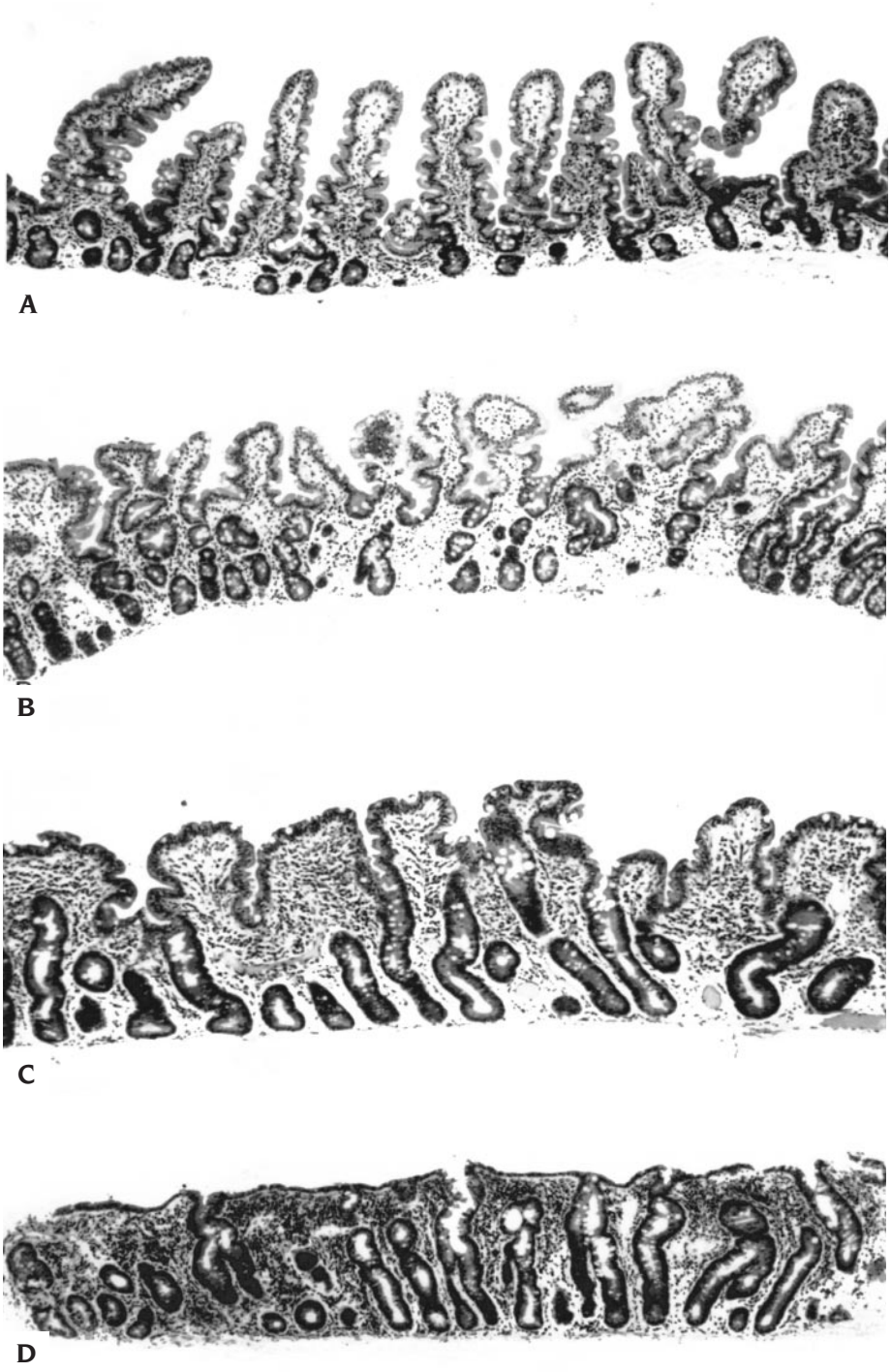

FIGURE 69-4 Light microscopic study: *A*, histologically normal small intestinal mucosa (female infant, 12 months, failure to thrive); *B*, minor enteropathy (female infant, 5 months, chronic diarrhea with failure to thrive); *C*, moderate enteropathy (boy, 6 years 9 months, celiac disease—postgluten challenge); *D*, severe enteropathy (girl, 6 years 10 months, untreated celiac disease) (hematoxylin and eosin; ×60 original magnification).

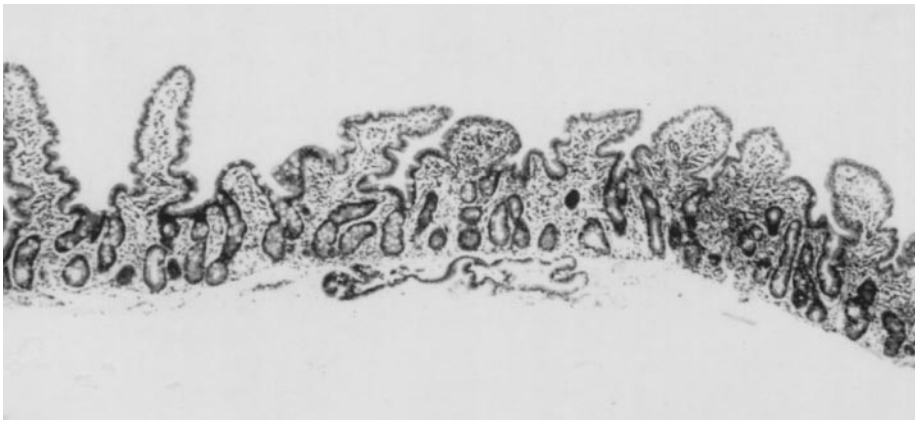

FIGURE 69-5 Light microscopic study: patchy enteropathy (girl, 2 years 2 months, diarrhea postcolectomy) (hematoxylin and eosin; ×60 original magnification).

the density of lymphocytes within the small intestinal villus or surface epithelium (number of lymphocytes per 100 epithelial cells) is of value for routine diagnostic use.[21] Children with normal histologic features (controls) have a mean value of 23,[22] which is similar to that described in adults.[23] Raised counts of intraepithelial lymphocytes (IELs) are found in children and adults with celiac disease, adults with untreated dermatitis herpetiformis or tropical sprue, and some cases of children with unexplained failure to thrive,[21] giardiasis,[21] cryptosporidiosis,[24] or CMSE.[22] IEL counts return to the normal range in cases of celiac disease when the patient is placed on a gluten-free diet, and they become lower than normal in patients with CMSE on a milk-free diet.[22] The absolute number of IEL may not alter with changes in morphologic features[25]; however, IEL density is not directly related to mucosal surface area.[26] Intraepithelial eosinophils have been reported to be increased in CMSE,[17] and intraepithelial neutrophils are seen in inflammatory bowel disease[27] and in EPEC infections[27] but may also occasionally be noticed in active celiac disease.

- *Lamina propria cellularity*. Study the lamina propria for the degree and nature of cellular infiltrate and the appearance of lacteals. This is usually done subjectively, but more reports are making use of readily available computer-based image analysis systems that provide a means of objective analysis. Recognition of basic cell types can be performed on HE-stained sections, but it is important to use immunohistochemical techniques to investigate cell phenotype (eg, lymphocyte subclass; see below) and for secretion of specific cytokines. Mucosal mast cells are best studied using Carnoy solution as a fixative[28,29] and chloroacetoesterase reaction for staining so that they can be clearly seen,[30] although this has limited diagnostic usefulness. Using this technique, Sanderson and others have demonstrated that a higher mast cell density exists in the ileum than in the colon.[31]
- *PAS staining*. This allows the preservation of the brush border to be visualized and demonstrates mucus-

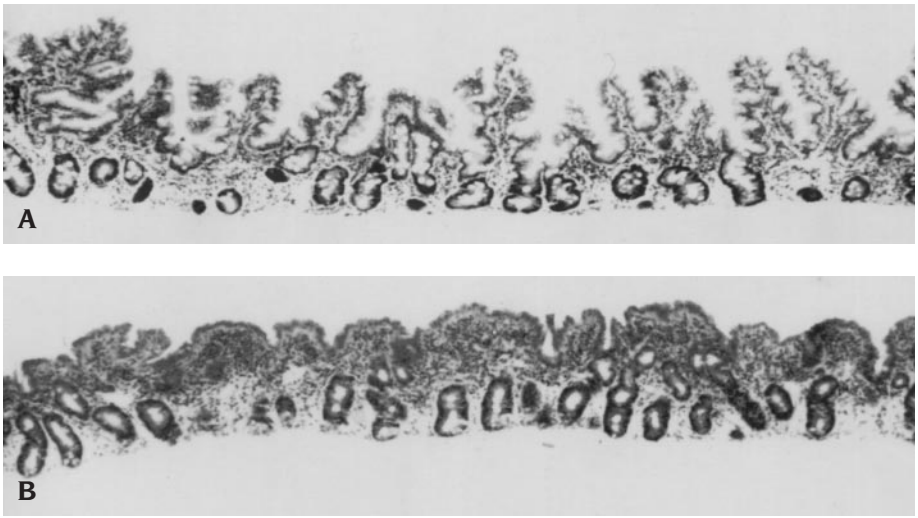

FIGURE 69-6 Light microscopic study: villous atrophy without marked crypt hyperplasia. *A,* Mild enteropathy (female infant, 1 year 7 months, cow's milk protein intolerance). *B,* Moderate enteropathy (male infant, 11 months, cow's milk protein intolerance) (hematoxylin and eosin; ×75 original magnification for both).

containing goblet cells. The presence of PAS-positive material in the apical cytoplasm of upper crypt and low villous epithelial cells indicates microvillous atrophy,[32] although this should not be confused with the granular epithelial staining seen in enteropathies, where it reflects an increased presence of lysosomal bodies. PAS-positive inspissated mucus in crypt lumina suggests cystic fibrosis.[33]

Electron Microscopy. Transmission electron microscopy and scanning electron microscopy have been used to study the morphologic characteristics of small intestinal biopsy tissue taken from children[34] and adults. It is important to consider whether electron microscopy will be of diagnostic value in advance of the biopsy so that glutaraldehyde fixation (2–3% glutaraldehyde in 0.1 M phosphate or cacodylate buffer is suitable) can be used to preserve the tissue samples and optimize interpretation. Ultrastructural studies are now routine in cases of protracted diarrhea. Disorders such as microvillous atrophy (Figure 69-7),[32] attaching and effacing *E. coli* (Figure 69-8),[35] and cryptosporidiosis (Figure 69-9)[24] are diagnostic possibilities. Small intestinal biopsy specimens from patients with acquired immune deficiency syndrome (AIDS) should also be studied by electron microscopic examination because many infectious agents are beyond the resolution of the light microscope (eg, microsporidiosis).

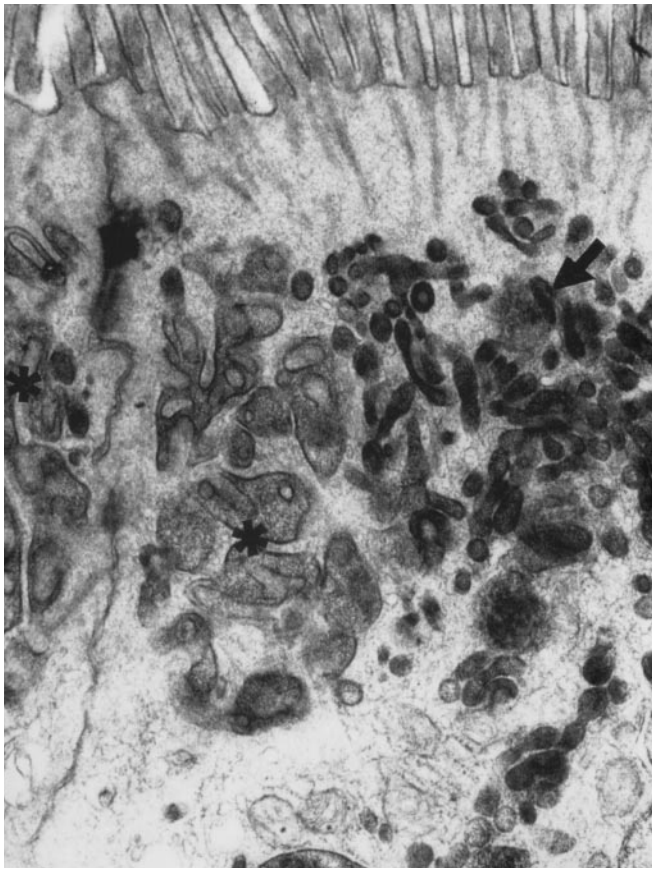

FIGURE 69-7 Electron microscopic study: microvillous atrophy (female infant, 14 months). Increased presence of secretory granules with membrane-bound inclusions containing microvillus-like projections (×40,000 original magnification).

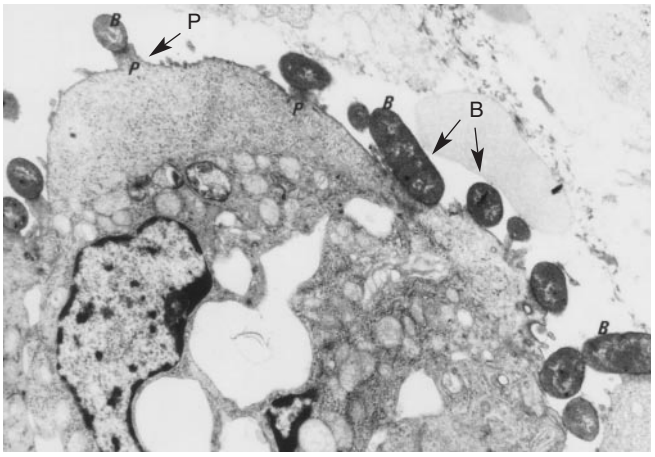

FIGURE 69-8 Electron microscopic study: enteropathogenic enteritis caused by *Escherichia coli* (male infant, 8 months). *E. coli* 0128 (B) attached to apical surface of epithelium in association with microvillous effacement and pedestal formation (P) (×18,000 original magnification).

Biochemistry. A mucosal sample should be taken and frozen for biochemical studies[36] in cases in which primary or secondary disaccharidase deficiencies are diagnostic possibilities. Although enzyme levels can be appreciated on histochemical study of frozen tissue sections[37] or on duodenal juice, the usual practice is to analyze tissue homogenates.[36,38] Breath tests can be used to indicate disaccharide malabsorption,[39] but this is not the same as disaccharidase deficiency. Other techniques are possible and may be required to diagnose rare disorders, such as the use of brush border membrane vesicles to diagnose defective sodium-proton transport.[40]

Immunohistochemistry. Conventional staining techniques give information about intestinal architecture and cellularity without shedding much light on the precise nature of the inflammatory response. All lymphocytes look similar on HE staining, and it is impossible to see whether

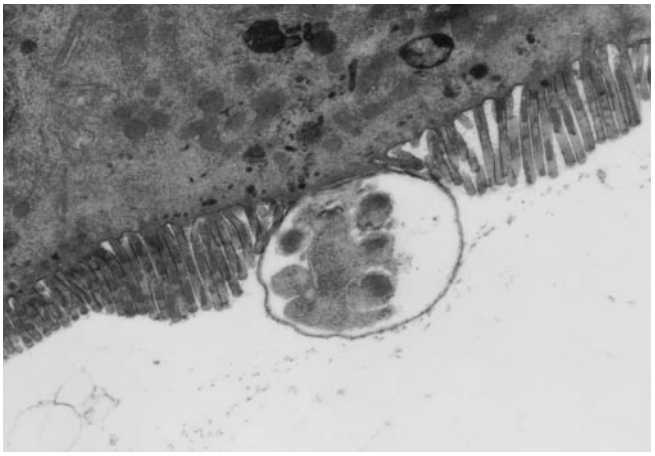

FIGURE 69-9 Electron microscopic study: cryptosporidiosis (male infant, 13 months). Cryptosporidial schizont adhering to the epithelial surface. Notice displacement of microvilli at site of attachment (×18,000 original magnification).

a lymphocyte infiltration represents oligoclonal expansion or a polyclonal response, whether an IEL has a particular T-cell receptor type,[41] or whether evidence exists of epithelial or endothelial inflammatory activation. Special techniques are required to understand disease mechanisms rather than simply describe appearances.

Immunohistochemical analysis may give highly important information in the diagnosis of enteropathy and study of disease mechanism. It is a relatively simple technique to perform, yet it is powerful because of the range of available antibodies that can give precise information on cell lineage, activation status, and functional protein expression. However, access to the full range of diagnostic markers requires the use of snap-frozen tissue because formalin fixation crosslinks tissue proteins and may alter tertiary structure enough to prevent antibody recognition. An important practical point is that any child with an unusual enteropathy should have biopsies snap-frozen at endoscopy in addition to those taken into formalin (ideally, a further biopsy should be taken into glutaraldehyde for possible electron microscopic studies). This is now accepted good practice for any infant with unexplained intractable diarrhea. The widespread use of endoscopic biopsy has removed the problems arising with capsule biopsy of balancing the need for proper histologic assessment and obtaining enough tissue for snap-freezing. Although immunohistochemistry may be considered a research tool for the majority of small bowel enteropathies, it is an important diagnostic aid for conditions such as autoimmune enteropathy, when activated mucosal lymphocytes may be seen in conjunction with class II major histocompatibility complex (MHC) expression (human leukocyte antigen [HLA]-DR) on the surface epithelium.[42] The detection of high numbers of T cells within the epithelium favors the diagnosis of celiac disease in a child with positive serology, even if the mucosa shows only subtle abnormalities otherwise.

Immunohistochemistry has been particularly useful in celiac disease, allowing the recognition of the primary role of lamina propria CD4 cells in the pathogenesis of this condition.[43,44] Immunohistochemical analysis may extend to the detection of secreted molecules such as cytokines, and most of the major immunoregulatory molecules can be demonstrated in this way.

Molecular Biology. Using molecular biologic techniques, it is possible to detect the presence of messenger ribonucleic acid (mRNA) for specific proteins. If whole biopsy specimens are homogenized, specific mRNA can be detected by Northern blotting or reverse transcriptase polymerase chain reaction. Such techniques allow detection and semiquantitative estimation of such small amounts of mRNA but do not allow localization within tissue. The technique is also dependent on expertise, and some centers have found difficulty in differentiating diseased tissue from controls because many cytokines are produced at low levels in healthy intestines and thus may be amplified to apparently similar levels. In contrast, it is possible to localize specific mRNA within individual cells using in situ hybridization, which is technically more difficult but which may also be combined on the same slide with tissue for immunohistochemical study. These techniques are still largely reserved for research, although detection of viral or bacterial pathogens such as cytomegalovirus or mycobacteria may be efficiently achieved.

In general, in situ hybridization requires the use of frozen tissue, although mRNA for some molecules may be detected in formalin-fixed tissue after proteolytic digestion to unmask ribonucleic acid sequences.[45] The use of in situ hybridization in studying intestinal function has been demonstrated best in animal work. The development of class II MHC molecules (required for antigen presentation to T lymphocytes) in mouse small intestinal epithelium was shown to be dependent on the introduction of a complex diet at weaning.[46] The expression of the Na-glucose cotransporter gene was maximal at the crypt-villus junction in rabbits, although the actual enzyme activity was greatest at the villus tip.[47]

The advent of laser capture microdissection now allows molecular biologic analysis of collections of individual cells so that transcriptional activity of discrete cell populations can be achieved.[48] Combined with knockout mouse technology, this has allowed functional genomic analysis of intestinal stem cells,[49] and these techniques promise important advances in understanding gut function.

Lymphocyte Isolation. In addition to the staining techniques available, it is possible to obtain individual inflammatory cells from whole biopsy samples. Epithelial cells and intraepithelial lymphocyte populations can be extracted initially by separation from the rest of the biopsy using ethylenediaminetetraacetic acid (EDTA). Lamina propria lymphocytes may then be obtained by digestion of the remaining tissue with collagenase. If necessary, the lymphocyte subpopulations may be separated further by density gradient centrifugation followed by magnetic bead extraction. Once separated, the inflammatory cells may be categorized on the basis of their surface markers by fluorescent-activated cell sorter analysis,[50] which may also be employed to demonstrate spontaneous cytokine secretion (Figure 69-10). Other techniques include measuring supernatant levels after the cells are stimulated and the use of the enzyme-linked immunospot.[51]

In Vitro Organ Culture Techniques. Although it is not possible to maintain the viability of biopsy tissue for any great length of time, individual explants (approximately 2 mm^2 pieces) will maintain morphologic features for 24 to 48 hours under appropriate culture conditions. Cultured small intestinal explants from celiac patients show improvement in morphologic features if cultured in the absence of gluten,[52] whereas the addition of gliadin fragments upregulates the expression of HLA-DR on epithelial cells in vitro.[53] Short-term culture (8 hours) is of particular value in experimental studies of microbial pathogenesis.[54]

Role of Small Intestinal Biopsy in Diagnosis

Currently a single, investigative, small intestinal biopsy has two main roles that are of value in making a diagnosis in clinical pediatrics. The first is to demonstrate the pres-

ence or absence of a proximal small intestinal enteropathy.[55] Enteropathy may be defined as an abnormality of the small intestinal mucosa that can be demonstrated with the light microscope. The second is to provide samples of small intestinal mucosa for other diagnostic purposes (eg, for disaccharidase assay).

Arranged small intestinal biopsies are sometimes required before a final diagnosis can be made. Such situations include organized dietary challenges with pre- and postchallenge biopsies (eg, in celiac disease or dietary loading), in which diagnostic features may be more easily distinguished (eg, lipoproteinemias, lymphangiectasia).

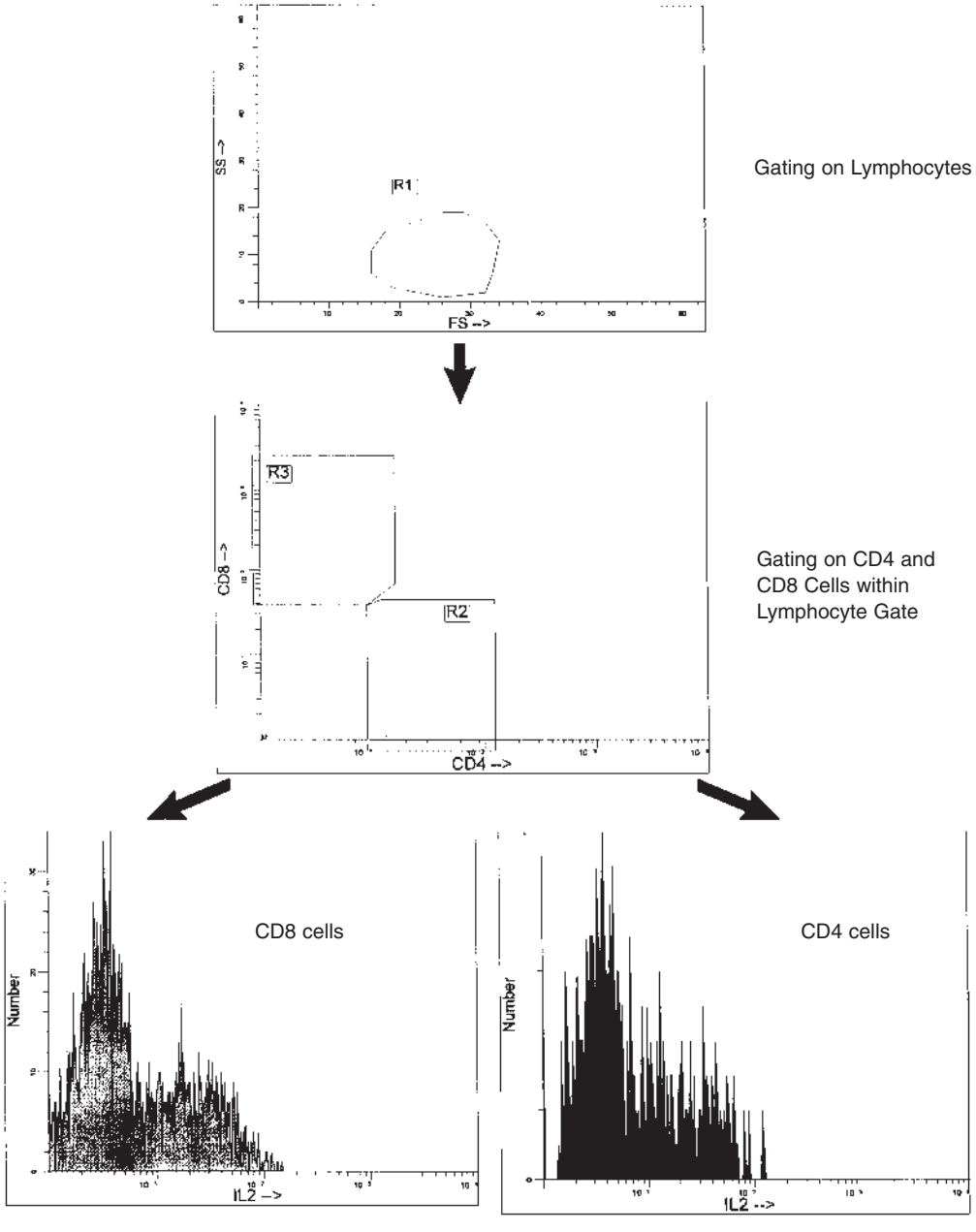

FIGURE 69-10 Fluorescent-activated cell sorter analysis (FACS). Use of flow cytometric (FACS) analysis to determine cytokine production at the single-cell level by mucosal lymphocytes. Intraepithelial lymphocytes may be studied separately from lamina propria lymphocytes, by separation of the entire epithelial compartment using ethylenediaminetetraacetic acid. Lamina propria lymphocytes are separated following collagenase digestion. The lymphocytes are first identified by "gating" on cells of appropriate size and density among the total population. Within that gate, cells expressing CD4 (T helper cells) or CD8 (cytotoxic/suppressor cells) can then be identified using monoclonal antibodies tagged with fluorescent markers—a whole panel of other cell lineage markers can be used if cell numbers and research budgets are adequate. Finally, the proportion of CD4 and CD8 cells spontaneously producing cytokines (here interleukin-2) can be identified.

The current ESPGHAN recommendations for the diagnosis of celiac disease suggest that dietary challenges are not necessary in older patients and that the demonstration of a flat mucosa with a good clinical response to gluten elimination affords a satisfactory diagnosis. However, young children under 2 years of age may require dietary challenge to confirm the diagnosis in view of other possible explanations for enteropathy in this age group.[56]

Those disorders in which small intestinal biopsy has a role in diagnosis may be placed into groups. First, there is a group of disorders for which biopsy is invariably of value in making a diagnosis (Table 69-1). These include disorders in which a proximal small intestinal enteropathy is a diagnostic prerequisite or in which there is a specific enzyme deficiency.

The demonstration of an enteropathy is an absolute requirement for the diagnosis of celiac disease, but it is not specific for this disorder. A flat small intestinal mucosa is characteristic of celiac disease, but there are other causes of a flat mucosa in childhood, and, on occasion, lesser degrees of mucosal abnormality may be found in children with celiac disease.

The enterocyte in abetalipoproteinemia cannot synthesize betalipoprotein, and, as a result, chylomicron formation is impaired. Thus, absorbed dietary fat is not properly mobilized from the enterocyte. As a result, the cytoplasm of those cells lining the upper half or two-thirds of the villi appears vacuolated in ordinary HE-stained sections. These cells can be shown to contain fat through the use of special stains on frozen sections. A similar appearance is seen in hypobetalipoproteinemia.

Children with agammaglobulinemia lack plasma cells in the lamina propria, but the mucosal architecture may range from a flat mucosa to a completely normal one.

In the enteropathy associated with multisystem autoimmune disease and the presence of circulating autoantibodies (autoimmune enteropathy) (Figure 69-11), the mucosa is severely abnormal at the time of diagnosis, sometimes with a flat mucosa. The demonstration of an enteropathy in the presence of circulating autoantibodies against the enterocyte in a child who has chronic diarrhea is essential for diagnosis of this syndrome. It is very important to do in-depth immunologic studies in these children to help identify therapeutic options.[20]

Microvillous atrophy can be diagnosed only by the demonstration of characteristic microvillous inclusions and an increase in secretory granules on electron microscopic examination of a small or large intestinal mucosal biopsy specimen.[32,57,58] An abnormal accumulation of PAS-positive material occurs within the apical cytoplasm of epithelial cells and corresponds to the increase in secretory granules seen on electron microscopic study.[32,57]

The presence of adhering bacteria can be seen on light microscopy; however, the resolution of transmission electron microscopy is required to recognize the microvillous loss and pedestal formation of the attaching and effacing lesion, which is typical of classic EPEC infections (see Figure 69-8).[35] EPEC infections are capable of causing severe protracted diarrhea and much morbidity and mortality. The recognition of an enteropathy with the presence of attaching– and effacing lesion–forming bacteria indicates that antibiotic therapy would be appropriate.[59] This is important because nonclassic serotypes of *E. coli*, so-called atypical *E. coli*, also can cause an attaching and effacing lesion,[60] and routine stool microbiologic study would not identify them.

Children who have either of the two primary disaccharide intolerances, namely congenital alactasia and sucrase-isomaltase deficiency, have normal small intestinal morphologic features, but the characteristic enzyme deficiencies are present on disaccharidase assay.

Second, when the lesion is nonuniform (ie, patchy) or when there is penetration of the mucosa by a parasite, biopsy may provide a specific diagnosis, but in this group of disorders, the absence of abnormality (ie, a normal mucosa) does not exclude the diagnosis (Table 69-2). It is possible in parasitic situations and infections with attaching and effacing *E. coli* that a more distal site of the intestine is affected, and the diagnosis is made on stool microbiology.

The trophozoite of *G. lamblia* is often found in the duodenal juice of children with giardiasis but also may be found on section of small intestinal biopsy specimens. Similarly, in children with strongyloidiasis, larvae of *Strongyloides stercoralis* may be found in juice and on section of the mucosal biopsy specimens. Cryptosporidial schizonts may just be visible by light microscopic study, but the characteristic morphologic features are readily identifiable by electron microscopic examination.

Small intestinal lymphangiectasia may be diagnosed by biopsy of the small intestinal mucosa, but because the lesion is often patchy, it can be missed on a single biopsy; multiple biopsies may be indicated.

Small intestinal lymphoma are rarely diagnosed by biopsy if the lesion has invaded the mucosa.

Children with hypogammaglobulinemia may be found to have hyperplastic lymphoid follicles on small intestinal biopsy, as well as a diminished number of plasma cells and variable morphologic abnormalities. *G. lamblia* is often found in the duodenal juice of such children.

Third, in another group of disorders in which the lesion may also be patchy, the demonstration of an enteropathy is nonspecific (Table 69-3). However, the finding of mucosal abnormality is diagnostically useful in such patients because it indicates the presence of disease in the small intestine. Some disorders in this group (eg, CMSE) may be diagnosed by serial biopsy related to dietary protein withdrawal and challenge, although no pathologic fea-

TABLE 69-1 DISORDERS IN WHICH BIOPSY IS VALUABLE

MORPHOLOGIC FEATURES	
ABNORMAL	**NORMAL**
Celiac disease	Congenital alactasia
Agammaglobulinemia	Abetalipoproteinemia
Autoimmune enteropathy	Sucrase-isomaltase deficiency
Microvillous atrophy	
Attaching-effacing *Escherichia coli*	

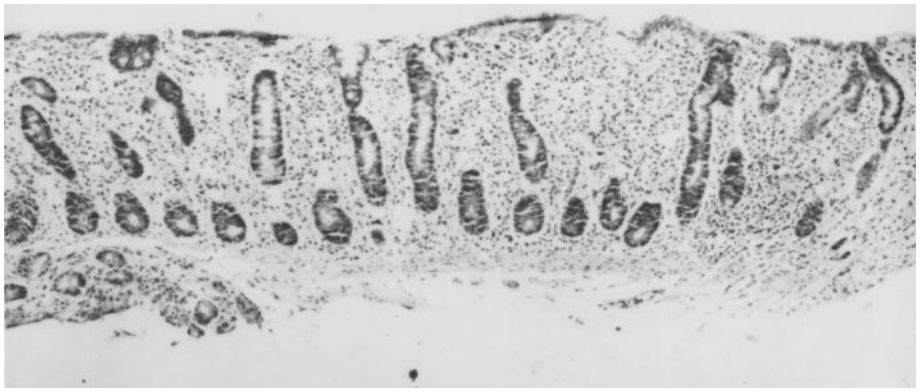

FIGURE 69-11 Light microscopic study: autoimmune enteropathy (male infant, 14 months), severe enteropathy (hematoxylin and eosin; ×120 original magnification). Reproduced with permission from Unsworth J, Hutchins P, Mitchell J, et al. Flat small intestinal musosa and autoantibodies against the gut epithelium. J Pediatr Gastroenterol Nutr 1982;1:503–13.

tures are specific for this disorder. It is unusual to perform challenges in CMSE, and diagnosis rests on a clinical response to an elimination diet with a return to normal histologic features before the reintroduction of cow's milk, when it is considered that the disease has resolved. AIDS must be added to the list of nonspecific abnormalities.

Finally, for completeness, a group of disorders in which small intestinal biopsy specimen is characteristically normal is listed in Table 69-4.

To conclude this section on the role of small intestinal biopsy in diagnosis, it is important to briefly review the diagnostic approach to a child thought to have small intestinal disease. This is summarized in Table 69-5.

The emphasis is not on demonstrating malabsorption (eg, steatorrhea or xylose malabsorption), as formerly was the case, but on pinpointing an anatomic abnormality of the small intestine, a structural abnormality of the small intestinal mucosa (ie, an enteropathy), or a specific infectious etiologic agent. Thus, barium studies, small intestinal biopsy, and stool examination are particularly important investigative tools. Hematologic investigations, such as a full blood count and serum folate levels, provide important evidence of a deficiency state that may need to be treated immediately or followed up as a marker of response to treatment. Radiologic studies are particularly important for diagnosing Crohn disease and congenital anatomic lesions of the small intestine.

LARGE INTESTINAL BIOPSY

Large intestinal pathology can be investigated using a variety of different types of biopsies of the bowel. For mucosal disease, mucosal biopsies taken during fiberoptic colonoscopy are employed. Suction rectal biopsies are suitable for the diagnosis of aganglionosis (Hirschsprung disease), but for other enteric neuromuscular diseases, full-thickness or seromuscular biopsies are required.

TABLE 69-2 DISORDERS IN WHICH BIOPSY MAY BE VALUABLE DIAGNOSTICALLY

Giardiasis
Strongyloidiasis
Small intestinal lymphangiectasia
Small intestinal lymphoma
Hypogammaglobulinemia

MUCOSAL BIOPSY TAKEN AT FIBEROPTIC COLONOSCOPY

Since its introduction in the early 1960s, the value of fiberoptic colonoscopy as a diagnostic procedure in adult patients has become well established. It is now clear that this is also a very useful technique in pediatric practice. One of its most useful aspects is in the provision of multiple large intestinal mucosal biopsies. The ileum can be entered in over three-quarters of cases with an experienced endoscopist,[61] affording an opportunity to assess the intactness of the mucosa and to study ileal histology. The availability of pediatric colonoscopy is restricted to pediatric gastroenterologic centers and is dependent on the availability of endoscopy skills. When these are available, this approach has largely overtaken the need for barium enema examination in the investigation of rectal bleeding and suspected chronic inflammatory bowel disease.[62]

The value of histologic assessment following full colonoscopy has been evaluated in the investigation of children when first presenting with inflammatory bowel disease.[61] Diagnosis of inflammatory bowel disease on morphologic criteria is possible in 85% of cases. Although sigmoid colonic and rectal biopsies can also confirm the diagnosis of inflammatory bowel disease in 65% of cases, over one-third would have been assigned to the wrong diagnostic category on the left-sided findings alone. This is particularly relevant in pediatric patients whose right colon is often affected without involvement of the rectosigmoid. Limited examination will also miss ileocecal Crohn disease and may miss multiple polyps. However, when colonoscopy is not available, rigid sigmoidoscopy must be used to obtain colonic and rectal biopsies.

The principal purpose of obtaining intestinal colonic biopsies in this context is for the diagnosis of chronic inflammatory bowel disease and Crohn disease in particular.

TABLE 69-3 DISORDERS IN WHICH BIOPSY RESULTS MAY BE ABNORMAL BUT ABNORMALITY IS NONSPECIFIC

Postenteritis syndrome	Tropical sprue
Cow's milk protein intolerance	Radiation enteritis
Transient-gluten intolerance	Drug-induced lesion, eg, by methotrexate
Soy protein intolerance	Protein-energy malnutrition
Intractable diarrhea syndrome	AIDS

TABLE 69-4 DISORDERS IN WHICH BIOPSY FINDINGS ARE NORMAL

Cirrhosis
Hepatitis
Exocrine pancreatic insufficiency
Toddler's diarrhea

However, there are other reasons, and Table 69-6 lists the indications for fiberoptic colonoscopy in children. The technique of colonoscopy is detailed in Chapter 67, "Gastrointestinal Endoscopy," and is not detailed here. An important diagnostic benefit from colonoscopy is the recognition of microscopic colitides that are apparent not on gross observation but only following histologic assessment of biopsies.

In recent years, rectal suction biopsy containing sufficient submucosa has replaced full-thickness biopsy for the diagnosis of Hirschsprung disease. This cannot be taken at routine colonoscopy and requires a special biopsy apparatus.[63] Full-thickness rectal biopsy or laparoscopic colonic seromuscular biopsies may still be required for the diagnosis of disorders involving the myenteric plexus or muscularis propria.[64–66]

Disorders Diagnosed by Large Intestinal Biopsy in Childhood

These disorders may be broadly grouped into inflammatory disorders and motility disorders. A general schematic approach to assessment of mucosal biopsies is recommended.[67] Questions to be asked are (1) if the tissue is normal or abnormal and (2) if changes suggest chronic inflammatory bowel disease whether they favor Crohn disease or ulcerative colitis. Alternatively, it is important to ask if the inflammation suggests an acute infective etiology or another form of inflammation, such as chronic granulomatous disease,[68,69] allergic colitis, tufting enteropathy involving the large bowel,[70] or intractable enterocolitis of infancy.[71]

In mucosal disease, the diagnostically important features to be assessed are the overall architecture, the cellularity of the lamina propria, the presence of a polymorphic neutrophilic or eosinophilic infiltrate, and epithelial changes. The histologic diagnosis of chronic inflammatory bowel disease in children specifically has been reported.[72,73] The principal features of histologic diagnosis are outlined below.

Mucosal Inflammatory Disorders. *Ulcerative Colitis*. This is a chronic intermittent disease that affects the large bowel mucosa. It is possible to make a suggested histopathologic diagnosis from biopsies taken at the initial presentation. Confident biopsy diagnoses can certainly be made in established disease. Sequential biopsies form part of the diagnostic workup and also monitor activity after treatment; symptomatic improvement may not be accompanied by endoscopic or histologic mucosal healing.[74] Ulcerative colitis usually commences in the rectum and may extend to involve the rest of the colon. Often inflammation may be absent or patchy in the rectum or sigmoid, particularly in the pediatric population.[75] Both the clinical picture and the histopathology may be considered in terms of three phases: active, resolving, and in remission (quiescent).[76]

TABLE 69–5 DIAGNOSTIC APPROACH TO CHILDREN THOUGHT TO HAVE SMALL INTESTINAL DISEASE

Initial assessment
Detailed case history
Physical examination
Analysis of centile charts for height and weight
Initial investigations
Full blood count and erythrocyte sedimentation rate in older child (? Crohn disease)
Serum and red cell folate
Antigliadin and antiendomesial antibodies
Stool culture for bacteria
Stool electron microscopy for viruses
Stool examination for *Giardia lamblia* and *Cryptosporidium*
Stool-reducing substances
Next stage
Small intestinal biopsy
Duodenal juice examination for *G. lamblia*
Bacterial culture for bacteria (anerobic and aerobic as indicated)
Barium follow-through
Gut autoantibodies
Response to elimination diet

Active Phase. The most specific feature is loss of crypt architecture with crypt distortion and branching (Figure 69-12). Polymorphonuclear neutrophils are present within the crypt epithelium, and there may be crypt abscesses (Figure 69-13). The epithelium shows varying amounts of degeneration and regeneration. There is mucin depletion and increased mitotic activity. The lamina propria shows capillary congestion and edema; it contains a heavy mixed inflammatory cell infiltrate. This is diffuse throughout the lamina propria and is composed of plasma cells, lymphocytes, and neutrophils. Eosinophils are also found and may be prominent, although the significance of this in relation to the natural history is not fully clear. Inflammatory cells may also be found in the submucosa; this may be associated with severe ulceration.

Resolving Phase. An important feature of ulcerative colitis is the fact that the biopsy appearances vary with time. In the resolving phase, the crypts remain distorted and branched; the surface may take on a villous appearance. Goblet cells reappear in the crypts but may be elongated. Inflammation in the lamina propria is reduced and may become focal; there is then a possibility of confusion with other disease, such as Crohn disease. There are few polymorphs at this stage.

TABLE 69-6 INDICATIONS FOR PROCEEDING TO FIBEROPTIC COLONOSCOPY IN THE PEDIATRIC PATIENT

Unexplained rectal bleeding
Bloody diarrhea in the absence of stool pathogens (with or without abdominal pain)
Abdominal pain associated with weight loss (with or without diarrhea)
Other features suggesting a diagnosis of chronic inflammatory bowel disease (eg, strictures, fistulae, disease activity extent as a guide to therapy)
Surveillance for malignancy (long-standing ulcerative colitis, polyposis coli, Peutz-Jehgers syndrome, Gardner syndrome)
Polypectomy

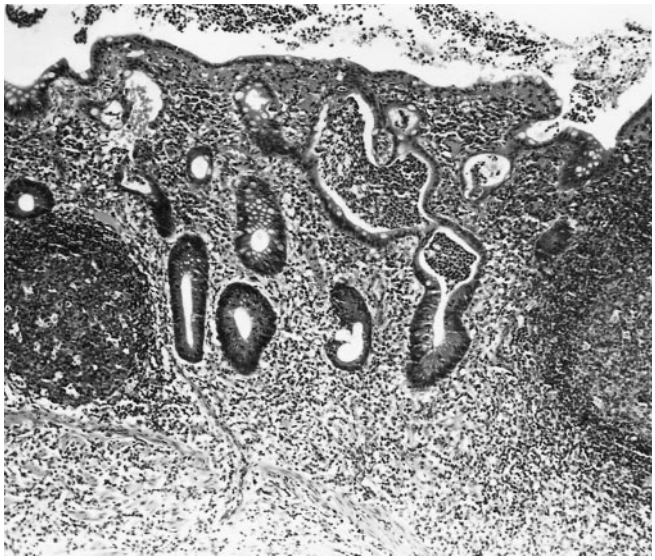

FIGURE 69-12 Chronic ulcerative colitis, active phase. Crypts are distorted and show mucus depletion; several contain neutrophils resulting in crypt abscesses. There is diffuse inflammation of the mucosa. Lymphoid follicles are also present (hematoxylin and eosin; ×100 original magnification).

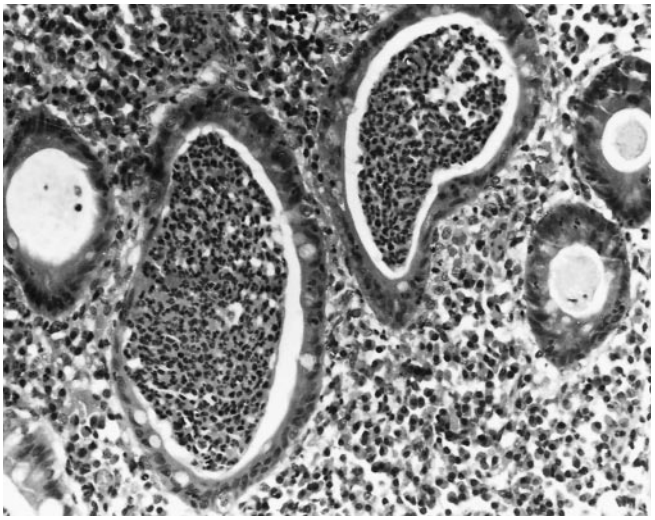

FIGURE 69-13 Chronic ulcerative colitis, active phase. High-power view showing crypt distortion, mucus depletion, and crypt abscesses. There is diffuse inflammation in the lamina propria (hematoxylin and eosin; ×250 original magnification).

Remission Phase. Here the crypts are atrophied and distorted. There is a gap between the muscularis mucosae and the crypt bases. Goblet cells are present, but there may be Paneth cell metaplasia and increased numbers of crypt endocrine cells.[77–79] There is no active inflammation; neutrophils are therefore absent.

Inflammatory Polyps (Pseudopolyps). These consist of granulation tissue, a mixture of glands and granulation tissue, or a tag of virtually normal mucosa. They are a frequent finding and indicate prior severe mucosal ulceration with irregular healing. Biopsy allows them to be distinguished from neoplastic polyps (adenomas).

Fulminant Acute Dilatation. This has been recorded in up to 13% of patients with ulcerative colitis in the early adult literature[80,81] but is probably much lower in pediatric practice today. The rectum is relatively spared, and the transverse colon is most severely affected. There are several misleading features in biopsies in this condition: inflammation may be transmural, and there may be fissuring ulceration; the crypt architecture is often intact, and inflammation may be mild.

Follicular Proctitis. Prominent lymphoid follicles are present, and there is an accompanying diffuse infiltrate of plasma cells and neutrophils. These features cause thickening of the mucosa. The crypt architecture is irregular. The differential diagnosis in this condition includes lymphoid polyps, malignant lymphoma, and lymphomatous polyposis.

Crohn Disease. Crohn disease may affect any part of the GI tract,[82–86] but most commonly it presents as regional ileitis,[87] ileocolitis, colitis, or perianal disease. It is characterized by its focal distribution and, unlike ulcerative colitis, often involves the full thickness of the bowel wall. The endoscopic appearances form an important part of the diagnosis in Crohn disease. The features include tiny aphthoid ulcers,[88] serpiginous ulceration, edema, linear ulceration ("cobblestoning"),[89,90] and, occasionally, inflammatory polyps. Areas of normal mucosa appear between abnormal areas and are thus termed skip lesions. Anal lesions consist of painless fissures, ulcers, fistulae, skin tags, and perianal abscesses.[91]

The crypt architecture and goblet cell population are usually preserved despite considerable inflammation (Figure 69-14).[92,93] However, there may be some crypt distortion close to areas of ulceration; such distortion may also occur in the early healing phase.[94] The inflammatory cell component consists of a mixture of lymphocytes, plasma cells, and polymorphs; their density varies across the biopsy.[95] Neutrophils are less conspicuous and more focal than in ulcerative colitis or infectious colitis, but crypt

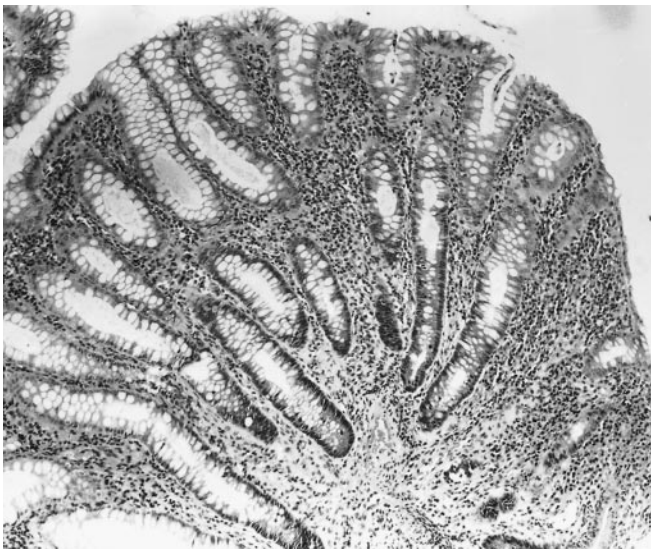

FIGURE 69-14 Rectal biopsy in Crohn disease. There is chronic inflammation in the lamina propria, but crypt architecture is well preserved (hematoxylin and eosin; ×100 original magnification).

abscesses may be found.[96] Small aggregates of lymphocytes occur adjacent to crypt bases.[97] Granulomas are also found; these are composed of collections of epithelioid histiocytes, multinucleate giant cells, and, frequently, a cuff of lymphocytes (Figure 69-15).

Granulomas occur throughout the bowel wall in Crohn disease and may be seen in both inflamed mucosa and endoscopically normal mucosa.[72,73,98] Microgranulomas consisting of clusters of histiocytes and small numbers of inflammatory cells also occur.[98] Confluent granulomas with florid central necrosis suggest a diagnosis of tuberculosis; however, a small focus of central necrosis may be seen in granulomas in Crohn disease.

The incidence of granulomas in biopsies is variable, and published figures in adults range from 0 to nearly 30%.[99–105] In the pediatric population with Crohn disease, epithelioid granulomas have been reported to be present in 36%[71] and 44%.[61] This is clearly an underestimate and is a question of sampling because in an analysis of 17 operative specimens from children with Crohn disease, 14 had noncaseating granulomas (82%) (see Figure 69-15).

Other features of biopsies in Crohn disease include aphthoid ulcers; these are seen as small areas of ulceration immediately over a lymphoid follicle.[97] Fissuring ulcers also occur; they penetrate down through the submucosa and are characteristic of Crohn disease. Fibrosing stenoses may also be found.[102] The muscularis mucosa may appear thickened.[103]

Biopsies in Crohn disease should include a portion of the superficial submucosa: this will often show "disproportionate inflammation"[94] with a mixed inflammatory cell infiltrate. This reflects the transmural nature of the inflammatory process in the disease. Granulomas in the submucosa are helpful in the diagnosis. The following features are considered to be the most helpful in the diagnosis of Crohn disease when granulomas are absent: the patchy nature of the inflammation, relatively little crypt distortion or goblet cell depletion, and the presence of basal lymphoid aggregates. Unfortunately, aphthoid ulceration and fissures are rare in biopsies.

No studies have yet been able to correlate specific features with disease activity.[104] As already mentioned, granulomas may occur in normal-appearing mucosa. Fibrosis in the submucosa and splitting up of the muscularis mucosae indicate long-standing disease. Granulomas have been claimed to indicate a favorable prognosis,[105,106] but not all studies are in agreement with this.[107,108] Ulceration and fissuring have been claimed to be indicative of a poor prognosis.[109] Overall, there appears to be no universally accepted prognostic microscopic feature.[94,110]

Infective Colitis. Infective colitis may be classified etiologically into bacterial, viral, protozoal, and fungal infections and infestation by helminths. The bacterial diarrheas cause the vast majority of diagnostic problems. *Salmonella* species, *Shigella* species, enteroinvasive *Escherichia coli*, and *Campylobacter* species may also produce similar histopathologic appearances, which have been termed infective biopsy pattern (Figure 69-16). It is important to be familiar with these histologic features so that patients with infectious disease are not mislabeled as having ulcerative colitis or Crohn disease. History of foreign travel is important to suggest more exotic infections; other clues may be the presence of numerous eosinophils or granulomas.

It is not usually possible from a biopsy to distinguish among the main causes of bacterial colitis.

Examination of the biopsy specimen indicates that the mucosa is widened by edema. Clusters of polymorphs are present throughout the biopsy but particularly in the more superficial portion and often adjacent to dilated capillaries or next to crypts. Polymorphs may be present between the crypt epithelial cells, and although crypt abscesses occur, they are less common than in active ulcerative colitis or Crohn disease.[111] Clusters of polymorphs also infiltrate

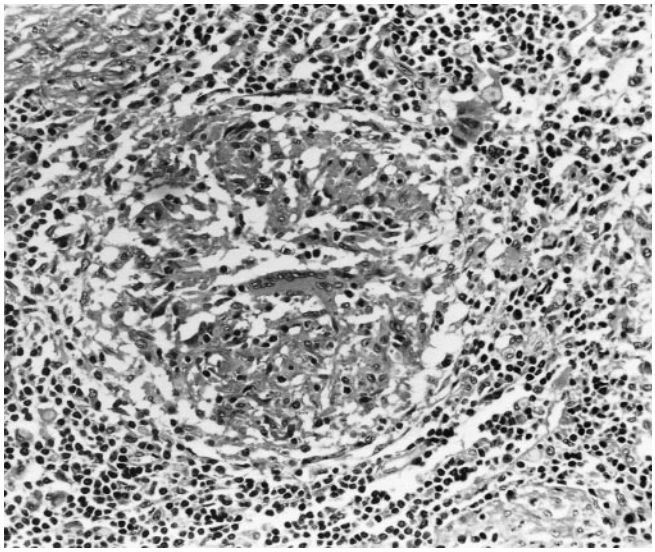

FIGURE 69-15 Crohn disease. A noncaseating granuloma in the submucosa (hematoxylin and eosin; × 250original magnification).

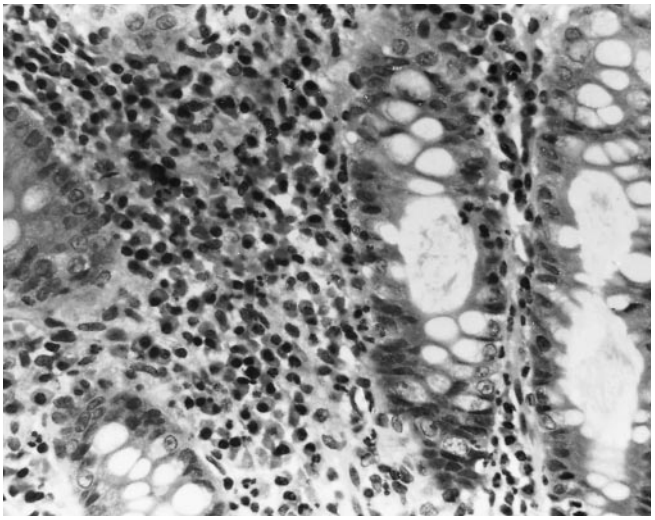

FIGURE 69-16 Infective colitis (*Campylobacter*). High-power view of a rectal biopsy showing patchy inflammation with neutrophil polymorphs in the lamina propria. One crypt is infiltrated by neutrophils (hematoxylin and eosin; ×400 original magnification).

between the cells of the surface epithelium. Although plasma cells and lymphocytes may be increased, this is often masked by edema, and polymorphonuclear neutrophils dominate the picture (see Figure 69-16).

The crypt pattern is regular, although the superficial crypt epithelium may show degenerative changes, and there is dilatation of luminal parts of the crypts (crypt "withering").[112,113] Mucin depletion and flattening of epithelial cells are also seen. Crypt destruction may be marked by a multinucleate giant cell. Other less specific abnormalities include luminal pus, margination of polymorphs, and capillary microthrombi.[114]

The above description represents a characteristic pattern and is most common in biopsies taken at the onset of symptoms or within the first 7 days.[112]

Differential Diagnosis of the Major Inflammatory Bowel Disease Entities. No single specific histologic feature is invariably present in one condition or absent from the others.[67] The concept of a spectrum of histologic appearances in chronic inflammatory bowel disease in childhood is useful and convenient for practical assessment.[72] This is particularly true in the early histologic appearance of children with Crohn disease when the definitive criteria may not be present. From the point of view of histologic assessment, particularly of mucosal biopsies, which are small in size, the histopathologist is faced with an apparent range of inflammatory changes falling within a continuous spectrum. This approach is not intended to imply that inflammatory bowel disease is generally a continuous spectrum of a single disease but reflects the difficulty of making a confident diagnosis by extrapolation from a very restricted sample of the organ in question. Although some findings may be highly suggestive of a particular diagnosis, in one study of children at first presentation of their illness, approximately one-third showed morphologic features suggestive of both conditions[61]—in particular, diffuse active chronic coloproctitis with crypt distortion suggestive of ulcerative colitis but also with granulomas and/or ileal disease too excessive for a backwash ileitis. It is not yet clear if these children will have a different natural history.

Key features of the various disorders are the following:

- *Ulcerative colitis*. There is crypt distortion, villous surface, goblet cell depletion, prominent crypt abscesses, and diffuse predominantly plasma cell infiltrate of the lamina propria.
- *Crohn disease*. Granulomas are present (25–28% of biopsies). Crypts remain well aligned with little mucin depletion despite a moderate inflammatory cell infiltrate. The infiltrate is often patchy. Basal lymphoid aggregates are helpful. Crypt abscesses and cryptitis are less constant than in ulcerative or infective colitis. There are microgranulomas (focal collections of inflammatory cells including histiocytes) and definite patchy inflammation.
- *Indeterminate colitis*. Although this term was first used in the histologic assessment of colectomy specimens, it has now gained general acceptance for mucosal biopsies.[67,72,115] It is used when features of chronic inflammatory bowel disease are present, but distinction as to type is not possible.
- *Infective colitis*. Crypts remain aligned but show degeneration. Polymorphs are the most conspicuous inflammatory cells; they cluster in the lamina propria and migrate between crypt epithelial cells, particularly in the superficial mucosa. Plasma cell infiltrate is light to moderate. Edema is prominent.

Other Recognized Abnormalities. The most common abnormality under this heading is a cellular infiltrate in the lamina propria with regular crypt architecture and variable goblet cell depletion. In some biopsies, a focal polymorph infiltrate in the lamina propria may be seen. A mild increase in plasma cells and lymphocytes may be an accompanying feature. The changes can be interpreted only after consideration of the clinical data (eg, Crohn disease may be suggested if there is evidence of disease at another site).[94] Some specific features that can easily be overlooked include the following:

1. *Microgranulomas in Crohn disease*.
2. *Spirochetosis*. This represents infection of colorectal epithelium by spirochetes that belong to the genus *Borrelia*. Histologically, this is seen as a basophilic fringe along the apical border of surface epithelial cells. It is not clear whether infection produces symptoms.
3. *Amoebae*. *Entamoeba histolytica* may cause large intestinal infection, which may closely resemble chronic inflammatory bowel disease in childhood.[116] Ulcers occur, which may result in perforation of the bowel wall. Amoebae are found on or just beneath the surface of ulcers, but in severe cases, they enter the inflamed bowel wall and may be seen within blood vessels. Diastase-PAS is a useful staining method for demonstrating them in histologic preparations.
4. *Cytomegalovirus*. This viral infection may occur in immunosuppressed children both in disease states such as AIDS or following organ or bone marrow transplant. Inclusions occur in endothelial cells, fibroblasts, and macrophages. More rarely, they are found in epithelial cells. Immunocytochemistry and polymerase chain reaction will increase the sensitivity of viral detection within the tissue.
5. *Chronic granulomatous disease*. This rare disorder is characterized by recurrent infections with catalase-positive organisms; the patients' neutrophils are unable to kill the organisms. Gastrointestinal involvement is well recognized; there may be narrowing of the gastric antrum owing to local granulomas, and perianal fistulae may occur. Clinically, the disease may mimic Crohn disease or tuberculosis, especially when there is ileal involvement with intestinal obstruction.[117,118] Histologically, the appearances consist of paucity of neutrophils and the presence of mainly eosinophilic inflammatory infiltrate, eosinophilic crypt abscesses, and pigmented (often reddish crystalline inclusions) macrophages in

the lamina propria of the mucosa.[68,69] Granulomas, if present, are exuberant

6. *Allergic colitis*. One manifestation of food protein allergies, in particular with cow's milk protein intolerance, is diarrhea and abdominal pain; perianal disease and constipation have also recently been reported.[119] Changes may be seen in the esophagus and duodenum and also within the colon. On endoscopy, the mucosa may appear edematous; histology shows intact architecture but large numbers of eosinophils in the lamina propria. Eosinophils may also show degranulation or infiltrate the crypt and surface epithelium.
7. *Microscopic colitis*. Histologic assessment allows separation into collagenous (a thickened [> 10 μm] collagenous band under the surface epithelium) and lymphocytic (increased intraepithelial lymphocytes) variants. Both are more common in adults and are associated with watery chronic diarrhea. This condition stresses the importance of performing biopsies in the absence of overt endoscopic abnormalities.
8. *Autism-associated colitis*. A number of children with autism and nonspecific GI symptoms have been described in whom there have been changes on endoscopy and on histology. This has included prominent lymphoid hyperplasia within the ileum and very focal active inflammation with more widespread chronic inflammation within the colon and rectum. The features are not thought typical for inflammatory bowel disease, but the findings have met with some controversy.[120]
9. *Intractable ulcerating enterocolitis of infancy*. This form of familial disorder was first described over a decade ago by Sanderson and colleagues.[71] The onset of disease is within the first few months from birth, and the condition responds poorly to immunosuppressive therapy requiring colectomies. Histologic appearances include deep well-circumscribed ulcers, which penetrate into the submucosa and reach even the muscularis propria. The mucosa between the ulcers shows only a mild increase in chronic inflammatory cells. The condition is associated with Epstein-Barr virus–related lymphoid proliferations, and patients can develop B-cell lymphomas (A. D. Ramsay, personal communication, July 2003).

Disorders of Motility

These are due to abnormalities in the enteric nervous system or defects in the enteric musculature, resulting in functional intestinal obstruction. Of these, Hirschsprung disease is the most common, with an incidence of about 1 in 4,500 live births. The condition results from congenital aganglionosis (absent enteric nervous system) of a variable length of distal bowel but can affect the entire colon and extend to involve the small intestine and beyond.[121]

Care should be taken with the use of the term intestinal neuronal dysplasia because it means different things to different people.[122]

Other neural disorders include hypoganglionosis and hyperganglionosis. The latter may be seen proximal to the aganglionic segment in some patients with Hirschsprung disease.[123] Only aganglionosis can be diagnosed on a suction rectal biopsy. For the diagnosis of hypo- or hyperganglionosis, the myenteric plexus must be examined.[124]

Suction Rectal Biopsy. *The Biopsy.* Ideally, three biopsies should be obtained 2 to 5 cm above the pectinate line and should include macroscopically obvious submucosa. The middle biopsy is processed into paraffin wax for 150 HE-stained 3 to 4 μm–thick serial sections to allow identification of any neurons present. A large number of sections must be examined to ensure that no neurons are missed even if the biopsy is taken from the physiologically hypoganglionic segment of the lower rectum.[125]

The other two are snap-frozen and cryostat sectioned alternately for HE (5–7 μm thick) and "spares" (10–12 μm thick). Two of the spares that include the most submucosa are incubated for acetylcholinesterase (AChE) activity by the standard method[126] or if the result is required urgently by the rapid method.[127]

In the case of an obstructed neonate, there is an urgency to confirm or exclude the diagnosis of Hirschsprung disease. The biopsies are usually taken not far from the dentate line. In this instance, the biopsies are snap-frozen, and serial cryostat sections are cut immediately and stained for HE. If no neurons are identified and the sections contain mucosa, muscularis mucosa, and submucosa, adjacent sections are cut and incubated for AChE activity initially using the rapid method. If the result is ambivalent, the standard method is applied on further sections. The remainder of the biopsy is fixed and processed into paraffin wax, and, subsequently, serial sections (as above) are cut and stained for HE.

Histologically normal rectal biopsy contains clusters of neurons in the submucosa in contrast to one from a patient with Hirschsprung disease, which has no neurons but has enlarged submucosal nerve trunks. However, biopsies taken from the physiologically hypoganglionic lower rectum close to the anal canal also have large nerves, particularly in association with large blood vessels. In this situation, one must not attempt to make the diagnosis of Hirschsprung disease. Of course, if an obvious neuron is found, then the diagnosis can be excluded. It is always wise to state, "Hirschsprung disease is excluded at the level examined" because the biopsy may have been taken above the aganglionic segment. Is not possible to know accurately how far from the anal verge the biopsy comes. On occasion, surgeons claim that the biopsy was performed at least 5 cm from the dentate line, but on histology, the biopsy contains anal skin instead of rectal mucosa.

AChE *Activity.* AChE-positive fibers are sparse and fine in the muscularis mucosae and lamina propria of the mucosa in normal rectum. There are, however, variations within normal limits. In Hirschsprung disease, at any age, there is an obvious increase in thick, knotted, crossing AChE-positive fibers in the muscularis mucosae and thick nerves in the submucosa, with no neurons present. With experience, it is possible to distinguish neurons from nerves on the AChE preparation, but it is safer to rely on the HE to do this. In an older baby with Hirschsprung disease, there is also an increase in the lamina propria in thick

AChE-positive fibers, which run not only vertically but also horizontally.

If no obvious neurons are found in the HE-stained cryostat section and the AChE pattern is not typical of Hirschsprung disease, it is vital to examine the 150 serial paraffin sections to be certain that the specimen is indeed aganglionic.

In rare cases of total colonic aganglionosis, AChE preparation may not be helpful. In these patients, there is no increase in AChE-positive fibers, and thick submucosal nerve trunks may also be absent. As many as possible serial HE-stained paraffin sections must be examined to identify any neurons present.

***Age of the* Patient.** In the newborn, the submucosal ganglia appear as small clusters of immature neurons, which are less easy to recognize than the larger neurons in an older baby or a child on an HE-stained section. Not differentiating the hematoxylin before the eosin helps, and if no neurons are seen, examination of the AChE pattern in the muscularis mucosae allows correct diagnosis.

Intestinal Ganglioneuromatosis. One serious condition one should be aware of is transmural intestinal ganglioneuromatosis. These appearances can be evident in the submucosa even in a suction rectal biopsy and consist of profound proliferations of neural tissue (neurons, supporting cells, and nerves) that appear as thickened nerve trunks embedded with mature nerve cells. If there is a suspicion of ganglioneuromatosis, this warrants further investigations to exclude multiple endocrine neoplasia type IIB (MEN IIB). This involves germline M918T and/or A883 F mutation analysis of the *RET* proto-oncogene.[128] Children with intestinal ganglioneuromatosis and the above-mentioned molecular diagnosis of MEN IIB inevitably develop medullary thyroid carcinoma. In our experience, monitoring the calcitonin concentrations and scanning for adrenal and thyroid masses are not sufficient because microscopic medullary thyroid carcinoma can be present without raised calcitonin concentrations, even with pentagastrin stimulation or without identifiable masses on imaging. A prophylactic thyroidectomy is recommended, as well as continued surveillance of the adrenal glands for evidence of pheochromocytoma.

Full-Thickness Intestinal and Seromuscular Biopsies. Identification of other intestinal neuromuscular disorders cannot be made on suction rectal biopsies. These require larger full-thickness bowel samples taken at the time of raising a stoma. However, seromuscular biopsies, if well oriented and of sufficient size, may be helpful in some myopathic disorders (fibrosis and/or abnormal contractile protein profile),[129] especially when using special stains and immunohistochemistry and acquired inflammatory conditions (ganglionitis or leiomyositis).[64,65,130]

Caution should be exercised when interpreting AChE preparations of biopsies sometimes taken from stoma sites, particularly from ileostomy sites. Small bowel normally has many AChE-positive fibers in the lamina propria, and AChE preparations on biopsies taken proximal to the splenic flexure may also be unreliable.[131]

HE-stained frozen sections of intraoperative seromuscular biopsies are used for positioning the stoma.

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CHAPTER 70

GASTROINTESTINAL MANOMETRY: METHODOLOGY AND INDICATIONS

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One of the most important functions of the gastrointestinal tract is to provide energy and nutrition. To accomplish this, the gastrointestinal tract has evolved not only specific functions for digestion and absorption but also complex mechanisms to allow for the ingestion of nutrients, their transport through different specialized areas, and the expulsion of unused portions at times when it is socially acceptable. The main result of this movement is the aboral transport or "propulsion" of gastrointestinal contents, and it results from the complex interaction between the muscles, the myenteric plexus, the peripheral nervous system. and the brain (see Chapter 4, "Motility").

Each area of the gastrointestinal tract has a specific pattern of motility. These patterns have been characterized for the esophagus, antroduodenum, jejunum and ileum, sphincter of Oddi (SO), colon, and anorectum. It has been shown that alterations in those patterns will produce functional dysfunction and clinical symptoms. By studying these alterations, it is often possible to determine if the problem that the patient has is related to motility dysfunction and if the dysfunction is occurring in the muscle or the intrinsic or extrinsic nerves.

When the symptoms of the patient suggest a motility disorder, careful exclusion of anatomic, mucosal, or metabolic disorders needs to be accomplished before motility studies are undertaken (Table 70-1).[1–3] The exclusion of an anatomic lesion is the initial and most important step, and the usual techniques that are used for this purpose are mainly radiologic or occasionally endoscopic and are reviewed in different chapters in this book.

Once a decision has been made that the problem is related to gastrointestinal motility dysfunction, transit through different segments can be evaluated, and the specific contractile or electrical activity may be studied (see Table 70-1). Research that is used to study transit includes mainly scintigraphic methods, although other techniques, such as different markers or ultrasonography, have been used. These techniques are discussed in other sections of this book. The main focus of this chapter is on tests that have been designed to study the myoelectric and motor phenomena of the gastrointestinal tract.[1]

GASTROINTESTINAL MANOMETRY

Gastrointestinal manometry has evolved during the past years and has changed from being a research technique to becoming a useful diagnostic tool.[1] The role of gastrointestinal manometry has been defined more clearly for

TABLE 70-1 APPROACH TO THE PATIENT WITH SUSPECTED MOTILITY DISORDER

1. Exclude anatomic problems (*the most important initial step, and it should be undertaken in all patients*)
   - Radiography
     - Plain films, upper gastrointestinal series with small bowel follow-through, barium enema, endoscopy
2. Exclude mucosal or metabolic disorders
   - Endoscopy and biopsy, laboratory tests (metabolic, endocrine, etc)
3. Evaluate transit (*provides actual information on how effective the motility is*)
   - pH probe/impedance
   - Scintigraphy
     - Gastric emptying
     - Esophageal emptying
     - Gallbladder emptying
     - Small bowel–colon transit
   - Barium
     - Videofluoroscopy barium swallow
     - Esophageal emptying
   - Other studies to evaluate transit
     - Marker-perfusion studies
     - Oroanal transit—color markers, radiopaque markers
     - Orocecal transit—lactulose breath test
     - Colonic transit—radiopaque markers
     - Ultrasonography
     - Breath tests
4. Evaluate contractile activity (*provides information on contractile activity and allows the detection of problems in smooth muscle, intrinsic or extrinsic nerves*)
   - Gastrointestinal manometry
5. Evaluate electrical activity (*provides information on the myoelectrical activity*)
   - Electrogastrography, electromyography
6. Establish etiology and other associated problems
   - Is this a primary or a secondary motility problem?
   - Exclude systemic illness (endocrine, connective tissue disease, metabolic, etc)
   - Associated abnormalities (autonomic dysfunction, muscle/nerve abnormalities, etc)

anorectal and esophageal manometry, and it has become better defined for small bowel and colonic motility.[1] In general, the manometric evaluation may detect aberrations that may be clinically insignificant,[4,5] and care needs to be exerted to avoid overinterpretation.[5] It is also important to remember that a dichotomy frequently exists between the use of manometry as an investigation technique or as a useful clinical test.

There are two main methods to perform gastrointestinal manometry. One requires the use of water perfusion with low-compliance systems, and the other uses miniature strain gauge pressure transducers mounted within thin catheters.[1] Most laboratories have water-perfused systems, in which the catheter is connected indirectly to a physiograph or computer by a series of transducers. The pressure is recorded from a predetermined opening in the catheters. The catheter is perfused with a pneumohydraulic pump at a predetermined rate, and pressure changes in the orifices are transmitted to pressure transducers with the use of low-compliance capillary tubing. The hydraulic capillary infusion system achieves high-fidelity recording of intraluminal pressure at infusion rates that go from 0.1 to 0.4 mL/min per port. Some disadvantages are that the system may not accurately reflect when measuring transient high-pressure events, the size of the catheters, and the amount of perfusate that can represent a large fluid bolus in small children and premature infants.

Solid-state catheters have strain gauge pressure transducers incorporated into specially designed catheters. The information is captured by digital recording systems and is later downloaded into computers for analysis. The main advantage of the solid-state catheter is that it does not have the risks associated with the perfusion, as well as the fact that it allows the performance of prolonged ambulatory studies. The main limitations have been the cost and the fact that it requires a minimum-size catheter, so these catheters cannot be used in small infants or children.

Independently of the method used, gastrointestinal manometry provides direct evidence about the contractile events of the organ that is being studied. Even though the study of gastrointestinal motility in children is similar to the study in adults, the performance of those studies in the pediatric population has certain important characteristics that make this more challenging. The first is related to the developmental aspects, the second to a paucity of studies in normal controls, and the third to technical difficulties.

The developmental aspects of gastrointestinal motility have been discussed in detail in previous chapters, but it must be remembered that any manometric findings need to be placed in that context before a study is considered abnormal.[1,6]

The lack of normal controls has been another important limiting factor for the establishment of normal motility patterns in children. This lack of control information can make the interpretation difficult and subjective and, as mentioned, may lead to overinterpretation.

Finally, there are special technical aspects that are unique to the performance of gastrointestinal manometry in children. Some are related to the size of the catheters, the amount of fluid delivered with the perfused systems, or the lack of cooperation that may be found in younger children.[1] The size of the catheters can be a limiting factor because the standard manometric catheters have a diameter of 4.5 to 5 mm. The recent development of silicone extrusion tubing has allowed the creation of very small catheters that may include sleeves.[6,7] Studies have shown that manometric channels with diameters of 0.35 mm have acceptable fidelity and pressure increase rates with perfusion rates as low as 0.02 mL/min.[6] Multilumen catheter assemblies that measure less than 2 mm have been developed,[6] including the use of sleeves.[6–8]

APPROACH TO THE PATIENT

It is important to remember that for manometric studies to be successful, there must be a certain degree of patient cooperation. The understanding and degree of cooperation will vary according to the developmental age and previous experiences of the child. Results and patterns are difficult to interpret when there is crying or movement artifact, which can obscure pressure tracings.[1] Age and developmentally appropriate techniques should be used. The parents should be allowed to stay during the tests, and they can be an important resource for the child. The interaction between the parents and the children can be observed and can be important in understanding the child's symptoms.[1]

Young children may require sedation for the performance of the studies.[1] Medications that have been employed principally include chloral hydrate and midazolam. There are limited data on their effect on motility function. Studies have found no difference in esophageal or rectal manometry with chloral hydrate[9] or midazolam.[10]

SPECIFIC GASTROINTESTINAL MANOMETRY STUDIES

Recently, a pediatric task force of the American Motility Society (AMS) established minimum standards for the performance of manometric studies in children.[1] Standards for esophageal, antroduodenal, colonic, and anorectal manometry were published.[1]

Esophageal Manometry

Esophageal manometry is the gold standard for the diagnosis of primary motor disorders of the esophagus (Table 70-2).[4,11,12] It is most frequently performed in children with dysphagia who have no evidence of anatomic obstruction, and the clinical use of esophageal manometry is in defining the contractile characteristics of the esophagus.[1]

There are three functional regions of the esophagus: the upper esophageal sphincter (UES), the esophageal body, and the lower esophageal sphincter (LES). Both sphincter regions have a resting tone and relax in response to swallowing or other stimuli, and the body has contractions that propagate (Figure 70-1).

The UES is striated muscle that is maintained closed between swallows by tonic stimulation of the somatic nerves. It exhibits marked radial and axial asymmetry, with

TABLE 70-2 INDICATIONS FOR MANOMETRY STUDIES

Esophageal manometry
Esophageal dysfunction that is not explained by anatomic or well-defined problems
Dysphagia, odynophagia
Diagnosis of achalasia and other primary esophageal motor disorders
To support the diagnosis of connective tissue diseases or other systemic illness
Evaluation of patients with achalasia post-treatment and recurrent symptoms
Noncardiac chest pain
Patients with gastroesophageal reflux in whom the diagnosis is not clear (to exclude primary motility disorders)
Before a fundoplication when a severe motility disorder is suspected
To localize lower esophageal sphincter before pH probe placement in patients with abnormal anatomy (eg, hiatal hernia)
Anorectal manometry
To diagnose a nonrelaxing internal anal sphincter
To diagnose pelvic floor dyssynergia
To evaluate postoperative patients with Hirschsprung disease who have obstructive symptoms and to evaluate the effect of botulinum toxin
To evaluate patients with fecal incontinence
To evaluate postoperative patients after imperforate anus repair
To decide if the patient is a candidate for biofeedback therapy
Antroduodenal manometry
To establish the presence of pseudo-obstruction
To classify pseudo-obstruction into myopathic or neuropathic forms
To exclude a motility problem as the basis of the patients' symptoms; showing normal findings in children with "apparent intestinal failure"
Evaluation of unexplained nausea and vomiting
To distinguish between rumination and vomiting
To exclude generalized motility dysfunction in patients with dysmotility elsewhere (eg, before colectomy)
Indicated in patients with pseudo-obstruction being considered for intestinal transplant
May be useful to predict outcome after feeding or after drug use in patients with pseudo-obstruction
May suggest unexpected obstruction
Colonic motility manometry
In the evaluation of selected patients with intractable constipation because it can be helpful to differentiate functional fecal retention from colonic pseudo-obstruction[78]
Evaluation of children with pseudo-obstruction to establish presence of colonic involvement and to characterize the relationship between motor activity and persistent symptoms
To establish the pathophysiology of persistent symptoms in selected children with Hirschsprung disease, imperforate anus, and other colorectal problems
To assess colonic motor activity prior to intestinal transplant

Adapted from DiLorenzo C et al.[1]

greater values anteriorly and posteriorly than laterally.[4] It measures 0.5 to 1 cm at birth and increases in length to 3 cm in the adult.[13] UES relaxation occurs with swallowing. The manometric study of the UES has been difficult because the brisk movement of the larynx and sphincter is discordant with the movement of the intraluminal recording device. With the use of systems that can record changes greater than 400 mm Hg/s or sleeve devices, accurate measurements have become more feasible.[4] In adults, there is a wide range of reported UES pressure measurements, from 40 to 193 mm Hg, depending on the method used.[4] The information in normal children is very limited. It was reported to vary from 18 to 44 cm H_2O in one study.[14]

The esophageal body varies in length depending on the age of the patient. It is composed of striated and smooth muscle. In adults, approximately the upper 5% is exclusively striated muscle, the middle 35 to 40% is mixed, and the distal 50 to 60% is entirely smooth muscle.[15] No similar information is available in children. The resting pressure is usually lower than the gastric pressure and varies with respiration. Different types of esophageal contractions can occur in the esophageal body. *Primary peristalsis* is the one that occurs after swallowing. It is an orderly and progressive series of peristaltic contractions that begins in the pharynx and advances aborally (see Figure 70-1).[4,13] *Secondary peristalsis* can be elicited in response to luminal distention with air, liquid, or a balloon. *Tertiary contractions* consist of random, spontaneous, usually simultaneous, nonperistaltic contractions.

During primary peristalsis, the contractions usually progress at a speed of 2 to 4 cm/s, and there is a closely coordinated process between the UES and the LES. The mechanical effect is a stripping wave that milks the esophagus clean from the proximal to the distal end.[4] In general, a pressure complex in the distal esophagus with an amplitude < 35 mm Hg is considered hypotensive,[4,16] whereas contractions > 180 mm Hg are considered hypertensive.[4,16] Based on simultaneous manometric and videofluoroscopic studies, a cutoff of 30 mm Hg is now used to separate effective from ineffective peristalsis.[15,17] Failed peristalsis can occur in 4 to 15% of swallows in normal adult volunteers.[16] The typical duration of peristaltic contractions is around 4 seconds,[16] and double-peak contractions can occasionally occur in normal controls.[16] No similar information is available in healthy children.

The LES is tonically contracted and found at the distal end of the esophageal body (see Figure 70-1). LES pressure varies depending on the individuals and the methods used. Wide ranges have been reported. For example, Hillemeier and colleagues reported a mean LES pressure of 22.4 ± 4.7 mm Hg,[18] Cucchiara and colleagues reported 15 ± 2 mm

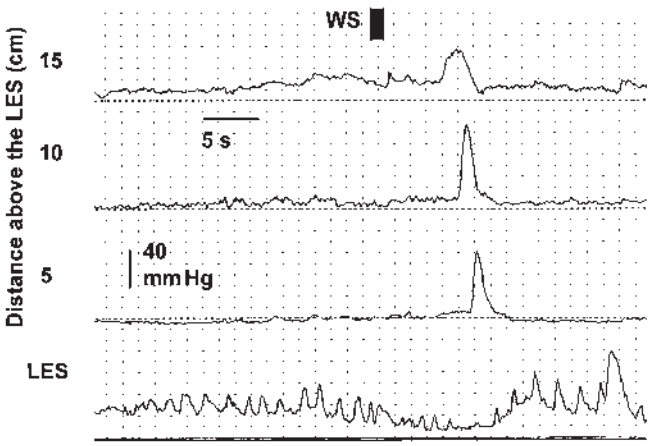

FIGURE 70-1 Normal esophageal manometry. The tracing shows the normal response to a wet swallow (WS). A peristaltic wave traveling aborally from 15 cm above the lower esophageal sphincter (LES) to the lower esophagus can be observed. Also, a normal LES relaxation after swallowing can be noted.

Hg,[19] and Moroz and colleagues reported 29.1 ± 2.4 mm Hg.[20] In adults, LES pressure varies from 10 to 45 mm Hg.[15] The LES is also asymmetric, with maximal pressures in the left lateral aspect. LES relaxation usually occurs with swallowing (see Figure 70-1), although it has been shown that it can also occur transiently when no swallowing is occurring.[15] An increased amount of transient relaxations has been reported both in adults and children with gastroesophageal reflux (GER).[15,19] The measurement of LES pressure is always performed relative to intragastric pressure. LES relaxation needs to be coordinated for more than 90% of wet swallows and complete with a drop to intragastric pressure. Different methods that have been used to measure basal sphincter pressure include either the midrespiratory or end-expiratory points. The midexpiratory pressure is the mean pressure at the midpoint of amplitude of the phasic respiratory component, whereas the end-expiratory pressure is when the tonic component is used alone.[4]

The manometry catheter is usually placed nasally, although, if necessary, it can also be placed orally, particularly in premature infants. In older children, nasal topical anesthesia with topical cocaine or viscous lidocaine is frequently used. Medications known to affect gastrointestinal motility (eg, prokinetics, anticholinergics, narcotics) are held for 48 hours before the procedure, and children are asked to fast for 4 to 6 hours, depending on the age and the need for sedation.

There is no standardized protocol to perform esophageal manometry in children. Adding to the difficulties is the fact that esophageal length varies with age, making the adoption of a standard catheter that can provide all of the information impossible. The pediatric task force of the AMS recommended the use of the slow pull-through technique.[1] Usually, detailed analyses of the LES and of the esophageal body are then done.[1] Even though UES measurements can be obtained, it is not clear that manometric findings are sensitive enough to have a clear impact on patient management.[4]

The responses to swallowing are observed. If possible, a swallowing marker is used, although, particularly in young children, careful observation and manual recording are employed. It is preferable to do "wet" swallows of water at room temperature (approximately 1 mL in infants and 3 to 5 mL in older children).[1] It has been shown that swallows with water give a more consistent peristaltic response than those that occur only with saliva.[16] In a typical study, 10 wet swallows are evaluated.

In young children, it is difficult to obtain swallows, and different techniques have been employed. Gently blowing air in the child's face (the Santmeyer reflex) may induce swallowing in young infants and neurologically abnormal children.[21]

In some centers, sleeve catheters are used to assess the LES. The sleeves are more accurate because they straddle the sphincter over 3 to 5 cm. Esophageal peristalsis is then measured by positioning the catheter at different segments of the esophageal body and using wet swallows. The amplitude, duration, and peristaltic characteristics of the contractions are measured.[16]

The UES is evaluated at the end. Techniques to measure it are the same as for the LES, although the recording speed may need to be increased. The UES relaxes to baseline, and the relationship between pharyngeal contractions and UES relaxation is determined. The clinical utility of UES/pharyngeal measurements is the least established.

Developmental Aspects. Recent advances with the use of microperfusion techniques and catheter size have allowed the study of even small premature babies.[6,8,22] It has been shown that in premature infants 26 to 33 weeks gestational age, esophageal pressure waves triggered by dry swallows were predominantly peristaltic (84%) in propagation sequence.[6] Swallow-unrelated pressure waves were usually nonperistaltic, and the researchers concluded that in infants 26 weeks or older, the control of swallow-induced peristalsis is fully developed.[6] All had tonic LES contraction with a mean resting pressure of 13.6 ± 4.2 mm Hg (range 5.0 ± 4.1 to 20.0 ± 4.8 mm Hg). In all infants, the LES relaxed in response to pharyngeal swallows. The duration of LES relaxation was 5.8 ± 3 seconds, and transient LES relaxations occurred an average of 2.6 ± 1.6 times per study. Those patterns are similar to the ones described in older premature infants.[23] Premature infants > 33 weeks of age had a mean LES pressure of 20.5 ± 1.7 mm Hg preprandially and 13.7 ± 1.3 mm Hg postprandially. It has also been shown that the patterns of LES relaxation were similar to those seen in healthy adults.[8]

Clinical Significance. Esophageal motility is mandatory for the diagnosis of primary motility disorders (Table 70-3) (see Chapter 26, "Other Motor Disorders").[1,2,4]

Esophageal motility remains the study of choice to make the diagnosis of achalasia.[1] Four manometric findings are characteristic of achalasia, as shown in Table 70-3 (see Figure 26-3 of Chapter 26).[24,25] The absence of peristalsis is the hallmark of the disease. Even though most patients with achalasia have incomplete or absent LES relaxation, the LES may occasionally show complete relaxation.[25] Manometry also provides quantitative information about the severity of the achalasia and the response to treatment.[1]

Esophageal manometry allows the diagnosis of diffuse esophageal spasm, nutcracker esophagus, or nonspecific esophageal motility disorders. The term nonspecific esophageal motility disorders has been used to describe manometric findings that are abnormal but not diagnostic of any established motility disorder.[26] Manometric features include apersitaltic, repetitive, or multipeaked contractions; low-amplitude contractions; intermittent segmental contractions; and prolonged contraction duration.[27]

Esophageal manometry is indicated to assess esophageal function in children and adolescents with dysphagia, odynophagia, and chest pain of noncardiac origin.[1]

Esophageal manometry can also be used to locate the LES for pH probe placement, particularly in patients with abnormal anatomy, such as a hiatal hernia.

Manometry has no role in the diagnosis of GER, and the exact role of esophageal motility in the preoperative evaluation of children is not clear. The only clear role it has

TABLE 70-3 DIAGNOSIS OF PRIMARY ESOPHAGEAL MOTOR DISORDERS

Achalasia* (four manometric findings are characteristic of achalasia[24,25])
Increased LES pressure
Absence of esophageal peristalsis (the hallmark of the disease; see Figure 70-3)
Incomplete or abnormal LES relaxation
Elevated intraesophageal pressure compared with intragastric pressure
Diffuse esophageal spasm†
Repetitive, simultaneous (nonperistaltic) contractions, at least 20% of wet swallows
Periods of normal peristaltic sequences
Alterations in the contraction waves (repetitive, increased duration and amplitude), although there are patients who can have normal amplitude
A normal LES in most patients, although incomplete LES relaxation or a hypertensive sphincter has been described[166–168]
Nutcracker esophagus‡
Increased distal peristaltic amplitude (180 mm Hg)
Increased distal peristaltic duration (> 6 s)
Nonspecific esophageal motor diosrders§[26,27]
Low-amplitude contractions (< 30 mm Hg)
Triple-peaked waves, spontaneous isolated contractions, retrograde contractions
Prolonged contractions (> 6 s)
Aperistalsis or nontransmitted contractions (during > 20% of wet swallows)
Simultaneous contractions (> 30% of wet swallows)

LES = lower esophageal sphincter.
*To make the diagnosis of achalasia, absence of esophageal body peristalsis is necessary; other criteria are often fulfilled but are not required.
†Primary finding is uncoordinated motility.
‡Primary finding is increased distal peristaltic amplitude.
§Abnormal manometric patterns that do not fit a defined entity.

in the diagnosis and medical management of GER is when there is uncertainty about the correct diagnosis and a primary motility problem such as achalasia is suspected.[4] In general, when used in the preoperative evaluation of patients with GER, it has not been shown to predict outcome.[4] The role that preoperative esophageal peristalsis plays in the development of dysphagia after fundoplication has been controversial.[28] Studies comparing preoperative patients with or without esophageal dysmotility have shown no difference in the prevalence of dysphagia after the operation.[28] At present, it can be concluded that insufficient data exist to evaluate the benefit of the routine preoperative assessment of peristaltic function.[4] However, it may have a role when there are severe preoperative dysphagia or atypical symptoms or when severe dysmotility is suspected. In children, the latter problem is commonly found in patients with scleroderma or tracheoesophageal fistulas,[29,30] in which a fundoplication may create a functional obstruction in a dysmotil esophagus.

Manometry may be useful in the diagnosis of connective tissue diseases, in which it may confirm the clinical suspicion.[1,15] Of all of the collagen vascular disorders, scleroderma shows the most marked esophageal abnormalities.[31,32] The manometric findings are quite suggestive of the diagnosis, although not pathognomonic, because severe reflux esophagitis can have the same manometric appearance.[32] The characteristic esophageal manometric findings are (1) an incompetent LES, (2) low-amplitude esophageal contractions in the smooth portion of the esophagus, and (3) later alterations in the striated muscle section.[31–33] Even though the esophageal motor abnormalities of scleroderma have been well characterized, other connective tissue diseases also have motor alterations,[33] particularly in patients with systemic lupus erythematosus and those with mixed connective tissue diseases.[33]

Esophageal manometry is useful in the evaluation of noncardiac chest pain.[1] Manometry should not be routinely used as the initial test[15] but may be useful when other tests have not provided an explanation. Manometric abnormalities are prevalent in patients with chest pain, dysphagia, or both. Achalasia or diffuse esophageal spasm accounts for the minority,[15] and most patients fall under the category of "nonspecific disorders." In a recent report of esophageal manometric findings in 154 children, the authors described the findings in 45 children with chest pain or dysphagia not associated with GER.[26] Esophageal manometry was described as abnormal in 30 (67%). Among those, a variety of motility disorders were diagnosed in 17 patients (achalasia in 12, pseudo-obstruction in 3, diffuse esophageal spasm in 1, and dysmotility after tracheoesophageal fistula repair in 1), whereas nonspecific esophageal motor disorders were diagnosed in 13 patients (8% from the initial 154). Those children had a higher incidence of food impaction.[26]

Recently, technical advances with solid-state catheters and digital recording devices have allowed the performance of prolonged monitoring of esophageal pressure.[34] There are no control values for children, and the number of controls in adults is limited. The manometry is performed with a solid-state catheter that has pressure transducers and usually one or two pH electrodes, allowing the correlation of symptoms with both motor events and reflux. Most software does not allow the measurement of continuous LES pressure. The primary indication is for the evaluation of patients with noncardiac chest pain[34,35] because it allows the demonstration of associated esophageal events (either motor or reflux related) with the pain.[34] Preliminary reports in children have shown that it is a technique that may be useful.[36,37]

The role that provocative tests play during esophageal manometry in children has not been established, so their use is not recommended.

The future for the study of esophageal physiology may be promising. The advent of mutlichannel intraluminal impedance combined with manometry has allowed the simultaneous evaluation of esophageal contractions and bolus transit.[17] Recent publications have established normal reference values for adults,[17] and there is still no information available for children. The results in adults have already shown that the manometric evidence of ineffective peristalsis may underestimate the true bolus clearance and that the combined impedance with manometry may be a more sensitive technique to assess esophageal function.[17] More information is needed before any firm conclusions can be established.

Indications. The primary indication continues to be in the evaluation of children with esophageal dysfunction that is not explained by anatomic or well-defined problems (see Table 70-2).[1,2]

Esophageal manometry is indicated when a primary motility disorder is suspected and to support the diagnosis of connective tissue diseases or other systemic illness. Manometry may be indicated in the evaluation of patients with achalasia post-treatment who develop recurrent symptoms when there is doubt about the physiopathology. Manometry is not indicated in the routine evaluation of GER, but it should be performed in those patients with GER in whom the diagnosis is not clear, as well as before a fundoplication when a severe motility disorder is suspected. It is also used to localize the LES before placement of a pH probe in patients with abnormal anatomy (eg, hiatal hernia). Esophageal manometry, particularly 24-hour recording, is indicated in the evaluation of children with noncardiac chest pain who have no diagnosis and have not responded to antireflux therapy.

Antroduodenal Manometry

Antroduodenal manometry measures the intraluminal pressure of the antrum and duodenum.[1,7,38,39] Antroduodenal motility has been clearly established as an important research tool, and even though it is being gradually introduced into clinical practice, the exact role that it plays in the evaluation of children and adults has not been firmly established. Manometric data are not necessary for patient management when there is a known underlying cause of dysmotility (see Table 70-2).[1]

Clinically, manometry provides useful information regarding contraction patterns in the region. In children, its utility has been mostly hampered by the lack of data that establish patterns in normal controls because it is not ethically possible to perform those tests in healthy children.[38] Therefore, reported controls have been derived by different authors from patients referred for antroduodenal motility who later were considered as not having upper gastrointestinal motility problems.[40] These difficulties in interpretation can be illustrated in reports in which abnormal manometric patterns occurred with equal frequency in healthy and symptomatic adults.[41] Therefore, definition of what constitutes a significant abnormality remains controversial. Because of this lack of controls in children, great care needs to be exercised in the interpretation of the studies to avoid overinterpretation of the findings.[5]

Small bowel motility can be performed with solid-state pressure transducers, impedance sensors, or perfused catheters.[1] The patient stops medications that can affect motility for at least 48 hours before the test. The catheter is introduced nasally or through an existing gastrostomy or jejunostomy. The catheter requires postpyloric placement and can be introduced either endoscopically or fluoroscopically, and, ideally, it is advanced beyond the angle of Treitz. Most importantly, however, the transducers need to be positioned across the antroduodenal junction.[42] The configuration of the catheters varies, but the minimum recommended recording ports include one in the antrum and three small bowel recording sites.[1] The distance for the duodenal/jejunal ports varies depending on the age of the patients, with a range that goes from 3 to 10 cm between ports. In most children, a distance of 3 cm is sufficient. The position of the catheter needs to be checked frequently during the performance of the test to ensure the correct position across the antroduodenal junction. It has been suggested that, on average, a stationary study requires an average five adjustments of tube location in the postprandial period to ensure accurate antral recordings.[3] At times, it is necessary to reconfirm placement during the test with radiography or fluoroscopy.

When perfused studies are performed, a close follow-up of the patient is necessary to avoid fluid overload, particularly in small children.[43] With the regular perfusion equipment available in most laboratories, steel capillary or other tubes that control the rate of flow are used, and the usual perfusion rates vary from 0.1 to 0.4 mL/min per port. If six ports are being used, and the test lasts 6 hours, a large amount of fluid is being administered. This could produce volume overload or, theoretically, hyponatremia. Even though in adult laboratories, the catheter is perfused with distilled water, in children, many centers use 0.2 to 0.5 normal saline or oral hydration solutions[1,2] to avoid hyponatremia. To be able to study premature and young infants, the system has been adapted by some investigators to decrease the perfusion rate to 0.01 to 0.02 mL/min.[7,44]

It is preferable to avoid anesthesia and sedation. The effects of benzodiazepines on motility recordings have not been evaluated,[42] but in some centers, it has been suggested that sedation with midazolam (2–5 mg) followed by reversal with intravenous flumazenil (0.2–0.4 mg) does not result in any appreciable change in motility recordings. The use of sedation or general anesthesia is usually necessary in children, particularly when the catheters are being placed endoscopically. To avoid the possible effects of the sedation or anesthesia, in most centers, the study is performed the day after the catheter has been placed.

The optimum duration of the test is not known. Even though most centers use 3 to 4 hours of fasting followed by 2 hours postprandially,[42,45] some authors have advocated the use of prolonged ambulatory studies. The pediatric task force of the AMS recommends at least 3 hours of fasting (or two migrating motor complexes [MMCs]) and at least 1 postprandial hour.[1] Soffer and Thongsawat have shown that prolonged tests, including sleep periods, enhance the diagnostic accuracy of the test.[45] Most of the improvement came from extra information gained by longer fasting periods. Because of frequent catheter displacement, ambulatory tests cannot be used reliably to assess postprandial antral activity.[3,42] Therefore, if the objective is to assess postprandial antral function, a stationary manometry is necessary.[3,42]

The analysis is usually performed by visual inspection.[1] Antroduodenal manometry provides identification of certain "patterns" and limited quantitative features.[42] At times, it may be possible to administer provocative medications and analyze the responses.

During fasting, the stomach and small bowel show a cyclic pattern, known as the MMC.[40] This cyclic activity is

usually divided into three phases. Phase III is the most characteristic and consists of regular rhythmic peristaltic contractions that start proximally and migrate down to the ileum. Phase III shows regular contractions that in the antrum occur at a rate of 2 to 3/min and in the small bowel at 11 to 12/min (Figure 70-2). The velocity of propagation of phase III decreases significantly, whereas the duration increases significantly with increasing distance from the mouth. Phase III is followed by a period of quiescence (phase I) that is interrupted by irregular motor activity (phase II). During phase I, contractions are not recorded. During phase II, the contractions vary in amplitude and periodicity. Phase I seems to be the most predominant in the antrum, whereas phase II is predominant in both the duodenum and the jejunum. In adults, phase I lasts from 12 to 20 minutes, phase II lasts from 30 to 130 minutes, and phase III lasts from 3 to 15 minutes.[3,42,46,47] There is also a large variation in cycle duration between individuals and within the same individual when studied on separate days.[42] There are no established standards for normal duration of the cycle in children, but in the series reported in the literature, the duration seems to be similar,[46] although it has been suggested that the cycle may be shorter.[40,48,49]

In one study of 18 children aged 2 to 12 years without upper gastrointestinal symptoms in whom antroduodenal manometry was performed, the authors found a phase III in 14 of the children during fasting, and it was induced in the other 4 children by erythromycin. Phase III propagation velocity increased with age, and the cycle length showed no age-dependent variation. Phase III occupied around 3%, phase I around 10%, and phase II around 87%.[40] These authors concluded that antroduodenal motility findings in those children were similar to those found in adults.[40]

After the ingestion of nutrients, the fasting pattern is interrupted by what has been denominated the fed pattern (Figure 70-3). The fed pattern is characterized by an irregular occurrence of contractions with various amplitudes.

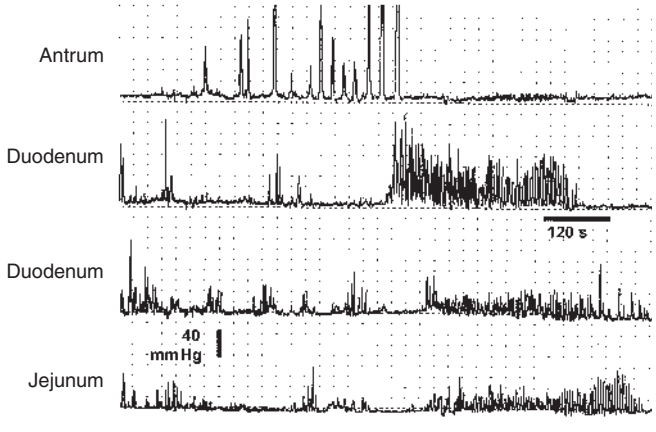

FIGURE 70-2 Normal fasting antroduodenal manometry. A normal phase III front originating in the antrum and migrating aborally along the duodenum into the jejunum can be observed. During phase III, the antrum contracts at a frequency of 3/min, whereas the small bowel contracts at a frequency of 11 to 12/min. Phase III is followed by a period of quiescence (phase I) and is preceded by intermittent irregular contractions (phase II).

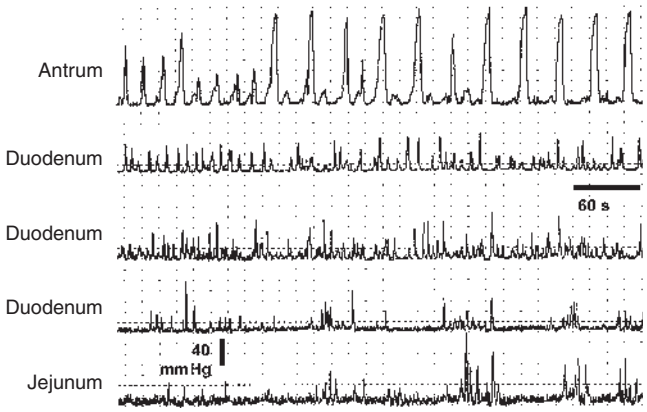

FIGURE 70-3 Normal postprandial pattern in a small bowel manometry. There are irregular persistent phasic contractions in the antrum and small bowel.

After solid meals, strong, repetitive contractions are often induced in the antrum, and the duodenal response looks similar to that of phase II, although the amplitude and frequency of contractions are greater in the fed state.[1]

An antral motility index has been used to calculate both the frequency and the amplitude of contractions. The following formula has been commonly used:

$$\text{Motility index} = \ln(\text{amplitude} \times \text{number of contractions} + 1)$$

with a normal value being 13.67 to 15.65 (5–95th percentile).[42,50] For this calculation to be useful and comparable, it must include 2 hours, assessed by pneumohydraulic perfusion manometry of the prepyloric antrum, and it must quantitate contractions 1 to 2 cm proximal to the pylorus.

The characteristics of the fed pattern vary with the type, composition, and amount of nutrients. For example, liquid nutrients decrease the amplitude of antral contractions and generate an irregular movement in the small bowel, whereas solid food produces high-amplitude contractions in the antrum and a pattern similar to that of liquids in the small bowel. In adults, the meal has been standardized to be at least 400 kcal to ensure a postprandial response of 2 hours duration.[42,51] The solid or liquid meal should be balanced and typical of an average US diet, with 20 to 25% fat, 20 to 25% protein, and 50 to 55% carbohydrate.[42] It has recently been shown that the caloric value of the meal regulates the duration of the fed pattern over a wide range of calories and that for caloric loads up to 1,100 kcal, there does not seem to be a maximum duration of postprandial motor activity. In children, no studies address this question, and there is no standardization, but most authors have used 5 to 10 mL/kg,[52] 20 cal/kg,[53] or 400 to 600 kcal. The AMS task force recommends that the type and size of the meal should be adjusted according to the patient's age and preference (at least 10 kcal/kg or 400 kcal; > 30% kcal from lipids).[1] The task force recommends administering the meal by mouth or intragastrically if possible.[1]

Development. Because of the recent technical advances described above, there is now a fair amount of information regarding normal patterns and age-dependent maturation

in preterm infants and full-term neonates.[7,43] Duodenal motility patterns differ in preterm and term infants. Gastric and duodenal MMCs are present after the 32 to 35 weeks of gestation. Approximately two-thirds of phase III activity starts in the duodenum, and its duration is similar to that in older children and adults. In contrast, premature infants with a postconceptual age less than 32 weeks do not have phase III activity, and there are no cyclic changes in the motility patterns. They show mostly sporadic and nonmigrating clusters of contractions. The number and amplitude of duodenal contractions increase significantly between 29 and 32 weeks, and there seems to be a gradual maturation of these parameters. With maturation, the frequency of these clusters decreases until a mature pattern is observed. As they develop phase IIIs, they differ from adults, with a slower migration velocity, a shorter interval between contractions, and a smaller peak amplitude of the contractions.

In premature and full-term infants, antral motor activity was similar to that in the fasting state. Antral motility consisted of isolated single contractions and clustered phasic contractions. There were no differences in the occurrence of antral activity, and the only differences observed were in duodenal activity. The proportion of antral clusters that were temporally associated with duodenal activity was also significantly lower in preterm infants than in term infants. After feeding, motor activity changes in both full-term and premature infants. It has been shown that in term infants, there is usually a mature response (similar to the one observed in adults), whereas only a third of preterm infants have a mature response. In the term infant, the number of antral waves, the duration of antral clusters, and the antral motility index decreased by one-third; the duodenal motility index and cluster activity increased significantly. This divergent response was also seen in premature infants.[43] In a study of premature infants, the conversion from fasted to fed patterns depended on caloric strength. The threshold concentration was about 22 cal/dL when 5 mL/kg of milk was given.

In infants, phase III does not appear right after feeding. There may be differences in the response depending on the nutrient. For example, in one study with healthy neonates, phase III appeared within 3 hours after breast milk in 10 of 12 breastfed infants but in 2 of 12 infants who were formula-fed.[54] The study of the fed patterns of antropyloric motility measured with the use of high-resolution manometric microperfusion techniques has indicated that the neuroregulatory mechanisms responsible for the coordination of motility and gastric emptying are well developed by 30 weeks gestation.[7]

It has been suggested that antroduodenal motility may be useful to establish feeding readiness in premature infants[44,55] or the best way to feed them.[56] It has been shown that in premature infants, continuous infusions produce better responses and tolerance than bolus feedings and that full-strength formula triggers adult-like motor activity.

Clinical Significance. Antroduodenal motility has been used to study the physiopathology of gastrointestinal motility disorders. Different patterns also allow the classification into neuropathic (Figure 70-4) or myopathic abnormalities (Figure 70-5).[1] They are defined based on qualitative abnormalities.[1] In general, most authors consider findings abnormal if there is abnormal propagation or configuration of the MMC, uncoordinated intestinal bursts of phasic pressure activity sustained over 30 minutes, uncoordinated intestinal pressure activity, and failure of the meal to produce a fed pattern (see Figures 70-4 to 70-6).

Based on these abnormalities, the following patterns can be seen:

1. *Antral hypomotility*. A reduced motility index of postprandial distal antral contractions is significantly correlated with the impaired gastric emptying of solids from the stomach (see Figure 70-6).[50,57]
2. *Myopathic disorders*. These disorders are characterized by low-amplitude contractions < 20 mm Hg[58] and, on average, are < 10 mm Hg. However, it is important to remember that amplitude measurements with a point sensor depend on luminal diameter and may be low because of a nonspecific dilatation (see Figure 70-5).[42]
3. *Neuropathic disorders*. These disorders have been associated with antral hypomotility, absence of phase III activity, abnormal propagation of MMC, bursts and sustained uncoordinated pressure activity (hypercontracility), and a lack of a fed response (see Figure 70-4).[59]
4. *Mechanical obstruction*. Even though mechanical obstruction needs to be diagnosed by other means, if undetected, it may be suggested by manometry. Two patterns suggestive of obstruction have been described: postprandial clustered contractions (> 30 minutes duration) separated by quiescence or simultaneous prolonged (> 8 seconds) or summated contractions.[42,60]

In an attempt to define normal fasting antroduodenal patterns in children, Tomamasa and colleagues compared the results obtained in 95 patients with symptoms suggesting a gastrointestinal motility disorder with 20 children

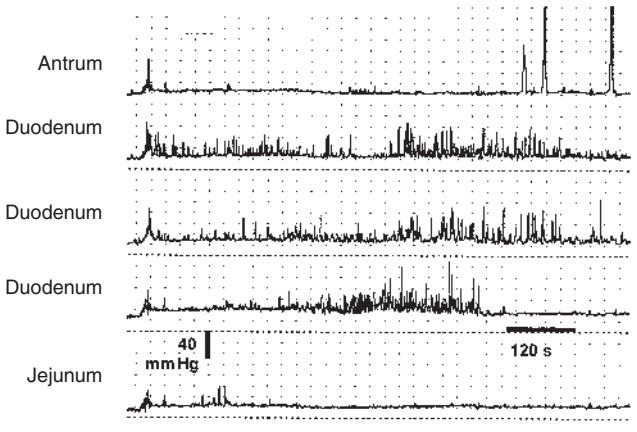

FIGURE 70-4 Antroduodenal manometry in a patient with neuropathy. The tracing shows abnormalities in the phase III of the migrating motor complex. Some uncoordinated clusters, as well as isolated irregular phasic contractions, can be observed. Throughout the study, there was no organized activity, and irregular phasic contractions were seen. No phase III could be observed, even after provocative medications.

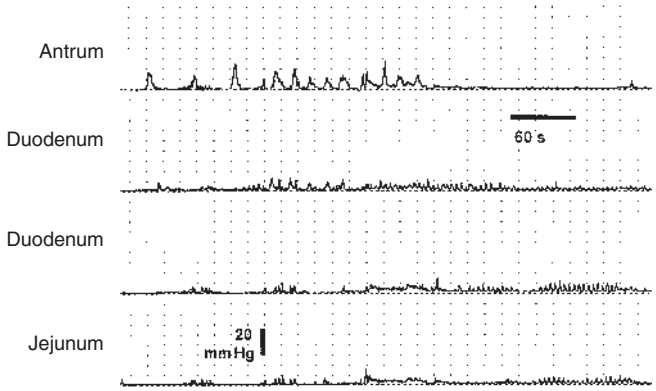

FIGURE 70-5 Antroduodenal manometry in a patient with visceral myopathy. The tracing shows the normal propagation of the interdigestive motor complex, although the amplitude is much lower than normal (see Figure 70-2).

without upper gastrointestinal disease.[38] They concluded that the following five manometric features have a clear association with pediatric gastrointestinal motility disorders: (1) absence of phase III of the MMC, (2) abnormal migration of phase III, (3) short intervals between phase III episodes, (4) persistent low-amplitude contractions, and (5) sustained tonic-phasic contractions. They mention that short or prolonged phase III, low amplitude of phase III in a single recording site, and clusters of contractions or prolonged propagating contractions during phase II were not more frequent in patients than in controls.

The presence of phase III activity appears to be a marker of neuromuscular integrity,[1,38,40,42,61] and it has been reported that the absence of phase III activity is abnormal (see Figure 70-4).[42] One-third to half of the activity front may commence distal to the stomach.[42,62,63] Hence, the absence of the antral component of phase III is not necessarily abnormal.[42,62]

In stationary studies, 66 to 75% of adult patients will have at least one duodenal/jejunal phase III activity during the 3-hour study.[42] Because even some normal adult subjects may have no phase III activity during stationary studies, this finding may have limitations in the interpretation of suspected neuropathic disorders.[41,45,64] Data from 24-hour ambulatory studies show that normal adult volunteers have at least one or more MMCs per 24 hours.[45,47,65] Therefore, the most definitive evidence of normal enteric neuromotor function can be obtained from prolonged studies.[45] In a study comparing the accuracy of short versus long manometry, the two analyses agreed in 81 of 91 cases. In 7 of 10 cases, a study was diagnosed as abnormal in the short recording but was considered normal after review of the long recording, whereas the opposite occurred in the remaining 3 cases.[45] There is no similar information in children, but it has been suggested that children have a shorter MMC cycle duration than do adults.[48,49]

If no spontaneous phase III activity is observed, intravenous erythromycin has been administered.[1,66] Erythromycin, at doses that are 10 to 20% of those used for its antibiotic properties, acts as a motilin receptor agonist.[66–68] In one study, erythromycin induced phase III activity when given to patients with functional gastrointestinal symptoms. It induced phase III in 18 of 20 children who had phase III activity during fasting, as opposed to 1 of 15 who did not. A dose of 3 mg/kg induced a higher antral motility index when compared with 1 mg/kg, although both doses were equally efficacious in inducing phase III. The lower dose was associated with less side effects.[66] In another study, the effect of erythromycin 3 mg/kg/h was compared among 10 controls, 7 patients with functional dyspepsia, and 6 patients with pseudo-obstruction.[68] In controls, erythromycin induced a premature activity front occurring 15 ± 3 minutes after starting the infusion. The propagation and duration did not differ from the spontaneous activity front. In patients with functional dyspepsia, erythromycin induced various patterns, such as a premature antroduodenal activity front, antral phase III–like patterns with short duodenal bursts, or prolonged phasic antral waves without duodenal activity. In patients with neurogenic pseudo-obstruction, rare or absent antral activity was present with uncoordinated or absent activity, and no contractions were elicited in those with myopathic pseudo-obstruction.[68] It has therefore been suggested that in children who have a normal MMC during fasting, erythromycin produces normal phase III, whereas it is abnormal in all patients with abnormal fasting motility. Therefore, some authors have suggested that in children, a 1-hour infusion of erythromycin could replace the 4-hour fasting portion of the test as a way to establish if there is presence or absence of phase III.[49] This observation has not been validated, and the disadvantage of a shorter test would be the inability to evaluate other aspects of fasting motility. The AMS task force recommended the use of erythromycin 1 mg/kg over 30 minutes if no MMC is recorded during fasting.[1]

The administration of erythromycin to full-term neonates induces phase III activity.[67,69] However, it induces a response only in premature infants older than 32 weeks.[70]

The usefulness of antroduodenal manometry to directing therapy has not been definitely established in adults. In children, it has been suggested that absent MMCs are an indicator of a poor response to enteral feeding[53] or to cis-

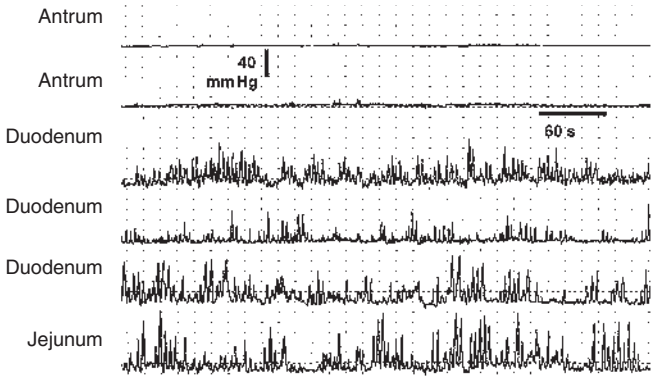

FIGURE 70-6 Antroduodenal manometry in a patient with antral hypomotility. The tracing shows normal postprandial activity in the small bowel and absent response in the antrum. This pattern is frequently seen in patients with gastroparesis.

apride.[71] Antroduodenal manometry may also be useful to study the effects of medications that may have a role in the treatment of children and adults with motility disturbances, such as octreotide.[72,73] Octreotide has been shown to induce phase III activity in the small bowel[72] and has varying effects on the antrum.

It has been suggested, however, that one of the most important contributions of the test may be in showing normal physiology in those patients with apparent intestinal failure (see Table 70-2).[1,5,38,40] Fell and colleagues tried to assess if antroduodenal manometry in the first 2 years of life helped define a neuropathic or myopatic etiology or clinical outcome in cases of pseudo-obstruction.[74] They studied 14 children histologically (5 with myopathy, 4 with neuropathy, and 5 unclassified) and manometrically. They found four abnormalities in the manometry: no detectable motor activity in 4 children, low-amplitude phase III activity in 5 children, poorly formed phase III activity in 3 children, and well-formed cyclic phase III activity with abnormal propagation in 2 children. The seven children with a low-amplitude phase III motility index < 10 pka/min had poor outcomes (death or dependence on total parenteral nutrition). The authors concluded that manometry was useful not only as an aid in diagnosing the etiology of pseudo-obstruction presenting in infancy but also in predicting outcome.[74]

Indications. Antroduodenal manometry is indicated when patients have unexplained upper gastrointestinal problems, such as nausea and vomiting or other symptoms suggestive of upper gastrointestinal dysmotility (see Table 70-2). Its main use had been in confirming or excluding the diagnosis of pseudo-obstruction or a motility disorder, and it helps to establish if the dysmotility is neuropathic or myopathic. Most importantly, a normal study may indicate that motor dysfunction is not the cause of the symptoms.[1,42] It is also used to decide if there is generalized dysmotility in patients with dysmotility elsewhere (eg, chronic constipation when surgery is contemplated, severe reflux with evidence of distal dysmotility when a fundoplication is being considered, or after a failed fundoplication that requires reintervention).[1]

It may be useful to distinguish between rumination and vomiting.[1] Some studies have also suggested that antroduodenal motility may be useful in patients with abnormal motility to predict outcome after drugs or ability to tolerate enteral feedings. It is also indicated in patients with pseudo-obstruction being considered for intestinal transplant and may suggest an unexpected obstruction.[1]

Colonic Manometry

Measurement of colonic intraluminal pressure has been performed for many years but has been confined mostly to the distal colon (anorectosigmoid segments). The study of colonic motility of more proximal segments is a technique that has recently been introduced.[75] Even though colonic intraluminal measurements alone do not have a clear role in clinical practice, the test has been shown to be useful in the evaluation of the pediatric patient with intractable constipation.[1] In adults, colonic motility does not seem to discriminate subgroups of chronic constipation more accurately than transit and pelvic floor tests. It may be helpful in confirming a diagnosis of slow-transit constipation (colonic inertia) in patients considered candidates for surgical treatment.

In humans, there is no interdigestive cyclic motor activity. The characteristics of colonic motility are the presence of irregular alterations of quiescence with nonpropagating and propagating contractions. In humans, colonic propagated events are basically of two types, arbitrarily defined on the basis of their amplitude: low-amplitude propagated contractions (LAPCs) and high-amplitude propagated contractions (HAPCs). LAPCs have been poorly investigated and are propagated waves of 5 to 40 mm Hg. Their exact physiologic significance is still unknown but may be involved in the transport of colonic contents and the occurrence of flatus.[75] In the fasting state, the motor activity is represented mostly by low-amplitude (5–50 mm Hg), nonpropulsive, segmental contractions; peristaltic movements seldom occur.[75] Some contractions last more than 30 seconds and are considered tonic; others are shorter and are considered phasic. Segmental nonpropagating contractions are rare in infants but are more common in toddlers.[76]

HAPCs have been defined as contractions of at least 80 to 100 mm Hg, lasting 10 seconds and propagating for at least 30 cm.[75,77] HAPCs can reach amplitudes > 200 mm Hg. HAPCs originate in the proximal colon and migrate distally > 95% of the time, usually stopping in the distal sigmoid colon (Figure 70-7).[75]

Food ingestion has an important influence on colonic motility.[75] The response lasts 2 to 3 hours and is mostly composed of segmental contractions; it is also accompanied by an increase in colonic smooth muscle tone and can also have HAPCs, which have also been described in pediatric patients.[1,78–80] The colonic response to eating is influenced by the caloric content and meal composition. Fat and carbohydrate represent an important stimulant, whereas protein may have an inhibitory effect.[75]

Another cyclic activity that can be found during colonic manometry occurs distal to the rectosigmoid junc-

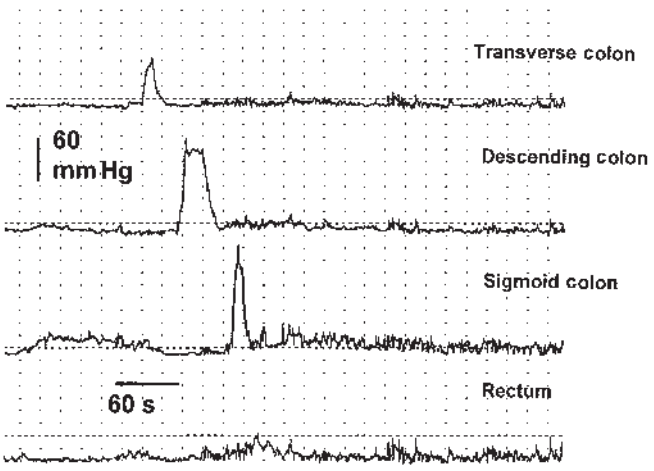

FIGURE 70-7 Normal colonic manometry. The tracing shows the presence of a high-amplitude propagating contraction that is originating in the transverse colon, advancing aborally along the large bowel, and stopping in the sigmoid colon.

tion and has been labeled the rectal motor complex.[75] This lasts an average of 10 minutes and has contractions with a mean frequency of two to four per minute and a wave amplitude of > 5 mm Hg. It occurs in adults every 90 to 300 minutes during the day and every 50 to 90 minutes at night.[75] Its physiologic role is unknown, although it is accompanied by a rise in anal canal pressure and probably represents a mechanism to preserve fecal continence, particularly during sleep.

It has been suggested that colon preparation alters colonic motility. Unfortunately, studies of colonic motility are difficult to perform in unprepared colons, so most data are available from studies in which the colon has been cleaned.[77] Usually, the patient's colon is cleaned with the use of a balanced electrolyte solution. In most studies performed in children, this is administered by a nasogastric tube. Usually, no enemas are given, no colonic preparation is given the day of the study, and medications that can influence motility are stopped at least 48 hours before the study.

The spacing of the recording ports varies, but they are generally separated by 10 to 15 cm. The catheter is placed with the use of colonoscopy. In general, a guidewire is placed into the transverse/hepatic flexure, and the colonoscope is withdrawn, leaving the guidewire in position. The motility catheter is then fed over the wire. The position of the tube is checked fluoroscopically.[49,77,79,80] The catheter can also be dragged with the colonoscope during the colonoscopy and placed directly in the desired location.

The colonoscopy is performed using either general anesthesia or intravenous sedation. When intravenous sedation is used, and only benzodiazepines are used, the motility study may be done on the same day, after the child has recovered from the sedation (mean of 3.5 hours).[77] However, the influence that the sedation or the colonoscopy may have on motility is not known. Therefore, in many cases, the catheter is placed on one day, and the study is performed on the next day.

A fasting recording is done for 2 hours, and then the patient is fed. There is no standardization of the meal. In adults, a 350 kcal meal did not stimulate colonic motility, but a 1,000 kcal meal did. In one pediatric study, patients received a combined liquid and solid meal (≥ 20 kcal/kg, with fat providing > 30% of the energy).[79] In another study, 240 mL of whole milk or formula was used in those enterally fed, and in those who were dependent on total parenteral nutrition, they gave the maximum amount of milk that the patients were able to tolerate without symptoms.[80] Usually, a motility index is calculated by measuring the area under the pressure for at least 30 to 60 minutes before and 30 to 60 minutes after the completion of the meal. HAPCs are excluded from the analysis.[77] It has been shown that meals stimulate colonic motility in healthy subjects.[79,80]

Clinical Significance. Constipated adults with slow-transit constipation have an impairment of colonic propagated activity, principally related to a decrease or an absence in HAPCs. The enteric neural program controlling colonic propagation and its ability to contract in response to exogenous stimulation may still be preserved in some patients, as shown by the elicitation of HAPCs after intraluminal instillation of bisacodyl. Patients may also have an abnormal response to eating,[75] which may be blunted or absent.

The patterns of colonic motility in healthy children have not been established. Most information comes from studies in which children referred for evaluation of neuropathy, constipation, or nonulcer dyspepsia have been studied.[76,77,79,80] From those studies, it has been suggested that in children, HAPCs also increase after meals. These authors have described that in the postprandial period, one HAPC is usually followed by others 3 to 4 minutes later.[76,77,79,80] In a study of 32 children with a median age of 5.5 years (15 with functional fecal retention, 10 with nonulcer dyspepsia, and 7 with Munchausen syndrome by proxy), it was suggested that there was an inverse correlation between the number of HAPCs and age (before and after administration of a meal) and that colonic contractions different from HAPCs increase with age.[77] The authors found at least one HAPC in 28 of 32 subjects, and the 4 subjects without it were > 8 years. HAPCs were also more frequent in the fasting period in those ≥ 4 years when compared with those who were older (8/13 vs 3/19). There were also differences in the number of HAPCs within 30 minutes of the meal (13/13 age 4 years vs 12/19 > 4 years), whereas there were no differences in the second 30-minute interval.

It has been suggested that colonic manometry offers a tool to differentiate children with different causes of constipation and to differentiate between myopathy and neuropathy.[1,80] In one study, it was suggested that children with neuropathy could be differentiated from those with functional fecal retention (see Figure 70-7) by a lack of HAPCs and a lack of increase in the postprandial motility index (Figure 70-8).[79] Di Lorenzo and colleagues have also suggested that in young children, the lack of HAPCs is a sensitive marker of disease, but these data require further validation.[77,79,80]

Intracolonic bisacodyl has been administered to try to shorten the duration of the motility study in ill children or in

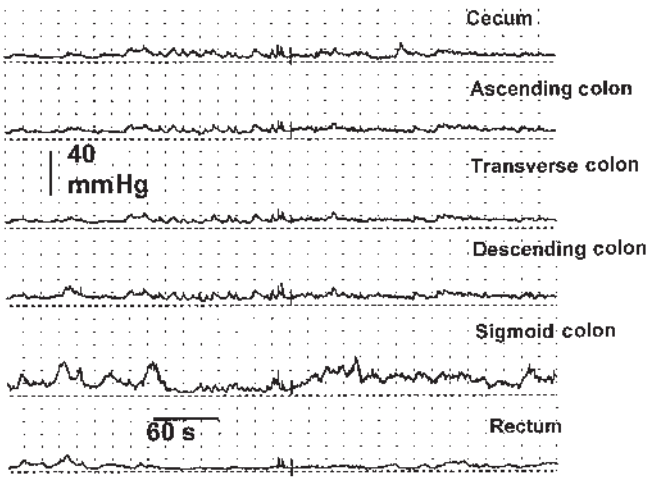

FIGURE 70-8 Abnormal colonic manometry. No high-amplitude propagating contractions (HAPCs) or changes in the motility index were seen in this patient with a colonic neuropathy. No HAPCs were seen even after the administration of bisacodyl.

those who cannot eat.[78] It has been shown that the HAPCs induced after bisacodyl were similar in amplitude, duration, propagation velocity, and sites of origin and extinction to naturally occurring HAPCs.[78] They found that the effect of intrarectal bisacodyl was similar to that of intracecal bisacodyl, except for a delay of 10 minutes in onset.[78] In their study of 28 patients with functional fecal retention, 22 had spontaneous HAPCs, whereas the 28 patients had HAPCs induced by bisacodyl. From 9 with pseudo-obstruction, none had spontaneous HAPCs, whereas 2 had HAPCs after bisacodyl. The interpretation of HAPCs in those 2 children is difficult because it raises the possibility that those children did not have pseudo-obstruction.

The use of colonic motility in children has also been found to be useful in the understanding of postoperative problems in patients with Hirschsprung disease[81] and imperforate anus.[82] It has also been suggested that colonic motility may help in the detection of abnormal colonic segments that may require surgical resection, but these findings need further validation.[81]

Indications. The information obtained from colonic motility still needs to be considered within the context of there being no normal controls in children and many unanswered questions about normal colonic function, the effects of colonic preparation on the results, and so on.[1]

Colonic motility is indicated in the evaluation of selected patients with intractable constipation because it can be helpful to differentiate functional fecal retention from colonic pseudo-obstruction.[1,78] It is also indicated in the evaluation of children with pseudo-obstruction to assess for the presence of colonic involvement and to characterize the relationship between motor abnormalities and symptoms, particularly when a colectomy is being considered. The test is also indicated to determine the relationship between motor activity and persistent symptoms following surgery for Hirschsprung disease and other colorectal problems. Colonic motility should also be performed to assess colonic motor activity prior to intestinal transplant.[1]

Anorectal Manometry

Anorectal manometry is used for the evaluation of children with defecation abnormalities (see Table 70-2)[1,2] and probably represents one of the most frequently performed motility tests in children. The main indication is for the exclusion of Hirschsprung disease,[1,2,83] but it also has an important role in the evaluation of children with fecal incontinence from various etiologies such as myelomeningocele or imperforate anus.[2]

Anorectal physiology has been studied because it has a vital role in maintaining continence. Normal continence and defecation are complex functions. Continence is maintained by the interaction of several mechanisms, including stool consistency, delivery of colonic contents to the rectum, rectal capacity, anorectal sensation, sphincteric function, and muscles and nerves of the pelvic floor. Intra-anal pressure is a combination of both internal and external anal sphincter interaction, with the former providing about 75 to 85% of the total pressure.[84] In adults, the proximal anal canal pressures are lower in the anterior quadrant, and, distally, the pressures are lower in the posterior quadrant. The internal anal sphincter (IAS) is smooth muscle and is in a state of continuous contraction.[85] The external anal sphincter (EAS) and the muscles of the pelvic floor also maintain continuous tone. When a fecal bolus enters the rectum, there is a reflex relaxation of the IAS (rectoanal inhibitory reflex [RAIR]) (Figure 70-9) and transient contraction of the EAS.[85] This relaxation is limited, and the sphincter reacquires its tone as the rectum accommodates to the distention. The relaxation of the IAS occurs independently of the spine and is lost when there is a lack of inhibitory ganglion cells (as in Hirschsprung disease) (Figure 70-10).[86] This relaxation enables the rectal contents to come into contact with the upper anal canal ("sampling reflex"), and normal people can discriminate among solids, liquids, or gas. The transient simultaneous contraction of the EAS allows time for the IAS to recuperate, avoiding incontinence. Further rectal distention with increasing volumes results in nonrecovery of the IAS. If it is socially acceptable, the person has a bowel movement; if not, the conscious contraction of the EAS and rectal compliance allow for postponement until it is socially acceptable.

Different techniques have been used to perform anorectal manometry. In children, the most common method used is with water-perfused catheters that have side holes at different levels of the longitudinal and radial axis.[1,86–89] A balloon is attached to the distal segment and inflated to produce rectal distention.[88] Usually, it is made of latex, and care should be taken when the test is performed in children who may be allergic to it. Solid-state catheters have also been used, but they are much more expensive. Another technique involves the use of a double-balloon device, but its use in pediatrics is limited, particularly because the size is too large for smaller children. The recent advent of micromanometric techniques with the use of sleeve sensors has allowed the accurate study of anal sphincters even in the very low birth weight patient.[90]

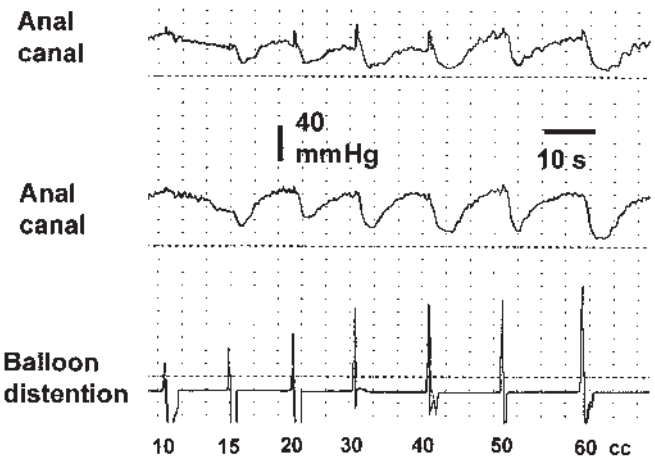

FIGURE 70-9 Normal anorectal manometry. The tracing shows the response of the internal anal sphincter to balloon distention. A normal sphincter relaxation after balloon distention can be observed. The relaxation follows a dose-response curve.

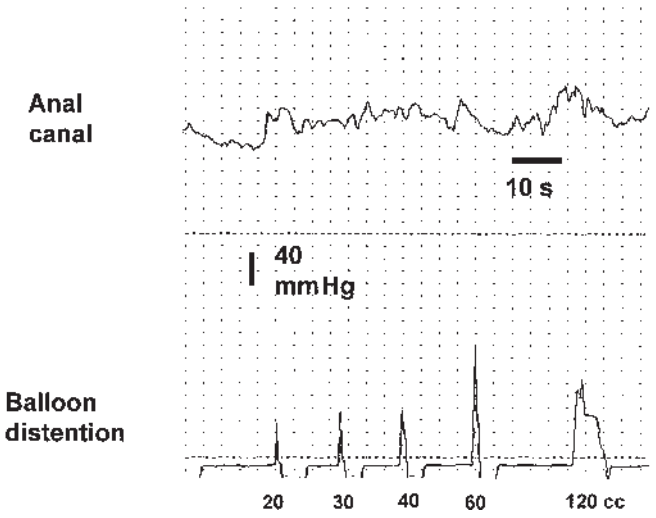

FIGURE 70-10 Anorectal manometry in a patient with Hirschsprung disease. Tracing in which balloon distention does not induce the rectoanal inhibitory reflex, even after large balloon volumes were used. This patient was later proven to have Hirschsprung disease.

In older patients, a phosphate enema is usually given the night before the manometry, and medications known to change anorectal function are stopped 48 hours before the test (eg, opiates, prokinetics, anticholinergics). If the patient is severely impacted, a cleanout is performed 2 to 3 days before the test. Children receive nothing by mouth for 4 to 6 hours before the procedure (depending on the age) if they will be sedated or when the primary indication for the test is to exclude Hirschsprung disease in case a rectal biopsy is needed.

During anorectal manometry in pediatric patients, a slow pull-through is performed in which the manometric assembly is pulled until a high-pressure zone is identified (intra-anal pressure). The probe is then positioned at the level of the maximum pressure, which is usually 1 to 2 cm above the anal verge. The balloon is then inflated to elicit the RAIR (see Figure 70-9).[1,86,88] The characteristics of the relaxation are studied. The minimum amount of air required to elicit a relaxation is determined (threshold of relaxation). The amount of relaxation is influenced not only by the volume but also by the speed of the inflated balloon, as well as by rectal resting volume and compliance. It is important to use large balloon volumes in patients with megarectum because a lack of sphincter relaxation may result from an inadequate distention of the rectal wall. The volume necessary for constant relaxation, which is the minimal amount of air that is necessary to produce a complete sustained relaxation of both the IAS and the EAS, is determined by adding air progressively until either there is constant relaxation or the patient has reached the critical volume (see below).[85]

Besides measuring the resting pressure of the anal canal, the patient is also asked to squeeze at each station. The squeeze pressure is then measured as the maximum pressure obtained above anal resting pressure.[1,88]

During manometric testing, some sensory information is obtained.[1] The most common technique is with the use of balloon distention. In cooperative children, the threshold required to perceive the distention is established (smallest volume of balloon distention) (threshold of sensation).[88,91,92] The threshold of sensation is usually determined with the use of a rectal balloon that is inflated with a handheld syringe. The air is rapidly injected and immediately withdrawn. The type of inflation (speed, phasic vs continuous), the size and shape of the balloon, or the distance of the balloon to the anal verge can affect the threshold. Critical volume has been defined as the minimum amount of air that produces a lasting urge to defecate[85,88] and the sensation of pain, which is defined as the maximum tolerable volume, although the clinical significance of those measurements is not clear.[85]

Compliance is measured as the ratio of pressure to volume at several distending volumes. Measurements are usually inaccurate and not reproducible unless a barostat is being used. Decreased compliance may be associated with an increase in stool frequency, rapid transit of stool in the rectum, and increased risk of fecal incontinence.[85] On the other hand, increased compliance may be found in patients with megarectum. There is a paucity of information on its use in pediatrics.

The study of defecation dynamics may be important (Figure 70-11).[1] This is done with the use of either electromyographic (EMG) surface electrodes or with the manometric assembly. Patients are asked to bear down, and the responses of the sphincters are recorded. Pelvic floor dyssynergia (anismus) is present when there is failure of relaxation or even an increase in pressure while attempting to defecate (Figure 70-12).[88,91,93] There has been some controversy as to whether this observed pattern is sufficient for the diagnosis. In one study, when the manometry indicated dyssynergia, the defecography was in agreement only 36% of the time, whereas when manometry was normal, defecography was normal in 88%.[94] No similar information is available in children.

Development. Studies of the developmental maturation of the rectoanal reflex have produced inconsistent results.

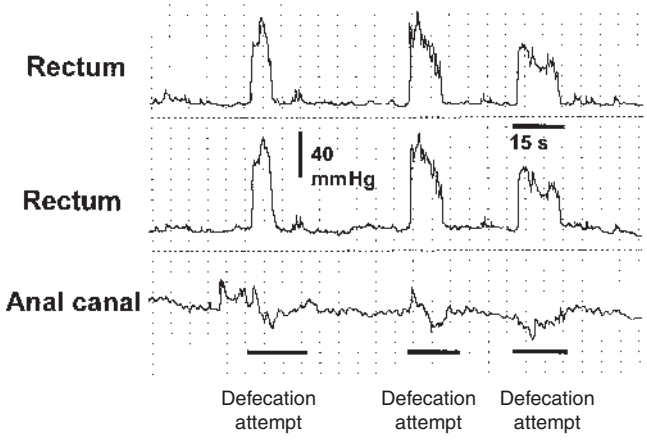

FIGURE 70-11 Normal defecation dynamics. The tracing shows the pressure changes in the rectum and anal canal when a normal child is trying to defecate. Note an increase in rectal pressure with a decrease in anal pressure.

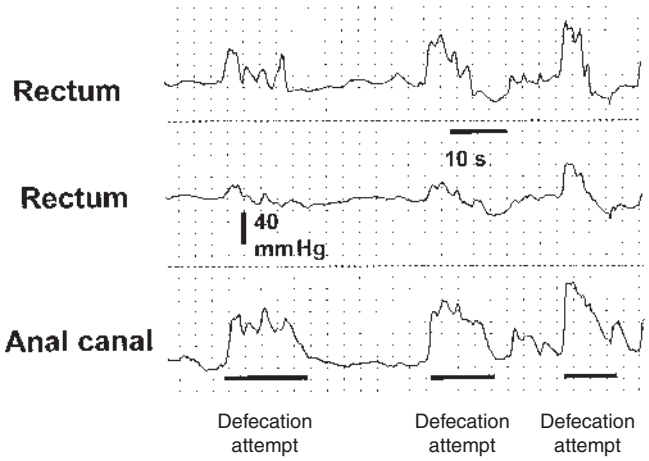

FIGURE 70-12 Abnormal defecation dynamics. The tracing shows the pressure changes in the rectum and anal canal when a child with anismus is trying to defecate. Note an increase in rectal pressure with a simultaneous increase in anal pressure.

The advent of micromanometric techniques and the use of sleeve sensors have allowed the accurate study of different sphincters even in the very low birth weight patient.[8,23,90]

Initial studies in premature infants have suggested that they do not have a RAIR. Some have shown that the RAIR may be physiologically absent up to 12 days of life in newborns, particularly if they are sick.[95] Ito and colleagues reported that the RAIR does not appear before a maturational age of 39 weeks.[96] Recently, however, other authors have found that the RAIR may be present in the first hours even in otherwise healthy premature infants,[86,89,97,98] although in three normal infants, there was absence relaxation at birth. On repeat manometries a few weeks later, the RAIR was preset, suggesting that there may be a maturational response. In a study of neonates, the range of anal resting tone was between 16 and 72 mm Hg.[86] The magnitude of anal tone was inversely correlated with weight but not gestational age. The amplitude of the RAIR in premature infants did not correlate with maturational age but correlated inversely with weight.[86]

A study of the anal inhibitory reflex in premature infants using micromanometric techniques and a sleeve has recently been published.[90] The authors studied 22 healthy neonates with a mean conceptual age of 32 weeks (30–38 weeks). They showed that the mean intra-anal pressure was 40 mm Hg (range 7–65 mm Hg), and they were able to induce a RAIR in 21 of 22 infants. The infant in which the RAIR was not present had a very low sphincter pressure (7 mm Hg), which made the determination of the reflex impossible. Resting anal pressure correlated significantly with postmenstrual age. The authors also found that gestational age, postnatal age, and time from birth to passage of the first stool did not correlate with any parameter of anorectal function. They asserted that air insufflation is the preferred method to produce the RAIR in children less than 34 weeks gestational age.[90]

Clinical Significance. Normal values for anorectal manometry in adults have been published.[85,99] The length of the anal canal was reported to range from 2.2 to 4.0 ± 1.0 cm in women and from 2.8 to 4.0 ± 1.0 in men. Anal resting tone varied from 49 ± 3 to 58 ± 3 mm Hg in women and from 49 ± 3 to 66 ± 6 mm Hg in men. Maximum squeeze ranged from 90 ± 9 to 159 ± 45 mm Hg in women and from 218 ± 18 to 238 ± 38 in men. The threshold for IAS relaxation varied from 14 ± 1 to 25 ± 2 mL, and the threshold for sensation varied from 12 ± 1 to 17 ± 9 mL.[99]

No similar comprehensive information is available for children. Values for normal controls have been published by different authors. The mean normal anal resting pressure in children ranges from 57 ± 10 mm Hg[88] to 67 ± 12 mm Hg.[100] The maximum squeeze pressure ranges from 118 ± 42 mm Hg[88] to 140 ± 52 mm Hg,[100] anal length is around 3.3 ± 0.8 cm,[100] the threshold to produce relaxation (RAIR) ranges from 5 ± 1 mL[88] to 11 ± 5 mL,[100] the threshold of rectal sensation ranges from 5 ± 2 mL[88] to 14 ± 7 mL,[100] the volume of constant relaxation is 104 ± 49 mL,[100] and the critical volume is 101 ± 39 mL.[100]

The main indication for anorectal manometry in pediatrics is to exclude the presence of a nonrelaxing IAS (see Table 70-2).[1] The finding of sphincteric relaxation (RAIR) excludes Hirschsprung disease (see Figure 70-9), particularly in older children,[101] and avoids the performance of more invasive testing such as a biopsy.[86] On the other hand, the lack of sphincteric relaxation strongly indicates the presence of Hirschsprung disease (see Figure 70-10), but a confirmatory biopsy is necessary.[86,100] In those patients in whom there are no technical difficulties and who have a nonrelaxing IAS with normal biopsies, a diagnosis of IAS achalasia needs to be considered.[102] The accuracy for the diagnosis of Hirschsprung disease by manometry varies with the age of the patients.[101,103] Anorectal manometry seems to be more accurate in older children,[104,105] for whom recent studies have suggested an accuracy of 90 to 100%. Most authors report that the accuracy in neonates is lower.[86,96,101,104–106]

A recent study of 59 patients (2–90 days) reported a sensitivity, specificity, positive predictive value, and negative predictive value of anorectal manometry for the diagnosis of Hirschsprung disease of 0.91, 0.56, 0.84, and 0.92, respectively.[103] In other studies, the overall accuracy, sensitivity, specificity, and positive and negative predictive values were 90%, 0.79, 0.97, 0.94, and 0.88, respectively, whereas in neonates, it was 90%, 0.86, 1, 1, 0.75, and in infants, it was 94%, 0.9, 1, 1, and 0.89, respectively.[106]

In the largest study of 229 manometries, there were 10 false-positive and 8 false-negative results, indicating an overall 7.8% error rate.[101] However, in a group of 38 neonates (including 7 premature infants), 26% had an inaccurate diagnosis. In 6 newborns with Hirschsprung disease, the manometry was normal, whereas in 4 normal newborns, there was no RAIR.[101] The numbers are even more inaccurate in premature infants. In that series, the diagnosis was incorrect in 71.4% of premature infants, in whom the authors found 2 children with proven Hirschsprung disease but 3 false-negative and 2 false-positive manometric findings.[101] In 39 children from 1 to 6 months of age (8 with Hirschsprung disease), there were

2 false-positive cases and 1 false-negative case, with a 7.7% error rate. From 41 children 6 months to 2 years of age (8 with Hirschsprung disease), there were 2 false-negative cases and 1 false-positive case, with a 7.3% error rate. In 47 children from 2 to 5 years of age (1 with Hirschsprung disease), there was 1 false-positive case, for an error rate of 2%. In 64 children from 5 to 15 years (2 with Hirschsprung disease), there was 1 false-negative case, for an error rate of 1.5%. This and other studies confirm the impression that accuracy increases as age increases.

Studies specifically performed looking at the experience in neonates have confirmed those observations. In some studies, it has been reported that manometry has led to the wrong diagnosis in up to 26% of neonates,[86,96,101] and it is generally reported that the diagnostic accuracy at that age varies from 70 to 80%.[86,104,105] Recently, a study in which 64 newborns (6 premature infants) with meconium delay or obstruction in whom repeated manometries were performed weekly for the first month of life was reported. There were 5 children with Hirschsprung disease in whom repeated manometries did not show a RAIR. There were, however, 3 newborns in whom the initial manometry did not show the RAIR, but when the study was repeated a few weeks later, it showed normal relaxation.[89] The opposite has been shown by other authors[106]: the manometry performed in the first 10 days of life may also give false-negative results (normal RAIR). In one report of 26 children with Hirschsprung disease, 3 initially had normal manometries that, when repeated later because of the persistence of symptoms, did not show a RAIR, and histology subsequently diagnosed Hirschsprung disease.[107] Therefore, anorectal manometry in the newborn may show more false-positive and false-negative results.

The false-negative results during anorectal manometry probably represent artifacts, such as movements of the probe, passage of flatus or feces, or relaxation of the EAS.[101] To avoid technical mistakes, it is necessary to have an empty rectum and correct position of the probe and to watch closely for movement (which could produce an artificial relaxation).

The false-positive manometries could be explained by different factors. First, there could be immaturity of the ganglion cells, particularly in premature infants and neonates. Other factors could include the following: some children have a high relaxation threshold, there could be technical errors in which the relaxation zone may be missed, and, finally, the presence of feces in the anorectum could interfere.[101]

Before a patient who has a nonrelaxing IAS and obstructive symptoms with normal biopsies is categorized as having a false-positive manometry, one has to decide if the patient suffers from IAS achalasia, or ultrashort-segment Hirschprung disease.[102,108–110] Anorectal manometry is the only way to diagnose this entity. In IAS achalasia, there is a nonrelaxing IAS, but the biopsies show normal ganglion cells and normal acetylcholinesterase.[102,108–110]

Another important use of anorectal manometry in children with constipation, after Hirschsprung disease has been excluded, is to detect those who have pelvic floor dyssynergia (see Table 70-2 and Figures 70-11 and 70-12).[92] Pelvic floor dyssynergia has been found in 30 to 53% of patients with constipation who have undergone manometry. The significance of this finding has been controversial, but it has been suggested that it may be associated with lower recovery rates. [91,111,112] Attempts to normalize defecation dynamics have been undertaken with biofeedback therapy.[93,113–115] In multiple open-label studies, biofeedback was shown to be effective, but the results of recent randomized trials have not demonstrated long-term efficacy.[93,113–115]

Changes in resting and squeeze pressure, as well as abnormal sensation, have been found inconsistently in patients with constipation by different authors and do not seem to have any major clinical implications.[1,88,111,116–118] Abnormal sensation has also been described in children with constipation,[88,92,112] and it is not known if the abnormality represents a primary problem or if it is secondary to the megarectum.

Manometry is useful in the evaluation of patients who have undergone surgery for Hirschsprung disease and continue to have incontinence or obstructive symptoms (see Table 70-2).[1] The presence of the RAIR in postoperative patients has been variable and in most studies does not seem to be correlated with outcome.[119–121] In a long-term follow-up study of adults, there was a positive correlation between functional outcome and anal resting pressure.[122] It has recently been suggested that those with obstructive symptoms and a high-pressure, nonrelaxing sphincter may benefit from procedures designed to decrease sphincter pressure. IAS myectomy[123] or, recently, botulinum toxin injection has been used.[102,124] Anorectal manometry is therefore a technique that allows detection of the nonrelaxing sphincter and may be useful to decide if the patient is a candidate for botulinum toxin or to evaluate patients after it has been applied.

The utility of anorectal manometry in diagnosing neuronal intestinal dysplasia is controversial.[125,126] The first difficulty arises because there is no consensus regarding the histologic diagnosis.[126] However, even in those reports in which a diagnosis of neuronal intestinal dysplasia has been made, the anorectal manometry has not been able to discriminate abnormal from normal cases.[125,127,128]

Anorectal manometry has been shown to allow some discrimination between patients with or without fecal incontinence (see Table 70-2).[1,129–132] In a study of 350 patients, 178 of whom were incontinent by history, it was found that squeeze pressure had the greatest sensitivity (60%) and specificity (78%) if a cutoff value of 60 mm Hg was used.[129] The measurement of the resting anal canal pressure was less sensitive and specific. In another study comparing 302 patients with fecal incontinence and 65 controls, it was found that by taking the mean – 2 SD for controls as the cutoff, the sensitivity of the maximum squeeze pressure to predict incontinence was 92% and the specificity was 97%. Basal anal resting tone had a sensitivity of only 37%.[130] An abnormal sensation has also been reported in patients with fecal incontinence,[131] and it has been suggested that the most important component of

biofeedback training for fecal incontinence is an improved ability to detect rectal distention.

In patients with imperforate anus, anorectal manometry is a useful technique to evaluate the state of intrarectal pressure and sensation, as well as of the voluntary muscles.[1,131,133–135] Some studies have found that patients with a repaired imperforate anus have significant abnormalities in anorectal function.[131,134,135] It has been shown that patients with more incontinence have lower squeeze pressures and sensation abnormalities.[131,133] In postoperative patients with fecal incontinence, the measurement of intraanal pressure is important, particularly when a reoperation is being considered. Also, a manometric evaluation may allow the detection of abnormalities that may be amenable to biofeedback training.[2,131,135,136]

Indications. The main indication is in the evaluation of children with constipation to exclude the presence of a nonrelaxing IAS (see Table 70-2).[1] Hirschsprung disease also has to be considered in older patients (including adults) with intractable constipation.[137] It is indicated in the evaluation of patients with fecal incontinence to evaluate sphincter function and weakness, as well as sensation.[1] In patients with imperforate anus repair or neurogenic problems, it may be useful to decide if the patient is a candidate for biofeedback.[1] It is also indicated in the evaluation of children with Hirschsprung disease who have postoperative obstructive symptoms to determine if they are candidates for botulinum toxin or after botulinum toxin has been applied and the symptoms recur (see Table 70-2).[1]

The study of defecation dynamics is also important, although the use of biofeedback in children has not been shown to be effective.[1,113]

Other Tests to Evaluate Anorectal Function. EMG of the pelvic muscles can be performed using either a needle electrode, a surface electrode, or an anal plug. This test allows the identification of areas of injury, establishes if the muscle contracts or relaxes, and identifies evidence of denervation-reinnervation. The needle electrodes that are used study the activity of a large number of motor units (concentric) or a single fiber. This requires the insertion of the needle in the perineum into the sphincter muscle, so it is painful, and its utility in children is limited. In children, it is most useful in the evaluation of postoperative patients of imperforate anus, in which it may be used to show the presence or absence of striated muscle around the anal opening. It is also useful in the establishment of nerve damage from spinal lesions. Because of its invasiveness and its limited usefulness, needle EMG is not routinely used in pediatrics. On the other hand, superficial EMG electrodes can be useful, particularly to evaluate for the presence of pelvic floor dyssynergia.[88,91] This causes less discomfort to the patient. It has also been shown that the number of motor units recruited during squeeze correlates with squeeze pressure. Therefore, surface electrodes have been successfully used in the assessment of defecation dynamics and squeeze pressure and can also be used to provide biofeedback training.[113]

Other tests, such as balloon expulsion and pudendal nerve terminal motor latency, have not been shown to have a clinical role in the evaluation of children.

OTHER STUDIES TO EVALUATE GASTROINTESTINAL NEUROMUSCULAR FUNCTION

Electrogastrography

Electrogastrography (EGG) is a technique in which the electrical activity generated by gastric smooth muscles is measured.[138–140] It is a noninvasive method of assessing the gastric myoelectrical activity that controls gastric motility.[140–144] This is now done by using surface cutaneous electrodes placed on the abdomen overlying the stomach.[138,141,142] Because of its noninvasive nature, it represents an attractive tool for the diagnosis of gastric motility problems in children.[138,139,141–143]

The cutaneous EGG usually uses three to four electrodes that are placed on the abdominal wall close to the antral region to reduce interference and to obtain a high signal-to-noise ratio.[138,144] Ideally, the electrodes are placed either with ultrasound or fluoroscopic guidance to identify the gastric contour.[140–144] In adults, it has been advocated that a standard configuration can be used without the need for imaging studies.[138,140,144] The first electrode is set above the antrum (located 1 to 3 cm right of the midline, between the xiphoid process and the umbilicus), the second 45° and 3 to 6 cm above and left of the first electrode (based on the size of the subject), and the third (reference) at the left flank, horizontal to the first electrode. In an effort to establish if it is possible to accurately place the electrodes without the use of ultrasonography in neonates, Patterson and colleagues compared the results obtained by both ultrasonography or blind placement of the skin electrodes and found no differences.[142] In another study in premature infants, the first electrode was located immediately below the left costal margin in the midclavicular line.[141] The third electrode was located between the umbilicus and the xiphoid process and the second electrode between the first and third electrodes.

The duration of an EGG recording session may vary depending on the center or the working diagnosis.[138,140,144] Most commonly, the test is performed with 1-hour fasting, then a standardized meal is given, and the activity is recorded for another 1 to 2 hours postprandially.[138,140–144] It has been suggested that for the major EGG parameters, 30 minutes in the fasting state is sufficient to produce reliable results, and in the postprandial period, recordings from 30 to 60 minutes adequately represent the change after meal ingestion.[140] The recordings are analyzed by computer using specialized software, and usually the fast Fourier transform is used to detect the dominant frequencies.[42,138,139,141,143]

The major EGG parameters that are analyzed include the following: the dominant frequency, the dominant power, the percentage of normal slow waves, the percentage of gastric dysrhythmias, and, in some centers, the dominant frequency instability coefficient (DFIC).[138,139,141,143]

Many studies have documented that the dominant frequency of the cutaneous EGG corresponds to the basal gastric rhythm or frequency of the gastric slow wave.[138,140,144] It is thought that the electrical activity reflects the gastric slow wave generated by the stomach's pacemaker, the interstitial cells of Cajal of the greater curvature.[55,140,143] The normal gastric slow-wave frequency is about 3 cycles per minute (cpm) (normal range 2–4 cpm).[138] The computer also determines the "power" for each of the signal frequencies. Power is a reflection of both the amplitude and the regularity of the EGG.[140,144] It has been shown that the EGG amplitude does not always correlate with the force of the gastric contractions,[144] and the absolute value is influenced by electrode placement, skin/fat thickness (distance between stomach and skin), and movement of the stomach.[140,144] Therefore, only relative changes are considered by comparing in the same individual the change of the power after a meal.[138,140,144] This can be calculated from the ratio of the postprandial power of the dominant frequency to the preprandial power of the dominant frequency.[139,143,144] Usually, the postprandial-to-fasting power ratio value is more than 1.[55,144] It has been suggested that a diminished or absent increase in the power ratio correlates to delayed gastric emptying and antral hypomotility.[144]

The percentage of normal slow waves reflects the regularity of the EGG.[140] It is defined as the percentage of time during which 2 to 4 cpm slow waves are present across the entire recording. Studies suggest that most healthy individuals display a value above 70% in fasting and postprandial states.[140]

A dysrhythmic index (percentage of dysrhythmic time during the recording) is usually reported.[138] It measures the percentage of time during the recording in which 2 to 4 cpm slow waves are absent. It has been suggested that abnormal rhythms of gastric myoelectrical activity may be detected. They are classified as tachygastria (rapid) usually > 4 cpm, bradygastria (slow) < 2 cycles/min; bradytachyarrhythmia (mixed), or absent activity.[139,143,144] Antral hypomotility has been associated with both brady- or tachyarrhythmia.[139,144] Values for a 1-hour recording in both fasting and fed states have been reported in 10 asymptomatic children and in 14 children with dyspepsia.[145] The fasting dysrhythmic index was 1.6% (range 1.6–33.3) in the controls versus 33.3% (range 10–48) in the dyspeptic patients. The fed dysrhythmic index was 2.7% (range 1.6–5.5) in controls and 15% (range 11–66) in patients.[145]

The DFIC specifies the stability of the gastric electrical peak visible on the running spectra plot and calculated as the percentage ratio of the frequency standard deviation to mean gastric frequency.[138] The DFIC reflects subtle changes in gastric slow waves.

In children, the validation of the technique is limited, but the use of EGG is growing. One of the biggest problems so far has been the fact that there is almost no information about the normal EGG patterns in healthy children, including a lack of information on developmental aspects.

A study in 114 normal children (6–12 years) found in the preprandial state a dominant frequency of 3.0 cpm (2.1–3.5), a DFIC of 26% (6.7–54.5), normal slow waves of 81.5% (59.3–100), bradygastria of 3.7% (0–22.2), and tachygastria of 9.4% (0–38.7).[146] The dominant frequency postprandially was 3.0 cpm (2.1–3.7), the DFIC was 30% (11–67; $p < .05$), normal slow waves were 76.9% (54.4–97.2), bradygastria was 4.9% (0–29.3), and tachygastria was 11.8 (0–35). The EGG power increased postprandially to a power ratio of 3.6 (0.8–19.7). The researchers found no age differences or effects of age, gender, and body mass index.[146]

In another study of 24 normal boys ranging from 1 to 11 years (mean 6 years), Cheng and Tam found that the percentage of bradygastria was higher in younger children, accounting for 34% of the recording.[147] The normal 3 cpm increased with age, whereas the bradygastria decreased. They suggested that by the end of the first decade, the EGG patterns are similar to those in the adult,

A recent study of 50 healthy volunteers ranging in age from 6 to 18 years described a mean dominant frequency of 2.9 ± 0.4 cpm preprandially and 3.1 ± 0.35 postprandially, with 80% ± 13% of test time spent in the normogastric range (2–4 cpm) before and 85% ± 11% after the test meal.[143] The authors concluded that there was a postprandial increase in rhythmicity and amplitude of the gastric slow waves and that key normative values are not dependent on age, gender, or body mass index.[143]

Development. Different authors have found that both preterm and newborn infants have a low percentage of gastric slow waves.[148,149] Chen and colleagues studied EGG in five different groups: premature infants, newborns, infants 2 to 6 months, children 4 to 11 years, and adults.[149] Children and adults showed similar activity.[149] Infants showed a percentage of normal slow wave that was significantly lower than that in adults but higher than in newborn and premature infants. The percentage of 2 to 4 cpm slow waves was 26 ± 4% in premature infants, 30 ± 4% in full-term infants, 70 ± 6 in 2- to 6-month-old infants, 85 ± 3% in children 4 to 11 years, and 89 ± 2% in adults.[149] As in other studies, both newborns and premature infants showed an absence or a decrease in normal slow waves.[148–150] The biggest limitation of the study is the small number of patients. Koch and colleagues also showed that in premature infants and newborns, there were no significant differences in activity after gavage feeding.[150] Bradygastria, 3 cpm, and tachygastria comprised 49%, 17%, and 29% of the preprandial recording and 51%, 15%, and 27% of the postprandial EGG.[150]

The developmental maturation of the gastric slow waves has recently been analyzed in a longitudinal study of 19 preterm infants followed from birth to 6 months. It was shown that the percentage of 2 to 4 cpm slow waves increased gradually over time to a value slightly lower than that in adults.[151] The rate of development was twice as fast in the first 2 months, and dominant peaks and an increase in power were not observed at birth in any of the infants but could be identified in 70% of the children at 6 months of age.[151] Patterson and colleagues studied 9 healthy neonates born at 34 weeks gestation and showed that their EGG dif-

fered from that of adults.[141] Tachygastria and bradygastria were seen more frequently, with fewer episodes of 3 cpm. There was an increase in 3 cpm episodes over time.[141]

Clinical Significance. Interest in EGG has been generated not only because it is a noninvasive technique but also because, in recent years, a correlation between disorders in gastric electrical rhythm and certain clinical syndromes characterized by alterations in gastrointestinal motility has been described.[138,140,146] Because normal motility of the stomach requires integrity of the enteric and extrinsic nerves and smooth muscle, any abnormality in them may result in dysfunction.[140] Therefore, there are some diseases in which EGG may be theoretically useful, and it is possible that it may be used as a noninvasive screening technique[140] or, most importantly, as a way to evaluate follow-up or the results of therapy, particularly when invasive techniques are the only other alternative. To date, however, no therapies have convincingly demonstrated in controlled studies that correcting abnormalities detected by EGG improves upper gastrointestinal symptoms.[138] Ideally, EGG could be used as a screening tool to detect those patients who may have underlying motility disorders. It may provide evidence that upper gastrointestinal symptoms are associated with gastric dysmotility. However, studies have shown that there are patients with gastric dysrhythmias who do not have abnormal motility or that not all patients with abnormal motility have dysrhythmias. In children, limited data have tried to validate the specificity and sensitivity of EGG to detect motility disorders in children. In a study in which simultaneous EGG was performed together with antroduodenal motility in 25 children, the authors found that EGG differentiated groups of children with normal manometry from others with neuropathic or myopathic change, but in some patients, there was an overlap of EGG results between children with normal and abnormal manometry.[55] The percentage of tachygastria time was higher in patients with mild (44 ± 16%) and severe neuropathy (48 ± 19%) compared with those with myopathy (20 ± 16%) or normal motility (23 ± 13%). There was considerable overlap in the percentage of tachygastria and total arrhythmia time among the different groups. Every child with a total arrhythmia time < 35% and a ratio of postprandial-to-preprandial power > 2.4 had normal motility.[55]

In children, EGG has also been used as a method to understand the physiopathology of upper gastrointestinal symptoms in patients with different medical problems, such as pseudo-obstruction,[152] GER after fundoplication,[153,154] with functional dyspepsia[139,140,145] or in children with systemic or generalized problems. Gastric dysrhythmias have been described in children with renal failure,[155] Noonan syndrome,[156] cyclic vomiting,[157] cystic fibrosis,[139] or neurologic handicaps.[154,158]

EGG has also been suggested as a noninvasive way in which to evaluate the effects of therapy for motor disorders. In a study to evaluate the effects of cisapride, Cucchiara and colleagues showed that after 8 weeks of therapy, the medication reversed the abnormal myoelectric activity in three children with nonulcer dyspepsia.[159]

Indications. Even though, at present, the use of EGG in children is still considered a research tool and future studies will be needed to continue to validate its usefulness, it is very likely that EGG will become a useful test in the evaluation and treatment of children with gastrointestinal motility disorders. The most likely indication will be in the evaluation of children with unexplained nausea, vomiting, and postprandial abdominal bloating or distention. It may also become a screening test to predict gastroparesis, to assess for gastric motor dysfunction in patients with other gastrointestinal symptoms (eg, constipation), to try to predict who will develop symptoms after fundoplication, or to follow the response to medications.[42,55,140,153,154,159] For now, however, the proposed clinical indications for the performance of the EGG in adult and pediatric patients with unexplained nausea, vomiting, and dyspeptic symptoms must be validated by prospective controlled investigations.[138]

Other Future Techniques

Other gastrointestinal motility studies are currently being performed mostly in adult units or research laboratories that have the potential to become clinically useful in pediatrics. Therefore, a brief description follows, although, at present, there is very limited information in children, and these studies have no role in the routine treatment of children with motility problems.

Manometry of the SO. The evaluation of manometry of the SO has been used mainly to evaluate patients for SO dysfunction. The intraluminal pressure recording from the billiary tree requires intubation with the use of endoscopic retrograde cholangiopancreatography (ERCP), which makes the technique very invasive. Also, the effects of anesthesia or sedation on manometric results have not been well established. In addition to the technical difficulties involved, the performance of SO manometry increases the incidence of post-ERCP pancreatitis and may result in pancreatitis in up to 15 to 20% of patients.[160]

When SO manometry is performed, a perfused system is usually used. A catheter with three lumens of 0.5 mm internal diameter making up a catheter of 1.7 mm or a tapering catheter of 1.5 outer diameter is usually introduced using the biopsy channel of the duodenoscope through the papilla and into either the bile duct or the pancreatic duct. The catheter is then withdrawn until all three recording ports are situated in the SO. The SO is characterized by a basal pressure on which prominent pressure peaks representing phasic contractions are superimposed. Usually, the basal SO pressure ranges from 3 to 35, with a median of 15 mm Hg.[161,162]

Over the past decade, different abnormalities of the SO have been described,[160,161] and abnormal pressure is the parameter most often employed.[161] The clinical significance of SO manometric findings is still debated. Some authors recommend SO manometry for the evaluation of recurrent pancreatitis or unexplained biliary pain and for the selection of those patients who may benefit from endoscopic therapy (eg, sphincterotomy).[160,161] In children, there is very limited information,[162] and there are no nor-

mal controls or validation of its clinical utility. Because of those limitations and the high incidence of pancreatitis associated with its performance, SO manometry should still be considered only a research tool, although it is possible that, in the future, it may have a role in the evaluation and treatment of children with unexplained right upper quadrant pain or recurrent pancreatitis.[162]

Barostat. This technique allows the study of a component of motor activity that cannot be evaluated by conventional manometry or EMG. The barostat is mainly used to measure intraluminal volume or pressure relationships and helps to establish sensory thresholds.[163,164] The instrument requires a high-compliance balloon, and it measures tone by monitoring the volume of air required to maintain a constant preselected pressure level in a flaccid bag. The system uses an electronically regulated air injection/aspiration device. The volume of the bag increases or decreases depending on intraluminal pressure, which is determined by the motor activity of the organ being studied. When used in the stomach, it uses a balloon designed for the fundus/body, in which relaxations and contractions are relatively slow.

The barostat also allows the quantification of sensory thresholds triggered by intraluminal distention. In children, the use of the barostat has allowed some insight into the pathophysiology of irritable bowel syndrome and abdominal pain.[163,164] At this point, the barostat remains a research tool that is being used to understand the physiopathology of irritable bowel syndrome or other gastrointestinal disorders and to evaluate the effects of therapy, and it is difficult to predict what role it will play in the routine evaluation and treatment of patients with gastrointestinal motility disorders.

TRAINING IN GASTROINTESTINAL MANOMETRY

The North American Society for Pediatric Gastroenterology Hepatology and Nutrition (NASPGHAN) has approved and published guidelines for training in gastrointestinal motility.[165] The guidelines recommend two levels of training. Level 1, or basic training, is expected from all trainees and includes understanding the pathophysiology of motility disorders, treatment of an adequate number of patients with these problems, and understanding of the rationale, usefulness, and limitations of the common tests used in the evaluation of the patient. Level 2, or advanced training, is recommended for those who are planning to perform specialized motility studies or act as consultants to other gastroenterologists. Table 70-4 shows the number of procedures recommended to achieve proficiency in the performance of the common gastrointestinal manometry studies.[165]

TABLE 70-4 NUMBER OF PROCEDURES TO ACHIEVE COMPETENCE IN GASTROINTESTINAL MANOMETRY*

STUDY TYPE	THRESHOLD FOR COMPETENCE
Esophageal manometry	20
Anorectal manometry	20
Antroduodenal manometry	25
Colonic manometry	25
Electrogastrography	25

Adapted from Rudolph C et al.[165]
*NASPGHN guidelines.

SUMMARY

The study of gastrointestinal motility in children continues to evolve and has changed from being a research technique to becoming a useful diagnostic tool. There continues to be a dichotomy between the use of manometry as an investigation technique or as a useful clinical test, and as new techniques are being developed and validated, the clinical indications are becoming better defined. The performance of gastrointestinal manometry in children is more challenging, and recent technical advances have allowed the study of younger and smaller babies, which has allowed the understanding of some developmental aspects.

The role of gastrointestinal manometry has been defined more clearly for anorectal and esophageal manometry, although small bowel motility is becoming clinically more accepted. Other studies are mainly performed in research laboratories and have still not been fully validated. Manometry is mainly useful for the diagnosis of primary motility disorders and can be useful in some cases in which the motility alterations are secondary to other illness. In general, the manometric evaluation may detect aberrations that may be clinically insignificant, so care needs to be exerted to avoid overinterpretation.

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CHAPTER 71

pH MEASUREMENT

Yvan Vandenplas, MD, PhD

WHY MONITOR THE PH IN THE ESOPHAGUS?

The idea that the measurement of the pH in the esophagus may be of great clinical importance started with the observation that acid perfusion–induced heartburn coincides with the fall of intraesophageal pH below 4.0.[1] Esophageal pH monitoring is often considered an investigation technique studying esophageal motility, which it obviously does not do because it does even not measure gastroesophageal reflux (GER). This being said, the major shortcoming of esophageal pH monitoring is obvious: the technique measures changes in esophageal pH, not GER. The first clinical tests were performed in the early 1960s by Miller.[2] Modern electronic technology has profoundly changed the practice of medicine, principally through its ability to monitor, record, and analyze large volumes of data. The introduction of computers has provided physicians with powerful tools to identify elusive and intermittent disorders, such as gastroesophageal reflux disease (GERD). Although a continuous investigation technique such as pH monitoring is extremely suitable for ambulatory outpatient application, many centers still hospitalize patients.

Major areas of indications for esophageal pH monitoring are (1) in clinical and laboratory research, (2) as a routine clinical procedure in the diagnosis of GERD, especially in children presenting with atypical GER manifestations (Table 71-1), and (3) in the evaluation of the efficacy of treatment of GERD on the frequency and duration of the presence of acid in the esophagus.[3,4]

HARDWARE AND SOFTWARE: PEDIATRIC NEEDS

Device

Purchase costs, system abilities, costs in use, number of measurements, and durability of the material are factors to consider before purchasing equipment. Of importance for pediatric use are a time indication on the display (ie, the number of data recorded, the real time, the duration of the investigation) and the protection of the event marker(s) to avoid erroneous use by the child.[4] A system should refuse to work if it has not been calibrated properly.

One of the advantages of pH monitoring is the possibility of realizing an ambulatory recording, even in young children. Therefore, the device should be small and light. Devices not larger than a credit card, although of course a little thicker, are commercially available.

Electrode

pH sensors or "electrodes" exist in several forms, of which the two most popular are glass and antimony. Ion-sensitive field effect pH electrodes are modified field effect transistors. Glass electrodes are generally considered to be the most accurate.[5,6] Clinical studies require a pH sensor that is both economical and reliable. Glass electrodes with an internal reference are "the best" but are expensive and have a rather large diameter (3.0–4.5 mm). Although the passage of such an electrode through the nostrils of a young baby is, most of the time, technically possible, it does not mean that it is well

TABLE 71-1 SYMPTOMS OF GASTROESOPHAGEAL REFLUX DISEASE

ESOPHAGEAL MANIFESTATIONS
Specific symptoms
Regurgitation
Nausea
Vomiting
Symptoms possibly related to reflux esophagitis
Symptoms related to anemia (iron deficiency anemia)
Hematemesis, melena
Dysphagia (as a symptom of esophagitis and/or due to stricture formation)
Weight loss and/or failure to thrive
Epigastric or retrosternal pain
"Noncardia angina-like" chest pain
Pyrosis or heartburn, pharyngeal burning
Belching, postprandial fullness
Irritable esophagus
General irritability in infants ("colic")
UNUSUAL PRESENTATIONS
GER related to chronic respiratory disease (eg, bronchitis, asthma, laryngitis, pharyngitis)
Cystic fibrosis
Sandifer Sutcliffe syndrome
Rumination
Apnea, apparent life-threatening event, sudden infant death syndrome

GER = gastroesophageal reflux.

tolerated and that it is the best option. From recent experience with combined pressure-pH recordings, it became clear that the larger the diameter of the electrode(s), the more the patient had to swallow (personal data). Thus, the pharyngeal presence of an (large size) electrode has a decreasing effect on GER episodes because the more the patient has to swallow, the more primary peristalsis is induced, and the better esophageal clearance becomes.

Because of their smaller diameter, antimony (2.1 mm) (Synectics Medical, Queluz, Portugal) or glass microelectrodes (1.2 mm) are preferable in infants. Antimony electrodes also exist with a diameter of about 1.5 mm for use in premature babies; these electrodes are too flexible for older babies. Single esophageal pH monitoring cannot detect alkaline reflux.[7] Glass electrodes have only one pH sensor. Antimony electrodes with multiple pH sensors may help to detect alkaline reflux episodes. Antimony electrodes with two sensors can also be helpful to evaluate the therapeutic efficacy of acid-reducing medication: the esophageal sensor measures the incidence of acid GER, whereas a gastric sensor measures the efficacy of the medication. Antimony is only poorly resistant to gastric acid, but the fact that acid should be reduced or minimalized in these patients minimizes the impact of this shortcoming.

Most antimony and all glass microelectrodes need an external cutaneous reference electrode, which is a possible cause of erroneous measurement resulting from transmucosal potential differences. If the environmental temperature is high or the patient sweats a lot, the conductivity of the contact gel will change, resulting in a less accurate conduction of the electric potential. Antimony electrodes with a diameter of about 2.0 mm with an internal reference electrode have been developed, providing comparable results (personal data, 1997; Table 71-2). This electrode is accurate, thin, flexible, and easy to place in the esophagus, and there is no longer need for a cutaneous reference electrode. However, in clinical reality, the purchase cost of the electrode and income of the pH monitoring will substantially influence the type of electrode used. Whatever the type of electrode chosen, each center should preferentially use one type or a limited number of electrodes.

Prior to each study, an in vitro two-point calibration must be carried out. The electrode and reference are placed in two buffer solutions (usually pH 1.0 and 7.0) at either room or body temperature until stabilization is reached. This calibration should be repeated on return of the patient to rule out electrode failure and to check for slow pH drift. A drift of less than 0.5 pH over the 24-hour period is acceptable. Calibration needs to be corrected according to room and body temperature.

LOCATION OF THE ELECTRODE

There is abundant evidence in the literature that the esophageal location of the electrode is of critical importance regarding the number and duration of acid reflux episodes recorded. It seems logical that the closer the electrode is located to the lower esophageal sphincter (LES), the more acid reflux episodes will be detected.[8,9] In adults, the electrode is, by consensus, positioned 5 cm above the proximal border of the LES. Also in adults, determination of the position of the LES by means of a standard stationary esophageal manometry study is generally regarded as the optimum method for pH probe localization.[6] In children, several methods have been proposed to determine the location of the electrode: fluoroscopy, calculation of the esophageal length according to the Strobel formula (distance from the nose to the cardia = 5 + 0.252 [length in cm]), manometry, and endoscopy. Ideally, as in adults, the electrode should be sited in reference to the manometrically determined LES. However, this has several inconveniences: (1) manometry in infants and children is time consuming, rather invasive, or at least unpleasant, and (2) this method would have the inconvenience that the electrode is located at a fixed distance to the LES, whereas the length of the esophagus increases from less than 10 cm in a newborn to over 25 cm in an adult. Moreover, manometry cannot be performed in all centers. Therefore, the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition Working Group on GER recommended the use of fluoroscopy to locate the electrode.[4] The radiation involved is minimal, and the method can be applied in each center. As the tip of the electrode moves with and during respiration, the tip should be positioned in such a way that it overlies the third vertebral body above the diaphragm throughout the respiration cycle (Figure 71-1). Dislocation by a curled electrode is also

TABLE 71-2 ADVANTAGES AND DISADVANTAGES OF PH MONITORING TO DIAGNOSE GASTROESOPHAGEAL REFLUX DISEASE

	ADVANTAGES	DISADVANTAGES
Technique	Physiologic conditions Normal ranges Good reproducibility Long duration (24 h)	Physiologic conditions Social discomfort (electrode)
Diagnosis	Quantification of number of pH changes Quantification of duration pH changes Time-relation symptom-pH change	pH change, not GER Alkaline GER (?) No neutral GER
Complications	Area under pH 4: related to esophagitis	No information tissue damage
Treatment	Contributes to choice of treatment Evaluation of treatment	No acid if H_2 blocker, PPI

GER = gastroesophageal reflux; PPI = proton pump inhibitor.

prevented with fluoroscopy. If the pH device is exposed to x-rays, the data and calibration may be erased.

PATIENT PREPARATION

No special patient preparation is required for pH monitoring, except for fasting. The patient should fast for at least 3 to 5 hours before the study, depending on the age, to avoid nausea and vomiting. If the child is able to communicate, it is important to reassure the child at the beginning of the study and explain what will happen. The child should understand that the passage of the catheter through the throat is uncomfortable, but after the first few swallows, it will feel better. To facilitate insertion, a silicone spray (eg, Silicone-spray, Alphamed, Brussels, Belgium) can be placed on the electrode (but not on the pH sensor!) and/or local anesthesia of the mucosa of the nostrils can be done. Sedation should not be used because the sedative may interfere with swallowing and influence pressures.

Histamine$_2$ (H_2) blockers or proton pump inhibitors should be stopped at least 3 or 7 days, respectively, before a diagnostic pH monitoring (on the condition that the investigation is not performed to evaluate the acid-buffering effect of the drug). Antacids are permitted up to 6 hours prior to the start of the recording. Prokinetics should be stopped at least 48 hours before the pH monitoring.[8] The continuation or discontinuation of drugs depends on the indication for the pH study: diagnosis of reflux or evaluation of efficacy of treatment.

It is best not to start a pH metry the same day an upper gastrointestinal tract endoscopy was performed because of the sedation, fasting, and inflated air. It is best to start pH metry at least 3 hours after a barium swallow or radionuclide gastric or esophageal studies.

PATIENT-RELATED INFLUENCING FACTORS: RECORDING CONDITIONS

Feeding, position, and physical activity are examples of patient-related factors influencing pH monitoring data. Patient-related factors that possibly influence the results of pH monitoring are a controversial topic.[4,8] The answer to the fundamental question if patient-related factors should be minimized and standardized is difficult and necessarily ambiguous. If the pH monitoring is performed as part of a diagnostic workup in a patient, it is interesting to study the patient during normal daily life, noting what is enhanced by unrestricted recording conditions. But if the pH monitoring is performed as part of a (clinical) research project, recording conditions should be standardized. Standardization of recording conditions inevitably causes a loss of patient-specific information.

DURATION OF THE RECORDING

The duration of the recording should be "as close as possible to 24 hours" and at least 18 hours, including a day and a night period.[4,10,11] If the pH monitoring is performed for diagnostic purpose, there is no indication for short-duration pH tests (eg, Tuttle and Bernstein tests, 3-hour postprandial recording). The first reports on the clinical use of pH monitoring concerned esophageal tests of short duration. Tuttle and Grossman developed the "standard acid reflux test."[12] This test was modified by Skinner and Booth[13] and Kantrowitz and colleagues,[14] demonstrating that pH tests can contribute to define abnormal GER. The Tuttle test was reported to have a sensitivity of 70%.[15] After great initial enthusiasm for this test, criticism became more and more common. The test is unphysiologic in requiring intragastric instillation of acid and various artificial maneuvers to raise intragastric pressure. In the early 1980s, it was reported that the false-positive rate might be as high as 4 to 20% and the false-negative rate as high as 40%.[16–18] Bernstein and Baker demonstrated in 1958 that heartburn could be provoked by infusing diluted hydrochloric acid into the esophagus in susceptible individuals.[19] This test was shown to be 100% positive in reflux patients.[20] A modified Bernstein test was used to illustrate the relationship between GER and apnea and stridor and between nonspecific chest pain and GER.[21,22] Provocative testing can be used in particular conditions to demonstrate the relationship between GER and specific symptoms (bradycardia in relation to acid in the esophagus). However, provocative testing has always had the inconvenience that the investigation conditions are unphysiologic. The latter might explain some discrepancies in literature. Ramet and colleagues showed a prolongation of the R-R interval in infants during provocative testing with acid instillation in the esophagus,[23] whereas others could not reproduce these findings in 24-hour recordings in physiologic conditions.[24,25]

There is now substantial evidence that in controls and in the majority of infants and children with classic symp-

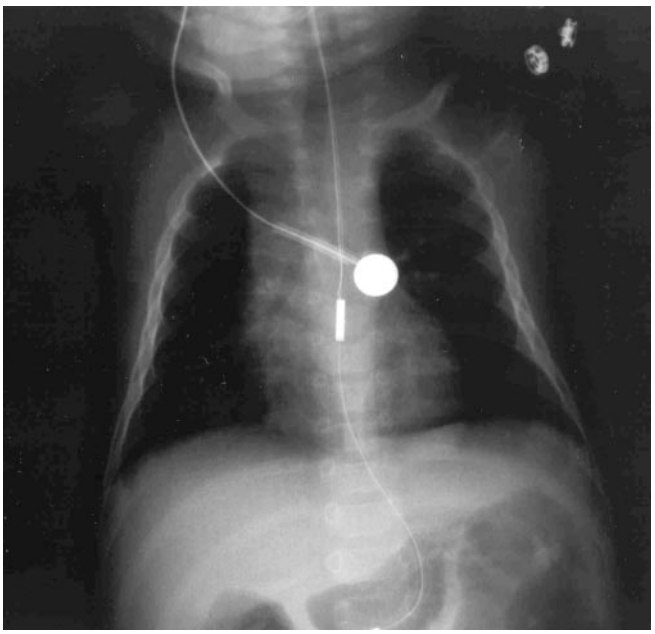

FIGURE 71-1 Radiograph of the thorax to show the localization of the pH electrode (third vertebra above the diaphragm). The radiograph shows a two-channel electrode with the distal electrode in the stomach and the proximal electrode at the third vertebra.

toms of GERD, esophageal acid exposure is highest during the day, probably because of provocation of GER by food ingestion and physical activity. Controls have more reflux upright than supine or more reflux awake than asleep.[26] The relationship between esophagitis and nocturnal reflux is far from clear.[27–29]

Feeding

Feeding during pH monitoring has been and still is an area of controversy. On the one hand, it seems logical to forbid the intake of acidic foods and drinks. However, many popular foods and beverages have a pH of < 5.0 (eg, cola drinks, fruit juice, tea, soup), resulting in a quite restricted diet. A too restricted diet might alter the patient's normal daily habits in such a way that the investigation is no longer performed in physiologic conditions. Electrodes are temperature sensitive; therefore, very hot and ice cold beverages and food (eg, coffee, tea, ice cream) should be avoided.[4] Ask the older child not to chew gum or eat hard candy because these will increase saliva production and thus will induce swallowing and peristalsis and tend to normalize the test results. In older children, alcohol intake and smoking should be recorded on the diary.

In infants, it has been suggested to replace one or several feedings during pH monitoring with apple juice.[30] This would solve the problem of gastric anacidity after a milk feeding, but apple juice has a pH of about 4.0 and a very rapid gastric emptying and is not part of normal infant feeding. In our unit, feeding is free, but parents and children are asked not to exaggerate "acid" ingestion and to avoid very hot and cold food and beverages and chewing gum. Although the ingestion of acid such as a cola drink might simulate a reflux episode, the duration of ingestion is limited to a few minutes and most of the time is irrelevant to the total 24-hour data. To minimize this effect, it is possible to eliminate these false reflux episodes with the help of a diary.

All of the above is true for diagnostic investigations. However, in research, the opposite might be valid: all factors possibly influencing the pH data should be controlled and standardized as much as possible.

The influence of a particular food on the incidence of acid GER episodes detected by pH monitoring might be opposite to its influence on the incidence of reflux episodes: a fat meal is known to provoke GER because of its delayed gastric emptying. Because the duration of postprandial gastric anacidity after a fat meal is prolonged, a meal with a high fat content will result in delayed gastric emptying and thus less acid reflux episodes detected by pH monitoring.[31,32] Some drugs influencing gastric emptying (eg, prokinetic agents) have a comparable effect on pH monitoring data: prokinetic drugs enhance gastric emptying, shorten the period of postprandial gastric anacidity, and prolong the periods during which acid GER can be detected.

Position

Different patterns of GER (upright, supine, combined) have been reported in adults and older children.[33] Orenstein and colleagues. demonstrated that the prone sleeping position is the preferred position for infants as far as GER is concerned because crying time is decreased if compared with the supine position.[34–36] There is evidence that the prone anti-Trendelenburg 30° sleeping position reduces GER in normal subjects and patients, although the position is difficult to apply and maintain correctly (infants have to be tied up in their bed). Meanwhile, the literature on sudden infant death syndrome (SIDS) in infants shows that infant mortality decreases if infants are put to sleep in the supine position. Accordingly, the prone anti-Trendelenburg position is no longer recommended in infants at risk for SIDS.[37,38] Thus, the position of the infant should be recorded on the diary during pH monitoring.

DATA ANALYSIS

Interpretation and Parameters

Interpretation starts with a visual appreciation of the pH tracing, what is subjective and difficult to standardize (Figure 71-2). Nevertheless, it is of the utmost importance "to have a look" at the tracing. The parameters that are classically analyzed are the total number of reflux episodes, the number of reflux episodes lasting more than 5 minutes, the duration of the longest reflux episode, and the reflux index that is the percentage of time of the entire duration of the investigation during which the pH is less than 4.0. From all "classic" parameters, the acid exposure time or reflux index is the most relevant. The correlation between all four parameters is good, and they are closely related to the reflux index.[39] Results should also be (automatically) calculated for periods of interest, such as sleep, wakefulness, feeding, postprandial fasting, and body position. A time relation between atypical manifestations (eg, cough, bradycardia, desaturation) and "changes" in pH (not necessarily a drop in pH below 4.0) should be searched for. The duration of reflux during sleep has been suggested to be a good selection criterion for reflux related to apnea in infancy (the "ZMD-score").[40] However, one should not forget that the response time of an electrode (the time needed to reach 95% of the exact pH) is at least about 5 seconds. The "area below pH 4.0" is a parameter considering the acidity of the reflux episodes,[41] which has been shown to correlate better with the presence of reflux esophagitis than with the reflux index.

Various complex reflux scoring systems (Johnson-Demeester Composite Score, Jolley, Branicki, Kaye, Boix-Ochoa scoring systems) have been developed. The majority of all of the parameters have been developed for assessing reflux esophagitis in adults. In marked contrast to these complex scoring systems is the simple recommendation by some investigators that the reflux index or total acid exposure time should be regarded as the most important, if not the only, variable in clinical practice.[39,41] Jolley and colleagues proposed a score for children.[42] Scores based on symptom indices are not applicable in infants and young children.

A major interfering factor in the interpretation of pH monitoring data is the "yes" or "no" interpretation of the data by computer software: a pH of 4.01 will be regarded as normal, whereas a pH of 3.99 will be considered as acid

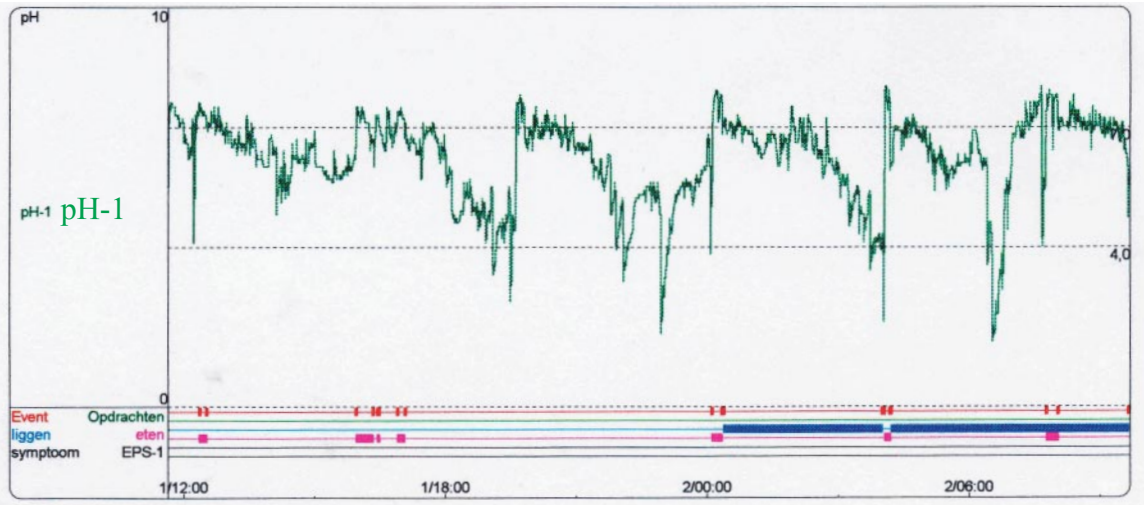

FIGURE 71-2 A 24-hour pH tracing, showing different acid and nonacid reflux episodes, during periods of wakefulness and sleep (*dark line*). Events (coughing) are either nonrelated or occur just after a reflux episode.

reflux. Minimal changes in esophageal pH around pH 4.0 can be at the origin of different software interpretations, although without difference in clinical meaning. The oscillatory index, a parameter measuring the time the pH oscillates around pH 4.0, was developed to evaluate this risk for erroneous computer interpretation.[43]

Normal Ranges

As for any measurement, normal ranges are mandatory. However, because there is a continuum between physiologic and pathologic GER, normal ranges should be regarded as a guideline for interpretation. Because GER is a naturally occurring phenomenon, there will inevitably be an overlap between "normal" and "abnormal" data. Excellent reproducibility has been shown for various parameters. Intrasubject reproducibility supports the diagnostic use of continuous pH monitoring. In general, it can be stated that a reflux index above 10% should be considered as abnormal, a reflux index below 5% as normal, and a reflux index between 5 and 10% as between normal and abnormal. However, normal ranges are developed to separate patients at risk for esophagitis from those not at risk, which is not the major indication of the procedure. Normal ranges proposed by one group can be used by another group only if the investigations are performed and interpreted in a comparable way. This means that materials and methodology should be identical (Tables 71-3 and 71-4). For some individuals and in some clinical situations, it may be more important to relate "events" (eg, coughing, wheezing, apnea) to pH changes rather than to know if the data are within the normal range. It should be borne in mind that normal ranges for a group are not always applicable to an individual.

pH MONITORING AND OTHER INVESTIGATIONS

Many different techniques to evaluate GER do exist, focusing on different aspects, such as postprandial reflux (scintiscan, barium swallow, ultrasonography), histologic abnormalities (endoscopy), continuous measurements that are pH dependent (pH monitoring) or not (impedance), and pathophysiology by measuring the relaxations of the LES (manometry). Because there is no one investigation for GER that is accepted to provide a clear-cut discrimination between normal and abnormal, there is no reason why pH monitoring should do so. Abnormal pH monitoring does not accurately predict the risk for esophagitis.[30,44] In a group of reflux patients with esophagitis, the sensitivity of pH metry is 88% and of scintigraphy is 36%.[45] In a group of patients with abnormal scintigraphy, the sensitivity of pH monitoring is 82%, of endoscopy is 64%, and of manometry of the LES is 33%.[46] Nonacid reflux my be inoffensive (postprandial) reflux at a neutral pH but may also contain bile, which is also toxic for the esophageal mucosa.[46] There is little experience with esophageal bile monitoring in children. The overall correlation between scintiscanning and pH monitoring is acceptable ($r = .78$).[47] However, during simultaneous pH recording and scintiscanning, only 6 of 123 reflux episodes were recorded simultaneously.[48] There is no correlation between the number of reflux episodes detected with scintigraphy and pH monitoring.[49] Barium studies seem to have a much lower sensitivity to pick up reflux episodes if pH monitor-

TABLE 71-3 COMPARISON BETWEEN DATA RECORDED WITH AN ANTIMONY ELECTRODE WITH INTERNAL REFERENCE ELECTRODE AND A GLASS MICROELECTRODE WITH EXTERNAL REFERENCE ELECTRODE IN 20 PATIENTS WITH TWO GASTROGRAPH MARK II* DEVICES

	ANTIMONY	GLASS
Reflux index	3.94 ± 3.68	3.61 ± 3.25
Number episodes	15.15 ± 11.09	17.45 ± 10.41
Mean pH/24 h	5.46 ± 0.39	5.33 ± 0.25

*Fresenius, Medical Intruments Corporation.
No significant difference between the different parameters studied.

TABLE 71-4 DEPENDABILITY OF ESOPHAGEAL PH MONITORING DATA ON THE SOFTWARE PROGRAM (37 RECORDINGS)

	PROGRAM		
PARAMETER	**1**	**2**	**3**
Reflux index (% < 4.0)	4.85 ± 3.84	4.86 ± 3.90	5.02 ± 4.15
Number episodes/24 h	87.38 ± 149.14 (a)	16.05 ± 10.38 (b, c)	19.05 ± 9.85 (a, b, c)
N° Ep > 5 min/24 h	2.32 ± 2.42 (b)	2.27 ± 2.25 (c)	2.92 ± 2.78 (b, c)
Duration Lo Ep (min)	18.54 ± 18.07	17.45 ± 16.84	15.33 ± 11.17

Program 1: Medical Instruments Cooperation program for Gastrograph Mark II (Fresenius, Medical Instruments Corporation): four measurements per second; the program calculates 360 medians per 24 hours; program 2: MIC program for Gastrograph Mark II: four measurements per second; the program calculates 43,200 medians per 24 hours; program 3: program for Gastrograph Mark II, developed by another company.
(a) : $p < .001$; (b) and (c) : $p < .05$.

ing is regarded as the "gold standard."[48] According to many authors, there is a high incidence of false-positive and false-negative studies with barium studies that is related to the short investigation time on the one hand and the intensity of reflux-provoking maneuvers on the other hand. Fifteen-minute postprandial period color Doppler ultrasonography was compared with 24-hour pH monitoring, showing agreement in 81.5%.[50] However, if pH monitoring was considered the "gold standard," the specificity of the color Doppler ultrasonography was as low as 11%, and there was no correlation between the incidence of reflux episodes measured with both techniques.[50] A far higher number of reflux episodes is detected with impedance in comparison with pH monitoring because only 14.9% of all reflux episodes are acid.[52] But only 57% of acid reflux episodes are detected with impedance.[51]

CONCLUSION

Esophageal pH monitoring measures pH in the esophagus, not GER. The miniaturization of devices and electrodes has made pH monitoring a procedure that is easy to perform, even in the youngest children. Patient-related factors such as feeding and physical activity influence pH monitoring results. Hardware- and software-related factors, as well as patient-related factors and recording conditions, determine the results. In clinical practice, pH monitoring is of interest in those patients in whom GERD is suspected who present without clear regurgitation or emesis and to measure the efficacy of treatment.

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CHAPTER 72

PANCREATIC FUNCTION TESTS

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Exocrine pancreatic function is notoriously difficult to assess. In practical terms, the organ and its secretions are relatively inaccessible, and direct assessment requires duodenal intubation to collect pancreatic secretions. The other obstacle rendering assessment difficult is the enormous functional reserve capacity of the exocrine pancreas. Digestive enzymes are synthesized and secreted by the pancreatic acini in considerable excess. Marked reduction of exocrine pancreatic function must occur before nutrients are malassimilated and the functional loss becomes a homeostatic threat. In pediatric patients with cystic fibrosis and Shwachman-Diamond syndrome, Gaskin and colleagues found that lipase and colipase outputs had to be less than 2% and 1% of normal values, respectively, before steatorrhea was apparent.[1] The corollary is that between 98 and 99% of pancreatic reserve for lipase and colipase must be lost before fat maldigestion occurs.

Steatorrhea is a useful indicator of pancreatic function. Steatorrhea is defined as a fecal fat output in excess of 7% of ingested fat in patients over 6 months of age and in excess of 15% in patients under 6 months of age. Patients are pancreatic insufficient if steatorrhea is present. Patients are pancreatic sufficient if steatorrhea is absent and may have pancreatic function in excess of 2% of normal. Pancreatic-insufficient subjects can be detected reliably by a variety of tests. The challenge has been to develop a test that evaluates the range of function in pancreatic-sufficient subjects.

TESTS OF EXOCRINE PANCREATIC FUNCTION

Test Categories

There are three categories of exocrine pancreatic function tests (Table 72-1). Direct tests assess the secretory capacity of the exocrine pancreas. Pancreatic secretions are collected via intubation of the small intestine, usually under stimulated conditions, and are analyzed for the output of water, ions, and enzymes. Stimulation of the pancreas allows the pancreatic functional reserve to be assessed. Collection of unstimulated secretions from a rested organ provides little information.

Indirect tests detect abnormalities secondary to loss of pancreatic function, such as the maldigestion and consequent malabsorption of fat and/or nitrogen. Alternatively, these tests depend on the ability of pancreatic enzymes to cleave specific synthetic substrates, generating absorbable, measurable end products that are detectable in breath, serum, or urine. Additionally, pancreatic enzymes such as chymotrypsin and elastase 1 are relatively biostable and can be detected in the stool.

Blood tests rely on the fact that small but significant amounts of the enzymes and enteroendocrine hormones synthesized by the pancreas are normally present in the systemic circulation. In certain circumstances, the serum concentration of specific pancreatic enzymes (such as immunoreactive trypsinogen) and specific hormones (such as pancreatic polypeptide) may reflect residual exocrine pancreatic function.

The criteria for an ideal pancreatic function test are listed in Table 72-2. All currently available tests have one drawback, and many have several. Direct tests provide the most sensitive and specific measurements of exocrine pancreatic function and are useful in the detection of mild to moderate dysfunction. However, these tests are invasive and expensive, and the results are poorly reproducible between laboratories owing to different study protocols. Indirect and blood tests, although cheaper and easier to administer, are less sensitive and specific and, as a whole, lack an ability to differentiate between mild and moderate exocrine dysfunction.

Indications for Pancreatic Function Tests

1. Differentiate pancreatogenous malabsorption from other causes of malabsorption.
2. Diagnose and study the natural history of disorders affecting exocrine pancreatic function.
3. Assess the efficacy of pancreatic enzyme replacement in children with malabsorption secondary to exocrine pancreatic dysfunction.

DIRECT TESTS

The exocrine pancreas secretes fluid and ions in response to endogenous secretin and enzymes in response to endogenous cholecystokinin (CCK). Endogenous secretin and CCK are released from small intestinal mucosa in response to nutrients and/or gastric acid. The pancreas is also stimulated by neural pathways. It is supplied by vagal efferents that act on muscarinic receptors. Intestinal nutrients provoke stimulation of enzyme secretion via this pathway as well as by CCK release. Stimulation of the exocrine

TABLE 72-1 TESTS OF EXOCRINE PANCREATIC FUNCTION

DIRECT TESTS
Exogenous hormonal stimulants
Cholecystokinin*†
Cerulein*
Bombesin*
Secretin†
Nutrient stimulants
Lundh test meal
Fatty acids
Amino acids
Other
Selenium 75 methionine incorporation and release
Pure pancreatic juice
INDIRECT TESTS
Stool
Microscopy—fat, meat fibers†
Acid steatocrit†
Fecal balance†
Iodine 131 triolein excretion
Dual-radiolabeled fat
Trypsin,† chymotrypsin,† lipase, elastase†
Breath tests
Carbon 14 lipids
Carbon 13 lipids†
Carbon 14 cholesterol octanoate
Starch breath hydrogen
Urinary/plasma markers
Bentiromide†
Fluorescein dilaurate (pancreolauryl)†
Oral tolerance (fat and vitamins)
Dual-label Schilling
Urinary lactulose
BLOOD TESTS
Total amylase or lipase
Isomylase
Cationic/anionic trypsinogen†
Pancreatic polypeptide
Amino acids

*Used in various dose combinations with or without secretin.
†Test currently used in pediatric practice.

pancreas is undertaken by using one or both of these pathways by supplying either exogenous hormones or intestinal nutrients.

Successful quantitation of human pancreatic exocrine function is contingent on the following conditions:

1. The development of appropriate intravenously administered hormonal stimuli or appropriate nutrient delivery to the small intestine
2. The ability to quantitatively measure pancreatic secretions
3. The ability to exclude gastric acid and pepsin

Exogenous Hormonal Stimulation

There is no standard method of hormonal stimulation. Consequently, techniques vary between centers, and each laboratory is required to establish its own range of normal values. The doses of hormones used, the mode of administration (intravenous bolus or intravenous infusion), the duration of infusion, and, in the case of a combined secretin CCK infusion, the sequence of administration may all differ. Little information exists regarding optimum doses in children, especially for synthetic secretin and CCK; in most cases, doses have been extrapolated from adult data on a weight per kilogram basis. Current sources of supply of pancreatic secretagogues are listed in Table 72-3. Synthetic preparations of secretin and CCK are preferable to animal extracts in that they are not contaminated with other gut-derived peptides and because they are less allergenic. Supply of agents approved for human use has been problematic in recent years. Combined stimulation is optimal because there is evidence that CCK or similar hormones act synergistically with secretin.[2] Other investigators have used cerulein (a decapeptide) or bombesin (a tetradecapeptide) alone or in combination with secretin because these peptides have effects on the exocrine pancreas similar to those of CCK. No published information exists regarding their use in children.

Quantification of secretions requires that precise volume data be obtained. Two approaches are used: distal occlusion of the duodenum by a balloon[3] or continuous perfusion of a nonabsorbable marker,[4] allowing correction for distal losses. Balloon occlusion techniques are less physiologic in that they may cause luminal distention and possible stimulation by this means. Similarly, gastric acid and pepsin can be excluded either by continuous nasogastric suction or by a pyloric balloon.

The technique used at The Hospital for Sick Children in Toronto and at Australian centers employs practical solutions to the above conditions and is readily adaptable. The test is a quantitative technique modified from Go and colleagues[5] and is represented diagrammatically in Figure 72-1. Subjects should be fasting, and in the case of patients on pancreatic enzyme supplements, these supplements should be discontinued at least 48 hours prior to the test to remove any suppression of the exocrine pancreas by negative-feedback inhibition. Under fluoroscopic control, a double-lumen tube is inserted into the duodenum. The tube is constructed so that one lumen opens proximally at the ampulla of Vater, and the other lumen, which has several distal ports, is positioned 5 to 12 cm distally at the ligament of Treitz. Through the proximal lumen, a nonabsorbable marker solution (gentamicin, 20 mg/mL in 5% mannitol) is infused into the duodenum at a constant rate. Pancreatic juice mixed with infused marker solution is aspirated distally by intermittent low-pressure suction and

TABLE 72-2 CRITERIA FOR THE IDEAL PANCREATIC FUNCTION TEST

Inexpensive and easily performed
Noninvasive
Specific for pancreatic disease and able to exclude patients with other digestive disorders owing to small bowel mucosal disease, inherited defects of fat transport, or cholestasis
Defines the exact level of pancreatic function in subjects with pancreatic sufficiency and in whom partial impairment of exocrine function is present but nutrient assimilation is unaffected
Repeatable, reproducible between laboratories and able to monitor exocrine function longitudinally
No interference from exogenous pancreatic supplements

TABLE 72-3 SOURCES OF PANCREATIC SECRETAGOGUES

SECRETAGOGUE	SUPPLIER
Secretin, natural porcine; Secretin, Secrepan	Eisai (Bungkyo-Ku, Tokyo, Japan)*; www.eisai.co.jp
Synthetic porcine secretion	ChiRhoClin (Silver Springs)*†; www.chirhoclin.com
	Goldham-Bioglan Pharma GMBH* (Zusmarshausen, Germany)
Synthetic human secretion	Bachem AG (Bubendorb, Switzerland)*; www.bachem.com
	Calbiochem (La Jolla, CA); www.calbiochem.com
	Repligen (Needham, MA)*; www.repligen.com
	Research Plus (Bayonne, NJ); www.researchplus.com
	Sigma Aldrich (St Louis, MO); www.sigma-aldrich.com
Cholecystokinin octapeptide	Anaspec (San Jose, CA); www.anaspec.com
	Sigma Aldrich (St Louis, MO); www.sigma-aldrich.com
	Research Plus; www.researchplus.com
	Calbiochem (La Jolla, CA); www.calbiochem.com
	Anaspec (San Jose, CA); www.anaspec.com
Cerulein	Sigma Aldrich; www.sigma-aldrich.com
	Anaspec (San Jose, CA); www.anaspec.com
	Research Plus (Bayonne, NJ); www.researchplus.com
Bombesin	Research Plus (Bayonne, NJ); www.researchplus.com
	Calbiochem (La Jolla, CA); www.calbiochem.com
	Anaspec (San Jose, CA); www.anaspec.com

*Sources approved for human use (not necessarily in United States).
†Licensed to Repligen.

is collected over four 20-minute collection periods into flasks on ice. The first period allows equilibration of marker solution with pancreatic juice and also allows residual luminal pancreatic enzymes to be washed out. During the subsequent three periods, duodenal juice mixed with marker is collected while continuously and simultaneously infusing intravenous secretin and CCK at doses known to achieve maximal pancreatic stimulation. A separate nasogastric tube facilitates aspiration of gastric juice and minimizes contamination of duodenal contents with acid and pepsin.

This technique allows both the collection and quantification of pancreatic secretions. Although the biliary tree and the duodenal mucosa contribute to fluid secretion, the vast bulk of the secretory response is generated by the action of secretin on pancreatic ductular epithelium and acini and by the effect of CCK on acini. This fact, coupled with the use of a nonabsorbable marker, allows correction for distal losses of fluid and enzyme by the assumption that once equilibration has been attained, the degree of distal loss of the marker is the same as the degree of enzyme and fluid loss.[6] A simple volume correction factor can be calculated as follows:

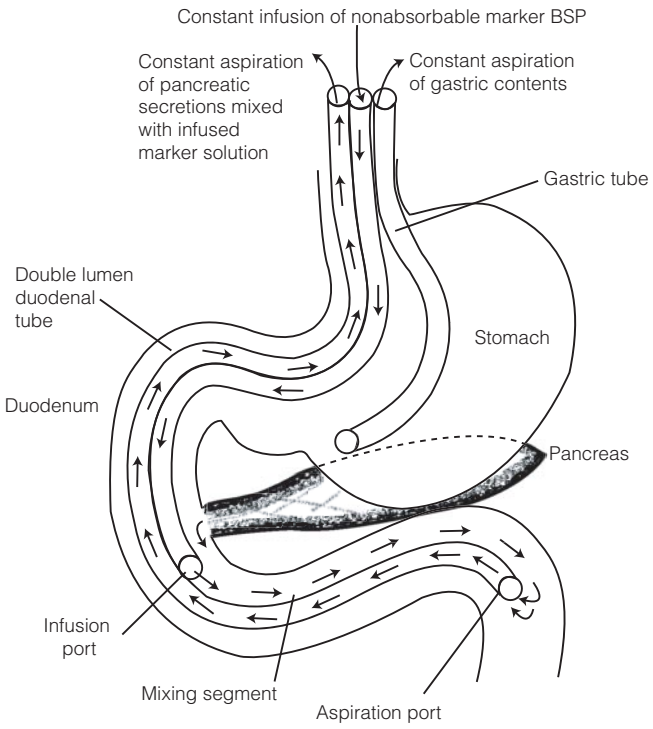

FIGURE 72-1 Scheme of the exogenous secretion/cholecystokinin pancreatic stimulation test with nonabsorbable marker perfusion. Gastric juice is removed via the nasogastric tube. Through the proximal lumen of the double-lumen intestinal tube, the nonabsorbable marker is perfused at a constant rate. Pancreatic secretions mix with the marker in the mixing segment, and the mixture is aspirated via the distal port. Pancreatic secretions are collected over a specific time period (60–80 minutes) while maximally stimulating pancreatic secretion with intravenous hormones (cholecystokinin and/or secretin). Adapted from Durie PR. Pancreatic function tests. Med Clin North Am 1988;20:3842–5.

$$\frac{\text{gentamicin in µg per period infused}}{\text{gentamicin in µg per period recovered}}$$

The determination of fluid, electrolyte, and enzymatic output can be adjusted accordingly. Trypsin, colipase, and total lipase outputs are measured routinely by titrimetric techniques, and bicarbonate output is measured by a colorimetric technique. Sodium, potassium, and chloride outputs are also measured. Other investigators have measured total protein, amylase, chymotrypsin, carboxypeptidase, elastase, cholesterol esterase, and deoxyribonuclease.

The techniques employed for enzymatic determination, especially for amylase and lipase, may differ. The substrates used for the colorimetric determination of amylase activity vary; consequently, the units used to express activity differ. In the case of lipase, results vary depending on whether a short-chain triglyceride such as glycerol tributyrate or a long-chain triglyceride such as olive oil is used as the substrate.

The invasiveness of this test tends to discourage routine clinical use, particularly in pancreatic-sufficient patients, the group in which it is most helpful. It is worthwhile reiterating that this test has helped to delineate pancreatic function in both healthy and diseased individuals. For example, the fact that colipase is the rate-controlling factor for lipolysis became apparent with the analysis of stimulated secretions from both normal individuals and patients with steatorrhea.[7] Additionally, in patients with cystic fibrosis, deficits in electrolyte secretion, particularly chloride[8] and bicarbonate secretion,[9] have been identified, which, in turn, may lead to reduced fluid secretion.[10]

Nutrient Stimulation

Nutrient stimulation of the exocrine pancreas can be undertaken by adapting the principles of the secretin-CCK stimulation test and substituting intraluminal nutrients for the intravenous secretagogues. This method is more physiologic in that the stimulus is provided both by the release of endogenous secretin and CCK directly into the splanchnic circulation and by a vagal mechanism that can be inhibited by atropine.

The most commonly used test meal has been that devised by Lundh for use in adult patients, consisting of milk powder, vegetable oil, and dextrose.[11] The total volume is 300 mL, with a final composition of 6% fat, 5% protein, and 15% carbohydrate. The Lundh meal is composed of intact nutrients. The presence of intact fat, protein, and carbohydrate renders the enzymatic determinations of lipase, protease, and amylase activity difficult. Lundh used a nonabsorbable marker to provide a reference for the absorption of nutrients, but the lack of continuous duodenal marker perfusion, coupled with the presence of salivary and gastric secretions, makes this test relatively qualitative. Although the test is more physiologic than exogenous hormonal stimulation, these practical difficulties have led other investigators to develop more quantitative methods of nutrient stimulation.

Alternative nutrients have been used. The most potent nutrient stimuli are essential amino acids, particularly phenylalanine, but methionine, valine, and tryptophan have also been shown to stimulate the pancreas.[12] Amino acids are usually given as duodenal infusions. Amino acids do not interfere with enzymatic or electrolyte determinations. For physiologic purposes, amino acid solutions should have a pH approximating that of the duodenal lumen because hydrogen ions neutralize bicarbonate, rendering assessment of secretin response difficult. A low-pH solution can also directly stimulate the pancreas through secretin release independent of the amino acid content. The volume of the infusions should be low because duodenogastric reflux of nutrients can stimulate the pancreas via gastrin release. The response of the pancreas to individual nutrients may depend in part on duodenal baroreceptors. Too low an infusion volume will result in a suboptimal stimulus.[13] With the exception of the Lundh meal, which is relatively unpalatable, nutrient stimulation has not been used clinically in children.

Other Direct Tests

A variety of alternative approaches have been used in adult patients. These include the use of radioisotopes to assess uptake, incorporation, and release of amino acids; direct ductal sampling of secretions; and other methods employing microtechniques to assess protease activity. Pancreatic synthetic capacity is measured by the ability to incorporate selenium 75 (^{75}Se)-labeled methionine.[14] In response to a CCK stimulus, ^{75}Se-labeled methionine is released into pancreatic secretions as a constitutent of enzymes and other proteins. Measurement of ^{75}Se activity serves as a guide to acinar function. ^{75}Se is a high-energy–emitting isotope, unsuitable for pediatric use. Endoscopic collections of pure pancreatic juice allow the assessment of uncontaminated samples.[15] Duodenal fluid collected at endoscopy and following intravenous infusion of secretin and CCK has been used to evaluate pancreatic function in children.[16] Unfortunately, a general anesthetic is required, and this may, in itself, suppress pancreatic function.

INDIRECT EXOCRINE PANCREATIC FUNCTION TESTS

The actions of individual pancreatic enzymes are assessed indirectly by quantifying the appearance of inappropriately increased amounts of specific nutrients in the feces (eg, fecal fat) or by measuring metabolic products in the blood, urine, or breath (eg, the bentiromide test and radiolabeled breath tests). Alternatively, the amount of enzymes such as chymotrypsin and elastase in the stool may reflect residual pancreatic function. Most of these tests cannot reliably assess the level of function in pancreatic-sufficient subjects or exclude biliary or intestinal causes of malabsorption. However, because they are relatively noninvasive and are indeed sometimes referred to as tubeless, some of the tests (such as that for fecal fat) can be used to evaluate the success of pancreatic enzyme supplementation in pancreatic-insufficient subjects.

Fecal Tests

Microscopic Examination. Microscopic examination of the stools may reveal meat fibers, neutral fat droplets, or

free fatty acid crystals, suggesting partial fat hydrolysis. Sudan III is the preferred stain for neutral fat, although the fat droplets can be seen quite easily without staining. Free fatty acid crystals are birefringent and are best visualized by a microscope with a polarizing filter. They can also be visualized by lowering the pH of the Sudan III stain.[17] If stool is obtained by rectal examination, lubricants containing oil or petroleum jelly should be avoided. Neutral fat droplets do not differentiate pancreatogenous steatorrhea from steatorrhea of intestinal or biliary origin.[18] Similarly, free fatty acid crystals do not rule out pancreatogenous malabsorption.

Attempts have been made to quantify the degree of steatorrhea by counting the number and determining the size of fat globules in a high-power field.[19] If, on cursory examination, steatorrhea is present, it is sensible to quantify fecal fat losses using balance studies. Microscopic examination of the stool should be mandatory in all cases of suspected malabsorption. However, it should not be regarded as more than a highly useful, albeit crude, screening test for malabsorption.

Steatocrit. The steatocrit, a measurement of fat malabsorption, works on the following principle: if homogenized feces are centrifuged, the lipid and liquid phases and stool residues separate, with the lipid phase on top of the liquid phase and the stool residue.[20] The lipid phase can be measured in a hematocrit tube if the tube is centrifuged at 15,000 rpm for 15 minutes. Reference values and ranges have recently been established for normal children.[21] The sensitivity of this test is markedly improved by the addition of perchloric acid to the fecal homogenate, and the acid steatocrit is determined.[22,23] This may prove to be a useful adjunct in laboratories with limited technical expertise, particularly in the third world, and may also provide a crude method for monitoring the response of patients receiving pancreatic enzyme supplements.

Pooled Stool Collections for Fat, Nitrogen, and Carbohydrate. Because of the functional reserve of the exocrine pancreas, these tests detect only pancreatic-insufficient subjects. All three nutrient classes—fat, protein, and carbohydrate—have been measured in stool to assess pancreatic function. Fecal fat analysis is the most widely used, the most informative, and the most homeostatically relevant of these tests. Pooled stool collections detect malabsorption but do not discriminate between patients with pancreatic and nonpancreatic malabsorption. Despite these limitations, fecal fat analysis is useful longitudinally, especially for assessing the efficacy of pancreatic enzyme supplements in patients with pancreatic insufficiency. Because of the odious nature of the test for both patients and laboratory technicians, it has fallen into disfavor in some circles. Alternative tests that rely on isotopic methods are more expensive, are almost as inconvenient, and still fail to differentiate the various causes of malassimilation.

The method most commonly used for the measurement of fecal fat is the titrimetric Van de Kamer method.[24] In adults, the test involves a diet containing 100 g of fat for 3 to 5 days.[25] Stools collected over 72 to 96 hours are pooled and refrigerated. The mechanics of collection can be improved by the use of a nonabsorbable marker at the start and the end of the diet. In children, the collection period is usually 3 days, although it is occasionally extended to 5 days. Because children find it difficult to adhere to a strictly regimented diet, meticulous weighing of food and careful dietary records are required to calculate the mean daily fat intake. Steatorrhea is present if more than 7% of ingested fat is excreted. Owing to the physiologic immaturity of the pancreatic and biliary secretions, infants under 6 months of age can excrete up to 15% of dietary fat.[26] The Van de Kamer method must be modified if the diet contains appreciable amounts of medium-chain triglycerides because these are not detected by the standard method. The potential for error is great because collections may be incomplete, fat intake may be inaccurately quantitated, and the occasional patient may have delayed intestinal transit. Other methods of estimating fat in feces, such as nuclear magnetic resonance spectrometry[27] and near-infrared reflectance spectroscopy,[28] may make laboratory analysis easier and less odious. The Van de Kamer method and these other methods potentially overestimate fecal fat excretion because they detect biliary lipids and complex lipids derived from intestinal cell turnover. Additionally, this test does not discriminate readily between mucosal, pancreatic, and other causes of fat absorption.

Fecal nitrogen has been used as an index of exocrine pancreatic function but does not provide further diagnostic information because it is unlikely that significant creatorrhea will occur if steatorrhea is absent. The same criticism can be made of fecal carbohydrate measurements. The most commonly used assessment of carbohydrate relies on the measurement of reducing sugars and does not assess total carbohydrate. The anthrone method, which assesses all hexose carbohydrates, has provided better quantitation of carbohydrate losses.[29] Carbohydrate measurements are likely to be elevated with small intestinal mucosal disease, and both nitrogen and carbohydrate are subject to variable colonic absorption and substrate use by fecal flora. For the above reasons, they are less accurate than neutral fat as a guide to pancreatic insufficiency.

Stool Isotopic Methods. Most of these methods are inappropriate for pediatric use because they use gamma ray–emitting isotopes. Single isotopes (iodine 131, iodine 125) bound to triglycerides are expensive, and the test necessitates a 3-day stool collection, but the need for strict dietary records is eliminated. Dual-isotope methods append markers to a nonabsorbable lipid such as glycerol triether and to a lipid subject to hydrolysis and absorption such as glycerol trioleate. This technique allows fat malabsorption to be estimated from single stool samples. Although some of the dual-labeling systems use β-emitting isotopes,[30] none of these methods has been adapted for pediatric use.

Fecal Trypsin, Chymotrypsin, Lipase, and Elastase 1. The capacity to measure both fecal trypsin and chymotrypsin has existed for 30 years. Fecal elastase 1 has been widely used only for the last decade. The initial tests

measured enzymatic activity by means of a laborious titrimetric estimation using low-molecular-weight substrates. A number of problems existed with these tests. The enzymes are subject to proteolytic degradation by both pancreatic and bacterial proteases; thus, interpretation varies with intestinal transit. Chymotrypsin was preferentially measured instead of trypsin because it is more resistant to inactivation by colonic bacteria. However, a high proportion of chymotrypsin is strongly bound to insoluble stool residue,[31] and this thwarted attempts to develop accurate and more convenient photometric methods. The early tests have been superseded by a photometric method, the BMC test developed by Boehringer Mannheim Corporation (Mannheim, Germany), which employs a detergent to solubilize chymotrypsin in stool[32] and is convenient, reproducible, and sensitive. Patients receiving pancreatic enzyme supplements should discontinue them at least 5 days prior to measurement. Fecal chymotrypsin reliably differentiates between pancreatic-insufficient and pancreatic-sufficient patients. However, it does not reliably discriminate between pancreatic-sufficient patients and normal subjects. Patients with pancreatic insufficiency can be differentiated from those with intestinal or biliary disease. This method has been validated for pediatric use by showing a good correlation between the 72-hour fecal output of chymotrypsin and the CCK-secretin–stimulated duodenal output of chymotrypsin.[33] Other observers have shown a good correlation in children between duodenal chymotrypsin output following CCK stimulation and three random stool samples collected within 72 hours of pancreatic stimulation with CCK.[34] Fecal chymotrypsin is relatively stable at 18°C for up to 72 hours and can thus be sent from peripheral centers to a reference laboratory. If random stool samples are used and a low value is obtained, repeating the test will eliminate most false-negative results.

Fecal immunoreactive lipase can be measured by using an enzyme-linked immunosorbent assay (ELISA) technique.[35,36] The test has limitations similar to those of the fecal chymotrypsin test and is both sensitive (87%) and specific (97%) in pediatric patients with cystic fibrosis.[36]

The sensitivity is reduced comparably, presumably because of the inclusion of pancreatic-sufficient subjects. The technique is absolutely specific for human pancreatic lipase, and the results are not confounded by concomitant use of pancreatic enzyme supplements. It has also been found to be useful in the first 6 months of life, when lipase secretion is traditionally held to be low owing to ontogenic immaturity of the acini.

Elastase 1 is a member of the acidic elastase family. It is a sterol-binding protein as well as an endoprotease.[37] Elastase 1 is both a human- and pancreas-specific enzyme that is stable during abnormal intestinal transport.[38] Consequently, porcine pancreatic enzyme supplements do not alter the measurement of elastase 1. Age-related increase of elastase occurs only in the first 3 months of life, probably because the ontogenic development of elastase 1 is completed in early postnatal life.[39] An ELISA has been used to assess elastase 1 as an indirect measure of pancreatic function in both children and adults. This test is both more sensitive and specific than fecal chymotrypsin in detecting pancreatic insufficiency, but it will not delineate patients who are pancreatic sufficient.[40,41] However a decline in fecal elastase concentrations precedes fat malabsorption in patients with pancreatic-sufficient cystic fibrosis who become pancreatic insufficient.[42] False-positive results may occur as a result of villous damage secondary to several conditions, including celiac disease, cow's milk protein, enteropathy, and bacterial overgrowth.[43] It is possible that these patients may have a secondary pancreatic insufficiency owing to impairment of mucosal release of pancreatic secretagogues. Diarrheal disease and short-gut syndrome may result in false-negatives owing to a dilutional effect.[44,45] This problem can be overcome by lyophilization of stool samples, which removes stool water content as a confounding variable.[46] False-negatives are occasionally seen in pancreatic-sufficient patients with Shwachman-Diamond syndrome.[45] Despite these limitations, the stability of this enzyme allows storage at room temperature for at least a week, which facilitates mailing of fecal samples, and the absolute specificity of this test makes it one of the most attractive "tubeless" pancreatic function tests.[37,40] The use of this test is becoming more prevalent in pediatric practice.

Breath Tests

Radiolabeled and Stable Isotope Breath Tests. The technique and principles of breath testing are described in Chapter 73, "Breath Analysis." Ingested lipids are predominantly hydrolyzed by pancreatic lipases in the small intestine, absorbed as free fatty acids and monoglycerides, and transported to the liver, where oxidative metabolism liberates carbon dioxide (CO_2). The radiolabeled breath tests take advantage of this fact by substituting either carbon 14 (^{14}C) or 13 (^{13}C) for carbon 12 in a triglyceride molecule. The three triglycerides of different carbon chain lengths that have been commonly used are trioctanoin, tripalmitate, and triolein. All three substrates labeled with ^{14}C are sensitive in detecting fat malabsorption.[47] Triolein is more specific than either trioctanoin or tripalmitate for fat malabsorption; however, it does not differentiate between pancreatic and nonpancreatic causes of fat malabsorption. Normal release of CO_2 from triolein and tripalmitate requires adequate lipolysis, bile salt solubilization, and an adequate mucosal surface and transport capability. The release of CO_2 from trioctanoin is limited by lipolysis alone and can distinguish pancreatic insufficiency from bile salt deficiency and mucosal defects. Using these substrates in combination with one another (eg, testing with triolein and repeating the test with trioctanoin) improves specificity but not sensitivity. Other confounding variables are the action of lingual and gastric lipases on the substrate, varying individual lipid pool sizes, and the variable respiratory excretion of CO_2 in chronic respiratory disease. ^{14}C labeling mandates that these tests not be used in children.

The specificity of these tests may be improved by repeating them after administering pancreatic enzyme supplements. The same compounds have been labeled with ^{13}C, a stable isotope that is measurable by mass spec-

troscopy, and similar results have been obtained in children.[48] These tests have also recently been adapted to assess gastric emptying in children. Recently, ^{14}C cholesteryl octanoate, which is hydrolyzed by the pancreatic-specific cholesterol esterase, has been used as a substrate.[49] Studies suggest that hydrolysis by cholesterol esterase is the rate-limiting step.[50] The test is adaptable to ^{13}C labeling, allowing its use in children.[50] A synthetic mixed triglyceride (1,3-distearyl 2[^{13}C] octanolyl glycerol) has also been used as a substrate.[51] Excretion of ^{13}C-labeled CO_2 ($^{13}CO_2$) is slower than that seen with cholesteryl octanoate. Stearyl hydrolysis by pancreatic lipase is the rate-limiting step.

Release of CO_2 from ^{13}C-labeled starch has been assessed in adults.[52] This test works on the principle that hydrolysis of starch by pancreatic isoamylase is the rate-limiting step in carbohydrate metabolism. Test specificity is improved by also measuring CO_2 release after ^{13}C-labeled glucose. The ratio of $^{13}CO_2$ excretion after starch ingestion to $^{13}CO_2$ excretion after glucose ingestion corrects for differences in oxidative metabolism. Even after correction, the test is relatively insensitive and will only detect pancreatic-insufficient subjects.

Release of CO^2 from ^{13}C-labeled egg white has been assessed in adults with pancreatic disease. This test is a crude test of proteolytic activity and could be adapted to pediatric subjects.[53]

Because stable isotopes and mass spectroscopy are expensive, these breath tests have not found a niche for routine pediatric use. However, benchtop devices for measuring ^{13}C have become more affordable, making these tests more accessible.

Hydrogen Breath Test. This test measures breath hydrogen excretion following starch ingestion. Starch is normally cleaved enzymatically into oligosaccharides by pancreatic isoamylase prior to further cleavage by brush border disaccharidases. When amylase secretion is impaired, undigested starch is digested by colonic bacteria, generating hydrogen, which is absorbed and excreted in the breath. A two-stage test with concomitant ingestion of oral pancreatic enzymes results in reduced breath hydrogen. This test is extremely nonspecific; false-positive results may occur in blind loop syndromes and also when small intestinal transit time is reduced. False-negative results may occur when the colon is colonized with non–hydrogen-producing bacteria and in subjects who have recently received antibiotics. Currently, there are no pediatric data.

Urinary/Plasma Markers

Bentiromide Test. Bentiromide is a nonabsorbable synthetic peptide (*N*-benzoyl-L-tyrosyl-*p*-aminobenzoic acid) that is specifically cleaved by pancreatic chymotrypsin in the upper small intestine. This results in the release of *p*-aminobenzoic acid (PABA), which serves as a marker and which is rapidly absorbed, conjugated in the liver, and excreted in the urine. Colorimetric assay can measure PABA in both blood and urine, and the detection and quantification of PABA form the basis of the test. Falsely abnormal results have been demonstrated in subjects with bowel, liver, or renal disease owing to defects in the absorption, conjugation, or excretion of PABA. Additionally, both intestinal bacteria and the intestinal brush border may demonstrate chymotrypsin-like activity, reducing specificity. Ingestion of a number of drugs such as sulfonamides, diuretics, acetaminophen, and chloramphenicol and foods such as prunes and cranberries may result in elevated aromatic amines that may interfere with laboratory determinations of PABA.[54] Recently, high-pressure liquid chromatography techniques have been developed to sensitively detect PABA and its metabolites, and these techniques may prove to be superior to colorimetric assay because they eliminate interference from drug and dietary amines.[55]

The bentiromide test was introduced in 1972, and initial reports relied on a one-stage test with a urinary collection.[56] The method involved collections over varying time periods and varying doses of substrate. Consequently, reports of test specificity and sensitivity varied widely. In North America, the recommended method for adults entails the patient receiving a 500 mg dose of bentiromide (170 mg of PABA), ingesting sufficient fluid to maintain an adequate diuresis, and collecting urine for a period of 6 hours.[57] The urinary recovery of PABA is expressed as a percentage of the orally ingested PABA. Less than 50% PABA excretion purportedly reflects pancreatic insufficiency. To correct for potential defects of absorption, hepatic conjugation, or excretion, a two-stage test has been suggested with an equivalent dose of free PABA administered subsequently and the urine collected for an identical time period.[58] This allows the urinary recovery of PABA after bentiromide to be corrected for the urinary recovery of equimolar free PABA. The results are expressed as a PABA excretion index (PEI):

$$\text{PEI} = \frac{\text{PABA recovered after bentiromide (\%)}}{\text{PABA recovered after free PABA (\%)}}$$

This maneuver improves sensitivity and specificity, but the test is cumbersome and time-consuming. Additionally, timed urine collections make the test awkward to perform in infants. In adults, this drawback has been circumvented by the simultaneous administration of ^{14}C-free PABA[59] or a free structural analog of PABA, *p*-aminosalicylic acid (PAS).[60] The ^{14}C-PABA method is impractical for pediatric use. A ^{13}C-PABA method that could be adapted for use in children has been described.[61] The PAS method has been used in the pediatric age group and has improved the sensitivity of the test.[62]

The initial pediatric experience with the bentiromide test concentrated on timed urine collections.[63] However, the specificity and sensitivity of the test have been improved with the development of methods to measure plasma PABA,[55] and the need for dual collections and urinary collections has been eliminated. The recommended pediatric dose of bentiromide (15 mg/kg) has been used in older children and is based on extrapolation from adult data. For the first 3 hours following ingestion of the dose, plasma PABA concentrations rise, and optimal discrimination between normal adolescent controls and patients with pancreatic

insufficiency is obtained at the 90- and 120-minute points.[64] Reliable detection was not obtained in patients with cystic fibrosis and pancreatic sufficiency (between 5 and 10% of normal pancreatic chymotrypsin output as measured by the secretin-CCK test). In patients with Shwachman-Diamond syndrome, none of whom had malabsorption, the plasma test failed to detect pancreatic dysfunction in those patients with an enzyme output as low as 1% of normal. Bentiromide (15 mg/kg) is not useful for assessment in infants if clear fluids are given with the dose. Test sensitivity is improved by using a liquid meal and by increasing the dose to 30 mg/kg.[65] The bentiromide test may discriminate between pancreatic steatorrhea and steatorrhea from other causes and could potentially provide a method of monitoring the effect of pancreatic enzyme supplementation.

4-(*N*-Acetyl-L-tyrosyl) aminobenzoic acid has been used in adults and children[66,67] and has reportedly allowed better differentiation between controls and patients with chronic pancreatitis in comparison with the standard bentiromide test.[66] A modified Lundh meal was used in the pediatric study, and extremely good separation was obtained between normal controls and patients with cystic fibrosis.[67] However, no information exists on its usefulness in subjects who have pancreatic sufficiency but reduced functional reserve.

Fluorescein Dilaurate (Pancreolauryl) Test. This test is based on a principle similar to that of the bentiromide test. Orally administered fluorescein dilaurate is hydrolyzed by pancreatic cholesterol esterase, liberating lauric acid and free water-soluble fluorescein. Fluorescein is readily absorbed in the small intestine, partially conjugated in the liver, and excreted in the urine, predominantly as fluorescein diglucuronide. Fluorescein is nontoxic and can be easily measured in both serum and urine by spectrophotometric or fluorometric techniques.

The commercial version of this test in adult patients involves the ingestion of 0.5 mmol of fluorescein dilaurate with a standard meal. To enhance diuresis, 1 L of unsweetened tea is consumed between the third and fifth hour of the test. All urine is collected over a 10-hour period. To correct for individual differences in intestinal absorption, conjugation, and urinary excretion, the test is repeated using equimolar free fluorescein after an interval of at least 24 hours. The results are expressed as a ratio of the fluorescein detected on the test and the control days. A ratio of greater than 30% is considered normal, a ratio of between 20 and 30% is equivocal, and a ratio of less than 20% is abnormal.[54] Equivocal results should be repeated. The dose can be modified for pediatric purposes.[68] Caution should be used in interpreting this test in diabetic patients in whom glucose interferes owing to the formation of fluorescein glucuronide.[69]

The serum test is more convenient because it is less time-consuming and because the need for urine collection is eliminated. Peak serum levels occur at approximately 210 minutes after absorption, and the best cutoff point for discriminating between pancreatic exocrine-insufficient patients and controls appears to be between 240 and 300 minutes.[70] Concomitant administration of mannitol, which is transported in a similar fashion to free fluorescein, permits completion of the test in 1 day. The results are expressed as a fluorescein-to-mannitol ratio and are equivalent to those of the more cumbersome 2-day test. This method has been used successfully in pediatric subjects.[71] This test has some advantages over the bentiromide test but is not capable of detecting subtle impairment of function in pancreatic-sufficient subjects. Analysis is easier, and there is less interference by exogenous compounds, although it is recommended that niacin and sulfasalazine be avoided prior to the test.[68] False-positive results can occur in patients with biliary tract and mucosal disorders. Cholesterol esterase is pancreatic specific, and the test is therefore not subject to the influence of brush border enzymes. However, bacterial overgrowth can influence the results because some bacteria (in particular, streptococci) are able to hydrolyze fluorescein dilaurate.[68]

Oral Tolerance Tests

Oral fat-loading tests may provide useful information in patients from whom a reliable stool sample cannot be obtained. Serum triglycerides and chylomicron levels are measured at 2, 3, and 5 hours following the ingestion of a meal consisting of 50 g of fat, containing equal amounts of butter and margarine, emulsified in 70 mL of water. Serum triglycerides usually peak at 3 hours after ingestion. An abnormal result consists of a serum triglyceride rise of less than 1.13 mmol/L, or less than 100% above the fasting level, and/or the appearance of less than 7% chylomicrons.[72] This test does not differentiate among patients with pancreatic disease, intestinal mucosal defects, and bile salt deficiency.

Attempts have been made to improve test specificity by using radiolabeled lipids. Initial tests in adults employed triolein labeled with iodine 131 (^{131}I). Subsequently, a dual-label lipid system was evaluated using a tritium (^{3}H)-labeled free fatty acid (oleic acid) and a ^{14}C-labeled triglyceride (triolein). The substrates are administered simultaneously, and the serum ^{3}H-to-^{14}C ratio is calculated.[72] Patients with pancreatic insufficiency have a higher ratio than normal patients or patients with mucosal disease. However, this test does not exclude patients with defects of bile salt delivery or synthesis. Labeling with radioisotopes precludes using this test in children.

Dual-Label Schilling Test

Patients with exocrine pancreatic insufficiency often have an abnormal Schilling test. Pancreatic enzymes are responsible for the cleavage of intrinsic factor from the R protein–intrinsic factor complex secreted by the gastric parietal cells. This step is required for intrinsic factor–cyanocobalamin binding to occur before ileal absorption. A dual-label Schilling test using this principle has been developed.[73] Cobalt 57 (^{57}Co) cobalamin–intrinsic factor complex is administered with cobalt 58 (^{58}Co) cobalamin–hog R protein complex. Free human intrinsic factor and a cobalamin analog are administered to prevent endogenous human R protein from stripping ^{57}Co cobalamin from intrinsic factor. The excretion of ^{58}Co and ^{57}Co is measured in the urine and expressed as a ratio. A low ratio is said to denote

severe pancreatic insufficiency. Because transfer of cobalamin from R protein to intrinsic factor is pH dependent, this test is capable of detecting pancreatic-sufficient patients with impaired pancreatic bicarbonate secretion.[74] Unfortunately, this test is not suitable for pediatric use owing to the radiation dose.

Urinary Lactulose. Lactulose is a poorly absorbed and nonmetabolized disaccharide. Increased small intestinal permeability to lactulose, reflected by increased urinary lactulose excretion as measured by thin-layer chromatography, has been demonstrated in patients with pancreatic insufficiency owing to cystic fibrosis and Shwachman-Diamond syndrome.[75] Less pronounced increases in lactulose excretion were also seen in pancreatic-sufficient patients. The mechanisms responsible for this finding are unknown, but the test could prove to be a useful screening test for pancreatic exocrine insufficiency. Unfortunately, it does not exclude mucosal defects such as celiac disease. Lactulose excretion could also vary with intestinal transit; this could be a problem in cystic fibrosis, where increased intestinal transit time has been noted. Although intestinal transit should be factored into this test, altered intestinal transit has been shown to be a major contributor to increased urinary lactulose excretion.[76]

BLOOD TESTS

All pancreatic enzymes are detectable in small quantities (ng/mL) in the sera of normal individuals. Some enzymes, such as lipase and amylase, are released as active enzymes, whereas others, such as trypsin, are released as the zymogen or proenzyme trypsinogen. Excessive quantities of circulated pancreatic enzymes are seen in the following three circumstances:

1. *Acute pancreatitis*. Enzymes and proenzymes may be released directly into the circulation as a consequence of inflammation.
2. *Ductal obstruction*. Obstruction of pancreatic enzymatic outflow may result in elevated levels of pancreatic enzymes in sera in the absence of inflammation. The mechanism responsible is thought to be regurgitant release of enzymes from the acini or ducts.
3. *Impaired renal function*. Pancreatic enzymes are cleared from the circulation by the kidneys. Impaired renal function may result in significant elevations of pancreatic enzymes in the absence of pancreatic disease.

Theoretically, in the absence of inflammation, ductal obstruction, or impaired renal function, the serum level of a particular enzyme should reflect the amount of functioning acinar tissue, and this consideration forms the rationale for enzyme determination in sera. However, until recently, two considerations have prevented this goal from being attained. The first is a lack of test specificity. Biochemical determinations of enzymes in sera (total amylase, in particular) have been used for many years as a crude screening test for acute pancreatitis. The major limitation of enzymatic techniques has been the lack of substrate specificity. For example, the traditional starch and iodine method does not distinguish between salivary and pancreatic isoamylases. Similarly, trypsin substrates are subject to degradation by other circulating serine proteases. Immunoassay techniques have been developed that sensitively detect and measure specific pancreatic enzymes and that should circumvent these problems. Because techniques vary, it is vital that each laboratory establish its own normative data.

The second constraint on serum enzyme determination is the variable maturation (ontogeny) of pancreatic enzymes. Concentration of serum enzymes varies with age, especially in early infancy. In most instances, serum enzyme levels increase with age and reflect the ongoing maturation of the exocrine pancreas and consequent pancreatic parenchymal enzyme levels. For example, at birth, the pancreas synthesizes and secretes very little amylase and continues to produce very little during the first year of life. In contrast, trypsin(ogen) production is relatively mature, and comparatively larger amounts of trypsin are secreted.[77] Serum trypsinogen levels change relatively little during childhood, whereas serum amylase levels increase markedly. The different rates of maturation of pancreatic enzymes lead to varying degrees of usefulness of serum enzyme determinations for diagnosing pancreatic disease or for determining function. An appreciation of the dynamics of enzyme maturation helps in the interpretation of serum enzyme data. These considerations are best addressed by detailed examination of the various tests.

Serum Amylase

Total amylase measurements are extremely nonspecific because the enzymatic determination does not distinguish between salivary and pancreatic isoenzymes. Refinement of amylase measurement has concentrated on distinguishing between pancreatic and salivary isoamylase. Biochemical methods include column chromatography, electrophoresis, isoelectric focusing, salivary isoenzyme inhibitors derived from wheat, and differential thermolability. In addition, highly specific monoclonal antibodies to the pancreatic isoenzyme have been raised, permitting the development of immunoassay techniques. The pancreatic isoenzyme peak on isoelectric focusing or electrophoresis appears to correlate with the level of function in older patients with cystic fibrosis and Shwachman-Diamond syndrome.[78] However, in patients with slight or moderate reduction of function, values are within the normal range. This test is therefore of little use in pancreatic-sufficient individuals. In addition, levels of pancreatic isoenzyme are low in both normal neonates and neonates with cystic fibrosis, and the levels rise throughout childhood.[79] This finding limits the interpretability of the test in younger patients.

Serum Lipase

The enzymatic measurement of serum lipase relies on a titrimetric or turbidometric method in which lipase hydrolyzes a triglyceride substrate, producing free fatty acids and glycerol. These methods are not conducive to the assessment of large sample numbers. A sensitive ELISA is available commercially and allows rapid determination of

lipase in sera from multiple patients. Cross-sectional evaluation of the usefulness of serum lipase as a measure of pancreatic exocrine function was undertaken in a population with cystic fibrosis and was compared with normal controls. The results were validated by fecal fat evaluation and/or a secretin-CCK stimulation test in younger patients (less than 5 years of age) and in older patients (greater than 5 years of age) with cystic fibrosis.[80,81] The patterns seen in each group are distinctive. In all cystic fibrosis patients, serum lipase is much higher than control values during the first year of life. In pancreatic-insufficient patients, the levels decline after the first year of life, gradually reaching a nadir of 25% of control values after 5 years of age. In pancreatic-sufficient subjects, levels also decline during early childhood, but after 5 years of age, they remain elevated approximately threefold above control levels. There is a wide scatter, however, and some pancreatic-sufficient patients have levels within the normal range. The elevated serum lipase in the first year of life has encouraged the adaptation of serum lipase as a screening test for cystic fibrosis. However, the test has not attained the same popularity as cationic trypsinogen. It is less sensitive, with a detection rate of 76% in the first year of life as opposed to a 90% detection rate with cationic trypsinogen. After 5 years of age, the test is reasonably sensitive and specific for the detection of pancreatic insufficiency (95% and 85%, respectively) but remains relatively imprecise for the detection of pancreatic-sufficient subjects. There is no information about the usefulness of serum lipase in delineating pancreatic insufficiency in other pancreatic diseases of childhood.

Serum Immunoreactive Trypsin(ogen)

Two forms of trypsin(ogen) (cationic and anionic trypsinogen) exist and are detectable in sera. Specific radioimmunoassays, particularly for the cationic form, have permitted the population screening of pediatric groups at risk for pancreatic disease. An ELISA method using a monoclonal antibody specific for the zymogen (proenzyme) trypsinogen is quicker, easier to perform, and less labor intensive.[82] Neonatal screening for cystic fibrosis by measuring immunoreactive trypsinogen in dried blood spots is now routine in some parts of the world.[83,84]

Serum immunoreactive trypsinogen levels have been evaluated both cross-sectionally and longitudinally in pediatric patients with cystic fibrosis[85] and also in children with exocrine pancreatic functional impairment attributable to other causes.[86] The findings have been validated in comparison with those in normal controls. In cystic fibrosis, two patterns emerge. In all individuals with cystic fibrosis, the serum immunoreactive trypsinogen level is grossly elevated during the first year of life.[87] In pancreatic-insufficient patients, a rapid decline is noted during the second year of life, with levels becoming subnormal by 6 years of age.[87] In pancreatic-sufficient patients with cystic fibrosis, no consistent pattern of decline is seen; indeed, many older patients continue to have elevated serum levels.[80] However, there is a wide scatter, and the test is of little value for predicting the degree of functional impairment in this group. The control group provides a reasonably narrow normal range, with individual values being unrelated to age. Serum immunoreactive trypsinogen measurement in cystic fibrosis is useful in two circumstances. In infants less than 1 year of age, the test is a sensitive diagnostic screening test; the detection rate is 90%. In patients over 7 years of age, depressed serum levels are highly predictive of pancreatic insufficiency. In 199 patients with cystic fibrosis over 7 years of age who had pancreatic insufficiency, only 9 had normal values and 3 had elevated values, resulting in a predictive rate of 94%.[85] Although this test does not distinguish pancreatic-sufficient subjects from normal individuals, it is a sensitive, relatively noninvasive method of screening for pancreatic insufficiency in older subjects. Below 7 years of age, a fecal fat determination is recommended.

In patients with other pancreatic diseases of childhood, this test has proved useful in distinguishing pancreatic steatorrhea from nonpancreatic steatorrhea. At The Hospital for Sick Children, this test provided absolute separation of 10 children with pancreatic steatorrhea from 22 children with other causes of steatorrhea (Figure 72-2).

The other causes of pancreatic steatorrhea included Shwachman-Diamond syndrome, insulin-dependent diabetes mellitus, idiopathic pancreatic insufficiency, and celiac disease with primary pancreatic insufficiency.

Serum Pancreatic Polypeptide

Pancreatic polypeptide, a 36–amino acid straight-chain peptide, is predominantly confined to the pancreatic islets of Langerhans and is also located between acinar cells. Pancreatic polypeptide is an inhibitor of pancreatic enzyme secretion and is released into the circulation in response to various stimuli, particularly protein meals and CCK.

A radioimmunoassay technique has been used to assess fasting pancreatic polypeptide levels or to assess serial responses of plasma pancreatic polypeptide evoked by CCK infusions or in response to various nutrients.[88,89] In adult patients with chronic pancreatitis, fasting plasma pancreatic polypeptide levels are low. Additionally, in response to CCK octapeptide, patients with chronic pancreatitis display either no rise in pancreatic polypeptide or a greatly limited rise compared with both normal controls and patients with other causes of steatorrhea. Thus, the test is capable of differentiating between patients with pancreatic steatorrhea and those with nonpancreatic steatorrhea. However, the test fails to discriminate between pancreatic-sufficient and pancreatic-insufficient subjects with chronic pancreatitis and, as such, gives no indication of actual pancreatic function.[89] This test has not been used in pediatric practice.

Amino Acids

Plasma amino acid levels decrease if the exocrine pancreas is stimulated. The amino acids are incorporated into enzymatic protein within minutes of hormonal stimulation. Both CCK and cerulein stimulation coupled with secretin result in a decrease of plasma amino acid levels in humans.[90,91] The magnitude of this decrease at 45 minutes after stimulation appears to be directly related to

pancreatic function and can differentiate patients who are pancreatic sufficient with decreased functional reserve as measured by stimulated chymotrypsin output.[91] Serine, valine, isoleucine, and histidine reduction may discriminate mild impairment of function better than total plasma amino acid reduction. More recently, others have not been able to replicate these results by using CCK alone.[92] This test is time-consuming and expensive and has not been used in pediatric practice.

FUTURE DIRECTIONS

Fecal elastase is the only new widely used pancreatic function test to be developed in the last 15 years. It is becoming increasingly unlikely that new stool urine and breath tests more specific and sensitive than current tests will be developed. Functional imaging techniques using new imaging technology have been developed and trialed in adult patients. These tests, which both image the pancreas and allow an estimate of function, may be adaptable in the future to pediatric patients.

The first of these tests to be reported uses magnetic resonance cholangiopancreatography after secretin stimulation.[93] This technique, which employs a negative bowel contrast agent, was evaluated in 34 adult patients who had undergone prior pancreatoduodenectomy. The caliber of the main pancreatic duct can be graded on a scale of 1 to 3, reflecting secretion. More recently, the technique has been further refined to calculate water secretion rates by correcting for signal intensity.[94] These gradings correlate well with clinical symptoms but do not correlate well with a urine bentiromide test. The poor correlation with the bentiromide test, the necessity for the patient to cooperate and stay still, and the expense of the technique suggest that it will not be adaptable for pediatric use.

More recently, biliary excretion has been assessed by secretin stimulation after injection of sodium bicarbonate ($NaH^{11}CO_3$) in adult patients.[95] This test, which is adaptable to pancreatic function, requires a cyclotron (to generate carbon 11) and a positron emission tomography scanner. Patients need to stay still for 10 minutes. Most pediatric units do not have access to this technology. Carbon 11 is radioactive but has a short half-life, which may allow the adaptation of this test to selected older pediatric patients.

SUMMARY, CONCLUSION, AND RECOMMENDATIONS

Clinical use of pancreatic function tests depends on three major variables: (1) the indications for pancreatic function testing, (2) how the test satisfies the criteria for ideal pancreatic function in the clinical circumstance in which it is being used, and (3) the age of the patient. If useful clinical information can be obtained that subverts the need for a pancreatic function test, this information should be obtained. For example, if a mucosal cause of malabsorption is suspected, it is sensible to perform an intestinal biopsy as the primary investigation. Similarly, if biliary disease is suspected, there are usually good clinical signs that will point in this direction, and pancreatic function testing is usually inappropriate.

Invasive tests are now used much less frequently in pediatric practice. The major use of the direct pancreatic function tests in pediatric practice was to delineate pancreatic-insufficient from pancreatic-sufficient cystic fibrosis and also to establish a diagnosis of cystic fibrosis in those cystic fibrosis patients in whom sweat tests and clinical signs were equivocal. Genetic testing and, to a lesser extent, nasal potential difference have rendered this indication partially obsolete. If we know a patient's genotype, we can, with a degree of certainty, predict whether they have cystic fibrosis and whether they either have or will develop pancreatic sufficiency.[96] The genetic diagnosis of other disorders, coupled with an awareness of the clinic constellation, also means that direct pancreatic function testing is seldom required. Genetic diagnosis is possible for lipase,[97] colipase,[98] trypsinogen,[99] and enterokinase[100] deficiency; Pearson bone marrow pancreas syndrome[101]; some forms of pancreatic aplasia[102]; and Shwachman syndrome.[103] It should soon be possible for Johanson-Blizzard syndrome. In these circumstances, the diagnosis should be sought, and the presence of malabsorption should be sought either by microscopy for fecal fat or a 3-day fecal fat collection in an older child or by fecal elastase 1 determination in a younger child.

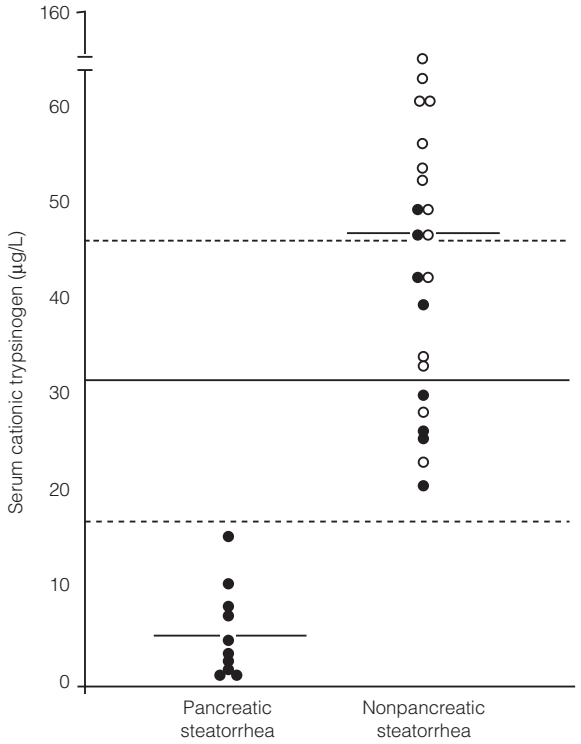

FIGURE 72-2 Serum cationic trypsinogen values in patients with pancreatic and nonpancreatic steatorrhea. The solid and interrupted horizontal lines indicate mean normal cationic trypsinogen of ±2 SD, respectively (31.4 ± 14.8 g/L). *Closed circles* = patients who underwent a pancreatic stimulation test. *Open circles* = patients with nonpancreatic steatorrhea who did not have a pancreatic stimulation test. Reproduced with permission from Moore DJ et al.[86]

TABLE 72-4 RECOMMENDED PANCREATIC FUNCTION TEST: CLINICAL INDICATIONS

Determination of pancreatogenous malabsorption
Fecal elastase 1
^{13}C octanoin breath test
^{13}C cholesterol octanoate
^{13}C mixed synthetic triglyceride
Serum fluorescein dilaurate/mannitol collection
Serum bentiromide/PAS collection or ^{13}C label
Direct pancreatic stimulation—CCK
Direct pancreatic stimulation—CCK + secretion
Natural history of disease
Fecal elastase
Serum cationic trypsinogen
Acid steatocrit
Fecal fat 3 or 5 d (near-infrared reflectance spectroscopy or magnetic resonance spectrometry analysis)
^{13}C octanoin breath test
^{13}C cholesterol octanoate
^{13}C mixed synthetic triglyceride
Serum fluorescein dilaurate/mannitol
Serum bentiromide/PAS collection
Direct pancreatic stimulation
Effectiveness of pancreatic enzyme supplements
Acid steatocrit
3- to 5-d fecal fat
^{13}C octanoin and other breath tests
Serum fluorescein dilaurate/mannitol collection
Serum bentiromide/PAS collection or ^{13}C PABA

CCK = cholecystokinin; PABA = *p*-aminobenzoic acid; PAS = *p*-aminosalicylic acid.

TABLE 72-5 RECOMMENDED PANCREATIC TESTS BY AGE GROUP

Age < 1 yr
Fecal elastase
Serum cationic trypsinogen
^{13}C-labeled breath tests
Age > 1 yr–toilet training
Fecal elastase
^{13}C-labeled breath tests
Acid steatocrit
Direct pancreatic function tests
Toilet training to 7 yr of age
Fecal elastase
^{13}C-labeled breath tests
Acid steatocrit
3- to 5-d fecal fat
Fluorescein dilaurate test bentiromide + PAS or ^{13}C PABA modification
Direct pancreatic function tests
Seven yr of age and older
Fecal elastase
^{3}C-labeled breath tests
Acid steatocrit
3- to 5-d fecal fat
Fluorescein dilaurate
Serum bentiromide/PAS collection or ^{13}C PABA
Direct pancreatic function test
Lundh meal
Teenagers
All the above tests and MRCP with secretin

MRCP = magnetic resonance cholangiopancreatography; PABA = *p*-aminobenzoic acid; PAS = *p*-aminosalicylic acid.

The other major consideration for pancreatic function testing is to assess the efficacy of enzyme supplements, and in these circumstances, the more dynamic tests that rely on proteolysis or lipolysis, such as the bentiromide tests, the pancreolauryl tests, and breath-stable isotope-labeled tests, are more appropriate.

The most appropriate tests for the clinical indications are listed in Table 72-4, and the most appropriate tests for the various pediatric age groups are listed in Table 72-5. The principle employed in listing these tests is the axiom *primum non nocere*. The tests are listed pragmatically, with the easiest and most applicable test listed first and the most difficult, invasive, and expensive test last.

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CHAPTER 73

BREATH ANALYSIS

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The analysis of components of the breath to assess gastrointestinal function has undergone a resurgence in recent years. Dynamic function testing using expired breath to better understand both physiology and pathology depends on the measurement of freely diffusible gases that are either produced endogenously or in response to an orally administered substrate. In the former case, the gas is produced as either a bacterial metabolite or in response to cellular damage following oxidant stress or inflammation. The gas detected can then indicate the presence or absence of an infection or an inflammatory process and the intactness of digestive processes such as fat absorption or gut motility (Figure 73-1).

This chapter discusses the current status of breath testing, which is rapidly evolving; thus, many of the tests discussed are not yet in routine clinical practice.

HISTORY

The noninvasive nature of breath tests makes them particularly useful for application in pediatric settings; nonetheless, no clinical applications were to be realized for almost 40 years after alveolar carbon dioxide (CO_2) was first measured.[1] The two major gases in expired air for understanding and investigating gastrointestinal function are hydrogen (H_2) and CO_2. However, other gases, such as methane (CH_4), ethane, pentane, and mercaptans, are also likely to be important indicators of disease and gastrointestinal malfunction in the future. The principles of measuring labeled CO_2 using radioactive substrates tagged with carbon (^{14}C) were introduced in the 1960s.[2] In the early 1970s, use of nonradioactive breath tests, led by H_2 assessment,[3] ushered in a new era for diagnosis, particularly in childhood for the assessment of lactose and other carbohydrate intolerances. For breath tests using expired CO_2 as the end point, several technological advances and observations have redefined the potential scope of clinical use. The first was the observation that an individual at rest produces a roughly constant amount of CO_2 output per unit time.[4] This allowed interval breath sampling for estimating labeled CO_2 in expired breath as a paradigm for the determination of absorption and subsequent metabolism of a test substrate. The second was the availability of a wide variety of substrates labeled with the radioactive isotope of carbon, ^{14}C. The third more recent development has been the introduction of user-friendly mass spectrometers (isotope ratio mass spectrometry) for the detection of the stable isotope of carbon, ^{13}C, in expired air. For H_2 breath testing, the important demonstration of a direct relationship between expired H_2 and H_2 produced by the microflora in the large bowel formed the basis of this test for diagnosis of sugar intolerances.[5]

ANALYTIC TECHNIQUES

The variety of techniques for measurement of particular gases primarily rely on gas chromatography to separate the gases as a first step prior to detection by selected detectors (eg, thermal conductivity, mass spectrometry). This is not the case for detection of radioactively labeled ^{14}C, in which the CO_2 is trapped using an alkaline liquid scintillation fluid for adsorption of the CO_2 to a solid substrate, and the disintegrations per minute are subsequently assessed using a scintillation counter or similar device. The implications of using the radioactive ^{14}C versus the stable isotope (^{13}C) are discussed below. For mea-

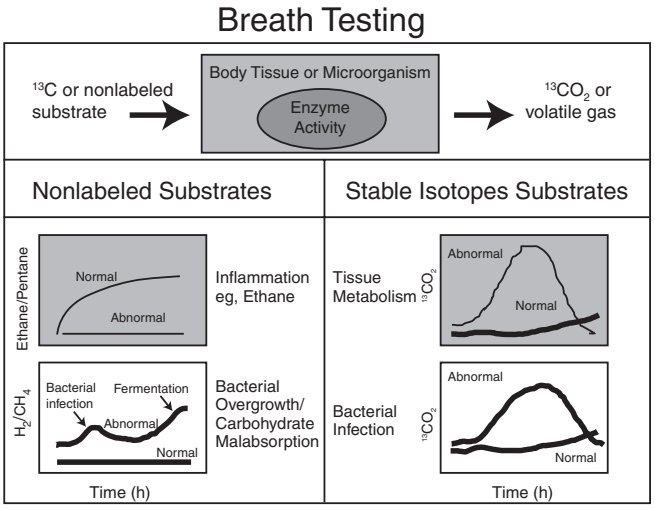

FIGURE 73-1 Principles of $^{13}CO_2$ breath tests, nonlabeled substrate breath tests, and volatile gas breath tests. Theoretic curves are presented for presence or absence of various gases where nonlabeled substrates or no substrates (eg, ethane) are used. Where ^{13}C substrates are used, the curves presented are $^{13}CO_2$ expiration. Ideal excretion curves are illustrated for tissue tests, inflammation, bacterial overgrowth, and fermentation.

surement of H_2, CH_4, and other hydrocarbons (eg, ethane and pentane), gas chromatography followed by flame ionization detection, thermal conductivity, or solid-state sensing devices is commonly used.

BREATH SAMPLING AND COLLECTION TECHNIQUES

Although interval sampling methods are less precise than closed continuous collection techniques, they are certainly adequate for patient management and clinical research. Most clinical applications only need the concentration of the specific gas at particular time points from which an estimate of output over time can be made (usually expressed as an area under the curve generated) and not the absolute quantity excreted. In pediatric populations, well-tolerated collection systems have been developed that can be used in infants and toddlers who cannot actively cooperate. The most successful method for this is the nasal prong, which is held at the nostril by the patient or collector.[6] As the patient expires, the collector aspirates 2 to 4 mL during the latter period of expiration until a sample sufficient for analysis has been obtained. Correction for contamination with ambient air using either O_2 or CO_2 is mandatory when collecting expired gases using this technique.[7,8] For older children and adults, samples can be collected by simply blowing through a straw into an evacuated glass tube or a suitable gas-tight bag (eg, Mylar, Teflon).

SAFETY ISSUES

Depending on the substrate, a wide variety of breath tests are unquestionably safe. For breath tests that do not involve the ingestion of an isotopically labeled substrate, the major probes used to date have been sugars, both natural and synthetic. Of the synthetic sugars, the disaccharide lactulose (constituent sugars galactose and fructose), generally derived from lactose, is the most studied carbohydrate probe. This sugar is a laxative and therefore requires some care in the dose used. This has been well characterized in more than 20 years of use as a diagnostic tool. Routinely, a dose of less than 10 g is administered to children.

For isotopic studies, the situation is different, particularly in women of childbearing age and children in whom the use of a radioactive substrate is contraindicated. This is both on environmental grounds and to protect the patient from unnecessary exposure to radiation no matter how small the actual measured and the biologically active dose. Thus, ^{13}C substrates should be used for breath testing. The instruments required for measurement of $^{13}CO_2$ are now much more user-friendly and are becoming more widely available. Indeed, more stable isotopes are also now on the market, and the use of naturally enriched substrates is likely to open up many more possibilities for studies in nutrition[9] and in the clinical setting (lactose).[10]

SAMPLE STORAGE

In many cases, sample storage is not required; however, where collection is carried out remotely from the analytic unit, then evacuated glass tubes that have not been irradiated (because this releases gases into the evacuated glass tubes from the rubber seals) can be employed. Indeed, these collection tubes are used in most isotope ratio mass spectrometers and thus afford a standardized system for collection of H_2, CH_4, and ^{13}C-labeled CO_2. Other hydrocarbons may also be stored and collected in these containers provided that special attention is paid to the type of seal used (eg, silicon or silicon plus Teflon seals may be necessary for storing samples other than H_2 and CH_4).

H_2, CH_4, and $^{13}CO_2$ can be collected and stored at room temperature for at least 30 days[6] and, in our experience in the latter case, for 6 months or longer.

NONLABELED COMPONENTS OF BREATH

The clinical applications of nonlabeled components of breath H_2 testing are outlined in Table 73-1. This does not include tests such as breath CH_4, which is still being evaluated. The use of breath H_2 test for carbohydrate malabsorption was the first clinical application of breath testing and is still the only one used continually in clinical practice. Most of the information on the efficacy of breath H_2 testing relates to lactose malabsorption owing to its high prevalence in most parts of the world. Other components of the breath, such as methane, ethane, and pentane, have been studied but have not yet been validated for use in routine clinical practice.

HYDROGEN BREATH TEST

The criterion for H_2 breath test positivity is a sustained rise in H_2 of greater than 10 ppm above baseline, which is usually seen 60 to 120 minutes after sugar ingestion.[7,11] Others have suggested a rise of 20 ppm, but this may lead to decreased sensitivity.[3]

TABLE 73-1 CLINICAL APPLICATIONS OF NONLABELED COMPONENTS OF BREATH

CLINICAL QUESTION	SUBSTRATE	DOSE	INTERVAL/DURATION	REFERENCE
Lactose malabsorption	Lactose	2 g/kg; maximum 20 g in 20% solution	Every 30 min for 3 h	7, 11
Sucrose malabsorption	Sucrose	2 g/kg; maximum 20 g in 20% solution	Every 30 min for 3 h	6, 27–29
Glucose malabsorption	Glucose	1 g/kg; maximum 20 g in 20% solution	Every 30 min for 4 h	41
Fructose malabsorption	Fructose	0.3–0.5 g/kg; maximum 20 g in 20% solution	Every 30 min for 3 h	33
Bacterial overgrowth	Glucose	20 g	Every 20 min for first hour	51, 46, 47
	Lactulose	6.68 g (10 mL Duphalac)	then every 30 min for 2 h	

In some cases, the baseline H_2 is high (> 10 ppm) but drops to lower levels within the first 30 minutes. In this situation, the 30-minute sample is considered as the baseline. A persistently elevated fasting H_2 may indicate either inadequate fasting or a high nonabsorbable carbohydrate diet in the previous 24 hours.[12] The possibility of gastrointestinal stasis and bacterial overgrowth also needs to be considered, as discussed below.[13]

A false-negative result may be obtained because of factors affecting H_2 production, which requires a colonic bacterial flora capable of fermenting carbohydrate. Failure to detect H_2 occurs in 2 to 40% of subjects.[3,7,14,15] The prevalence of true nonhydrogen detection in breath may, however, be quite low and dependent on the time of collection, as shown by Strocchi and colleagues, who collected for up to 8 hours.[12] Antibiotics may also suppress H_2 production, which may last for several months, although, again, this may be questioned.[12] In a recent study of patients with bacterial overgrowth, H_2 production was significantly decreased after 1 week of antibiotic therapy.[16] Rapid ventilation can also reduce H_2 in expired air,[17] whereas cigarette smoking can produce a falsely elevated H_2.[18] To avoid a false-negative result, any subject on recent antibiotic therapy and all negative results should have a lactulose breath test to confirm H_2 detection.

CARBOHYDRATE MALABSORPTION

Lactose

The principal clinical use of the breath H_2 test is for the diagnosis of lactose malabsorption, and it has been clearly shown to be the most accurate indirect test of lactase deficiency[19] and is used widely in pediatric practice.[20,21] Because it assesses the function of the small intestine as a whole, it is also more reliable than the rather invasive small intestinal biopsy,[22] which is probably required only to prove a congenital absence of lactase.

The importance of testing for carbohydrate malabsorption rather than relying on clinical symptoms (abdominal pain, distention, and diarrhea) is highlighted by studies showing that patients with these symptoms were unaware that they were due to lactose ingestion,[23] and, alternatively, those who believed themselves to be severely intolerant were shown to be breath test negative.[24] The use of clinical symptoms as the basis to invoke dietary change is thus unreliable and may contribute to unnecessary parental anxiety and the possibility of dietary insufficiency.[25,26] It is, therefore, best clinical practice to prove the diagnosis of lactase deficiency before embarking on dietary change.

Sucrose

Both primary and secondary sucrase deficiency can be diagnosed using breath H_2 testing.[6,27–29] In primary sucrose malabsorption (congenital sucrase-isomaltase deficiency), the diagnosis can be suspected with clinical improvement following removal of sucrose from the diet but needs to be proven by showing sucrase deficiency in an intestinal mucosal biopsy sample. In a recent study of children with cystic fibrosis and abnormal bowel function, breath testing showed a high prevalence of secondary sucrose malabsorption.[29]

Monosaccharides

Fructose malabsorption has been demonstrated in children with diarrhea and abdominal pain using the breath H_2 test.[30,31] Both children and adults may physiologically malabsorb fructose owing to a limited absorptive capacity.[31–33] Fructose absorption can be facilitated by equimolar doses of glucose and especially L-alanine, although the mechanism remains unclear.[34] Without the underlying knowledge of the rate-limiting step in fructose absorption, it is difficult to apply a standardized breath H_2 test to investigate for possible fructose malabsorption.[33,35]

Sorbitol, which is present naturally in fruits and juices and is also used as a sweetener in low-joule products, has a similar structure to fructose and has also been shown to be malabsorbed in children.[36] Like fructose, sorbitol is commonly malabsorbed in healthy individuals; therefore, a breath H_2 test cannot be used to simply demonstrate abnormality.[37] Although the demonstration of fructose or sorbitol malabsorption, even in patients with abdominal symptoms, cannot of itself be considered pathologic,[38,39] there may be a subgroup of patients with functional bowel symptoms who can be identified and improved by a fructose-sorbitol–free diet.[40] Breath testing with fructose and sorbitol may help identify those patients.

Glucose malabsorption as an isolated event is extremely rare and probably occurs only in association with severe mucosal injury except in the case of congenital glucose-galactose malabsorption. Douwes and colleagues were able to use the breath H_2 test to confirm this diagnosis in a breastfed infant with severe diarrhea.[41]

Bacterial Overgrowth

Its noninvasive nature and lack of a radioactive label prompted the use of the H_2 breath test for diagnosis of bacterial overgrowth. Lactulose was used as the initial substrate because it is fermented rapidly, appearing in the breath within 8 minutes of bacterial contact. The first study in adults showed an early peak within 30 minutes of lactulose administration followed by a later peak when the rest of the lactulose bolus reached the colon.[42] Several subsequent studies in children confirmed the double peak as a diagnostic feature.[15,43] These children presented with symptoms of chronic diarrhea and abdominal pain, and bacterial overgrowth was unsuspected.[43] Lactulose was given in a dose of 6.68 g (10 mL) in 100 mL of water, and samples were collected every 20 minutes for the first 60 minutes and at 30-minute intervals for the next 2 hours. The finding in this study and others of an elevated fasting breath H_2 may be a useful indicator of small bowel bacterial overgrowth, but it is not specific, and the diagnosis needs to be confirmed with an oral glucose or lactulose challenge.[43–45] The combination of an elevated fasting breath H_2 concentration and increased breath H_2 excretion after a sugar load has been reported to have a 100% specificity for small bowel bacterial overgrowth.[44]

Most recently, Pimentel and colleagues have shown evidence of small bowel bacterial overgrowth in patients with irritable bowel syndrome using lactulose as the substrate.[45]

Glucose has also been shown to be an effective substrate for the diagnosis of bacterial overgrowth in both adults[46] and children.[47] In the latter study, glucose was given in a dose of 1 g/kg, and samples were collected at 20, 40, 60, and 90 minutes after ingestion. Recently, glucose has been used as the substrate to diagnose small bowel strictures and secondary bacterial overgrowth in Crohn disease.[48]

As with other breath H_2 tests, the major limitation is the possibility of non-H_2 detection, which is quite variable, occurring in 5 to 42% of patients.[7,14] The sensitivities of both lactulose (68%) and glucose (52%) breath H_2 tests to detect bacterial overgrowth have also been shown to be low when compared to jejunal culture in one study.[46] Despite these potential shortcomings, the noninvasive nature of the test, the ease of performance, the ability to repeat the test following therapy, and the lack of a suitable alternative make this approach worthwhile until more accurate tests become available.

Methane

Methane is produced in the left colon by reduction of H_2 and CO_2. Thus, delivery of an increased carbohydrate load to the colon may provide extra substrate for H_2 and CO_2 production in intolerant individuals, thus increasing the substrate for methanogenic bacteria and hence an increase in breath CH_4. Some studies have suggested that low or absent H_2 production following lactulose ingestion is related to H_2 consumption by methanogenic bacteria.[49] At present, it is unclear whether CH_4 measurement can usefully contribute to routine studies of carbohydrate malabsorption, particularly lactose. It has been shown that the prevalence of CH_4 production in lactose absorbers and malabsorbers was identical, and the same proportion of subjects were lactose malabsorbers regardless of methane status.[50] It has also been reported that the percentage of children with lactose intolerance who produce CH_4 is similar to that of normal children.[51] Most recently, Myo-Khin and colleagues showed breath CH_4 to be ineffective as a test for lactose malabsorption.[52]

Although the role of CH_4 as a useful tool in assessing large bowel bacterial metabolism is unclear, an interesting recent report showed that CH_4 production during a lactulose breath test was positive in 100% of constipation-predominant irritable bowel syndrome patients compared with diarrheal conditions such as ulcerative colitis and Crohn disease.[53]

Ethane/Pentane

The alkanes ethane and pentane are the major volatile hydrocarbons resulting from in vivo peroxidation of unsaturated fatty acids, especially those in lipid bilayers of cell membranes, and can be measured in breath. Ethane may be the better marker of lipid peroxidation because it passes through the liver intact, whereas pentane undergoes extensive hepatic oxidation. Both of these gases have been used to study the conditions that could affect lipid peroxidation in intact animals and humans. Vitamin E status in children has been studied noninvasively using both ethane[54] and pentane[55] as markers. More recently, an adult study has shown a reduction in oxidative stress in Crohn disease following vitamin E and C supplementation using ethane and pentane as breath markers.[56]

Excessive lipid peroxidation is probably an important pathogenic factor in inflammatory bowel disease that can now be assessed noninvasively by breath measurement.[57] Both breath ethane[58] and pentane[59] have also been shown to correlate with disease activity in ulcerative colitis.

Stable Isotope Breath Tests

The majority of stable isotope breath tests depend on the measurement of changes in labeled breath CO_2 concentration. CO_2 can be labeled radioactively with ^{14}C or ^{13}C, which is the nonradioactive stable isotope of carbon. The latter is the preferred isotope in children and women of childbearing age. ^{13}C is a naturally occurring isotope consisting of approximately 1.1% of total carbon. It is not radioactive and can be detected by a number of techniques (isotope ratio mass spectrometry, infrared spectroscopy, laser resonance spectroscopy), of which isotope ratio mass spectrometry is the traditional method used for breath tests because it has a high accuracy for a low level of enrichment (0.001–0.01 atom %). To enable suitable precision, stability, and comparable data, the ^{13}C abundance is always measured against a universal reference standard, which is carbon from Pee Dee Belemnite limestone.

The substrates most commonly used for CO_2 breath tests include carbohydrate, starch, fatty acids, bile acids, amino acids, and urea. The only test currently used widely in clinical practice is the ^{13}C urea breath test (UBT) for the diagnosis of *Helicobacter pylori* infection. Other potential clinical applications include evaluation of mucosal function, bacterial overgrowth, gastrointestinal motility, carbohydrate absorption, bile acid absorption, lipid absorption and pancreatic lipase activity, hepatic function, and protein absorption (Table 73-2). This review only discusses the ^{13}C breath tests because they are safe and suitable for use in children, unlike the radioactively labeled isotopes.

^{13}C Urea Breath Test

H. pylori is a gastric infection acquired in childhood that has been shown to be the major cause of gastritis in humans. The mode of acquisition and the evolution of the infection and disease associations must be studied in childhood and therefore require a safe, reliable, noninvasive diagnostic test. The ^{13}C UBT meets these requirements because it has been demonstrated to have a sensitivity of 100% and a specificity from 90 to 98%.[60]

The principle of the test relies on the presence of *H. pylori* infection in the gastric mucosa, a urease-producing organism that splits the orally administered ^{13}C-labeled urea into ammonia and labeled bicarbonate. The bicarbonate is absorbed, and labeled CO_2 is excreted in expired breath. Expired breath samples can be collected either using a nasal prong or by blowing through a straw into an evacuated glass tube. Analysis of the ^{13}C content of breath

TABLE 73-2 STABLE ISOTOPE BREATH TESTS IN CURRENT USE

FUNCTIONAL TEST	SUBSTRATE	REFERENCES
Motility		
Gastric emptying	^{13}C-octanoate	80–83
Orocecal transit	^{13}C–lactose ureide	86
Digestion and absorption		
Lipase	^{13}C–mixed triglyceride	69, 72, 76, 77
Amylase	^{13}C-starch	9, 74, 87, 88
Lactase	^{13}C-lactose	10, 78, 79
Sucrase	^{13}C-sucrose	No published information
Protein	^{13}C–L-leucine-enriched proteins	97–99
Carbohydrate	^{13}C-fructose, glucose, galactose	33, 35, 78
Lipid	^{13}C-triolein	66, 67
Malabsorption		
Protein	^{13}C-labeled protein (^{13}C-leucine)	97–99
Lipids	^{13}C–lipids	66, 67–73
Hepatic		
Liver glycogen storage	^{13}C-carbohydrate	95, 96
Hepatic cytochrome P-450	^{13}C-aminopyrine	95, 96
Hepatic cytochrome P-448	^{13}C-caffeine	95, 96
Infection		
*Helicobacter pylori**	^{13}Urea	60–65
Small intestinal bacterial overgrowth	^{13}C-xylose	94

*Only test currently widely accepted in clinical practice.

can be carried out using isotope ratio mass spectrometry. Other techniques, such as laser or infrared spectroscopy, may prove to be cheaper but have the disadvantage of requiring larger breath samples.[61]

The test can be performed after a 2-hour fast, and no test meal is required.[61] The oral dose of urea varies between 50 and 100 mg ^{13}C urea and can be administered dissolved in 10 mL of glucose polymer solution or citric acid solution. Citric acid is preferred to orange juice because the latter reduces the diagnostic accuracy of the tests.[62] Citric acid may also reduce contamination of the test by oral flora. Samples are collected before and 30 minutes after ingestion of the substrate. The ratio of ^{13}C to ^{12}C in the two samples is measured, with a positive test being above a cutoff value of 3.5%.

One of the most important advantages of the ^{13}C UBT, apart from its safety, is that it samples the whole stomach and is thus not prone to sampling error, as are biopsy-based tests. More experience is now being acquired with the use of this test in younger children[63] and children in developing countries.[64] It is suitable for epidemiologic studies, in any clinical condition in which endoscopy is not required and for assessing the efficacy of eradication therapy. It is also possible that the level of $^{13}CO_2$ in expired breath using the ^{13}C UBT may be a predictor of *H. pylori* bacterial load and the severity of gastritis.[65]

Pancreatic Function and Fat Malabsorption

^{13}C-labeled substrates offer a safe, noninvasive, attractive alternative to 3-day fecal fat collections because they provide a simple test that can be performed in a single day with minimal discomfort to the child.

The tests use lipids labeled with ^{13}C in the carboxyl moiety using either triolein,[66] hiolein,[67] or mixed triglyceride (MTG).[68–70] Recovery of labeled CO_2 in breath in amounts within a range established in healthy individuals is assumed to indicate normal fat digestion and absorption and, as such, an indirect measure of lipolysis within the small intestine.

A number of factors can theoretically affect the absorption or metabolism of labeled triglycerides and hence cause discordant or false results. These include gastric emptying, lung disorders reducing CO_2 excretion, disorders affecting fat metabolism, or oxidations such as obesity, hyperlypidemia, hepatic disease, thyroid disease, and small bowel transit time.

The MTG breath test molecule contains both long- and medium-chain fatty acids with stearic acid in the 1 and 3 position forming free fatty acids after lipase hydrolysis and ^{13}C octanoic acid at the 2 position forming the monoglyceride. MTG has advantages over other triglycerides such as trioctanoin and triolein because the rate-limiting step in its digestion is hydrolysis of the two stearyl groups by pancreatic lipase. Octanoic acid is rapidly absorbed and oxidized by the liver and in adults has been shown to correlate closely with pancreatic lipase activity. Normal values for this test have been obtained in children[69] and adults.[71] It may be used to compare the level of fat digestion of patients with pancreatic disease with healthy controls[71] and optimize the use of pancreatic enzyme replacement therapy in children with steatorrhea or poor growth.[72] The ^{13}C hiolein breath test, on the other hand, will assess pancreatic steatorrhea but does not reflect pancreatic function, and its primary clinical value would be in diagnosing pancreatic steatorrhea and monitoring efficacy of pancreatic enzyme replacement therapy.[68] This probably also applies to the ^{13}C triolein breath test. The ^{13}C cholesteryl-octanoate breath test has also been used to study pancreatic insufficiency, which, as in the MTG breath test, uses octanoic acid, in this case released by pancreatic cholesterol esterase activity.[73] Its specificity and sensitivity for pancreatic insufficiency are similar to those of other tube-

less pancreatic function tests. Unlike the other breath tests, it can be shortened to 3 hours.

The ^{13}C starch breath test has also been used in children to assess starch digestion in cystic fibrosis.[74] Corn provides a naturally enriched ^{13}C labeled starch and is thus cheaply and readily available. The study showed evidence of impaired starch digestion[74] but is not a suitable test for pancreatic function in clinical practice because of limited specificity and sensitivity.[75]

The above breath tests, particularly the ^{13}C MTG, represent a simple, safe, noninvasive, and repeatable method to study fat digestion and monitor pancreatic enzyme replacement therapy in children.[68,69,72] There is a need for studies aimed at standardizing the test parameters, which include the dose of isotope, type of meal, collection periods, exercise, and the length of fasting required.[76] Another unresolved issue is the significant discrepancy between the dose of isotope given and the percentage recovered, which can be as high as 80%.[77]

Carbohydrate Absorption

Unlike the H_2 breath test, which measures carbohydrate malabsorption, ^{13}C-labeled carbohydrates can measure carbohydrate absorption, providing a noninvasive analysis of enzyme or transport activity. Naturally enriched ^{13}C lactose has been the most widely used, with studies in both adults and children.[10,78,79] The test has also been used in combination with the H_2 breath test and was found to be more sensitive than the H_2 breath test alone.[79]

Fructose absorption has also been studied using combined breath H_2 and breath $^{13}CO_2$ using ^{13}C-labeled fructose.[35] Unfortunately, $^{13}CO_2$ in breath reflects both the absorbed fructose fraction and also the fraction formed as a result of colonic fermentation of unabsorbed fructose, and because of the inability to separate these over time, the test is of limited value in studies of fructose absorption. Thus, the H_2 breath test, although it only reflects malabsorption, may provide a more reproducible method for studying intestinal fructose transport.[35] The ^{13}C breath tests to study carbohydrate absorption are simple to use in clinical practice but are only in their infancy at present. They do, however, have the potential to provide the information needed to allow breath tests to stand alone as routine diagnostic tools.[78]

MOTILITY

Gastric Emptying

The understanding of both normal and aberrant gastrointestinal motility has been hampered by the invasiveness and lack of portability of scintigraphic techniques. Impaired gastric emptying causes significant morbidity in the pediatric population, with different presentations in various age groups. For example, determining the optimum time to institute enteral feeding in premature infants and in children and adolescents dyspepsia may involve altered gastric emptying.[80] Methods to study gastric emptying used in adults are not readily applicable to pediatrics. The results of noninvasive techniques such as applied potential tomography and ultrasonography are difficult to quantify. Breath testing to probe gut motility using ^{13}C-labeled substrates is now being increasingly applied to infants and children to measure gastric emptying.

Ghoos and colleagues pioneered the use of ^{13}C octanoic acid in adults and children,[81] and this has now been used in neonates[80] and premature infants.[82] Gastric emptying of liquids initially received more attention largely because it is easier to assess than gastric emptying of solids.[83] However, studies on the development of reproducible techniques for estimating emptying of solids have now increased.[84] Specific aspects of the methodology, including the test meal and sampling techniques, require standardization for different age groups. Research on gastric emptying in cystic fibrosis and other diseases is progressing but is not yet completely standardized for routine clinical use outside specialist centers.[77]

Small Intestinal Transit Time

Although administration of lactulose and measurement of breath H_2 has been widely used to obtain an estimate of small intestinal transit time, because it is a laxative, at the doses administered, the probe accelerates transit time.[85] The stable isotope probe lactose ^{13}C ureide, at a dose lacking the osmotic properties of lactulose, is likely to be more applicable in the future in a clinical setting.[86]

The use of naturally enriched substrates is also likely to become important for nutritional studies in children in which the ability to follow the transit of functional foods such as labeled starch[87] and nonstarch polysaccharides[88] will enable us to understand both normal and pathologic dynamics of handling and assimilation of different components of a meal. Furthermore, the development of naturally derived substrates with sufficient enrichment for detection may also provide a more affordable array of useful substrates for a variety of different breath tests.[87]

Bacterial Overgrowth

The efficacy of breath tests to detect small intestinal bacterial overgrowth is, at present, controversial. The ^{14}C-labeled bile acid breath test was originally designed to demonstrate bacterial overgrowth in the jejunum and ileum but also measures ileal function[89]; its efficacy as a marker of small intestinal bacterial overgrowth has been questioned,[90] and it is largely not used. However, significant differences between young and old individuals with respect to bile acid metabolism have been shown using this test. Whether a ^{13}C-labeled bile acid breath test can also be used to assess either bacterial overgrowth or ileal dysfunction has also been questioned.[91] The most acceptable labeled breath test is the use of xylose.[92,93] This has also been performed with ^{13}C xylose, and results are promising, but more studies need to be done.[94]

Combination breath tests have been suggested where, for example, ^{13}C xylose is combined with lactulose and the measurement of $^{13}CO_2$ and products of bacterial fermentation is assessed simultaneously. This potentially enhances the precision and accuracy of a test for bacterial overgrowth. Application of stable isotope breath tests has been limited in pediatrics.[94]

LIVER FUNCTION

A number of different stable isotope probes of liver function have been assessed experimentally in children (and adults), but none has yet been adopted in routine clinical practice. These tests assess liver function by determining the integrity of different metabolic pathways.[95] However, before application to clinical practice, tests need to be validated for normality, specific liver function, and variability.[96] The challenge now is to identify substrates that can be used to measure liver function validly and to compare these in prospective studies with conventional liver function tests.

PROTEIN ABSORPTION

Labeled amino acids have been used for some time to study protein metabolism in vivo in animals and humans. These studies mainly involved the measurement of protein synthesis and breakdown. Only recently have stable isotope labeled proteins become available for the study of protein digestion and absorption following a meal. Milk[97] and egg proteins[98] have been labelled with L-(1-^{13}C) leucine. Simultaneous measurement of ^{13}C enrichment of breath and enrichment of plasma in L-(1-^{13}C) leucine after ingestion of a ^{13}C-labeled egg white protein meal in healthy volunteers and patients with pancreatic insufficiency has confirmed that assimilation kinetics are accurately reflected by the breath test.[99] Although this test can be considered as showing promise for the evaluation and follow-up of patients with pancreatic insufficiency and studies of protein absorption in other disease states, more work is required. This relates particularly to an appropriately labeled test meal and establishing the sensitivity and specificity of the test and the effects of other parameters, such as gastric emptying.

SUMMARY

Because of their simplicity in application, particularly in the pediatric age group, breath tests provide the ideal noninvasive investigative modality for gastrointestinal function in health and disease. It is apparent that they are significantly underused in routine practice despite the amount of information supporting their veracity. This has been recently highlighted in a review of all English language abstracts from 1966 to March 2001, suggesting that breath tests are valuable tools and are generally underused, particularly in the pediatric setting.[100]

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CHAPTER 74

IMAGING

1. *Plain Radiographs and Contrast Studies*

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Radiologic investigations are frequently required in the diagnosis of pediatric gastrointestinal disease. Close cooperation between clinicians and radiologists is essential in selecting and interpreting those tests, which will contribute meaningful information to the diagnostic process. To avoid unnecessary repeat examinations, all of the patient's previous imaging should be available for review. The motto should be do it once, properly. To comply with radiation exposure regulations, each medical exposure should be justified. The referring clinician is obliged to provide sufficient and accurate clinical information.[1]

All radiologic modalities (including plain films, fluoroscopy, ultrasonography, computed tomography [CT], magnetic resonance imaging [MRI], and radioisotope studies) have applications in pediatric gastroenterology. This chapter outlines the use of plain radiographs and contrast studies of the gastrointestinal tract.

Although not strictly a gastroenterologic investigation, a plain chest radiograph is an important part of the initial assessment of an ill child. It should be remembered that erect chest radiography is not possible in very young children; therefore, the absence of free subdiaphragmatic air on the chest film cannot always rule out a perforation.

Children with chest infections do not expectorate but swallow sputum, subsequently presenting with vomiting. Abdominal pain, mimicking appendicitis, may be the clinical presentation of basal lung consolidation (Figure 74.1-1).

ABDOMINAL RADIOGRAPH

INDICATIONS

An abdominal radiograph is a useful starting point in the investigation of acute abdominal pain,[2] although it may be unhelpful in up to half of such cases.[3] It is essential in suspected intestinal obstruction or perforation. Ingestion of foreign bodies does not necessitate an abdominal radiograph unless the object is sharp or potentially dangerous (eg, batteries, which may leak),[4,5] although a chest film is important to exclude aspiration (Figure 74.1-2).

The plain film is particularly useful in neonates with obstruction, in whom the distribution of bowel gas is a

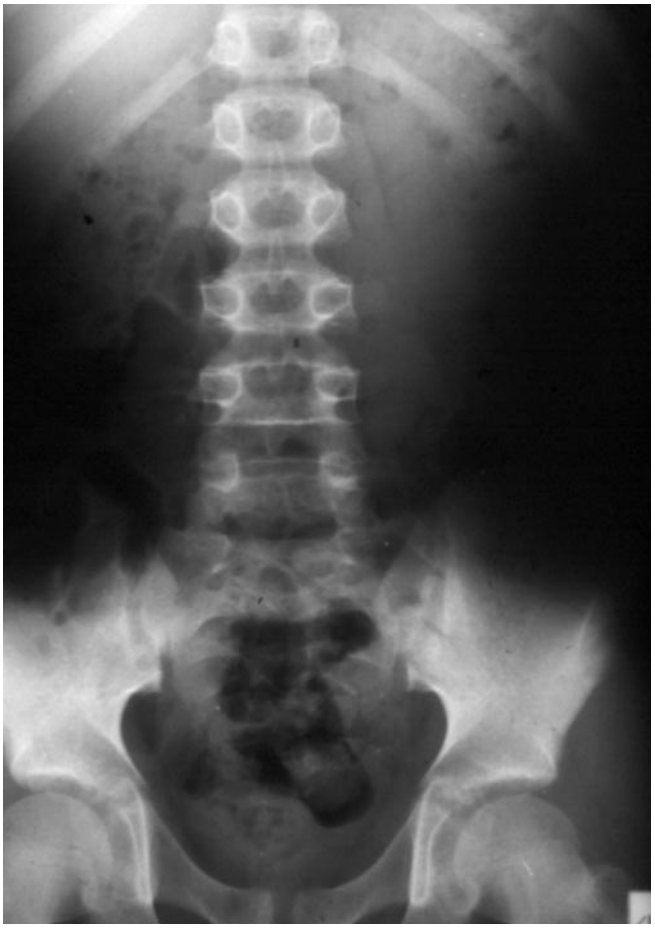

FIGURE 74.1-1 Abdominal radiograph of a child presenting with abdominal pain. There is right lower lobe consolidation.

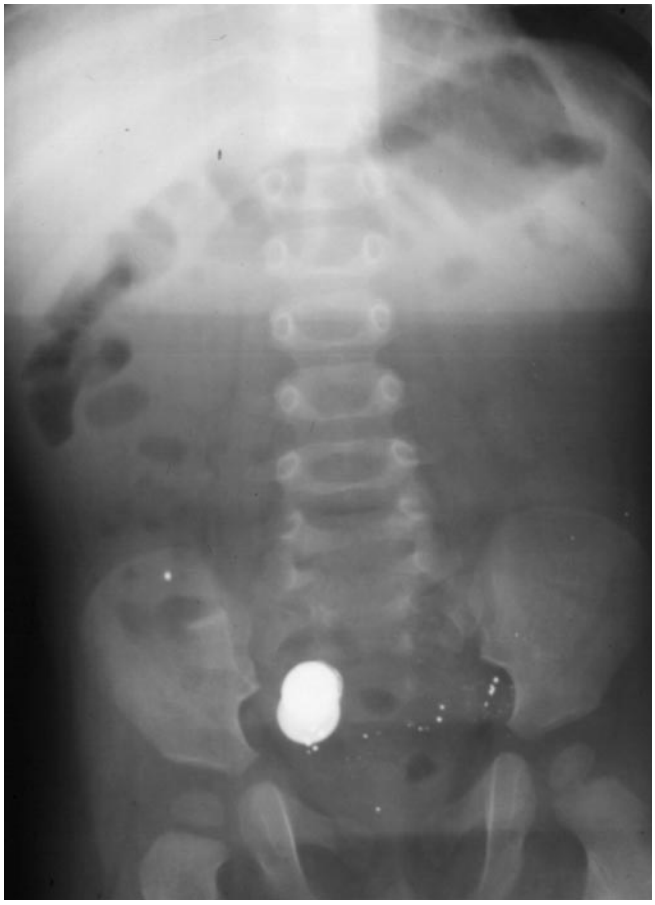

FIGURE 74.1-2 Foreign body ingestion: this patient had swallowed a battery.

clue to the level of obstruction, for example, differentiating esophageal from distal atresia. Associated findings such as the bubbly appearance of gas in meconium ileus, calcification following antenatal perforation, or gas in the bladder in a high anorectal malformation may give further clues as to the cause of the obstruction.[6] In modern practice, ultrasonography is often used in addition to the plain film and can be particularly useful in the diagnosis of intussusception, hypertrophic pyloric stenosis, and appendicitis.

The role of the plain film in chronic abdominal pain is less certain. Its main use here is to exclude pathology such as calcification in the renal tracts or biliary tree. It is diagnostically helpful when such calcification is found. Other features that may be suspected on the plain abdominal film are malrotation (easily missed on ultrasonography), impacted foreign bodies in a Meckel diverticulum, foreign bodies in the colon from compulsive pica, bezoars, and, rarely, duplication cysts seen as a mass or air-fluid levels in a focal area of ileal dysgenesis, which is sometimes called a giant Meckel diverticulum.[7]

Technique

A supine film is adequate in the majority of cases. The lung bases and hernial orifices must be included on the film.[2,3] The radiation dose can be reduced by the use of computed radiography,[8] the addition of extra filtration,[9] and not using antiscatter grids.[10] Specialized pediatric departments consistently record lower doses than do general hospitals,[10] not least because of familiarity with the unique challenges involved in gaining the cooperation of children and their parents: getting it right the first time avoids repeat films and unnecessary additional radiation.

Gonad shielding should not be used in girls because it may obscure important signs within the pelvis.[2] Indeed, studies have shown that lead shields are seldom appropriately placed to protect the ovaries in any case.[11,12]

In adult practice, erect films of the abdomen are sometimes performed to identify free intraperitoneal air and to look for fluid levels in intestinal obstruction. The incidence of intestinal perforation after the neonatal period is extremely low in children. Free air in the neonate is usually of large volume and easy to see on a supine film. Intestinal obstruction, after the neonatal period, usually occurs only with intussusception, postoperative adhesive obstruction, inguinal hernias, and sepsis. In most instances, the diagnosis is clinically obvious—hence the relatively limited value of a routine additional erect or decubitus film. If the diagnosis of intestinal perforation is in doubt, a lateral decubitus film (or, in neonates, a horizontal beam "shoot-through" with the patient supine) should clearly delineate free intraperitoneal gas, which will "float" above dependent fluid levels in the peritoneal space.

Interpretation

All abdominal radiographs should be reviewed in a systematic fashion. The following approach will ensure that nothing is missed:

1. Check the name, date, and left and right markers.
2. Identify the liver and stomach bubble to exclude situs inversus.
3. Check bowel gas distribution: masses or fluid collections will displace the bowel from its normal position. Identify, if possible, the cecum in the right iliac fossa (Figure 74.1-3).
4. Look for any gas that does not lie within the stomach or bowel; triangles, arcs, or straight lines of gas usually denote a perforation. The falciform ligament may be seen near the midline, outlined, or either side with free gas (Figures 74.1-4 and 74.1-5). Mottled or patchy areas of gas may lie within an abscess. Retroperitoneal air is notoriously difficult to see on a plain film but may be seen outlining the kidneys (Figures 74.1-6 and 74.1-7).
5. If distended bowel loops are present, identify the level to which they extend and the presence of any distal gas. In general, the jejunum has a feathery pattern owing to the plenitude of mucosal folds, whereas the ileum is more featureless. Haustral folds are present in the colon. An important caveat in neonates is that the colon has poorly developed haustral folds and can be mistaken for small bowel.[13] Owing to the absence of a pelvic cavity in an infant, the sigmoid colon may lie on the right side of the abdomen or even abut the liver. Assess the thickness of the bowel wall and the separation of bowel loops.
6. Identify both renal outlines, the liver and the spleen. Are there any unexplained masses? Renal outlines are

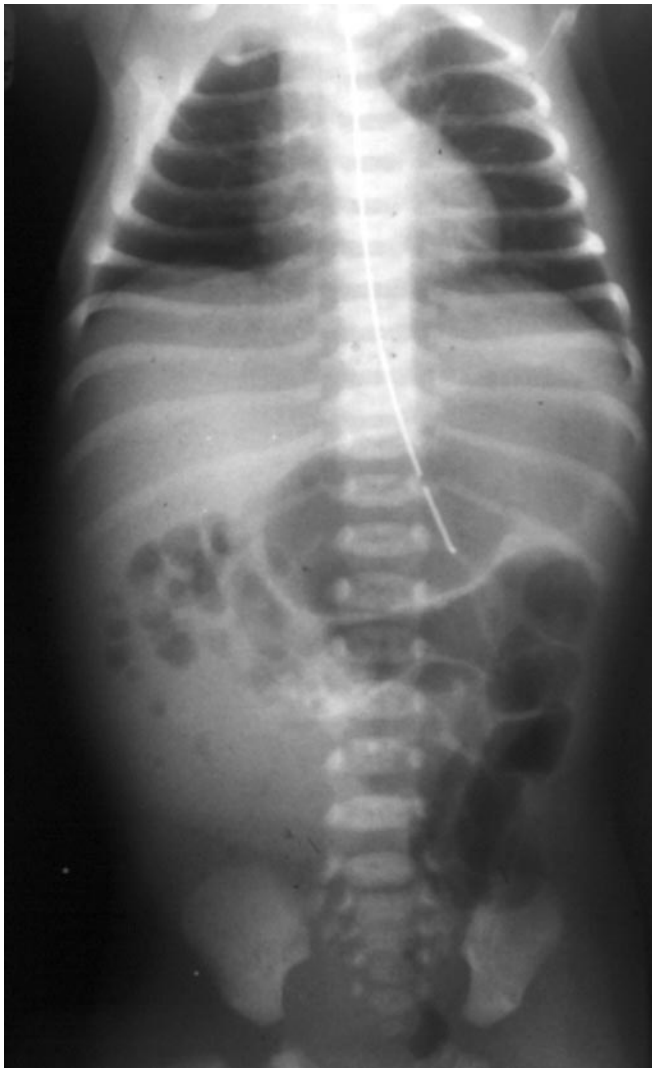

FIGURE 74.1-3 Malrotation: abnormal distribution of bowel gas with the small bowel lying to the right of the midline and the cecum clearly in the left iliac fossa.

poorly seen in children owing to the paucity of perirenal fat, but, as a rule of thumb, the kidneys should each measure about three and a half vertebral bodies in length. Liver size is poorly assessed on abdominal radiography. A Riedel lobe is a normal variant that may extend to the right iliac fossa. Spleen size may be reliably assessed; if the tip of the spleen is seen below the ribs, it is likely to be enlarged (Figure 74.1-8).

7. Look for any calcifications and decide on their location: renal tract, a mass lesion, lymph nodes?
8. Check the corners of the film (ie, lung bases and hernial orifices). Abdominal pain may be referred from above the diaphragm, and strangulated hernias are a common cause of bowel obstruction.
9. Examine the bones: vertebral anomalies are a clue to congenital anomalies such as tracheoesophageal fistulae and renal and anorectal malformations (Figure 74.1-9). Check sites of tubes and lines (see below).
10. Identify the properitoneal fat lines, which are normally convex medially beyond infant age. Distention of these, particularly if coupled with separation from bowel

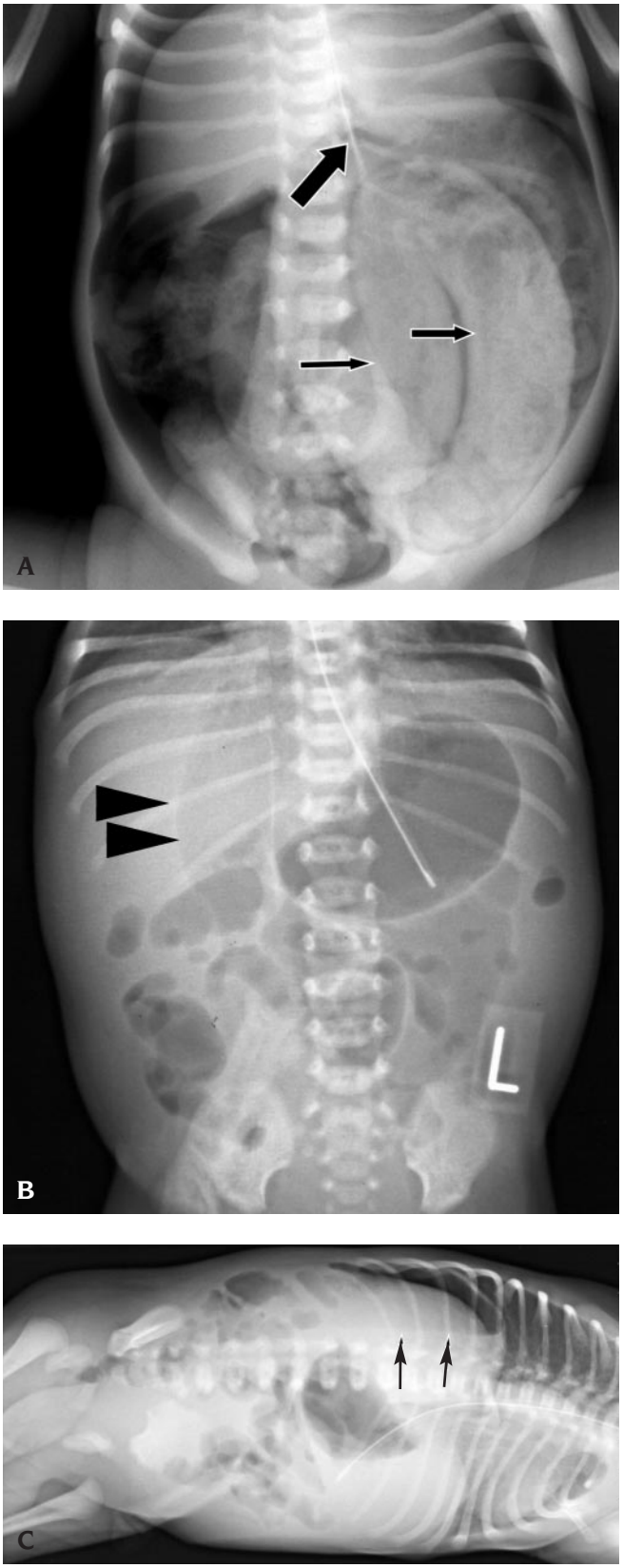

FIGURE 74.1-4 *A*, Perforation in a neonate with meconium ileus. Gas outlines the liver and the falciform ligament (*large arrow*) and distends the flanks (so-called "football sign"). Loops of bowel filled with granular-looking meconium are seen (*small arrows*). *B*, A more subtle example of perforation: an abnormal gas lucency lies centrally in the abdomen (*arrows*). Again, the falciform ligament is clearly seen. *C*, Decubitus film of the baby in *B*. A horizontal air-fluid level is now visible (*arrows*).

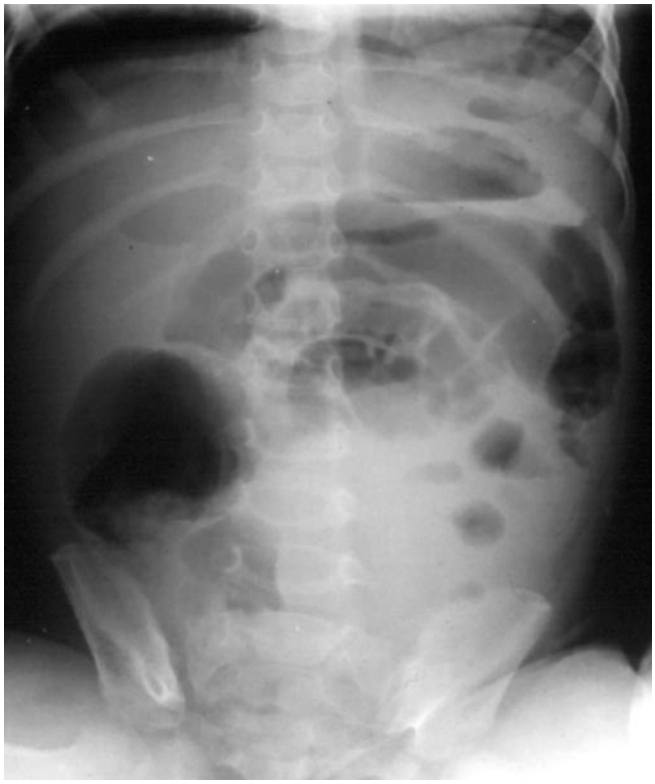

FIGURE 74.1-5 Perforation: crescents of air are seen beneath both hemidiaphragms.

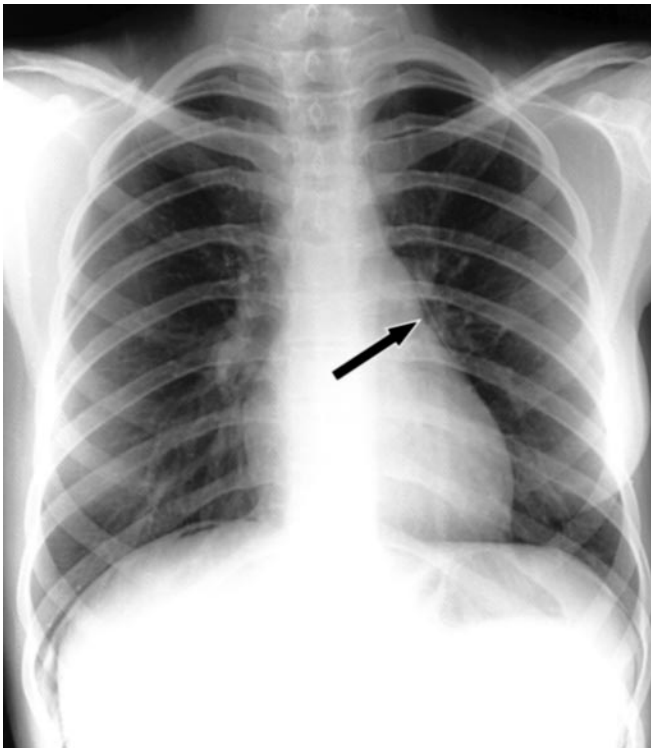

FIGURE 74.1-7 Chest radiograph of the patient in Figure 74.1-6. Air has tracked through the diaphragmatic hiatus into the mediastinum (*arrow*).

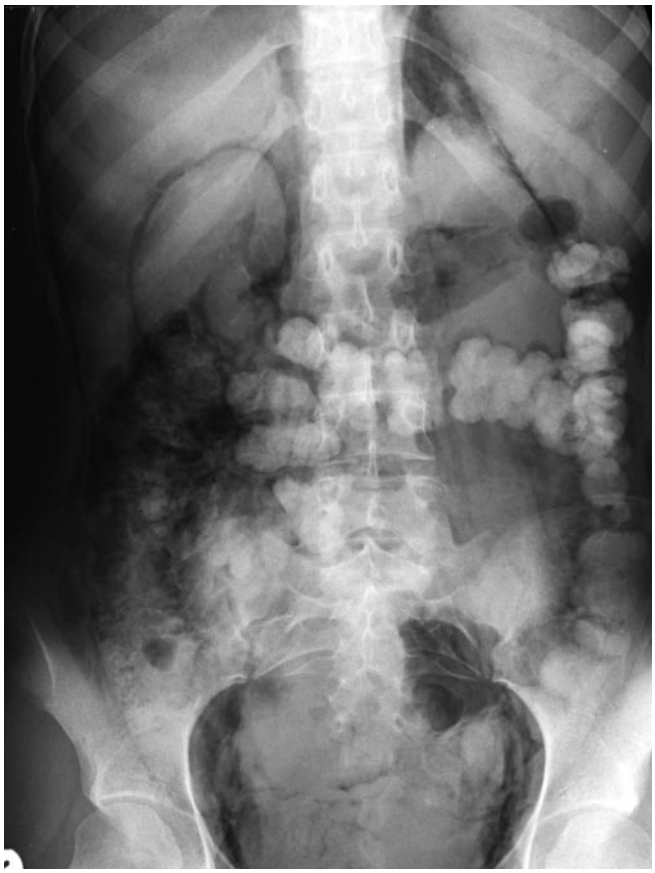

FIGURE 74.1-6 Retroperitoneal air following iatrogenic duodenal perforation. (Contrast in the colon is from a recent fluoroscopic examination.)

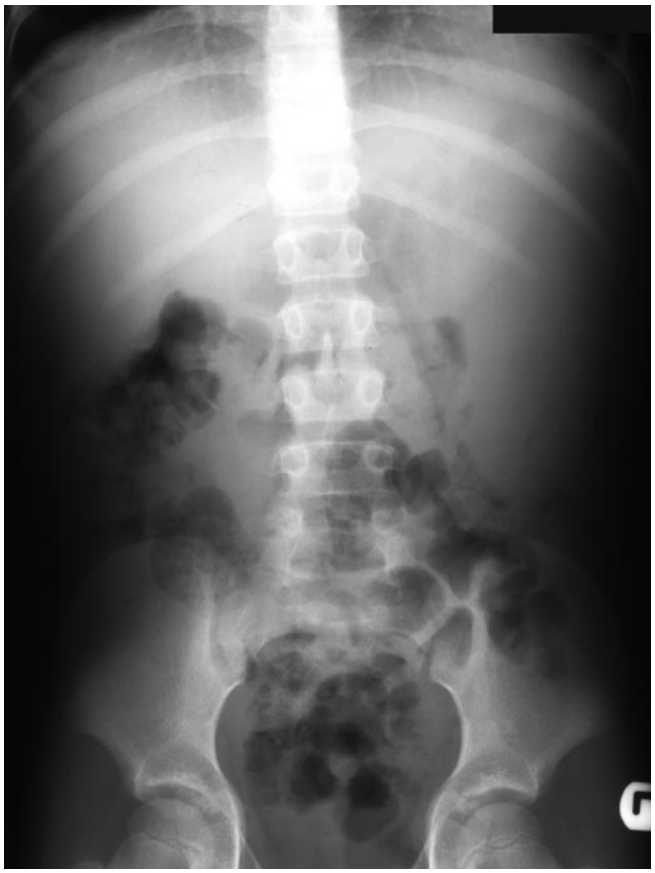

FIGURE 74.1-8 Splenomegaly in a patient with liver cirrhosis owing to cystic fibrosis.

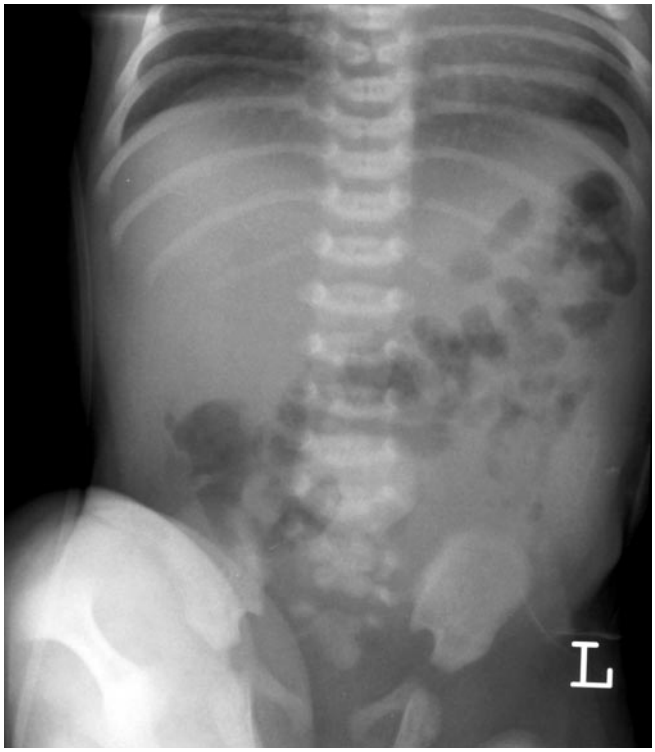

FIGURE 74.1-9 Vertebral anomalies in a patient with an anorectal malformation.

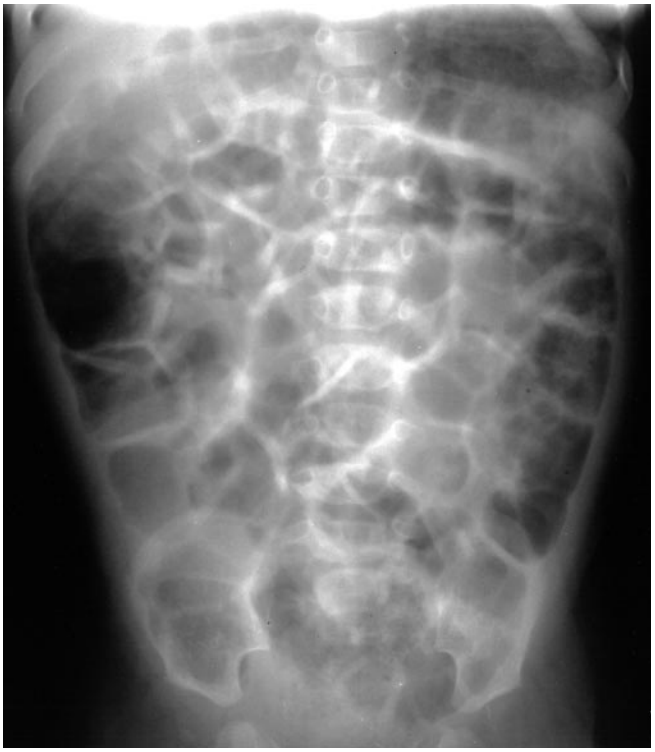

FIGURE 74.1-10 Gaseous distention in a patient with a tracheoesophageal fistula.

loops, may indicate ascites (although this is much more easily identified on ultrasonography). Look for the psoas outline, which is rendered visible by a fat plane. This may become obscured in intra-abdominal sepsis, which is classically described with perforated appendix. However, the psoas outline may be obscured in normal children owing to rotation of the film.

The following conditions have characteristic appearances and may be diagnosed on plain films:

Congenital Obstruction. The level of obstruction can usually be deduced from the plain film. Esophageal atresia could be expected to produce a gasless abdomen, but in practice, there is often a large amount of bowel gas present owing to a tracheoesophageal fistula distal to the esophageal atresia (Figure 74.1-10). Duodenal atresia produces the "double bubble" sign, with air in the distended stomach and proximal duodenum (Figure 74.1-11). Jejunal obstruction produces several dilated loops of small bowel in the left upper quadrant (Figure 74.1-12). The more distal the obstruction, the more loops of distended bowel are seen. Distal small bowel obstruction may be difficult to distinguish from large bowel obstruction.[14]

Anorectal Anomalies. The diagnosis of anorectal anomalies is clinical. However, a plain abdominal film is required to assess the sacrum for associated anomalies. A prone lateral "shoot-through" of the rectum will help to delineate the level of the anorectal atresia, but meticulous technique is essential. The baby should be laid prone with its bottom elevated for 30 minutes prior to the film being taken to allow air to rise to the most distal portion of the rectum. A radiopaque marker should be placed on the skin at the anal dimple. Rarely, there is air in the bladder owing to a colovesical fistula.

Acquired Bowel Obstruction. The plain film in acquired obstruction typically shows dilated loops of

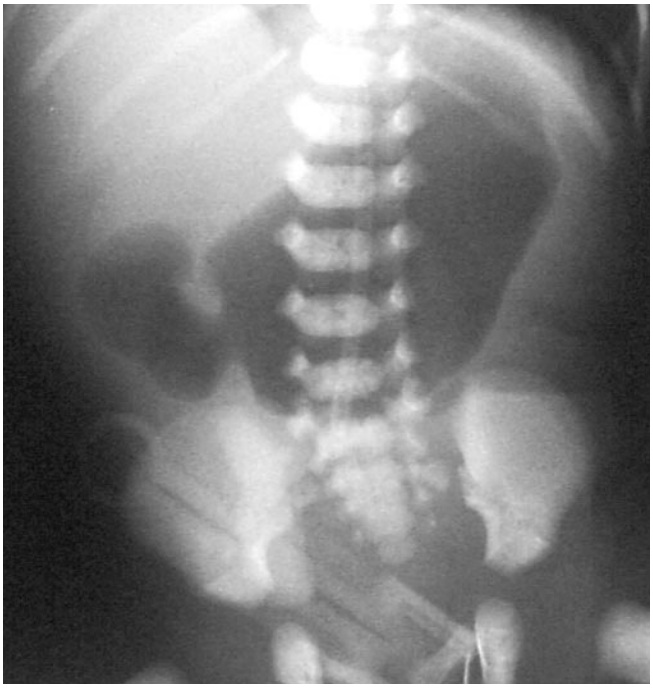

FIGURE 74.1-11 "Double bubble" sign in duodenal atresia.

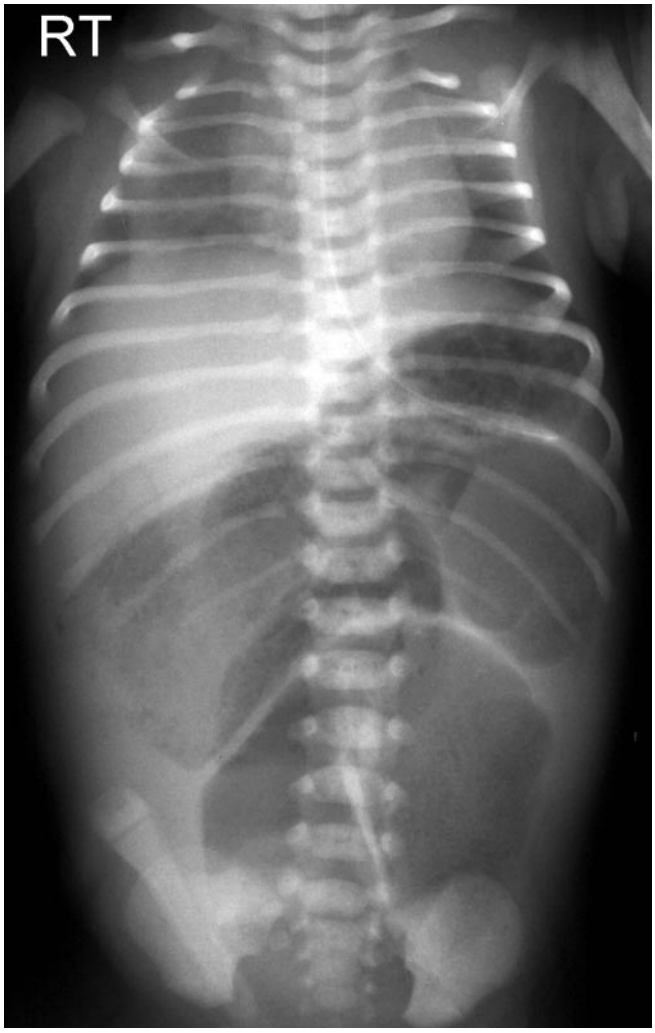

FIGURE 74.1-12 Jejunal atresia.

bowel proximal to the obstruction (Figure 74.1-13). If the obstruction is of recent onset, there may still be gas in the bowel distal to the obstruction. Multiple fluid levels within dilated loops form a "ladder" pattern.[2] In high-level obstruction, or when the bowel is very full of fluid, such as in closed-loop obstruction, air-fluid levels may not be present. Nasogastric suction may also alter the pattern of fluid levels.

Ileus. In paralytic ileus, there is an absence of peristalsis rather than mechanical obstruction. Therefore, both small and large bowel are dilated (Figure 74.1-14). The individual loops tend to be less distended than in mechanical obstruction, and fluid levels are longer.[2] Gas is seen as far as the rectum. Confusion may arise, however, in cases of prolonged mechanical obstruction, when ileus may ensue. Since sepsis is a common cause of ileus in children, ultrasonography is supplementary to identify any intra-abdominal or pelvic collections.

Intra-abdominal Abscess. Large abscesses will be visible on the plain radiograph as mass lesions with displacement of bowel loops. There is often gas within the abscess; depending on its location, this may be a subtle finding, such as in a subphrenic collection, in which the gas overlies the dome of the liver, and may form a gas-fluid level. A localized ileus may cause a few loops of distended bowel adjacent to the abscess.

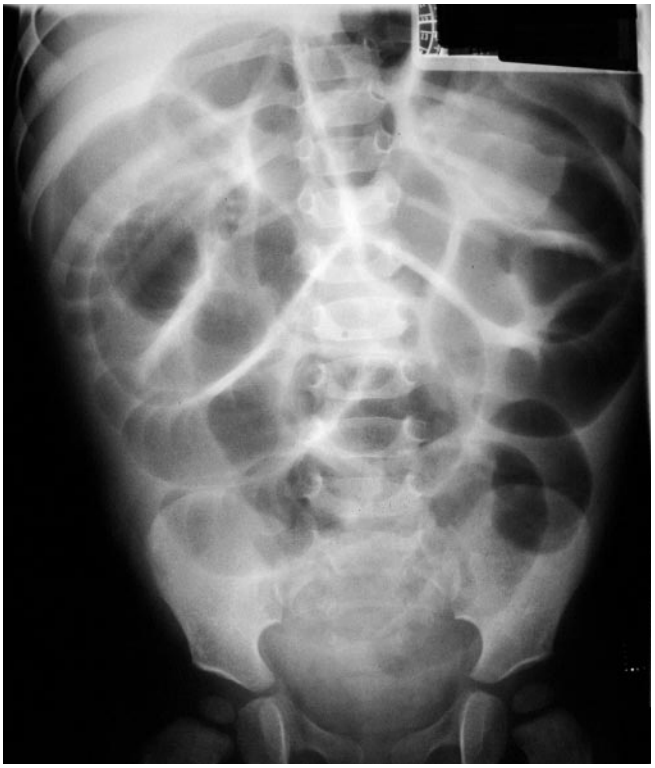

FIGURE 74.1-13 Small bowel obstruction. Note absence of gas in the rectum.

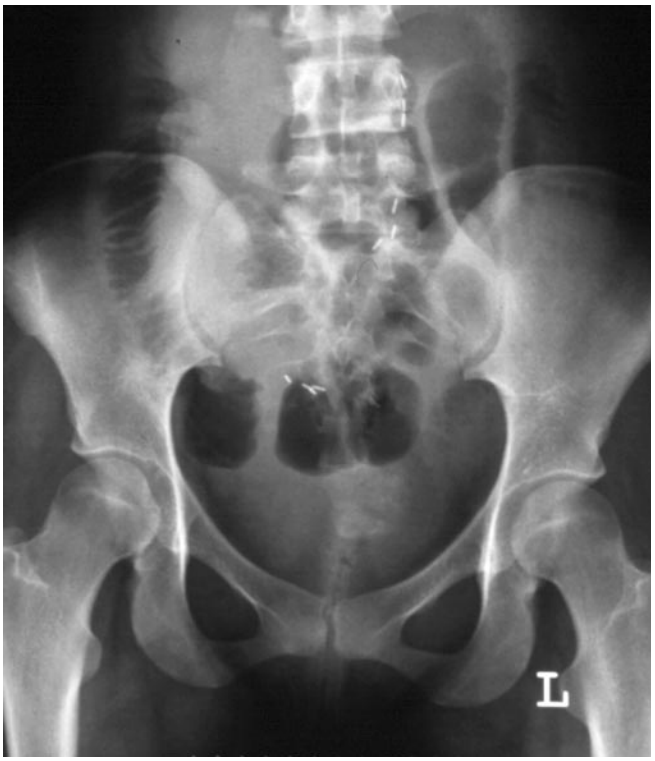

FIGURE 74.1-14 Postoperative ileus. Note the surgical clips and several loops of dilated small bowel.

Necrotizing Enterocolitis. The abdominal radiograph in the neonate with necrotizing enterocolitis will display nonspecific signs such as widespread dilatation of bowel loops and separation of loops. The radiologic hallmark, however, is intramural gas. This may be present at any level from the esophagus to the rectum and may be linear or bubbly in appearance (Figure 74.1-15). Portal venous gas may be present and is no longer regarded as an ominous prognostic sign.[13,15] It must be differentiated from air in the biliary tree, which tends to be more centrally located (around the porta hepatis) and displays a branching pattern. Portal venous gas occasionally may be introduced via an umbilical vein catheter.

Bowel Wall Thickening. Edematous, inflamed bowel wall appears thickened, and loops lie slightly separated from one another. "Thumbprinting" occurs with ischemia and may also be due to hemorrhage into the bowel wall, such as occurs in hemolytic uremic syndrome, Henoch-Schönlein purpura, or thrombocytopenia (Figure 74.1-16). Toxic megacolon, when the transverse colon is unduly dilated and inflamed, is rare in children.

Hernias. Gas within the scrotal sac in boys (or below the inguinal ligament in girls) indicates a hernia; however, in strangulated hernias, the gas may be absent, but there will be asymmetry of the scrotal shadow (Figure 74.1-17). Umbilical hernias may cause unusual gas shadows on plain films owing to air interposed between the hernial sac and the anterior abdominal wall (Figure 74.1-18).

Foreign Bodies. Incidental foreign bodies are frequently seen in children, particularly in the colon. Most will pass without incident. Swallowed dental amalgam

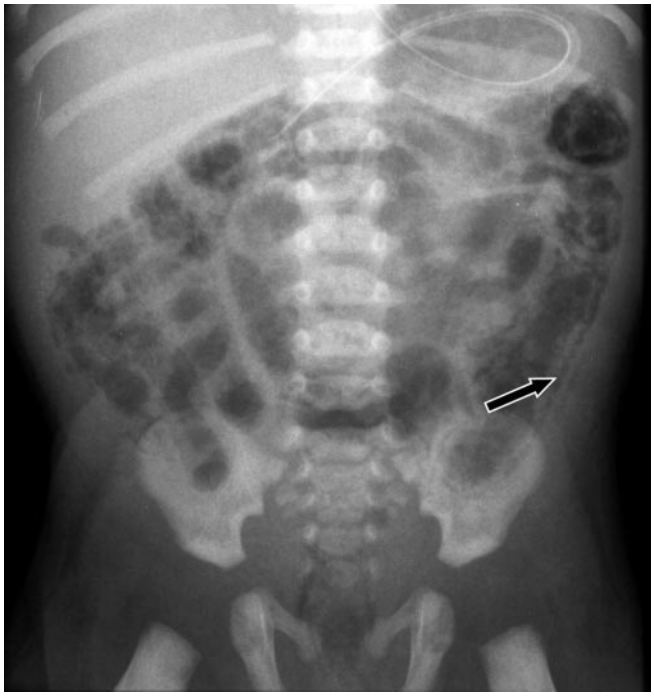

FIGURE 74.1-15 Necrotizing enterocolitis resulting in linear gas lucencies within the thickened bowel wall (*arrow*).

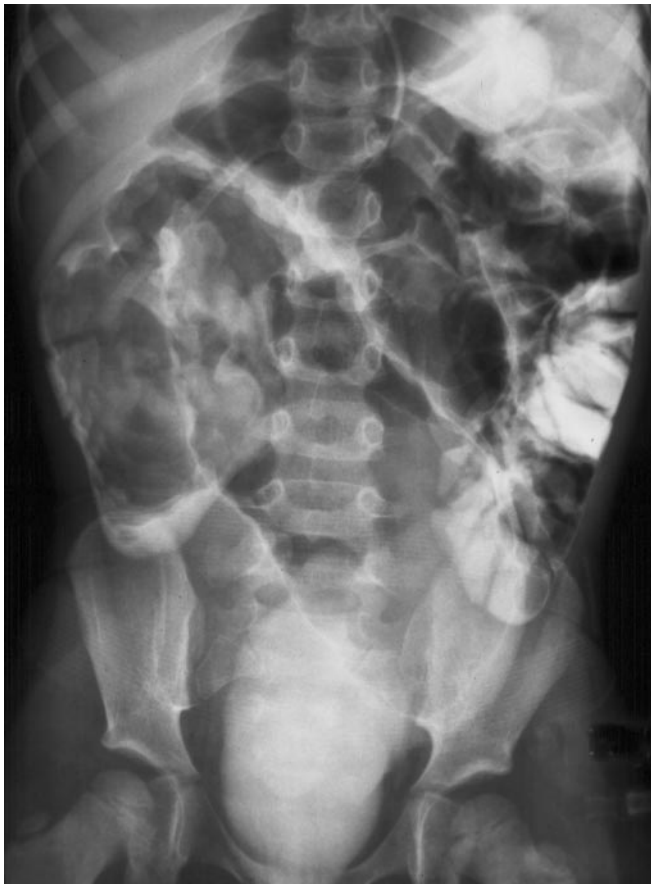

FIGURE 74.1-16 "Thumbprinting" of the colonic mucosa in a patient with Hirschsprung disease and necrotizing enterocolitis.

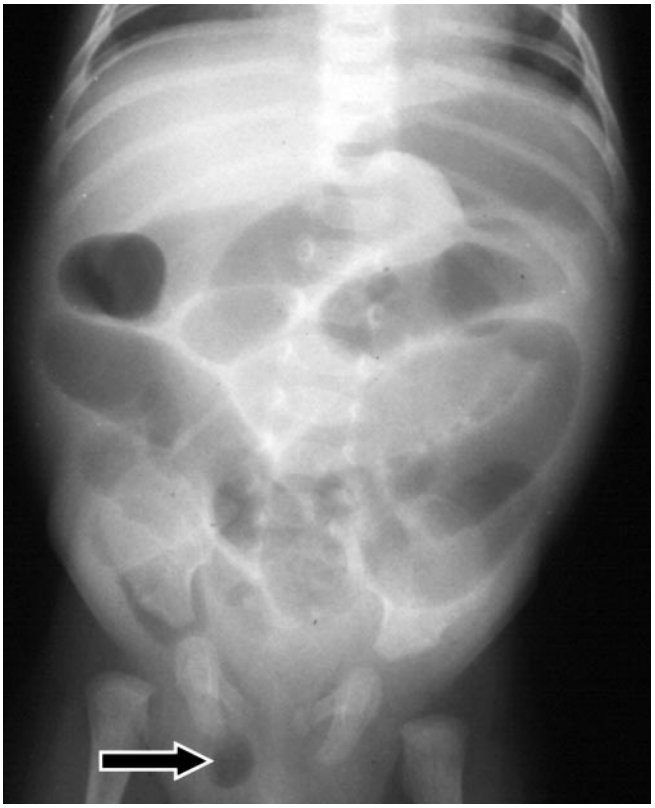

FIGURE 74.1-17 Small bowel obstruction owing to an inguinal hernia. There is gas in the scrotum (*arrow*).

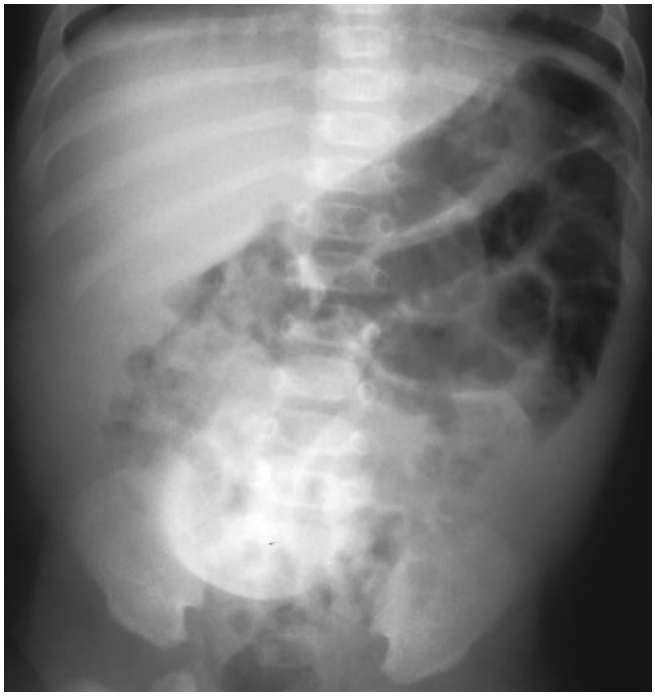

FIGURE 74.1-18 Umbilical hernia.

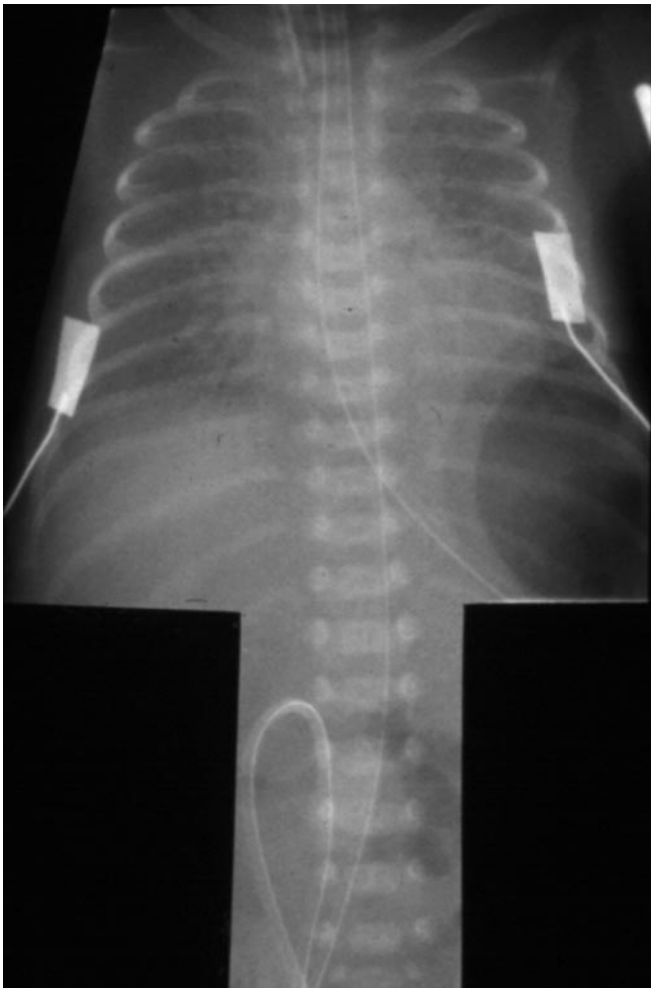

FIGURE 74.1-19 The umbilical vein catheter has been placed too high; its tip lies in a neck vein. Note endotracheal tube and nasogastric tube in a satisfactory position.

results in a characteristic pattern of small flakes distributed throughout the colon.

Tubes and Lines. The tip of a nasogastric tube should lie in the body of the stomach.

An umbilical vein catheter should lie in the right atrium[16]; a lateral film may be required to confirm this because malposition in the left atrium is associated with an increased incidence of intracardiac thrombus formation.[17] An umbilical artery catheter should lie between T6 and T10 (or L3–L5 if the low position is used) (Figures 74.1-19 and 74.1-20).[16] The positions of lines will vary in neonates with congenital diaphragmatic hernias and may give clues as to the contents of the hernia.[18] Ventriculoperitoneal shunts should terminate within the peritoneal cavity but may migrate upward as the child grows. If there is doubt as to the position of the line tip, a lateral film may be useful. If the tip is projected below the costal margin on the frontal radiograph, a lateral radiograph may show it to be superficial to the liver, where it may pass in and out of the peritoneal cavity, creating a valve effect, resulting in intermittent hydrocephalus.

Calcification. It is important to determine exactly where calcification on an abdominal radiograph is and to decide on its cause. Adrenal calcification commonly follows neonatal adrenal hemorrhage and is seen on either side of the vertebral column, at the top of the renal outlines. Antenatal intestinal perforation results in calcified meconium in the peritoneal cavity. Calcification within a mass lesion should always raise suspicion: neuroblastomas commonly calcify, and calcification is seen rarely in Wilms tumor, hepatoblastoma, and liver hemangioma (Figure 74.1-21).[19] In older children with cystic fibrosis, pancreatic calcification may be seen extending across the midline at the L1 level. Previous tuberculous infection may cause calcification in mesenteric lymph nodes, liver, and spleen; treatment of lymphoma with radiotherapy may also result in lymph node calcification. Fecoliths are common incidental findings, but their presence should be reported because elective appendectomy is indicated.[2] Renal calcification should be differentiated into parenchymal versus pelvic: medullary sponge kidney, renal papillary necrosis, and hyperparathyroidism are among the causes of the former, whereas calcification within the lumen of the renal pelvis, ureters, or bladder is likely to represent a true "stone." A transplant kidney may calcify owing to chronic rejection. In older children, phleboliths within the pelvic veins are easily mistaken for ureteric stones. In general, phleboliths are rounded with a slightly lucent center, whereas urinary tract calculi tend to be more angular in shape. An intravenous urogram, possibly including an oblique film of the bladder area, may be required to show whether a calcific focus lies along the path of the ureter.

Appendicitis. A child with unequivocal clinical signs of appendicitis does not require imaging. However, in cases in which the diagnosis is in doubt, radiologic input may be

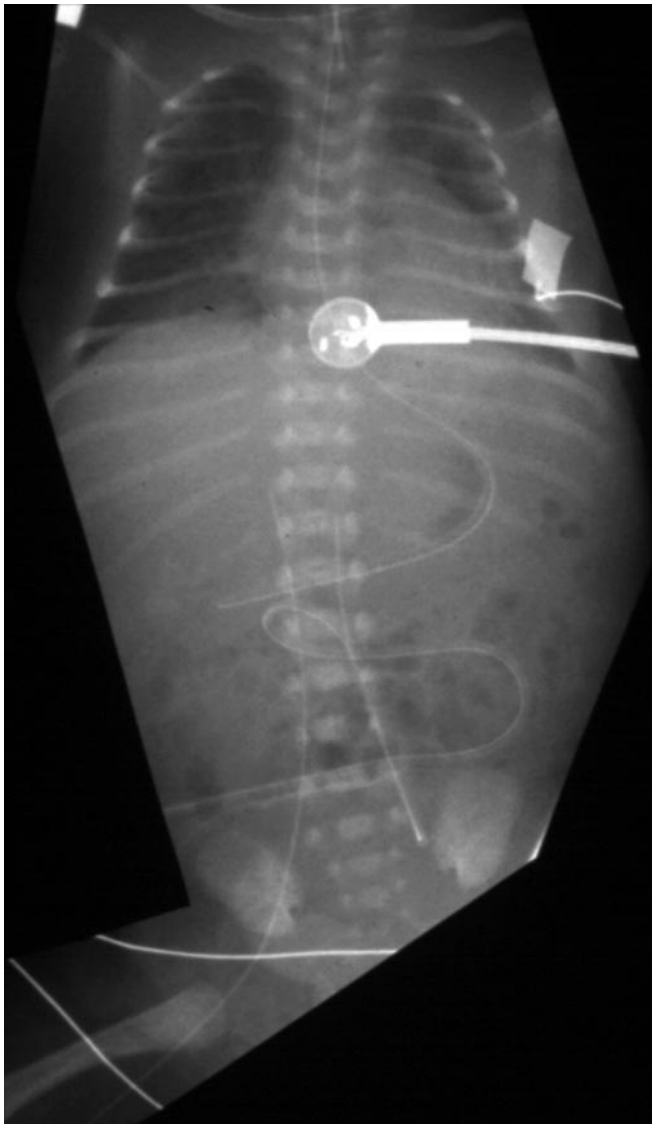

FIGURE 74.1-20 The umbilical artery catheter has been placed too low.

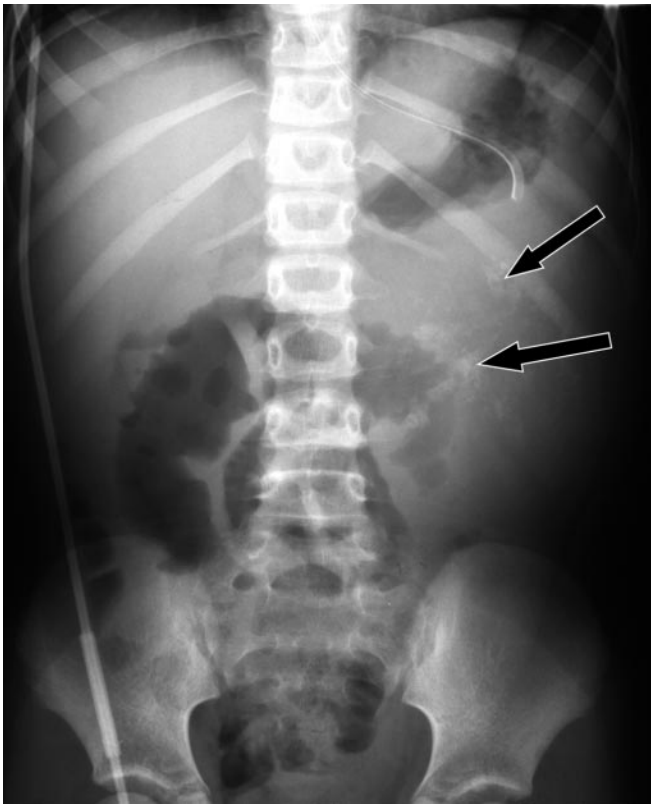

FIGURE 74.1-21 Left-sided abdominal mass containing calcification (*arrows*). This was a neuroblastoma.

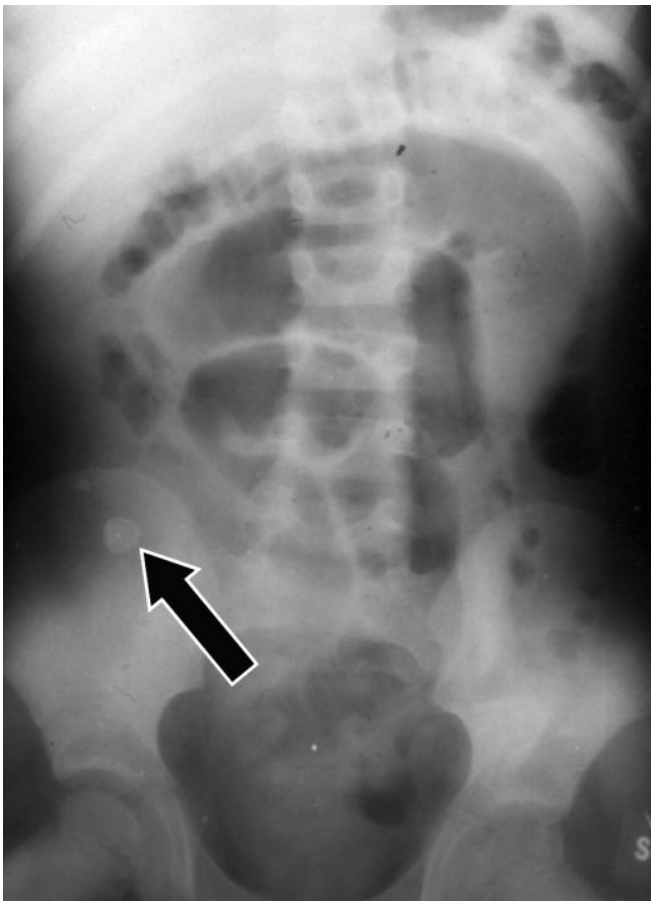

FIGURE 74.1-22 Appendicitis: there is a fecolith in the right iliac fossa (*arrow*).

valuable. The plain abdominal film is frequently normal in appendicitis. In about 10% of cases, a calcified fecolith in the right iliac fossa will confirm the diagnosis (Figure 74.1-22).[2] If the appendix is retrocecal, the fecolith may lie more superiorly and may even mimic a gallstone. Other plain film findings that have been described in appendicitis include a mass in the right iliac fossa with displacement of bowel loops and possibly containing gas (Figure 74.1-23), a localized ileus or even complete small bowel obstruction, and blurring of the peritoneal fat lines. In modern practice, ultrasonography is the first-line investigation in suspected appendicitis[20] and has the considerable advantage of not using ionizing radiation. There is also increasing evidence that CT in selected cases can significantly reduce the rate of negative appendectomies (Figure 74.1-24).[21,22]

Typhlitis. This severe form of colitis is seen in neutropenic and immunocompromised children. The name strictly refers to cecal inflammation, but the whole colon may be involved. Plain film signs include bowel wall edema

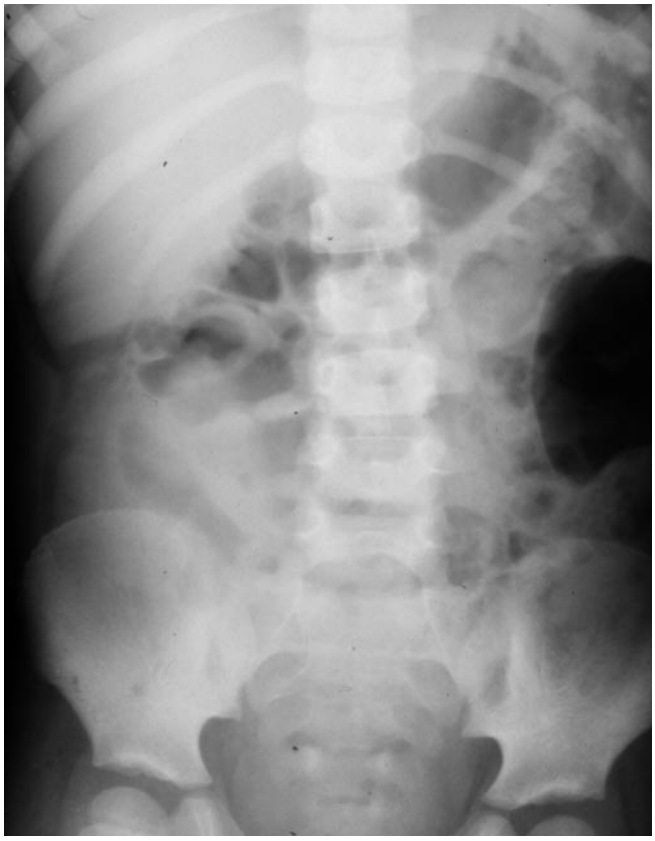

FIGURE 74.1-23 Appendicitis: a rounded soft tissue density in the right iliac fossa displaces bowel loops.

and "thumbprinting"; in severe cases, intramural gas may be present. Secondary dilatation of the small bowel may occur.[2] As with appendicitis, ultrasonography and CT are playing an increasingly important role in the diagnosis of typhlitis. It is particularly important to distinguish this condition from appendicitis because surgical intervention is not required in typhlitis unless complications (such as perforation) occur.[23,24]

Intussusception. The plain film may be entirely normal in cases of intussusception. The typical finding of a soft tissue mass indenting the colon (usually seen in the transverse colon) is seen in only about 25% of patients (Figure 74.1-25).[2,25] Less specific findings include the absence of gas in the right upper quadrant (with failure to identify the cecum and the typical hepatic flexure gas pattern) and a vague right-sided mass. Varying degrees of small bowel obstruction may be present, depending on the time elapsed since the onset of symptoms. Ultrasonography is sensitive in the diagnosis of intussusception and should be performed before proceeding to a contrast study and attempts at reduction.[26]

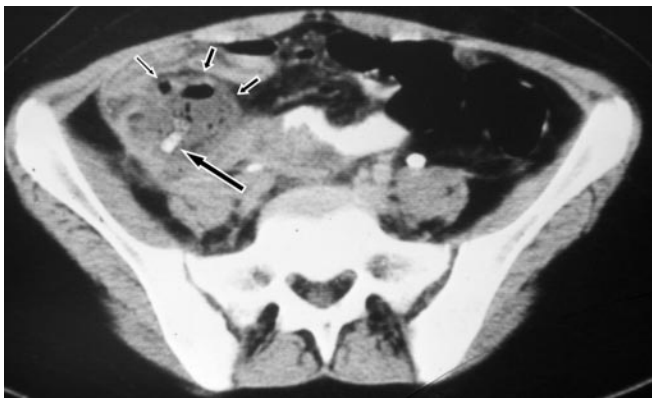

FIGURE 74.1-24 Computed tomographic scan of appendicitis: there is a fecolith (*large arrow*) and a rounded fluid collection (*small arrows*) containing flecks of gas.

Pitfalls for the Unwary. While assessing a pediatric abdominal radiograph, the clinician should be aware of several "oddities" that may masquerade as pathology. Films taken on the intensive care unit often include various tubes and equipment, which, in fact, lie outside the patient. The access hatch of an incubator may cause a mysterious circular lucency in the center of the film. Similarly, the umbilical cord, particularly if there is no clamp, may simulate a mass. The same effect can be produced by the penis seen end on. The tip of the normal coccyx may be misinterpreted as abnormal calcification within the pelvis. When assessing suspected intestinal obstruction, it is important to remember that air seen in the rectum may have been introduced by digital rectal examination and therefore cannot be relied on as a sign of patency of the distal bowel.

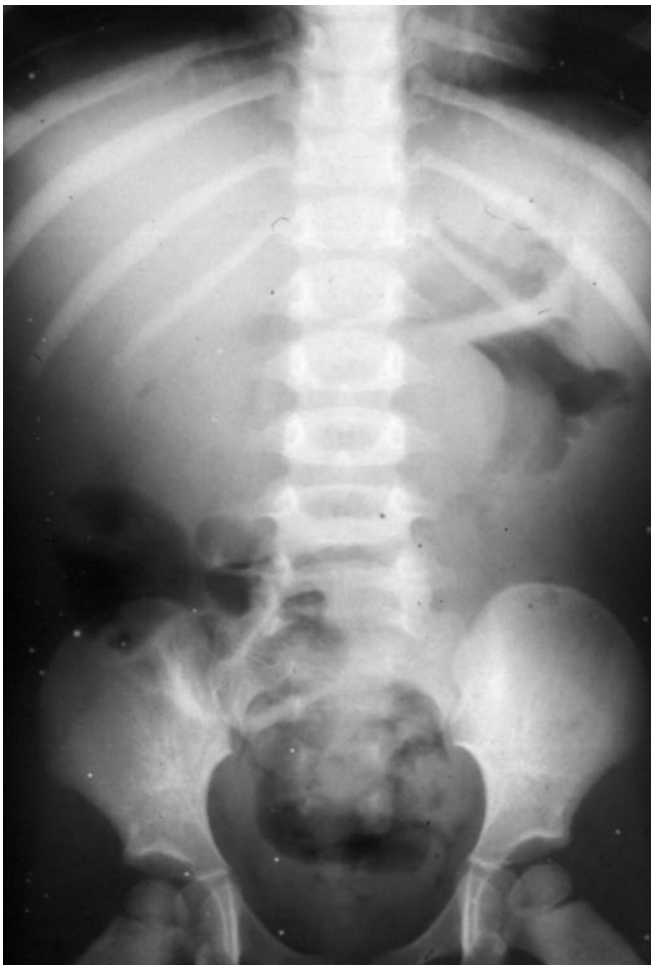

FIGURE 74.1-25 Intussusception.

CONTRAST STUDIES

Indications

Contrast studies of the gastrointestinal tract, when tailored specifically to the clinical indications, can provide the clinician with anatomic detail and functional information about motility and gastric emptying. The spectrum of disease in children differs from that in adults; ulcers and tumors are rare, and congenital anomalies, such as malrotation, are more frequent. Thus, the emphasis is on anatomy and function rather than mucosal detail, making a fluoroscopic contrast study a potentially more useful diagnostic tool than endoscopy in many cases. Contrast studies are well tolerated by the vast majority of children, whereas endoscopy requires sedation or a general anesthetic. However, contrast studies of the esophagus, stomach and duodenum, and colon have largely been replaced by endoscopy in specialist pediatric units. Contrast studies of the small bowel now make up a larger proportion of the work in a pediatric radiology department, although ultrasonography and CT are often complementary investigations in the diagnosis of small bowel lesions (Table 74.1-1 and Figure 74.1-26).

As well as the conventional techniques listed above, there is frequently the need for specific investigations in specific circumstances: "loopograms" or sinograms, which are intended to answer a particular clinical question, most often in surgical patients (Figure 74.1-27). It cannot be overemphasized that communication between the clinician and the radiologist is of the utmost importance to ensure that the appropriate test is performed. In some cases, CT may be used in conjunction with fluoroscopy, for example, giving more detail about the track of a sinus or fistula in three dimensions.

Contrast studies have been used in the past for the investigation of the gallbladder and biliary tract in children. Ultrasonography is extremely reliable in the diagnosis of biliary tract dilatation, choledochal cysts, and gallstones. More recently, MRI has advanced to the point where magnetic resonance cholangiopancreatography (MRCP) can provide high-resolution images of the biliary tree, gallbladder, and pancreatic duct, obviating the need for invasive procedures such as percutaneous transhepatic cholangiography or endoscopic retrograde cholangiopancreatography. MRCP has proved useful in the investigation of acute pancreatitis, primary sclerosing cholangitis, choledochal cyst, and congenital anomalies, such as pancreas divisum.[27–30] New techniques are continually being explored in this exciting field, and in the near future, virtual endoscopy of the pancreatic and biliary ducts using data from MRCP studies is likely to become routinely available.[31]

Choice of Contrast

Barium sulfate is the traditional gastrointestinal contrast medium of choice. It is cheap and readily available, and its high density provides excellent contrast and definition. It is not absorbed by the gut and can, therefore, be used anywhere in the bowel. It remains the contrast medium of choice for small bowel studies and for double-contrast enemas on the rare occasions that these are done. However, if any of the following contraindications exist, a water-soluble contrast medium (see below) should be used:

- Suspected perforation (barium excites an aggressive inflammatory response if allowed to escape into the mediastinum or peritoneum).
- Possibility of aspiration, for example, in neurologically impaired children (although aspirated barium is usually well tolerated, serious respiratory impairment can occur).
- Because barium is contraindicated, water-soluble media are now the contrast of choice for neonates.
- Barium may become inspissated in defunctioned bowel (eg, loopograms postsurgical resection), and there is a risk of impaction in Hirschprung disease and cystic fibrosis.

Gastrografin should not be used in the upper gastrointestinal tract because it is very hyperosmolar and can precipitate pulmonary edema if aspirated. It is used for

TABLE 74.1-1 GASTROINTESTINAL CONTRAST STUDIES PERFORMED IN CHILDREN AND SOME OF THEIR INDICATIONS

Speech study/phonetics
Identification of palatal movement and choanal closure
Pre- and postsurgical repair of cleft palate
Speech problems with nasal escape
Nasal reflux of food
Swallowing study
Oropharyngeal incoordination, mainly in children with neurologic impairment
Contrast swallow
Vascular rings and other extrinsic masses affecting swallowing
Tracheoesophageal fistula (including follow-up postsurgical repair) (see Figure 74.1-26)
Gastroesophageal reflux
Foreign body ingestion
Strictures
Upper gastrointestinal study
Usually follows contrast swallow
Demonstrates anatomy (eg, duodenojejunal flexure) and motility
Pyloric stenosis (now largely replaced by ultrasonography)
Gastrostomy study
Contrast instilled via gastrostomy tube
Assesses gastric emptying and reflux
Small bowel studies
Assesses the bowel, which is not within reach of endoscopy
Performed as follow-through or enteroclysis (see below)
Crohn disease
Small bowel strictures (traumatic/postsurgical)
Dysmotility
Barium enema
Now rarely performed
A limited study may be performed in severe constipation to exclude Hirschsprung disease
Defecating proctography
To assess pelvic floor dysfunction in obstructed defecation
Fistulography/sinography
Identification of tracks
Water-soluble contrast enema
Performed in neonates
To identify cause of intestinal obstruction, eg, Hirschsprung disease, meconium ileus, meconium plug, intussusception

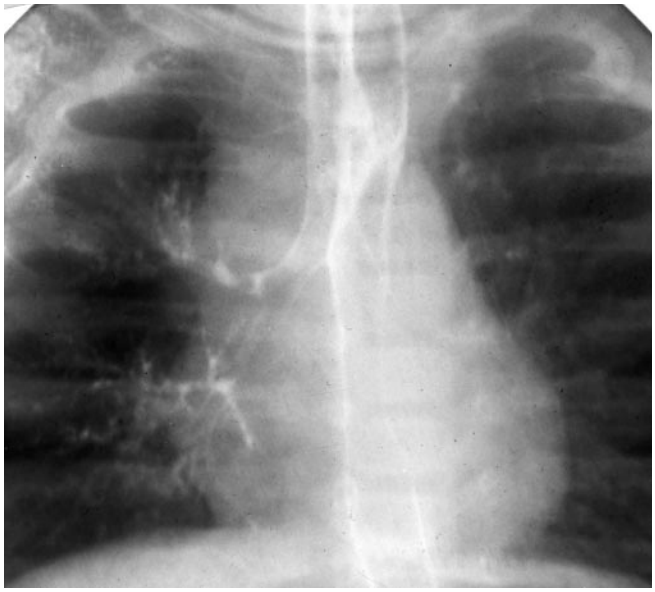

FIGURE 74.1-26 Contrast swallow in a patient with H-type tracheoesophageal fistula. Contrast is seen outlining the trachea and bronchial tree.

therapeutic enemas in meconium ileus, where it draws fluid into the bowel lumen to aid disimpaction of the sticky meconium. However, it may cause dehydration by drawing excessive amounts of water into the bowel,[6] particularly in a vulnerable neonate, and great care should be taken to ensure adequate fluid replacement. The radiologist must inform the clinician that Gastrografin has been used.

The iso-osmolar contrast agents are more expensive than Gastrografin but are safe if aspirated and have no effect on the peritoneum or mediastinum. Perhaps surprisingly, there is no evidence that iso-osmolar agents are any less effective in the treatment of meconium ileus than hyperosmolar media.[6]

Double-contrast studies of the gastrointestinal tract are performed in adult practice and provide excellent mucosal detail. The technique uses a thin coating of barium to define the mucosa, combined with distention of the viscus with air. With the advent of endoscopy, double-contrast studies are now rarely required in children.

Air is now the medium of choice for intussusception reduction.[32,33] It is introduced into the colon at pressures of up to 120 mm of mercury. Perforation is a rare complication, but if it occurs, a large pneumoperitoneum will result, with respiratory compromise. Accordingly, a large trochar should be available for immediate decompression of the peritoneum.[2]

TECHNIQUE

General Considerations. A pediatric "barium" list presents a unique challenge. Flexibility and ingenuity are essential so that each study is specifically tailored to the patient and the clinical problem.

Steps should always be taken to minimize the radiation dose to children undergoing fluoroscopic examinations. Digital fluoroscopy delivers a lower dose than older cine-film systems, and the dose can be reduced further by using pulsed screening.[34,35] A last-image-hold ("frame grab") facility allows images to be recorded without a static exposure being made. Grids should not be used routinely.

Video recordings should be made of all gastrointestinal contrast studies. This allows dynamic processes such as swallowing, gastroesophageal reflux, or gastric transit to be viewed in real time, or frame by frame. If an uncooperative child has allowed only an incomplete study to be performed, the video footage will often contain sufficient information even if no static images have been recorded, thus obviating the need for a repeat study.

Specific Techniques. *Speech and Swallowing Studies.* Speech and swallowing difficulties frequently coexist in neurologically impaired children. A multidisciplinary approach is essential in their management, and videofluoroscopy swallowing studies should be carried out with the speech therapist. Videofluoroscopy swallowing studies provide more detailed and objective evidence of swallowing dysfunction than traditional bedside evaluation.[36] The aim is to assess the phases of swallowing and to ensure that there is complete glottic closure without aspiration. Specific observable problems include poor tongue movements, delayed swallow reflex, reduced laryngeal elevation, and silent aspiration.[37] Aspiration is more likely to occur with thin fluids; therefore, swallowing of thicker consistencies should be assessed first, using, for example, bread or bis-

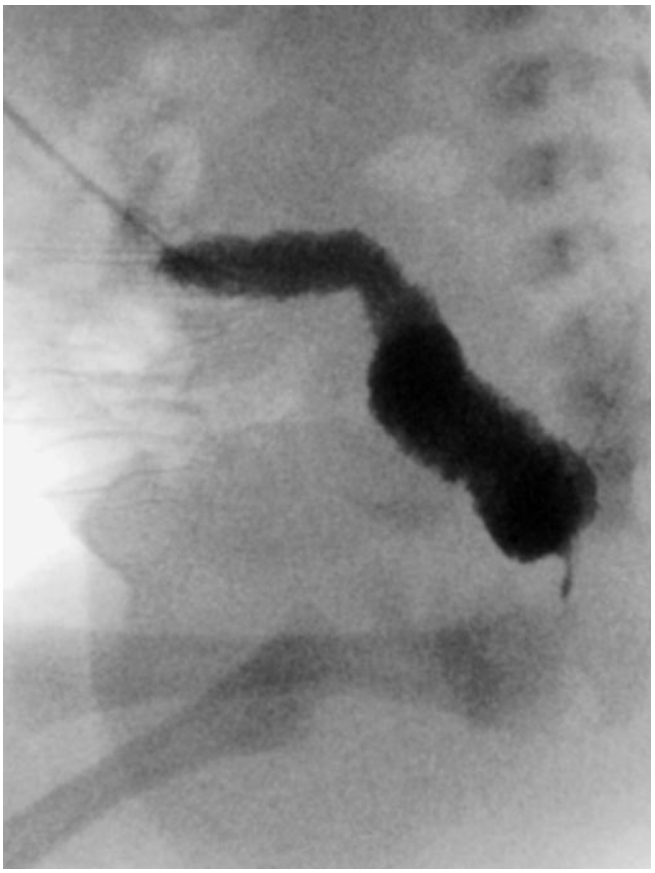

FIGURE 74.1-27 "Loopogram." Contrast has been introduced via the defunctioning stoma to demonstrate the level of the anorectal atresia.

cuits dipped in barium or yogurt mixed with barium for infants who cannot yet manage solids. Appropriate commercial thickening agents are available. The result of these studies can be of vital importance in the future management of the child, for example, determining the safest position for feeding, which will help prevent long-term aspiration and malnutrition.[38,39]

Upper Gastrointestinal Studies. Infants whose last feed has been withheld are usually quite willing to drink unflavored barium or (amazingly!) Omnipaque from a bottle. For older children, barium can be flavored to make a relatively pleasant milkshake-style drink; Omnipaque is effectively disguised in orange juice. To assess swallowing of solids, marshmallows can be soaked in barium.

Contrast swallows have historically been performed mainly for the detection of gastroesophageal reflux. However, the sensitivity of radiologic studies for reflux has been reported to be as low as 52%[40]; therefore, a negative study is of little diagnostic value. Barium swallows continue to be useful in the diagnosis of structural causes of dysphagia, such as vascular rings, strictures following caustic ingestion, long-standing reflux disease, or postsurgical repair of tracheoesophageal fistulae, as well as functional disorders such as achalasia and globus hystericus. Views should always be taken in the supine oblique position to fully distend the lower esophagus. A careful history is important: establish exactly what provokes the child's symptoms and try to reproduce these conditions as closely as possible during the study.

In children with feeding gastrostomies, the parent or caregiver should be asked to replicate the child's usual feeding process, using contrast mixed with food. Again, the conditions that produce symptoms should be reproduced; for example, if the patient habitually vomits after 200 mL of food have been given, a study using only a small volume of contrast will not be helpful.

The radiologist should take the opportunity during all upper gastrointestinal studies in children to observe the passage of contrast around the duodenal loop to the duodenojejunal flexure. An image of the correctly sited flexure (to the left of the spinal column, at the level of L1) will rule out 98% of cases of malrotation (Figure 74.1-28). In some centers, a contrast enema (rather than an upper gastrointestinal study) is the investigation of choice to rule out malrotation; however, in 16% of children with malrotation, the cecum is normally sited in the right iliac fossa. Furthermore, the cecum may be displaced from the right iliac fossa in 15% of normal children.[6] In cases of strong clinical suspicion of malrotation, where the duodenal position appears normal or equivocal, it is logical to proceed to an enema study to evaluate the cecal position.

All radiologic studies for malrotation are limited by the fact that only the bowel is imaged, not the mesenteric fixation, which is what actually determines the malrotation. It is also well recognized that the normal duodenum may be markedly mobile, especially in neonates, and this may lead to further confusion.[41]

Small Bowel Follow-through. Most would agree that the initial investigation of the small bowel should be by follow-through, with enteroclysis (see below) being reserved for selected difficult cases in which a follow-through study has failed to make a diagnosis.[42] In the follow-through technique, the child drinks about 500 mL of dilute barium after a 6-hour fast. An immediate supine film is taken, followed by prone films (the prone position separates bowel loops) at intervals until the ileocecal junction has been visualized. The films should be carefully examined as they are done, and fluoroscopy should be performed to clarify any doubtful areas, for example, to confirm fixation or separation of bowel loops. The terminal ileum should always be screened to ensure that underfilling is not misinterpreted as a stricture. If the colon is loaded with feces, the passage of barium will be slowed; this may often be overcome by giving the child a meal once contrast has reached the ileum.

Enteroclysis. Enteroclysis (or small bowel enema) involves introduction of contrast directly into the proximal jejunum via a long, wide-bore oro- or nasojejunal tube. A bolus of dilute barium is used, followed by water or methylcellulose for a double-contrast effect, and is monitored with fluoroscopy throughout the small bowel. The technique is more sensitive than follow-through studies, particularly for the demonstration of polyps, but is often poorly tolerated by children. Intubation may be difficult, and if the tube is too proximal, barium will reflux into the stomach and result in vomiting.

Peroral Pneumocolon. This technique is supplementary to a small bowel follow-through study. Once barium has reached the terminal ileum, air is insufflated per rectum to distend the colon and terminal ileum. This gives good mucosal detail of the terminal ileum but is relatively more invasive.

Characteristic Appearances of Selected Conditions on Contrast Studies. The reader is referred to the individual chapters for a more complete discussion of these conditions.

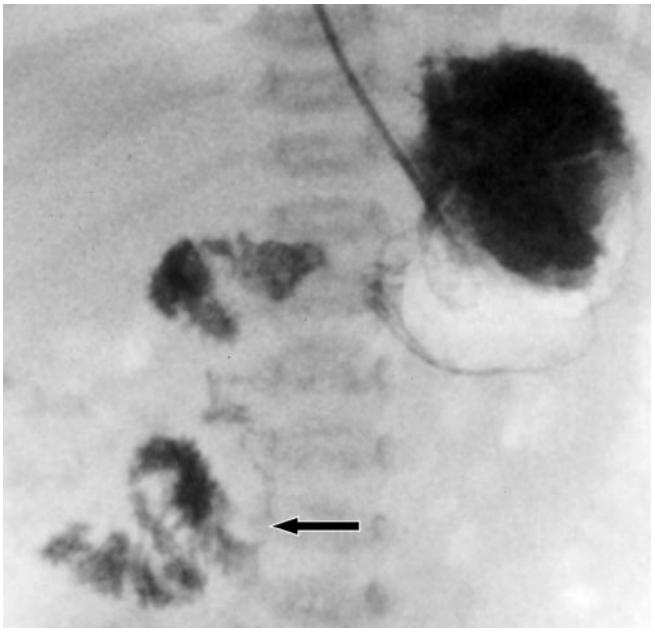

FIGURE 74.1-28 Malrotation: upper gastrointestinal contrast study showing abnormal position of the duodenojejunal flexure, which lies to the right of the midline (*arrow*).

Vascular Rings. The lateral view of the esophagus during a contrast swallow can predict the type of vascular anomaly in most cases. The plain chest radiograph will provide additional information about the aortic arch and tracheal position. In modern practice, the definitive diagnosis is usually made by MRI.

There are four patterns of esophageal and/or tracheal compression on the contrast swallow; these correspond to four major vascular anomalies. A double aortic arch causes posterior esophageal compression together with anterior tracheal compression. (A similar pattern occurs with a right-sided arch in combination with a left ductus arteriosus and aberrant left subclavian artery.) Compression of the trachea by a prominent innominate artery causes an anterior impression on the trachea but a normal esophagus. Aberrant right subclavian artery (the most common anomaly of the aortic arch) results in a posterior impression on the esophagus with a normal trachea. Finally, a posterior impression on the trachea, coupled with anterior compression of the esophagus, is caused by an aberrant left pulmonary artery, arising from the right pulmonary artery and passing between the trachea and esophagus.[14,43]

Achalasia. Videofluoroscopy in achalasia shows abnormal motility and failure of relaxation of the lower esophageal sphincter. The esophagus becomes dilated in long-standing cases and may be filled with food debris. The narrowed lower esophageal sphincter has a characteristic "rat-tail" appearance.[14]

Hiatal Hernia and Gastroesophageal Reflux. If the gastroesophageal junction lies above the diaphragm, a hiatal hernia is present. The significance of hiatal hernia in reflux disease has yet to be established.[44] A small amount of reflux is a normal finding in children. A clinical history of repeated vomiting, failure to thrive, and recurrent chest infections (owing to aspiration) are clues that significant reflux is present. If the gastroesophageal junction is widened and the esophagus appears "baggy," significant reflux is likely to be present. Any contrast refluxing above the gastroesophageal junction should be noted, as well as the level to which it ascends and the frequency of episodes during the study.[2] Failure to demonstrate reflux on videofluoroscopy does not exclude the diagnosis (see under "Technique" above). In chronic reflux disease, an esophageal stricture may develop, which is usually smooth and tapering in appearance.

Duodenal Obstruction. Complete duodenal atresia is usually diagnosed on the plain film, which typically shows the "double bubble" sign. Partial duodenal obstruction may be due to congenital webs, annular pancreas, Ladd bands, or a preduodenal portal vein. A web may appear as a linear filling defect within the duodenum, and the dilated, barium-filled distal duodenum with a convex end has been described as a "wind sock." Other causes of partial duodenal obstruction are difficult to distinguish from webs on barium studies (Figures 74.1-29 and 74.1-30). Cross-sectional imaging will be helpful in demonstrating pancreatic and portal vein anomalies.[14] However, pragmatically, this is not done in neonates because these children require surgery.

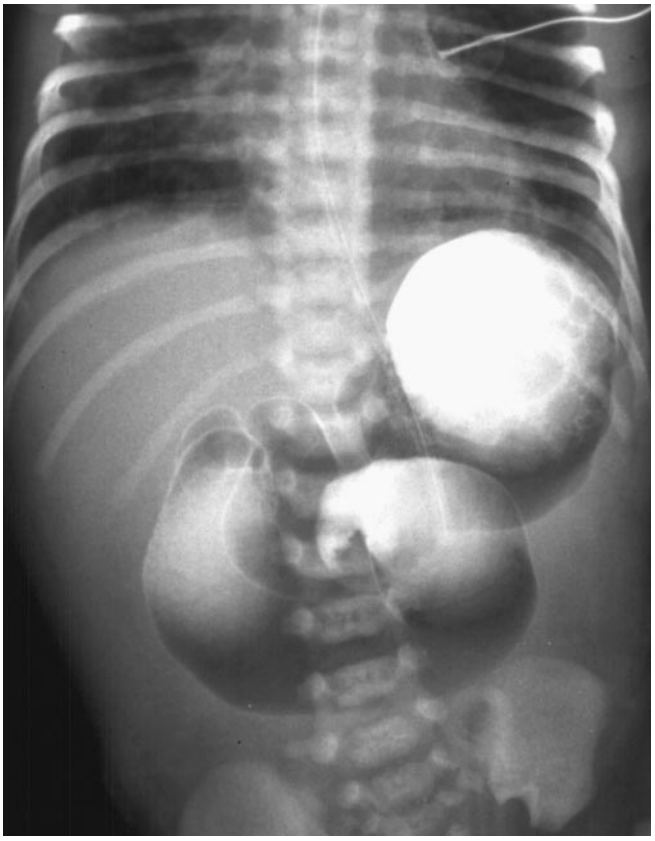

FIGURE 74.1-29 Contrast study demonstrating duodenal atresia.

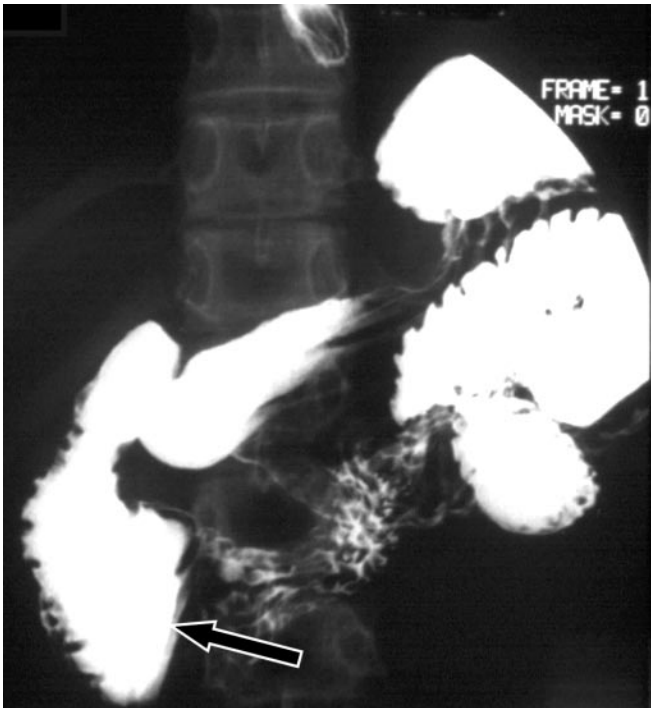

FIGURE 74.1-30 Partial duodenal obstruction, owing in this case to compression by the superior mesenteric artery. There is dilatation of the duodenum proximal to the obstruction (*arrow*).

Malrotation and Volvulus. As discussed under "Technique" above, contrast studies are not infallible in the diagnosis of malrotation. The major life-threatening complication of malrotation is volvulus of the small bowel around the superior mesenteric artery. If this is present, the contrast study may demonstrate a corkscrew or "twisted ribbon" appearance of the duodenum and jejunum, with thickened mucosal folds owing to edema of the bowel wall. Classically, the contrast terminates in a "bird's beak" at the point of obstruction; however, the obstruction may be rounded and appear to be similar to duodenal atresia.[14]

Malabsorption. Malabsorption attributable to any cause characteristically results in nonspecific changes in the small bowel contrast study: fragmentation of the barium column, flocculation of barium, and mild dilatation of the small bowel. Celiac disease is classically described as causing a featureless, smooth jejunum with an increase in mucosal folds in the ileum (so-called "jejunization" of the ileum) (Figure 74.1-31). Transient intussusceptions may be seen during the small bowel study.[2,45] There is an increased risk of small bowel lymphoma, so any mass should be treated with suspicion.

Crohn Disease. Crohn disease may affect any part of the gastrointestinal tract. In the small bowel, the terminal ileum is the most commonly affected region; however, up to 20% of children with small bowel Crohn disease will have a normal terminal ileum on imaging.[14,46] More typically, there is a segment of narrowed, rigid bowel with mucosal nodularity or "cobblestoning" and deep ulcers (Figure 74.1-32). If the stricture is tight, the proximal portion of bowel may be dilated. Several sections of bowel may be affected, with the intervening areas appearing normal ("skip lesions"). Fistulae may occur between adjacent loops of small bowel or between small bowel and colon or even stomach. In long-standing disease, eccentric scarring occurs, resulting in "pseudosacculations" on the antimesenteric border of the bowel. CT and ultrasonography have an important supplementary role in delineating thickened bowel loops and inflammatory masses.[47] In difficult cases, an isotope-labeled white cell scan may be required to identify an intra-abdominal site of inflammation.[2]

Crohn disease of the colon can be demonstrated on a double-contrast barium enema; however, these are rarely performed in modern pediatric practice because endoscopy is the mainstay of diagnosis for inflammatory diseases of the colon. The colon is usually asymmetrically involved (in contrast to ulcerative colitis), with predominantly the right colon being affected and less commonly the rectum.[14] In early Crohn colitis, there may be discrete aphthae (ulcers with a smooth raised edge); these later coalesce to form

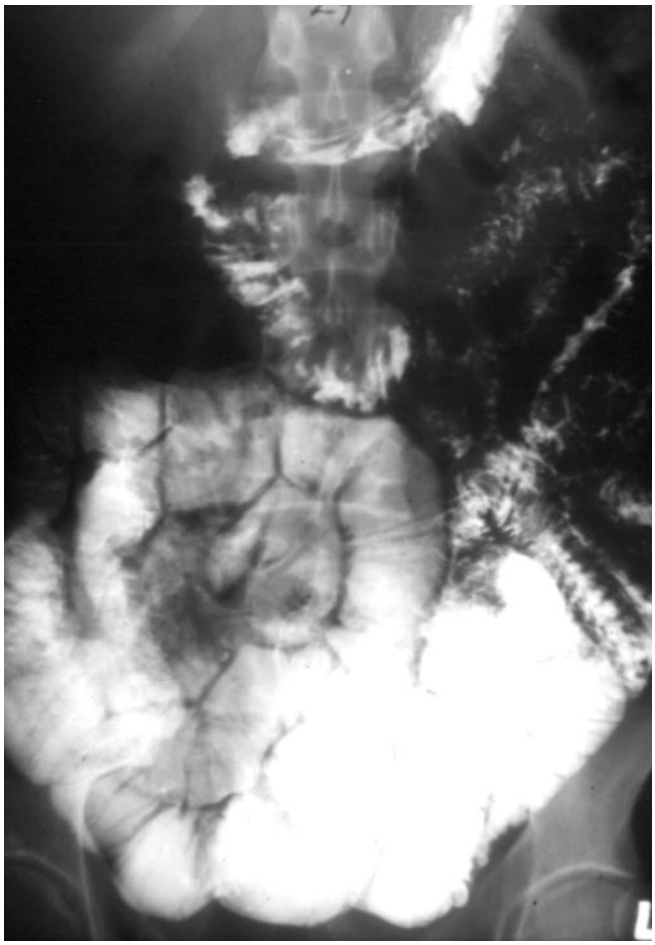

FIGURE 74.1-31 Small bowel meal in celiac disease. The jejunum is mildly dilated, smooth, and relatively featureless.

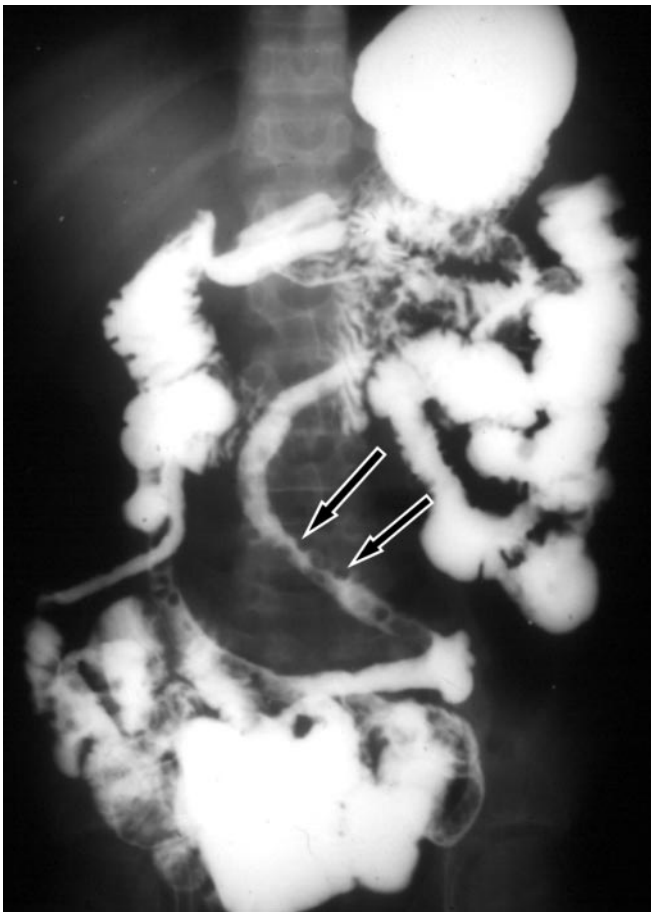

FIGURE 74.1-32 Crohn disease. There are several long strictures of the small bowel with "cobblestoning" and "rose-thorn" ulcers (*arrows*).

linear ulceration and may penetrate the bowel wall to form sinuses or fistulae. As in the small bowel, skip lesions are characteristic, and pseudosacculations may occur.

Intussusception. Intussusception may be ileoileal, ileocolic, or ileoileocolic (in which an ileoileal intussusception then invaginates into the colon). In all of these cases, the intussusceptum will be seen as a filling defect indenting the column of contrast or air (Figure 74.1-33). Following successful reduction, a residual filling defect is often seen in the cecum: this is the edematous ileocecal valve.[2,14]

Meconium Ileus. A contrast enema in meconium ileus will show a microcolon, and contrast refluxing into the terminal ileum will outline inspissated meconium (Figure 74.1-34). The colon is also of very small caliber in distal ileal atresia but tends to be of near-normal caliber in proximal ileal or jejunal atresia owing to a larger quantity of small intestinal secretions reaching the colon in fetal life.[14,48] However, in practice, the colon is seldom examined radiologically.

Meconium Plug. In meconium plug syndrome, there is functional immaturity of the colon. A contrast enema will demonstrate the meconium in a normal-caliber colon. A caliber change at or proximal to the splenic flexure, with the descending colon being narrowed, is known as small left colon syndrome but is a variant of meconium plug syndrome. A therapeutic enema with water-soluble contrast medium will aid the passage of meconium.[14]

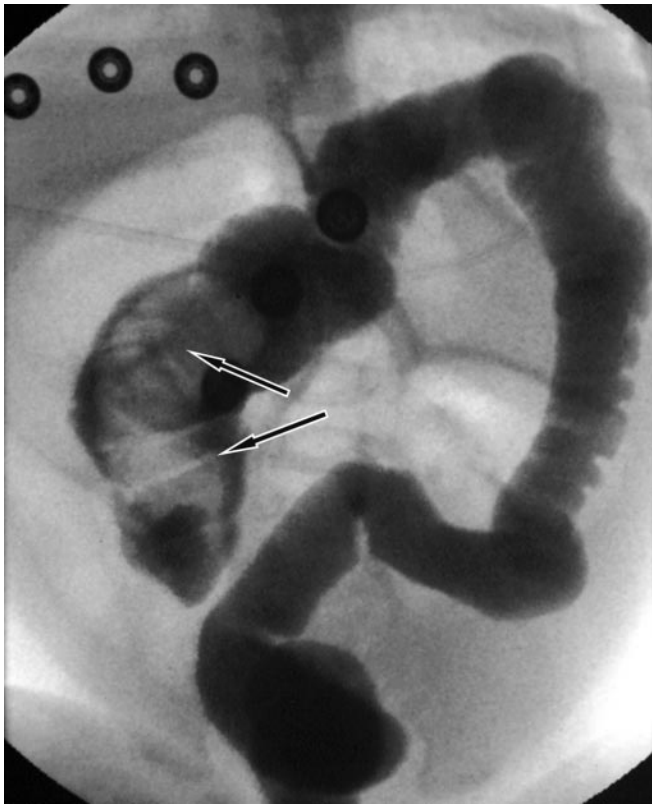

FIGURE 74.1-33 Contrast enema in intussusception. The intussusceptum shows as a filling defect (*arrows*).

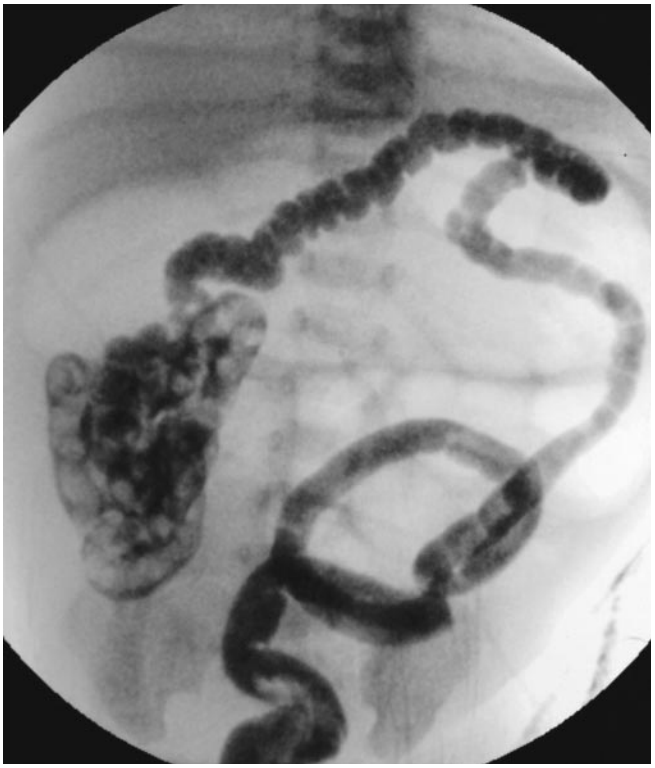

FIGURE 74.1-34 Contrast enema in meconium ileus. Note meconium within the terminal ileum.

Hirschsprung Disease. The characteristic indicator of Hirschsprung disease is a transitional zone between the proximal, dilated (normal) bowel and the distal, small-caliber aganglionic segment (Figure 74.1-35). Irregular

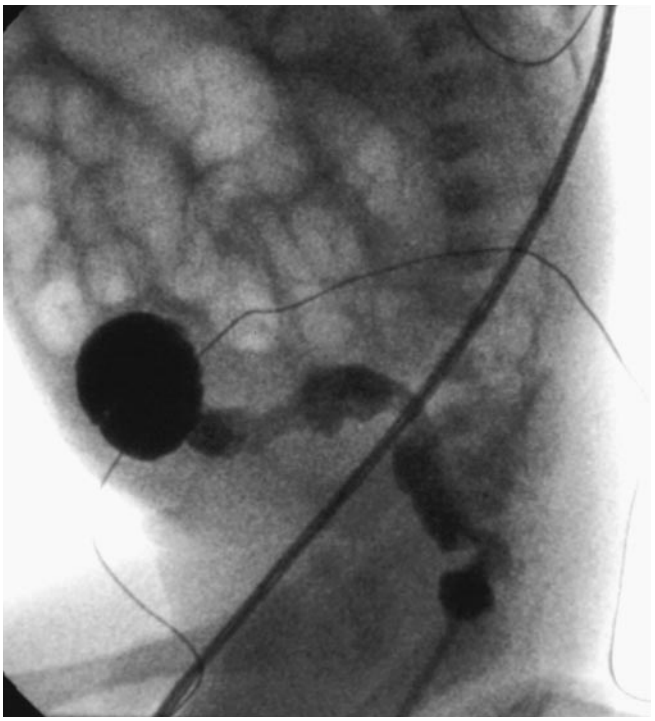

FIGURE 74.1-35 Contrast enema in Hirschsprung disease showing sharp caliber change at the transitional zone.

"sawtooth" contractions in the aganglionic segment may be seen.[14] A rectum that is narrower in caliber than the sigmoid colon is also highly suggestive of the diagnosis, although the value of this sign is debatable.[49] In rare cases of total colonic aganglionosis, there will be no transitional zone.

Ulcerative Colitis. As with Crohn colitis, endoscopy has largely replaced double-contrast barium enema in the diagnosis of ulcerative colitis in children. Barium studies show diffusely granular mucosa in early colitis. Unlike Crohn disease, involvement of the colon is continuous, usually extending proximally from the rectum. As the disease progresses, there is haustral thickening owing to edema, and deepening ulcers result in islands of normal mucosa appearing as "pseudopolyps." In long-standing cases, the colon may appear shortened and tubular—the "lead pipe" colon. Reflux ileitis may result in a patulous terminal ileum.[50]

Polyps. Polyps appear as sessile or pedunculated filling defects on barium studies. The appearances of the polyps in the various polyposis syndromes are nonspecific, but their distribution in the gastrointestinal tract, together with the associated clinical features, may suggest the diagnosis.[51] However, fiberoptic endoscopy is now the preferred diagnostic technique.

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2. Cross-Sectional Imaging: Ultrasonography, Computed Tomography, Magnetic Resonance Imaging

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The subspecialty of pediatric gastroenterology began almost 30 years ago, coinciding with revolutionary technological advances being made in the field of diagnostic imaging. Concurrent with the introduction of ultrasonography (US) in the early 1970s, gastrointestinal endoscopy was introduced. Rapidly following the introduction of US, computed tomography (CT) and then magnetic resonance imaging (MRI) were developed. These advances in imaging, which allow complex images to be stored, manipulated, and retrieved in microseconds, have now become routine radiology procedures rather than what might have been envisioned in science fiction stories 30 years ago. The detail of anatomic information gathered by these radiology examinations often approaches what is depicted at direct surgical exploration or pathology. Pediatric-sized endoscopes have made direct visualization and intervention possible even in very small children. It is now implausible to envision practicing the subspecialty of pediatric gastroenterology without these remarkable complementary technologies.

To successfully practice pediatric gastroenterology requires more than casual familiarity with cross-sectional imaging modalities. Although the clinical gastroenterologist is usually not required to perform or independently interpret these studies, an understanding of the indications, strengths, and limitations of particular investigative studies is extremely useful. In a rapidly evolving field, it is sometimes difficult to know the true benefit of a new technique until years later, when it has been fully applied and compared with other techniques. Especially important is to know when to use these often expensive technologies, which may involve exposure to ionizing radiation, the injection of potentially nephrotoxic contrast agents, and the risks of conscious sedation or general anesthesia. In this regard, there is no substitute for a good history and physical examination, and there is probably no more important a colleague than a trusted pediatric radiologist to help direct the imaging evaluation.

ULTRASONOGRAPHY

In children, US is often the initial choice of radiologic investigation because it does not require ionizing radiation, is not painful, requires little or no preparation, and is a relatively inexpensive examination. In children, especially younger infants, it is ideal because the general lack of internal body fat facilitates imaging. In certain settings, US is superior to other modalities, especially for delineation of cystic lesions. Generally, no sedation is needed, and even with young infants and children, little or no restraint is required.[1]

Technically, US uses sound above the audible range of frequencies of 20 kHz or 20,000 cycles per second. In practice, diagnostic US uses a much higher frequency, from 1 to 20 MHz (a million cycles per second). These sound waves are emitted from a transducer that contains crystals with piezoelectric properties. When the crystals are subjected to an electric current, they emit sound waves at a particular frequency depending on the size of the crystals and the current. Once the sound is emitted, it is directed through the body and is either reflected, refracted, scattered, or absorbed, depending on the properties of the tissues that are encountered, producing the equivalent of a reflected echo. This reflected echo, when returned to the transducer crystal, causes a vibration that generates an interpretable electric pulse. The returning sound beam will have a different speed and intensity from the original, which is referred to as attenuation, which depends on the properties of the encountered tissues.[2] "Real-time" images can be generated if rapid sound emissions are done at a rate of at least 15 image frames per second.[3]

US can also be used to determine blood flow moving through vessels and structures, based on the "Doppler shift principle." Sound reflecting off a moving target will change in frequency, proportional to the speed of the moving target. The returning echo can be detected as audible sound or as a traceable wave pattern depending on velocity. The use of color allows determination of direction of flow, conventionally with red indicating flow toward the transducer and blue indicating flow away from the transducer. Some investigators are using contrast agents such as stabilized intravascular microbubbles to accentuate detection of blood flow, but these techniques are not widely used in clinical practice.[4]

US has limitations that should be addressed and understood. US cannot penetrate air-containing structures well, as in bowel or lungs. Structures underlying bowel gas may

be completely obscured. US has little ability to penetrate bone, other calcified structures, and metal, such as sutures or plates.[5] The images are obtained in small sectors or wedges of information, and depth of penetration is limited, especially in older and larger patients. The spatial relationships and resolution are not as great as in CT or MRI, which sometimes leads nonradiologists to not fully understand or trust and therefore not fully appreciate the anatomic information offered by US.[3]

COMPUTED TOMOGRAPHY

Similar to routine radiographic studies, CT uses electromagnetic radiation to obtain images. A well-collimated beam is passed through the subject and, depending on the characteristics of the tissues and spaces, the x-ray will be subject to differing attenuation, making it possible to reconstruct an image via complex computer programs to create a cross-sectional image. Sophisticated hardware and software advances have enabled finely detailed images to be displayed by a grayscale, with a spectrum from air, seen as black, to water, seen as gray, to bone, seen as white. With use of the newer spiral and helical technology, the image is generated quickly and accurately as the table moves while the scan is being done. Now with the advent of multislice CT, the images can be acquired so quickly that CT "fluoroscopy" to assist biopsies and catheter insertions are possible. Vascular studies can be obtained from a single rapid injection in a matter of seconds. Traditionally, CT images are obtained in the axial plane, but images can be reconstructed in any plane desired at modern workstations after processing.[6]

Before ordering a CT scan, the clinician should be aware of several issues related to the risks versus the benefits of the modality. CT does involve exposure to radiation, raising important safety concerns that should be carefully considered, especially in children. Recent evidence postulating an increased cancer risk in adulthood in children exposed to diagnostic CT[7] has led to a complete revamping of the CT protocols used in pediatric studies to minimize dosage while still delivering high-quality images. Newer multislice CT scanners are now capable of better imaging with less radiation exposure than older machines and are preferable. Motion degrades the images, usually making some form of sedation or even anesthesia necessary in younger children. Intravenous injection of iodinated contrast is useful in most body cases, requiring adequate venous access. Although relatively safe, these agents can be nephrotoxic, and occasional allergic and anaphylactoid reactions are reported. Adequate ingestion of oral contrast, which is difficult to obtain in children who require sedation, increases the accuracy of the study. Young children and sedated subjects cannot maintain rectal contrast. The lack of internal body fat in children often makes interpretation of CT of the abdomen and pelvis challenging. CT is a more expensive test than US. The greatest advantage of CT is the high-resolution images it produces, including the ability to evaluate air-containing structures such as bowel that are relatively deep to the surface and may not be readily palpable or accessible to US.[8]

MAGNETIC RESONANCE IMAGING

This technique is based on the interaction between atomic nuclei of various tissues and radio waves that are directed through the body in a strong magnetic field. How the mobile nuclei are modified is labeled magnetic relaxation times (T_1 and T_2), which can be measured as a change in the electromagnetic field. The hydrogen atom is used for imaging purposes because it is the most abundant nucleus with a strong magnetic signal. A superconductor-type magnet generates the electromagnetic field by use of large coils reduced to near absolute zero to offer no resistance. In the presence of the strong magnetic field, the randomly oriented protons are aligned in the longitudinal axis. When subjected to a radio frequency in a perpendicular transverse axis, the protons are disrupted in their alignment. Once the pulse is discontinued, they will precess until they resume their alignment in the magnetized field, which can be detected as a voltage.[9] The rate of return to the magnetized state is the T_1 relaxation time and the rate of decay of the transverse signal is the T_2 parameter.[10]

Variations can be added to the magnetic field or the radio waves to highlight various features of the scan. The detail afforded by MRI allows precise characterization in different planes. With the injection of materials with distinct spectroscopic qualities, detailed images can be obtained. The most commonly used agent, gadolinium chelate, is useful in evaluation of solid organs, and enhancement characteristics often aid in differential diagnosis. With rapid injection of gadolinium, blood flow through various vessels can be highlighted, producing an angiography-like study.[11]

No ionizing radiation is involved in MRI, and there are no known biologic side effects from this imaging modality, making it excellent for pediatric applications. Images are, however, very affected by movement, often requiring conscious sedation or anesthesia, and, at times, extended breath-holding is needed to optimize certain acquisitions, something many young children cannot do. Even persistalsis from bowel can produce artifacts. Recent advances have reduced the acquisition times for MRI substantially, making MRI a more practical modality in pediatric settings.[12] Finally, MRI examinations are relatively expensive and sometimes less readily available than CT.

NORMAL APPEARANCE AND ANATOMY AS SEEN WITH CROSS-SECTIONAL IMAGING

Some baseline findings need to be appreciated before looking for abnormalities on any of these imaging modalities. Each modality has some advantages when compared with the other modalities, and they are very often complementary.

LIVER

Normal hepatic parenchyma appears homogeneous on US, with relative increased echogenicity compared with the renal cortex and echogenicity similar to that of the spleen. The vessels of the liver are determined by their point of origin and course.[13] Color Doppler US easily separates

vessels, which have slightly echogenic walls, from the biliary tree. On nonenhanced CT, normal liver parenchyma is slightly higher in attenuation than the spleen, with contrasting hypodense vessels. With contrast, the parenchyma enhances uniformly, with vessels that become hyperdense compared with the parenchyma. On MRI, the hepatic parenchyma varies with imaging sequences, with T_1-weighted images showing a higher signal intensity than the spleen and the reverse being true with T_2-weighted images.[14]

On previous classification schemata, the liver was broken down by anatomic lobar divisions; however, a more useful subdivision for the surgeon is based on the vessels that supply the various segments.[15] The right and left hepatic ducts are seen on all cross-sectional imaging studies, anterior to the portal vein bifurcation. The extrahepatic ducts should not measure more than 4 to 7 mm in diameter. In fact, the range within the pediatric population is substantially smaller, with normal ducts usually less than 4 mm.[16] The common bile duct is important to identify and can be seen at the level of the pancreas on MRI or CT. With US or magnetic resonance cholangiopancreatography (MRCP), the entire course of the common duct can be determined, although it is technically often difficult with US owing to overlying bowel gas. The gallbladder is generally pear shaped at the inferior border of the liver and in its normal state is thin walled and enhances after intravenous contrast on CT. On MRI, bile has the same signal as water but may have a higher intensity if it is concentrated in the gallbladder.[17] On heavily T_2-weighted images, the entire biliary tree can be imaged without the use of any contrast agents, producing MRCP of striking detail (Figure 74.2-1).

Spleen

The normal spleen on US shows a homogeneous echogenicity, similar to that of the liver, and lies adjacent to the left hemidiaphragm and stomach. The hilum is generally directed medially, and both the splenic vein and artery are easily seen on US. CT will also show homogeneous density with attenuation equal to or slightly lower than that of the liver. Rapid injection of contrast will show heteregeneous uptake initially as a manifestation of variable flow patterns.[18] On MRI, T_1-weighted images show signals of lower intensity than the liver, and T_2-weighted images will show brighter intensity. Intravenous gadolinium causes a similar enhancement pattern of the spleen, as seen on CT scan.

Pancreas

In children, the entire pancreas is usually not well defined on US because it lies obliquely or transversely in the retroperitoneum and is often obscured in parts by gas in the bowel. The normal pancreas is of uniform, cross-hatched echo-texture and of an echogenicity similar to that of the liver, but it can also be normal and hyperechoic or hypoechoic, especially in children. The normal pancreatic duct, if visualized, is a 2 mm or less tubular structure that runs through the pancreatic body and tail. The common bile duct, as well as either one or two gastroduodenal arteries[19] within the head of the pancreas, can be seen. On CT, the pancreas is better defined, with borders well highlighted when the adjacent bowel is opacified with oral contrast and blood vessels are highlighted by intravenous contrast, especially if there is retroperitoneal fat. The splenic vein and superior mesenteric vein can be seen posterior to the body. The lateral aspect of the head is nestled by the second and third portions of the duodenum, with the third and fourth portions of the duodenum extending inferior to the pancreas. The attenuation of the pancreas on CT is less than that of the liver.[20] On MRI, the pancreas can also be well displayed; however, thin-section CT is usually easier to obtain and requires shorter study times to produce high-resolution images.

Gastrointestinal Tract

The gastrointestinal tract is a hollow tube that is either fluid or air filled, and the gas pattern on plain film often dictates the imaging plan. On US, the mucosa of the bowel will appear as an echogenic interface with the echolucent muscularis. Bowel wall thickening can be appreciated, especially if the bowel is fluid filled. Although, more traditionally, the bowel is studied by upper gastrointestinal (UGI) and small bowel follow-through (SBFT) or by barium enema (BE), the bowel can be well studied by CT after the administration of oral and sometimes intravenous contrast. On CT, bowel wall thickening may be appreciated, and inflamed mucosa will enhance after the administration of intravenous contrast. The lumen can be delineated by oral contrast, but mucosal details are better

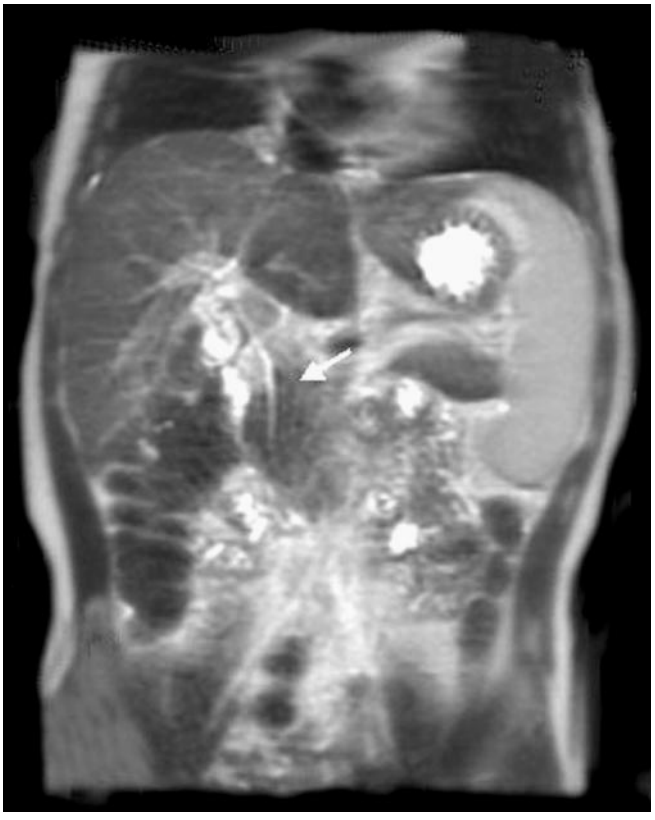

FIGURE 74.2-1 Normal coronal magnetic resonance cholangiopancreatogram reveals a normal extrahepatic bile duct (*arrow*).

defined by UGI, SBFT, or BE. Inflammatory changes in the mesentery and associated lymphadenopathy are well displayed. MRI is less useful at present for evaluating the bowel, but newer MRI techniques are being investigated to help better define the bowel.

Congenital Hepatobiliary Anomalies

Anatomic anomalies of the hepatic lobar structure do exist but generally are not of great clinical significance and can be evaluated by CT, US, or MRI. Atresia of a specific lobe of the liver is usually associated with hypertrophy of the remaining lobes and can be an isolated finding or may be associated with other anomalies.[21] Venous anomalies most frequently involve variations in the branching of the portal vein, with the only real clinical significance reserved for the technical aspects that arise during liver transplant. Color Doppler US is probably the best modality for demonstrating most of these vascular variations.[22] Congenital absence of the portal vein, which can also be documented by color Doppler US, is rare and is associated with a multitude of clinical associations, including cardiac defects, extrahepatic biliary atresia, and polysplenia.[23]

Also included under congenital vascular anomalies are vascular malformations, which consist mostly of hemangiomas and, less frequently, arteriovenous malformations. Both can present with high-output heart failure in the newborn period. Infantile hemangiotheliomas and cavernous hemangiomas are both mesenchymal tumors that demonstrate a considerable amount of variability of endothelial proliferation—hence their potential for both growth and involution.[24] Hemangioendotheliomas are the more common vascular liver tumors in infants, almost always presenting before 6 months of age with hepatomegaly and sometimes heart failure, massive bleeding, and uncontrollable coagulopathy. Hemangioendotheliomas can occur as solitary lesions or as multiple hepatic lesions, and probably half are associated with cutaneous hemangiomas.[25–27] Hemangioendotheliomas (Figure 74.2-2) on US are predominantly hypoechoic, whereas cavernous hemangiomas are hyperechoic, with a very high-velocity flow that can be demonstrated by color flow Doppler US.[28] On unenhanced CT scan, hemangioendotheliomas will show calcification in 40%. After the intravenous injection of contrast, both hemangioendotheliomas and hemangiomas classically show peripheral enhancement and delayed enhancement of the central areas (Figure 74.2-3).[29] Infarction can create considerable variations in enhancement patterns. Small lesions demonstrate homogeneous low signal intensity on T_1-weighted MRI, but larger lesions can be more heterogeneous (Figure 74.2-4). A T_2-weighted MRI will show high signal intensity of small lesions and more heterogeneity of large lesions (Figures 74.2-5 and 74.2-6).[30]

Anomalies of gallbladder position and number are more common than total agenesis, hypoplasia, duplication, and left-sided position. Although US is still the modality of choice to screen for biliary anomalies,[31] radionuclide scans and MRI can also be useful.

Choledochal cysts can be diagnosed at any age and are classified by location and shape. The most common are fusiform dilatation of the common duct (type I), but other variants, such as saccular diverticulum, choledochoceles that extend into the wall of the duodenum, and multiple cystic dilatations, as in Caroli disease (Figure 74.2-7), occur.[32] US is the study of choice for suspected cases (Figures 74.2-8 and 74.2-9), with MRCP used for confirmation and pre-preoperative definition, replacing percutaneous transhepatic cholangiography and endoscopic retrograde cholangiography (Figure 74.2-10).[33] The presentation of choledochal cysts during the neonatal period can be confused with biliary atresia, in which a cystic remnant of the atretic biliary system can remain. Choledochal cysts in later childhood can present with significant obstruction, and other causes of biliary dilatation, such as stone, stricture, or tumor, need to be excluded.[34]

US is often the preliminary study done when biliary

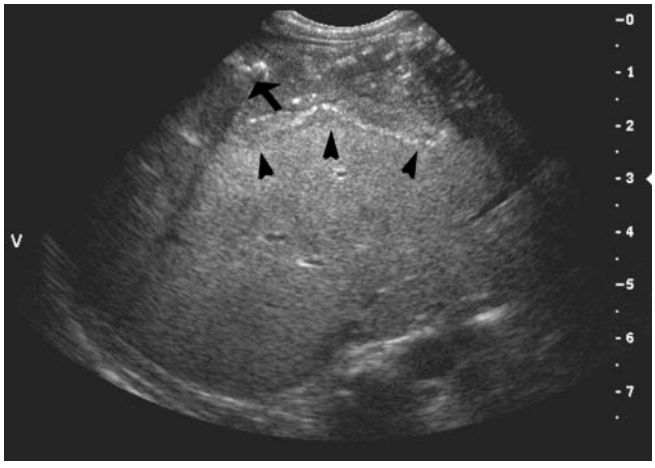

FIGURE 74.2-2 Transverse sonogram of the liver of a newborn infant with a hemangioendothelioma reveals a well-defined hypoechogenic mass with echogenic borders (*arrowheads*). Several echogenic foci with associated shadowing within the mass represent areas of calcification (*arrow*).

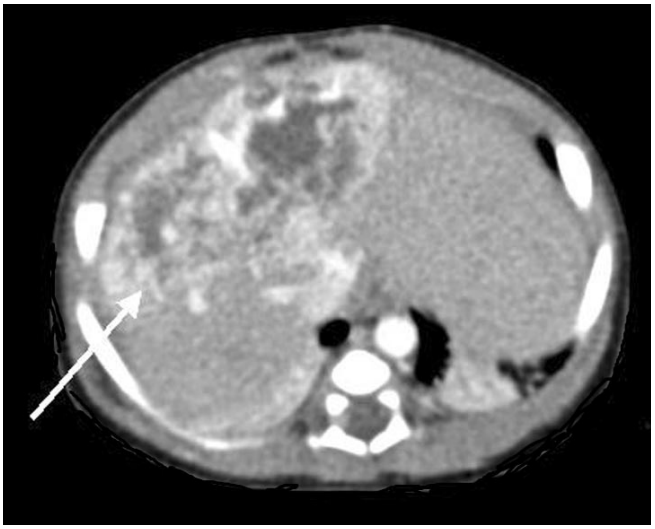

FIGURE 74.2-3 Contrast-enhanced axial computed tomographic scan of an infant with hemangioendothelioma reveals a large mass in the liver with peripheral enhancement (*arrow*).

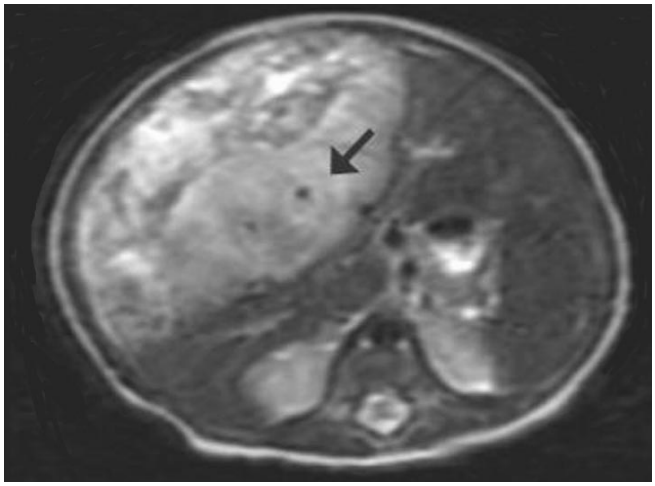

FIGURE 74.2-4 T_2-weighted magnetic resonance image of the infant in Figure 74.2-2 reveals the large, well-defined mass to have variable signal characteristics. Calcification appears as a signal void (*arrow*).

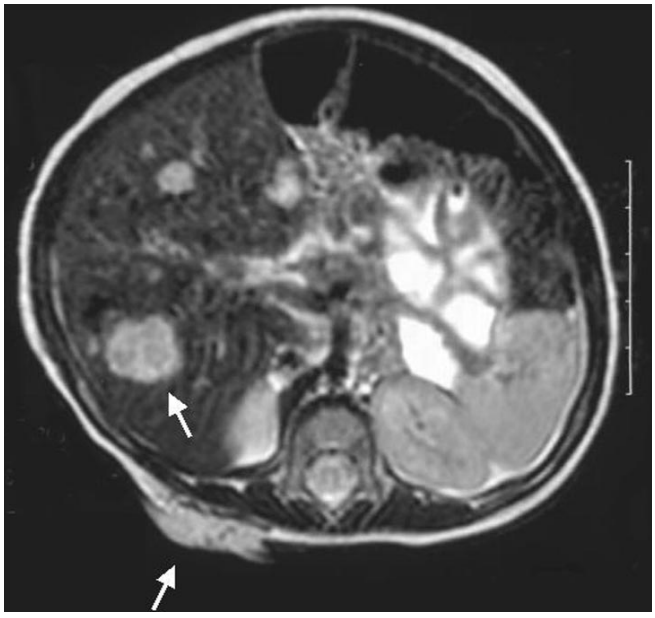

FIGURE 74.2-6 Axial T_2-weighted magnetic resonance image reveals that the lesions are of bright signal intensity (*arrows*).

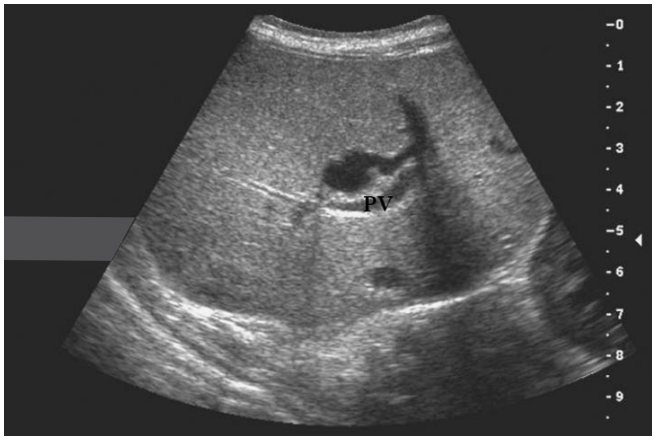

FIGURE 74.2-8 Transverse sonogram of the liver in an infant with a choledochal cyst reveals significant intrahepatic biliary dilatation, seen anterior to the portal vein (PV).

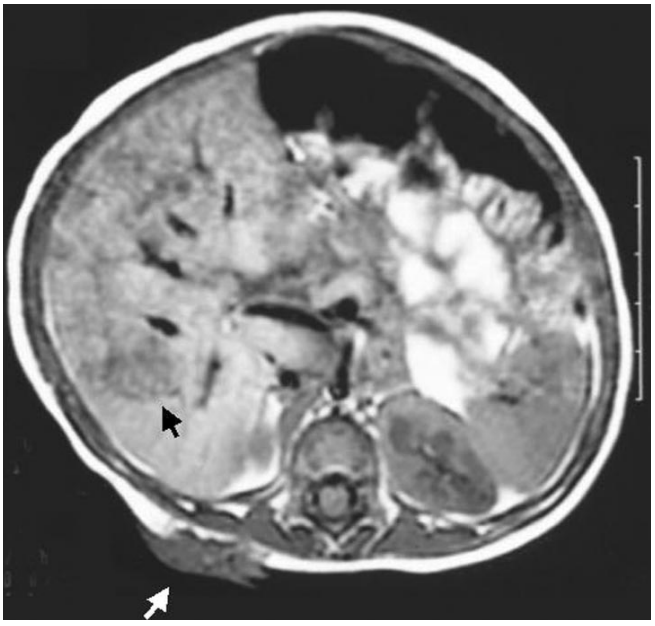

FIGURE 74.2-5 Axial T_1-weighted image of the liver of an infant with disseminated hemangiomas reveals multiple soft tissue masses in the liver (one indicated by a *black arrow*) and a soft tissue mass that arises from the posterior back (*white arrow*).

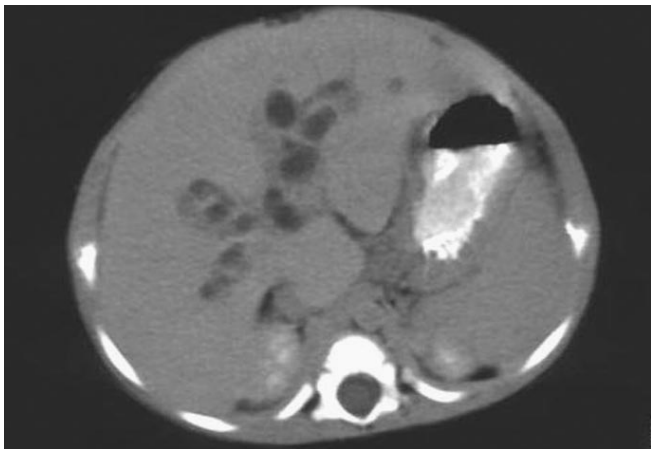

FIGURE 74.2-7 Axial contrast-enhanced computed tomographic scan of the abdomen shows multiple cystic branching structures within the liver in this patient with Caroli disease.

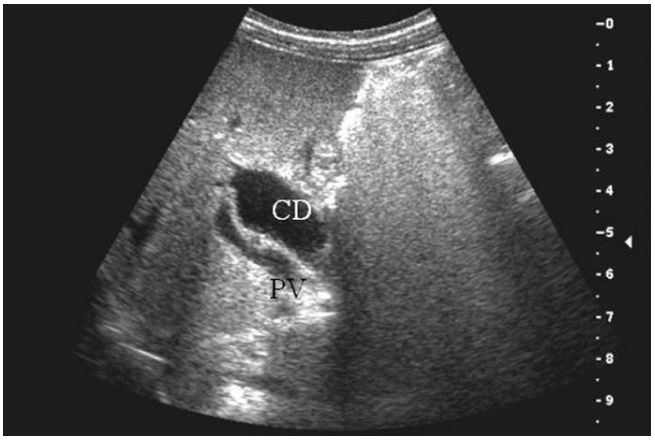

FIGURE 74.2-9 Sagittal sonogram of an infant with cholestasis reveals enlargement of the common duct (CD), consistent with a choledochal abnormality. PV = portal vein.

atresia is suspected; however, the absence of a normal-size gallbladder and failure to delineate the extrahepatic duct on US is suggestive but not conclusive. Documentation of the triangular cord, a periportal band of fibrotic tissue, both on US[35] and more recently on CT,[36] has been shown to correlate well with the documentation of biliary atresia at the time of surgical exploration. There was initial enthusiasm for MRCP for evaluating infants with cholestasis, but MRI is no more sensitive or specific than other modalities, including radionucleotide scanning, with a 75% positive predictive rate and roughly a 90% negative predictive rate[37] for biliary atresia. MRCP (Figure 74.2-11) does have the advantages of not requiring pretreatment and not using ionizing radiation,[38] and it has been shown to be extremely helpful in supporting the diagnosis of biliary atresia in late-presenters, in whom the morbidity and mortality of exploratory laparotomy are especially increased.[37]

Congenital Splenic Anomalies

The shape and position of the spleen can be quite variable and are not of clinical significance. Splenic clefts are common, as is persistent fetal lobulation, and both can occasionally be confused with lacerations.[39] US is generally adequate for most examinations, but CT will yield better images. The orientation of the hilum can be variable, and laxity of the ligamentous attachment can result in a mobile spleen. Occasionally, a "wandering spleen" can be confused for a neoplasm or can result in torsion.[40] Accessory spleens or splenules (Figure 74.2-12) occur in 10% or more of healthy individuals and are typically near the splenic hilum.[39] These are usually at least 2 cm in diameter to be visualized by radionucleotide scanning. Although not clinically significant, accessory spleens can hypertrophy after splenectomy or in patients with splenomegaly (Figure 74.2-13). Primary asplenia is not common and with polysplenia falls into the category of situs anomalies or heterotaxy syndromes (Figure 74.2-14), half of which are associated with cardiac and renal malformations and many with immunodeficiency. Because of the complexity and variability of the heterotaxy syndromes, US, followed by MRI, CT, and sometimes red blood cell–tagged radionuclide scanning, is helpful for full delineation.[41] True congenital splenic cysts are extremely rare, and those less than 5 cm can be managed conservatively.[42]

Pancreas

Most congenital anomalies of the pancreas involve anatomic variations of the ductal system. Numerous series have reported the inadequacies of US and CT and the need for endoscopic retrograde cholangiopancreatography (ERCP) to delineate ductal anatomy. Most recently, MRCP has proved useful in defining pancreatic duct anatomy.[43] Pancreas divisum (Figure 74.2-15) is a common anomaly that arguably may predispose the patient to pancreatitis.[44] Hypoplasia and agenesis of the pancreas have been reported but are extremely rare. Isolated congenital cysts and cystic disease of the pancreas are associated with polycystic disease of the kidneys and liver,[45] as well as the rare case of von Hippel-Lindau disease (Figures 74.2-16 and 74.2-17). Annular pancreas, which typically presents in the newborn period with bilious vomiting, is generally not diagnosed by cross-sectional imaging but rather by UGI series. Variations of annular pancreas have been reported in adults and can be imaged by MRI.

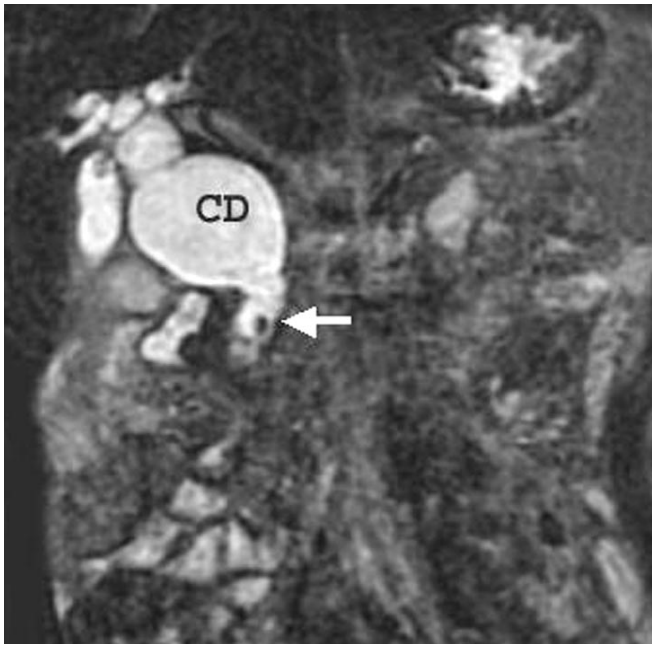

FIGURE 74.2-10 Coronal magnetic resonance cholangiopancreatogram of an infant with jaundice reveals marked enlargement of the extraheptic bile duct consistent with a type I choledochal cyst. A signal void in the distal end was caused by a stone (*arrow*). CD = common duct.

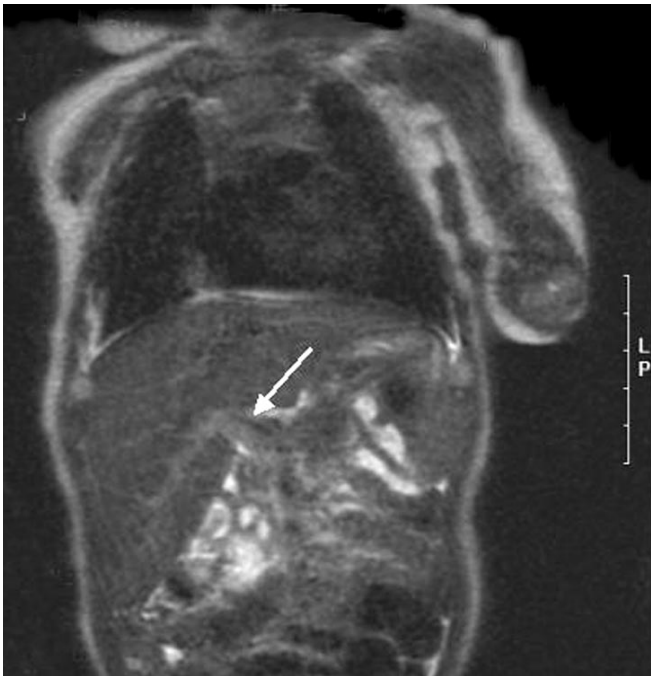

FIGURE 74.2-11 Coronal magnetic resonance cholangiopancreatography failed to reveal the extrahepatic biliary system in this infant evaluated for cholestasis. A chord of tissue was present in the anticipated location (*arrow*). Biliary atresia was confirmed at laparotomy.

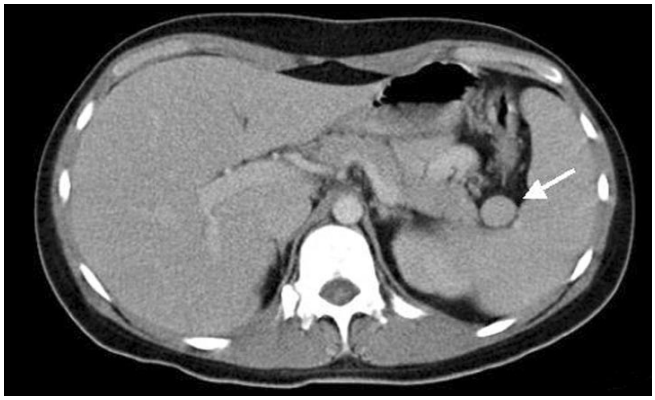

FIGURE 74.2-12 Axial contrast-enhanced computed tomographic scan of the abdomen reveals a small, well-defined mass (*arrow*) in the splenic hilum, of identical attenuation to normal spleen.

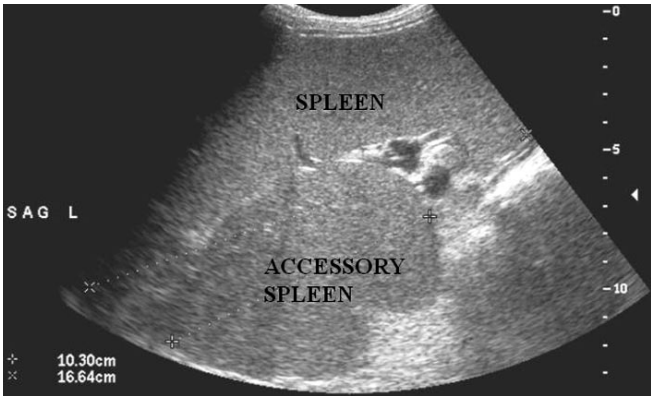

FIGURE 74.2-13 Sagittal sonogram of the left upper quadrant of a patient with Jeune syndrome, a rare cause of hepatic fibrosis, reveals an enlarged spleen. A large mass of identical echogenicity in the splenic hilum is consistent with an accessory spleen.

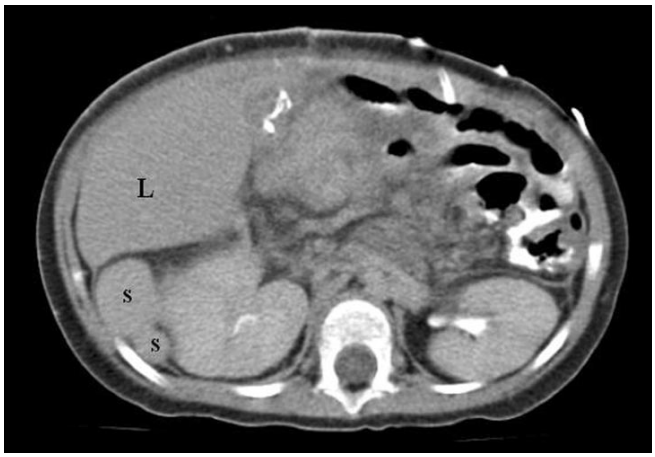

FIGURE 74.2-14 Axial contrast-enhanced computed tomographic scan of the abdomen reveals several small spleens (s) on the right, inferior to the liver (L) in this patient with heterotaxy syndrome.

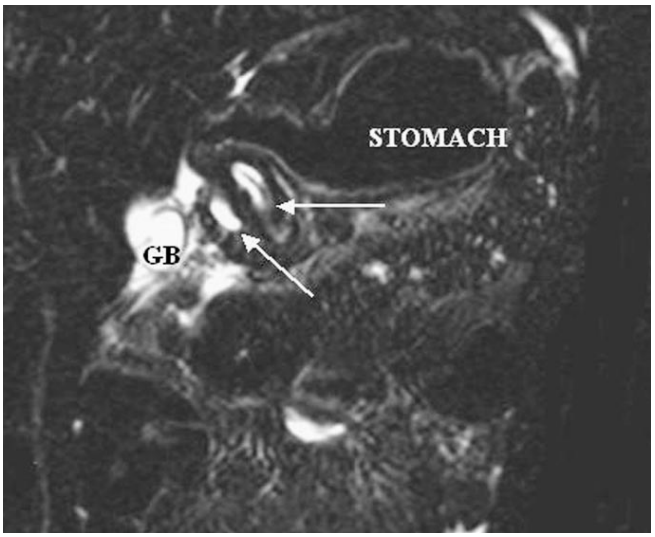

FIGURE 74.2-15 Coronal magnetic resonance cholangiopancreatography was performed in this 5-year-old female with recurrent pancreatitis. A dual drainage (*arrows*) system was seen in the head of the pancreas consistent with pancreas divisum. GB = gallbladder.

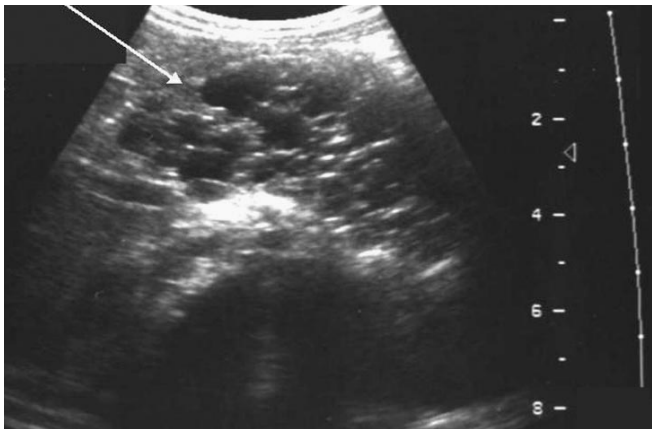

FIGURE 74.2-16 Transverse sonogram of a 7-month-old male with a palpable mass reveals that the pancreas is replaced by multiple cysts (*arrow*).

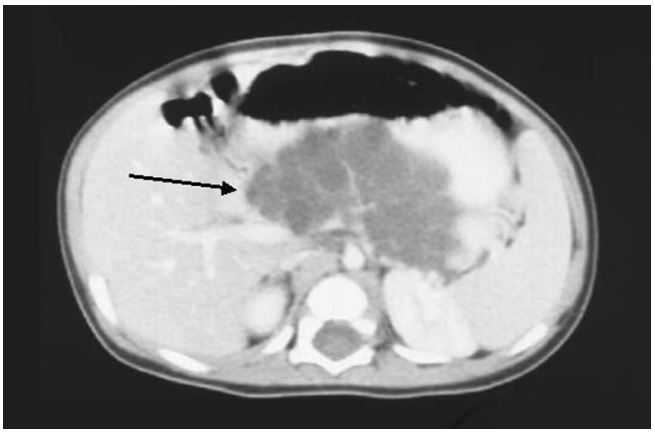

FIGURE 74.2-17 Follow-up contrast-enhanced axial computed tomographic scan of a 7-month-old boy confirms that the pancreas is replaced by multiple cysts (*arrow*) and no other cysts were present in other intra-abdominal organs. A diagnosis of von Hippple-Lindau disease was subsequently made.

Gastrointestinal Tract

Numerous congenital malformations of the esophagus and the remaining gastrointestinal tract exist, including esophageal duplication cysts, which may be detected on CT (Figure 74.2-18).[46] Vascular rings and impinging vessels, traditionally suggested by UGI series and confirmed by angiography, can now be well demonstrated by either CT or MRI (Figure 74.2-19).[47] Although antral webs and pyloric atresia can occasionally be seen on US as an intraluminal diaphragm of linear echo density within a fluid-filled cavity,[48] UGI or endoscopy remains the mainstay of diagnosis. High obstructions of the gastrointestinal tract, such as duodenal atresia, are readily suggested by a fluid-filled "double bubble" on prenatal US but are typically confirmed in the newborn period by UGI and not cross-sectional imaging. Low obstruction in the neonatal period may be caused by intrauterine perforation, resulting in meconium peritonitis, which calcifies and is evident on plain film. In this clinical situation, US is extremely useful in distinguishing active peritonitis and surgical emergency from a healed perforation by the detection of intra-abdominal fluid. Absence of fluid suggests recannalization and resolution in utero, allowing these infants to be fed and clinically monitored. Encysted collections of meconium will show a heterogeneous appearance on US.[49] Duplication and intestinal cysts, unless detected on prenatal US, generally present later in childhood. Duplication cysts most commonly occur in the distal ileum and esophagus, usually do not communicate with the intestinal lumen, and may or may not cause obstruction. US can be useful to define these fluid-filled lesions when intra-abdominal, and the presence of a double-walled sign helps distinguish gastrointestinal cysts from single-walled simple mesenteric cysts (Figure 74.2-20).[50] MRCP is helpful in distinguishing gastric duplication cysts from cysts of biliary origin (Figure 74.2-21).

Although hypertrophic pyloric stenosis does not usually present at birth but typically after the first month of

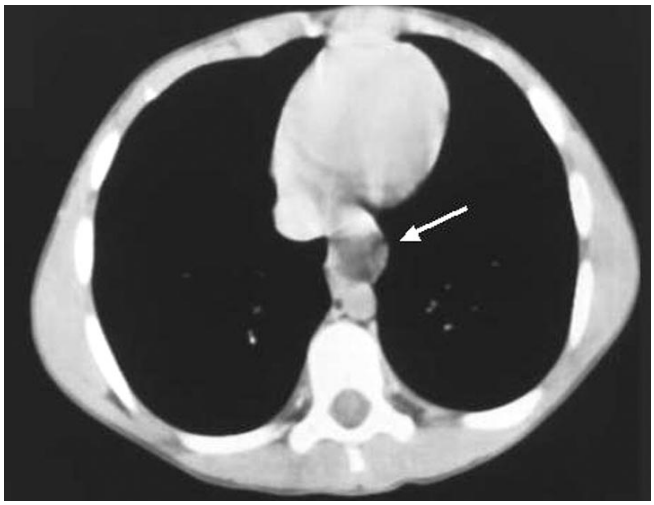

FIGURE 74.2-18 Axial computed tomographic scan of the lower chest of a 5-year-old boy with a history of dysphasia reveals a cystic mass adjacent to the esophagus (*arrow*), consistent with a duplication.

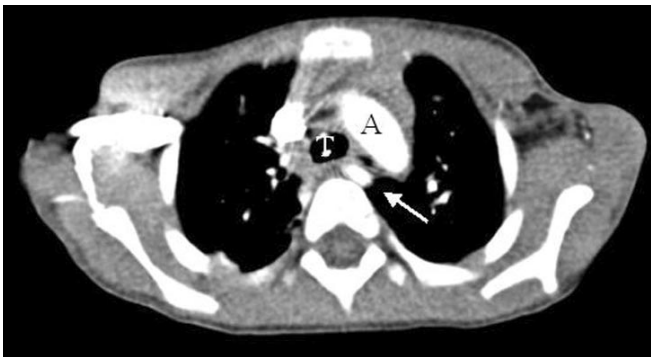

FIGURE 74.2-19 Contrast-enhanced multislice computed tomographic angiography demonstrating an aberrant subclavian artery.

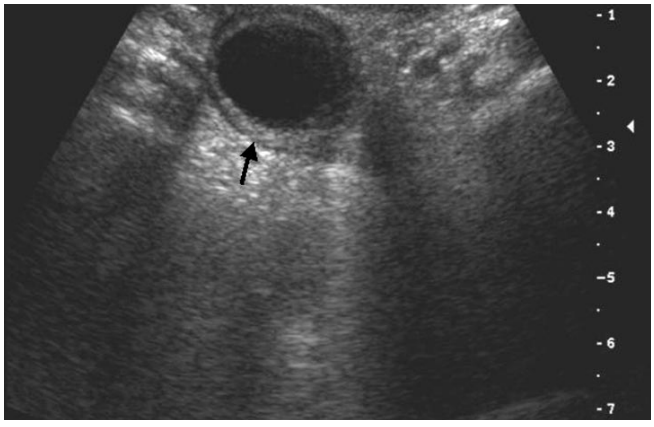

FIGURE 74.2-20 Sonogram of the right lower quadrant was performed on a newborn infant because of an abnormal prenatal sonogram. The presence of a cystic mass (*arrow*) was confirmed. The double wall suggests that it arises from the bowel as opposed to a simple cyst of another origin, such as the ovary or mesentery.

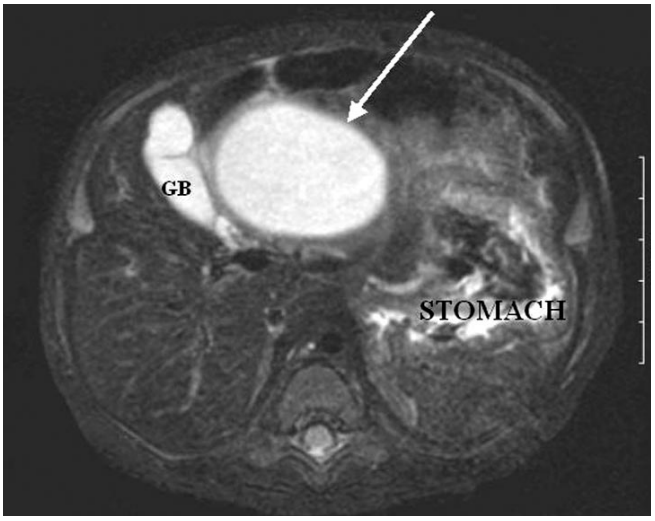

FIGURE 74.2-21 Axial magnetic resonance cholangiopancreatogram of a 4-month-old girl with a history of vomiting and cystic mass seen on ultrasonography demonstrates that the mass (*arrow*) arises in close proximity to the lesser curvature of the stomach and appeared separate from the gallbladder (GB), biliary system, and liver. A gastric duplication cyst was successfully resected.

life, it can be classified under congenital lesions of the stomach. The condition results from hypertrophy of the circular muscle of the pylorus, causing both thickening and elongating. The typical finding on physical examination is the palpated "olive" of hypertrophied muscle. When this cannot be felt, US is the study of choice to demonstrate the increased thickness and the increased length of the pyloric muscle (Figure 74.2-22).[51]

CROSS-SECTIONAL IMAGING IN ABDOMINAL TRAUMA

In pediatric settings, there is much less experience with penetrating injuries than with blunt trauma. With the increasing tendency toward conservative, nonoperative management of abdominal trauma, precise imaging of the abdomen is of the utmost importance. The liver is still the most frequently injured organ; however, in 20% of cases, multiple organs are involved. In general, contrast-enhanced CT is the recommended modality for diagnosing traumatic injury, with US reserved for serial follow-up examinations.[52] The use of imaging is both to detect specific injuries such as lacerations and subcapsular hematomas and to search for peritoneal fluid that might indicate impending clinical compromise.

Parenchymal disruption to the liver can vary from small lacerations to extensive fractures with significant extravasation of blood. Lacerations can be simple or complex and on CT (Figure 74.2-23) appear hypodense in either a linear, round, or stellate shape. Subscapular hematomas (Figure 74.2-24) vary in density according to the amount of blood loss and hematocrit, and tears in the capsule will allow blood to fill the peritoneal spaces. Traumatic injuries to the spleen (Figure 74.2-25) are very

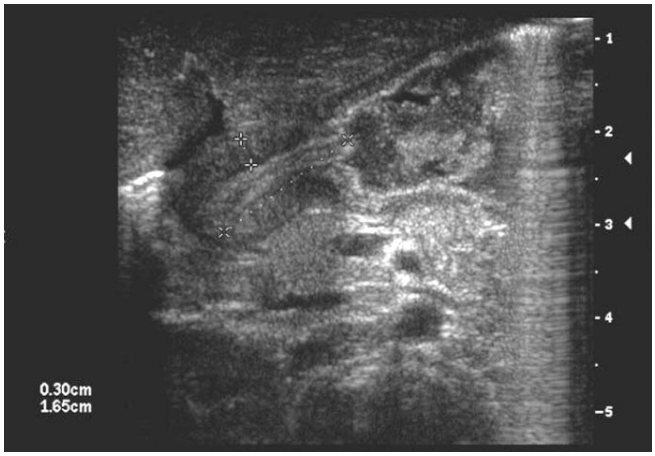

FIGURE 74.2-22 Hypertrophic pyloric stenosis. Transverse sonogram of the abdomen in an infant with nonbilious vomiting reveals both elongation of the pyloric channel (x) and thickening of the muscle (+), consistent with the diagnosis of hypertrophic pyloric stenosis.

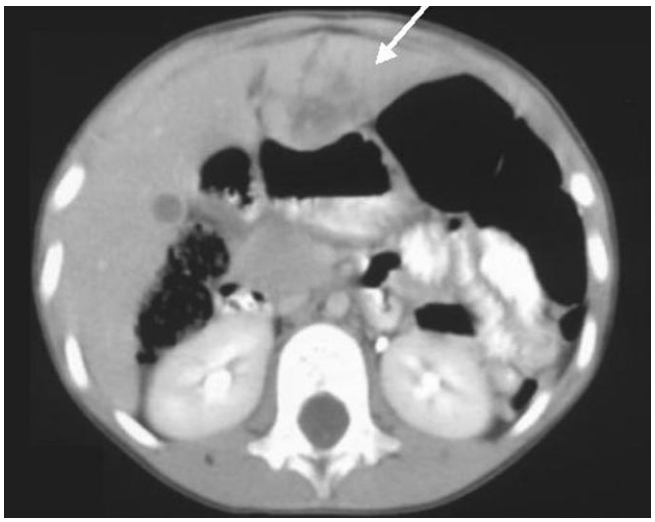

FIGURE 74.2-23 Axial contrast-enhanced computed tomographic scan of the abdomen was performed in this 11-year-old boy after a fall. Laceration of the left lobe of the liver is evident (*arrow*).

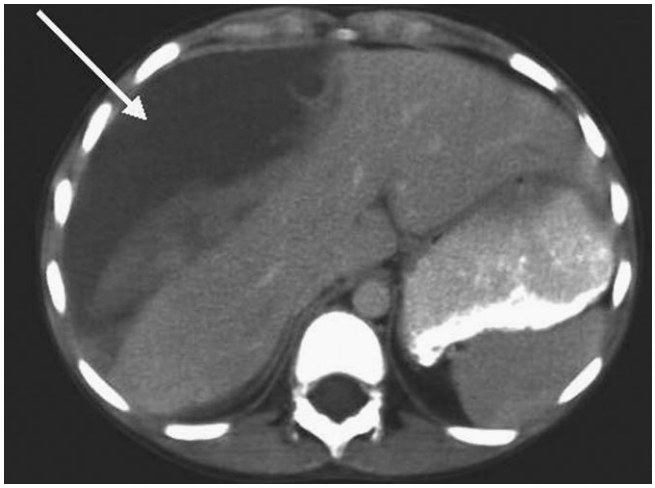

FIGURE 74.2-24 Contrast-enhanced computed tomographic scan of the liver of a 10 year old after a sledding accident reveals a large subcapsular hematoma peripheral to a lacerated liver (*arrow*).

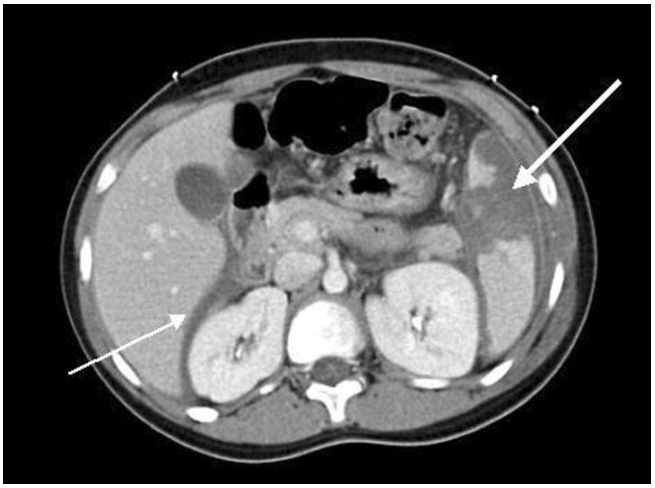

FIGURE 74.2-25 Contrast-enhanced axial computed tomographic scan performed after a motor vehicle accident on this 11-year-old boy reveals a fractured spleen (*thick arrow*) and free ascites, presumably blood, in Morrison pouch (*thin arrow*).

similar in CT appearance and are easily missed on initial US because acute injuries may appear isoechoic. US should be reserved for serial examinations of known injuries (Figure 74.2-26).

Much debate has surrounded the management of patients with blunt abdominal trauma. Most clinicians agree that in clinical settings in which surgical intervention is not necessary, there is little need for follow-up CT scans.[53] US is usually adequate for following the evolution from poorly defined and iso- or hyperechoic to less echogenic and smaller because liquefaction and organization occur over time.[54] Complications, such as the formation of cysts, bile lakes, infarctions, and calcifications, can also be determined by US[55] but are best defined by CT with contrast.

Contrast-enhanced multidetector CT angiography[56] has largely replaced angiography in the screening for arterial vascular injury, and interventional angiography with embolization has reduced the need for surgery in a significant number of cases.[57] Contrast-enhanced multidetector CT can be very useful in determining active hemorrhage but is inadequate in diagnosing disruption of the bile ducts, whereas radionucleotide scanning is useful in diagnosing suspected bile leaks.[58]

CT is the modality of choice for detecting trauma to the pancreas, which can be manifested by enlargement, edema, or complete transection (Figure 74.2-27), as well as pseudocyst formation. US is useful to follow pseudocysts, but pancreatic duct injury is best seen on MRCP.[59]

Hollow viscus gastrointestinal tract, namely the stomach and small and large intestine, is also subject to blunt trauma. CT is able to detect bowel and mesenteric injuries in greater than 90% of cases but cannot necessarily differentiate the need for surgical intervention.[60] Free air from perforation is readily identified on CT. Bowel wall hematomas are sometimes more readily seen on UGI and SBFT, and water-soluble studies should be obtained if clinically suspect.[61] Focused abdominal US for trauma has been advocated, but the high false-negative rate supports the use of CT for all significant injuries.[62]

TUMORS

Approximately 5% of all intra-abdominal masses, benign and malignant, occur in the liver, and of all pediatric malignancies, almost 2% are primary hepatic.[15] US is the preferred initial examination for lesions that are either purely cystic or typical for benign hemangiomata, seen as small, well-defined, echogenic masses. Serial US is recommended to follow these benign findings. Contrast-enhanced CT and MRI both provide further definition of all other liver masses. CT-guided biopsies may be used for diagnosis.[62,63]

Malignant Hepatic Tumors

Hepatoblastoma is the most common primary malignancy of the gastrointestinal tract in children and is the third most common abdominal malignancy after Wilms tumor and neuroblastoma.[15] Hepatoblastoma usually presents at a median of 1 year of age as a painless, solitary lesion, but it may be multifocal. Serum α-fetoprotein is elevated in 90% of patients, and metastasis occurs in up to 20%. Plain films will show calcifications in up to 55%. On US, there is usually a large, well-outlined, predominantly echogenic mass. CT will show any calcifications present in the mass well, and with intravenous contrast, there will be heterogeneous enhancement, usually related to hemorrhage and/or necrosis within the tumor. MRI also reveals a solid mass that typically enhances irregularly after the administration of gadolinium (Figures 74.2-28 and 74.2-29). Calcifications are not well seen on MRI but rather appear as signal voids.[64]

Hepatocellular carcinoma (HCC) (Figure 74.2-30) is more common in older children and is typically associated with some progressive, preexisting liver disease, such as chronic hepatitis, tyrosinemia, or glycogen storage disease. HCC is more often multifocal and infiltrative. In the setting of end-stage liver disease, it can be difficult to distinguish from diseased parenchyma on all imaging modalities. Small lesions are typically hyperechoic on US, whereas larger lesions are generally mixed. Color Doppler US is useful to demonstrate low-resistance tumor flow, as

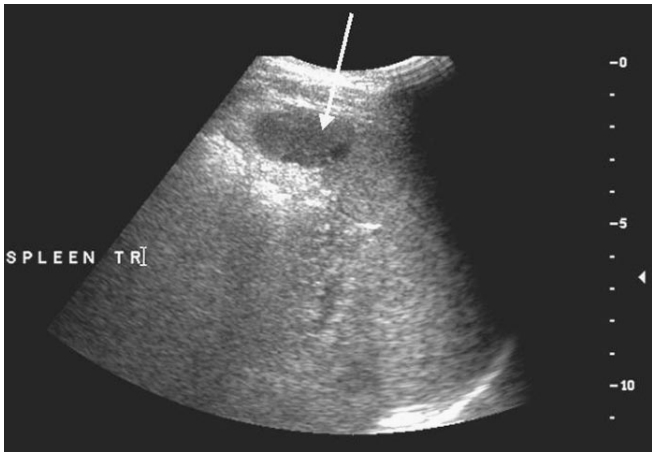

FIGURE 74.2-26 Transverse ultrasonography was used to follow the fractured spleen serially. Six months after the initial trauma, a hypoechoic area was still visualized (*arrow*) consistent with an organizing hematoma.

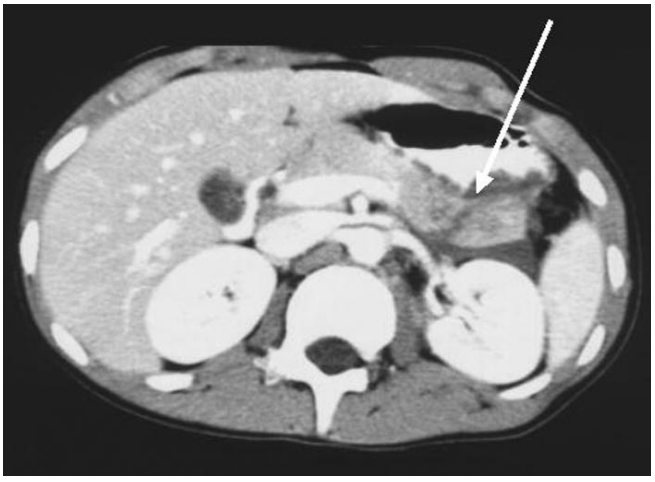

FIGURE 74.2-27 Contrast-enhanced axial computed tomographic scan of a 10-year-old boy who was struck by an automobile reveals a complete transection of the pancreas (*arrow*).

well as thrombosis of the adjacent veins by tumor extension if present.[65,66] Nonenhanced CT will show poorly defined masses, but after the intravenous administration of contrast, HCC usually demonstrates intense enhancement secondary to its hypervascular nature. Calcifications are unusual.[67] HCC is typically of low signal intensity on T_1-weighted MRI and hyperintense on T_2-weighted imaging. Gadolinium-enhanced imaging demonstrates a hypervascular tumor, similar to a contrast-enhanced CT scan.[68,69]

Fibrolamellar carcinoma is a very rare hepatic neoplasm but is often discussed in the differential diagnosis of HCC, despite very different features and presentation. In contrast to HCC, patients with fibrolamellar carcinoma are usually symptomatic, have normal serum α-fetoprotein, do not have predisposing liver disease, and carry a much poorer prognosis.[70] The tumors are usually solitary and lobulated, with a distinguishing central fibrous scar that is typically hyperechoic on US and at times calcified. On nonenhanced CT, the lesion, and especially the central scar, is well demarcated and of low attenuation relative to the surrounding normal liver. With the administration of intravenous contrast, the entire tumor, but not the central scar, will enhance. On MRI, the lesion will appear of low signal intensity on T_1-weighted images and of high signal intensity on T_2-weighted images, with failure of the central scar to enhance after the injection of gadolinium (Figure 74.2-31).[71] The presence of a central scar, although help-

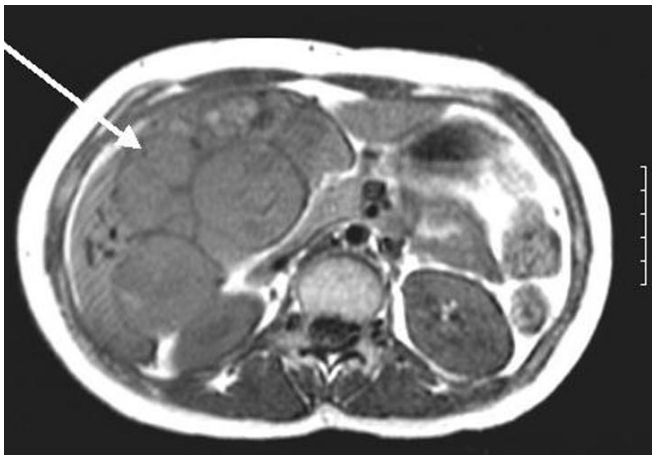

FIGURE 74.2-28 Axial T_1-weighted magnetic resonance image of an 11-year-old girl with a firm liver reveals a multilobulated solid mass within the liver (*arrow*), which was subsequently proven to be a hepatoblastoma.

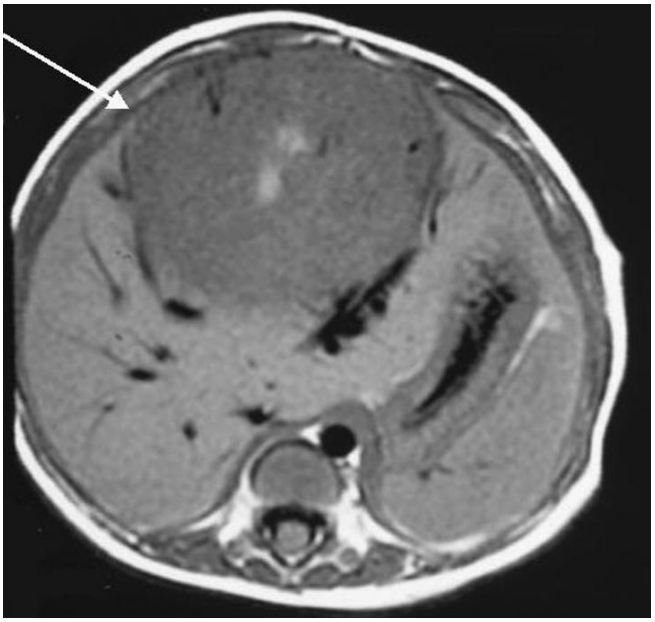

FIGURE 74.2-29 T_1-weighted axial magnetic resonance image of the abdomen of an infant reveals a large, well-defined mass (*arrow*) with a central area of high signal, which was proven to represent a hepatoblastoma with central hemorrhage.

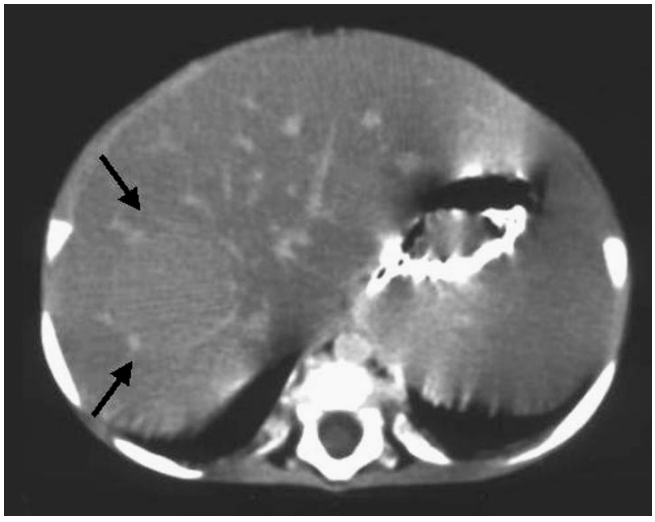

FIGURE 74.2-30 Axial contrast-enhanced computed tomographic scan of the abdomen of a 12-year-old girl who was hepatitis B positive reveals a well-defined mass in the posterior right lobe (*arrows*). Biopsy revealed hepatocellular carcinoma.

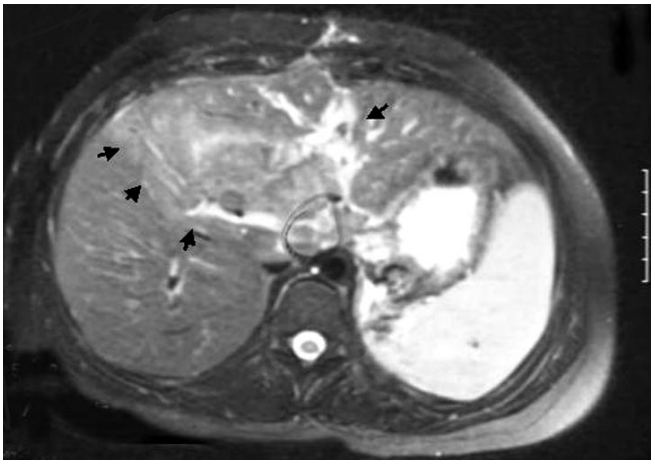

FIGURE 74.2-31 Axial T_2-weighted magnetic resonance image of a 16-year-old girl with a firm liver mass reveals a poorly defined mass (*arrows*) with a large central scar of increased signal after the administration of gadolinium. The patient underwent a computed tomography–guided biopsy that confirmed fibrolamellar carcinoma. Enhancement of the central scar is more typical for focal nodular hyperplasia.

ful, is not diagnostic because it can also be seen in focal nodular hyperplasia (FNH) and giant hemangiomas.

The fourth most common pediatric hepatic tumor is the undifferentiated embryonal sarcoma, which may be difficult to differentiate from a mesenchymal hamartoma. Unlike the latter, embryonal sarcoma often presents with symptoms of pain or mass. On US, it is usually solitary and echogenic, with small anechoic areas that probably represent cystic necrosis.[72] Nonenhanced CT shows a well-demarcated, low-attenuation mass. On contrast-enhanced CT, septations become evident. The lesion tends to be of low signal intensity on both T_1- and T_2-weighted MRIs, with enhancement of the tumor contrasting with low signal of the septa[73] after gadolinium administration.

As in adults, tumors of the biliary tree are much less common than hepatic tumors, and the prognosis is much worse. Embryonal rhabdomyosarcoma is probably the only one in this category that is seen at all in children, and it usually presents with jaundice. US will show biliary obstruction as well as an inhomogeneous echogenic mass, typically in the porta hepatis. CT shows a low-attenuation mass with some enhancement with contrast, and MRCP will show irregular, berry-like filling defects within the biliary system.[74]

Secondary malignant tumors of the liver occur, most commonly from neuroblastoma, Wilms tumor, lymphoma (Figure 74.2-32), and leukemia. All can be diffuse or focal. Immunosuppressed patients can develop post-transplant lymphoproliferative disease (PTLD) with various lesions that range from polyclonal B-cell hyperplasia to malignant lymphoma. Hepatic metastases from other tumors occur rather late in the disease process and carry a poor prognosis. The value of US in detecting lesions depends on the primary tumor and the pattern of metastasis, and even the presence of hepatomegaly is not useful.[75] CT with contrast can be more useful but may require dual-phase arterial and venous scanning for full evaluation. MRI with and without gadolinium is also sensitive for detection of focal liver lesions.

Benign Hepatic Tumors

FNH is an uncommon epithelial lesion in childhood, more often seen in middle-aged women. In children, it is usually an incidental finding, as a solitary lesion of less than 5 cm in size, without internal hemorrhage or necrosis. The distinguishing feature is a central fibrous scar, which is vascular and extends outward into fibrous septa. On US, FNH is a well-demarcated, either iso- or hyperechoic lesion, with a central scar demonstrated in 20% of cases. Associated calcifications are rare enough to suggest another pathology.[76] On nonenhanced CT, the lesion appears as a hypo- or isodense lesion that enhances after the administration of contrast but becomes isodense on delayed scans. The central scar in FNH will enhance in 70% of cases, helping to distinguish FNH from fibrolamellar carcinoma, in which the scar typically does not enhance. On T_1-weighted MRIs, FNH appears as a lesion of decreased or isointensity, and on T_2-weighted images, the signal intensity increases. After the administration of gadolinium, over 80% of cases will show enhancement of the central scar,[48] again distinguishing FNH from fibrolamellar carcinoma (Figures 74.2-33 and 74.2-34).

Hepatocellular adenoma is a rare epithelial tumor with an increasing incidence secondary to oral contraceptive and androgen use. Hepatocellular adenomas are also reported in association with glycogen storage disease type Ia and diabetes. In 80% of cases, hepatocellular adenoma is a solitary, well-circumscribed, and encapsulated mass. The US appearance can be variable, depending on the presence of lipid or internal hemorrhage.[77] On nonenhanced CT, the lesions are generally of decreased attenuation, with 50% containing calcifications (Figure 74.2-35). After the injection of intravenous contrast, feeding vessels may be identified.[78] The lesions have a variable appearance on MRI. Gadolinium is useful to identify feeding vessels.[79] Serial US

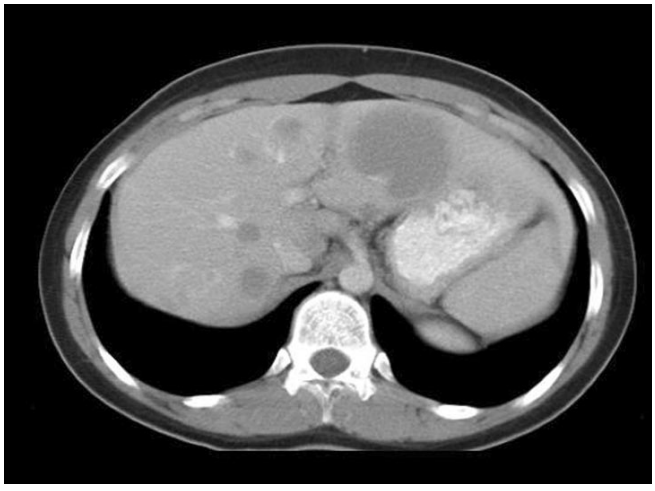

FIGURE 74.2-32 Axial contrast-enhanced computed tomographic scan of the abdomen of an 18-year-old female patient with night sweats reveals multiple low-attenuation masses within the liver. Bone marrow biopsy was consistent with B-cell lymphoma.

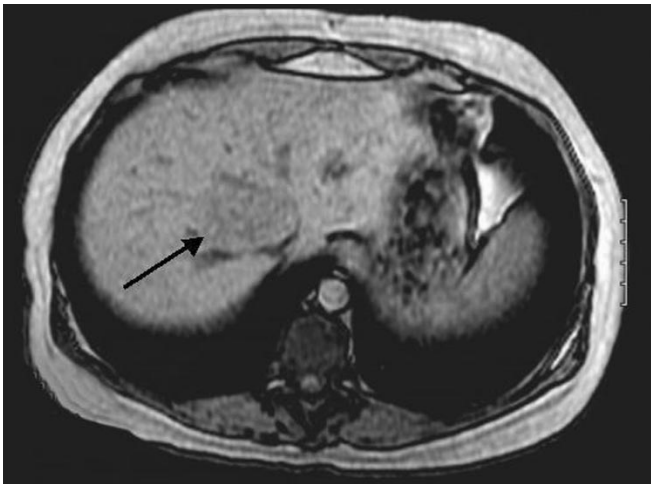

FIGURE 74.2-33 Axial magnetic resonance image of a 14-year-old asymptomatic girl followed for a liver mass discovered incidentally during renal ultrasonography shows a well-defined low signal lesion (*arrow*) with a central scar. Differential diagnosis was fibrolamellar carcinoma versus focal nodular hyperplasia.

examinations, in conjunction with monitoring the serum α-fetoprotein level, are recommended to screen for malignant degeneration, seen as sudden growth, necrosis, or a change to a heterogeneous appearance.[80]

Mesenchymal hamartomas, like most hamartomas, should probably be considered as developmental anomalies rather than true neoplasms. They contain gelatinous serous fluid in cystic spaces, intermixed with biliary ductal and connective tissue. On US, mesenchymal hamartomas are predominantly cystic, with multiple echogenic septa (Figure 74.2-36). Their appearance is similar on CT scan (Figure 74.2-37) and MRI (Figure 74.2-38).[81] Occasionally, mesenchymal hamartomas present as purely cystic lesions (Figure 74.2-39). Serial US examinations aid in distinguishing simple liver cysts (Figures 74.2-40 and 74.2-41) from cystic hamartoma; the latter tend to grow and change in appearance, and may develop more solid components with time.[82]

Related and more common entities are mesenchymal tumors, which include hemangiomas, hemangioendotheliomas, and lipomas. The first two have been discussed in part above. Cavernous hemangiomas are more commonly seen in adults than in children. Lipomas, benign masses of mature adipose tissue, are rarely seen in children.

Splenic Tumors

Splenic tumors are in general rare and can be divided between cystic lesions and solid neoplasia. Cysts are classified as true cysts, pseudocysts, and parasitic cysts. True cysts (Figures 74.2-42 and 74.2-43) have an epithelial lin-

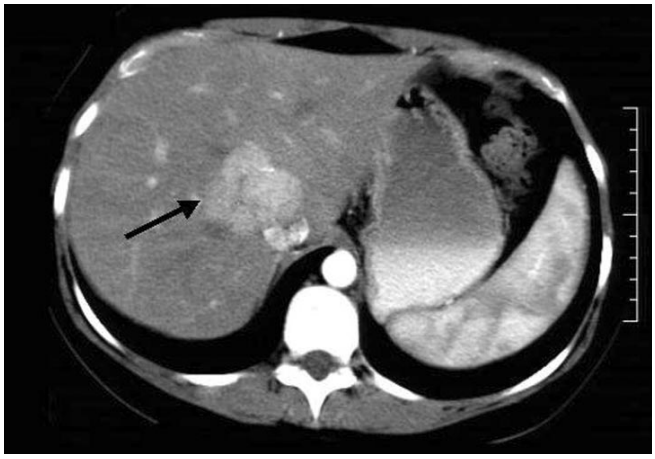

FIGURE 74.2-34 After the intravenous administration of gadolinium, there is intense enhancement of the mass (*arrow*) but not the central scar, a finding suggestive of fibrolamellar carcinoma rather than focal nodular hyperplasia. α-Fetoprotein remained normal, and the mass has remained stable in size for 5 years, findings more suggestive of focal nodular hyperplasia.

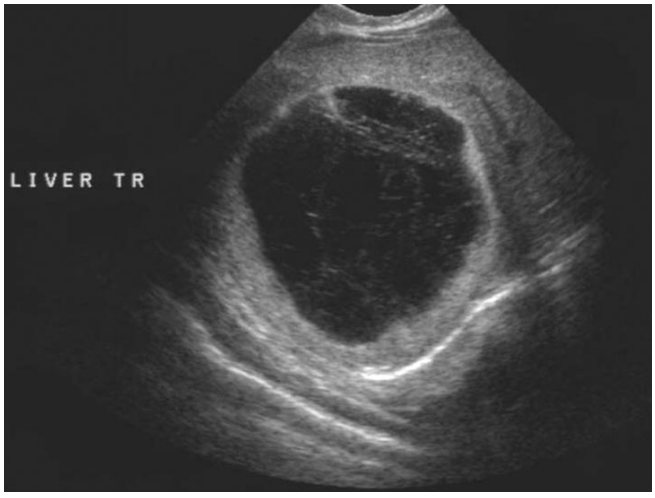

FIGURE 74.2-36 Sonogram of the liver of a newborn with an enlarged liver reveals a complex cyst within the liver, containing multiple septations. It was resected, and histopathology revealed a cystic hamartoma.

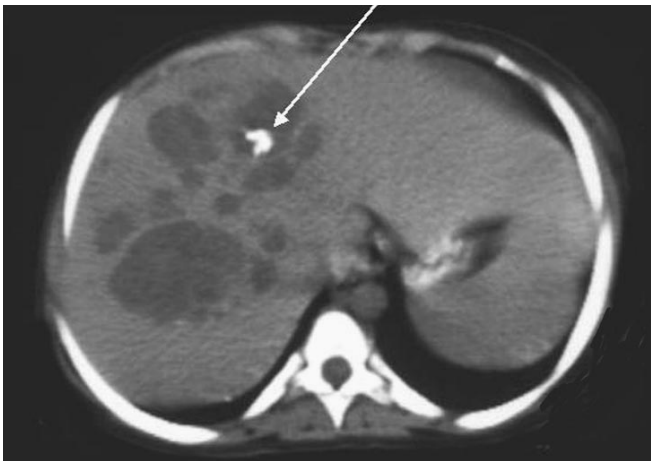

FIGURE 74.2-35 Non–contrast-enhanced computed tomographic scan of a 10-year-old girl with type IV glycogen storage disease reveals a large low-attenuation mass within the liver. A central calcification (*arrow*) was present. It was resected, and the diagnosis of giant adenoma was made.

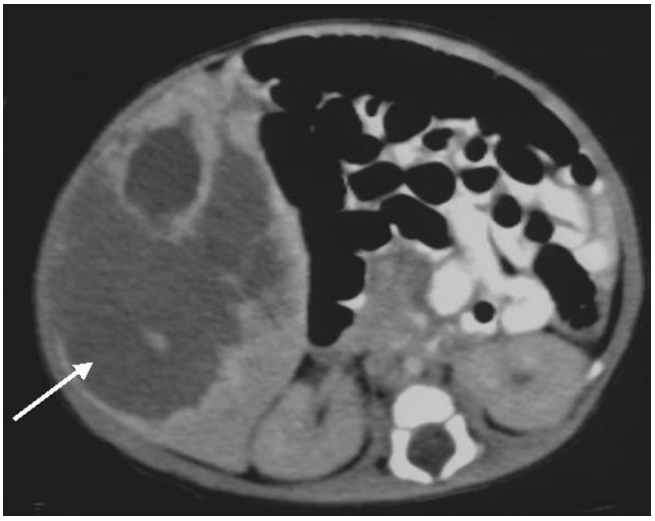

FIGURE 74.2-37 Contrast-enhanced computed tomographic scan of the liver demonstrates that a large portion of the liver has been replaced by cystic and solid tumor. Peripheral enhancement was seen. The patient underwent a three-quadrant resection for this hamartoma. The arrow demonstrates the cystic hamartoma within normal liver parenchyma.

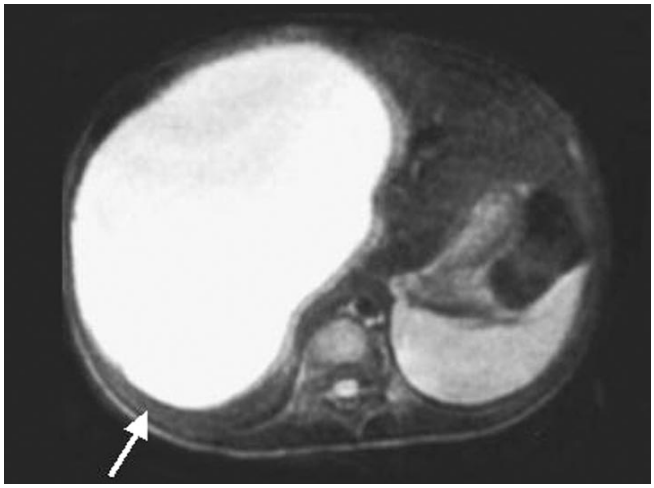

FIGURE 74.2-38 T_2-weighted axial magnetic resonance image of the abdomen of this infant with an enlarged liver reveals a large cystic mass (*arrow*). It was unroofed, but pathology was consistent with a cystic hamartoma rather than a simple liver cyst. It regrew with more solid components, and the patient underwent a successful wide resection of this tumor.

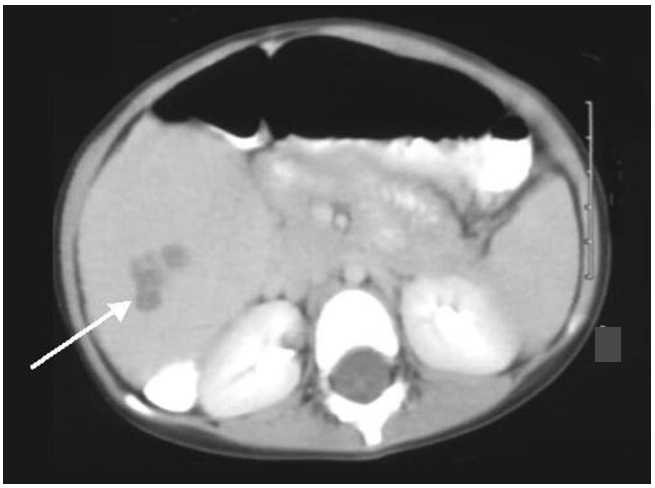

FIGURE 74.2-39 Axial contrast-enhanced computed tomographic scan of the abdomen of a 5 year old with right upper quadrant pain reveals a multilobulated cystic mass within the liver, which was subsequently proven to be a mesenchymal hamartoma.

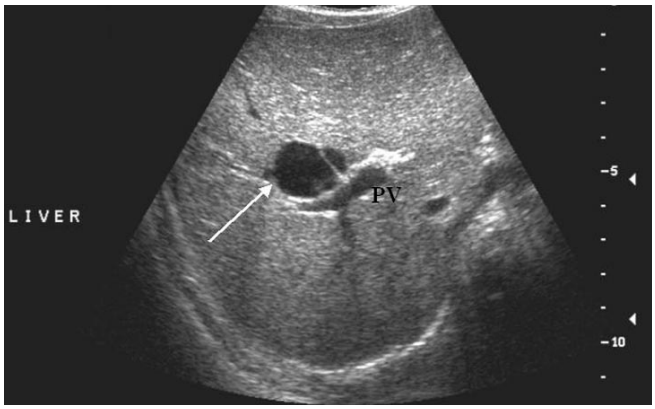

FIGURE 74.2-40 Transverse sonogram of the liver of an 8-month-old girl shows a multilobulated cyst within the liver above the portal vein (PV).

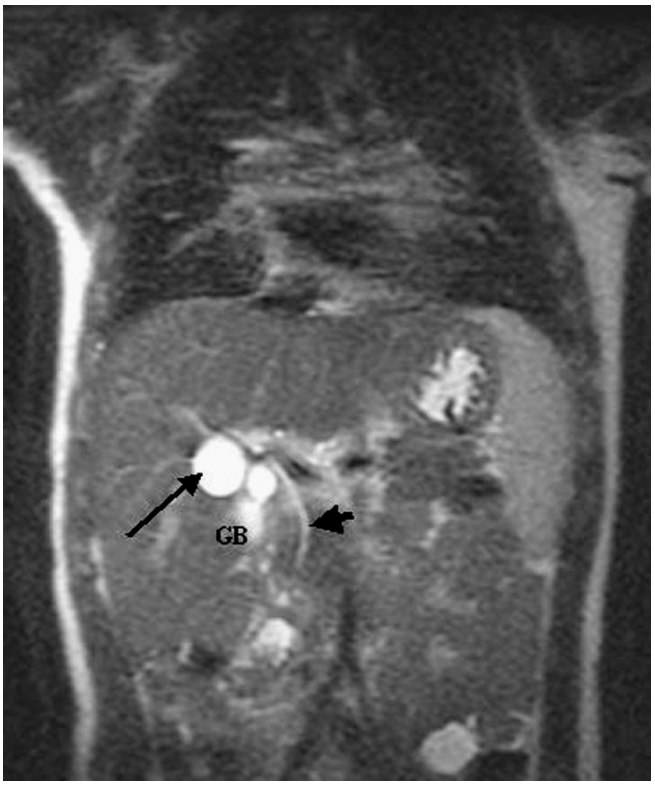

FIGURE 74.2-41 Follow-up coronal magnetic resonance cholangiopancreatogram confirms that the cysts (long *arrow*) are within the liver and separate from the biliary system. The normal extrahepatic duct is demarcated by the *short arrow*. A portion of the gallbladder (GB) is also seen.

ing and are sometimes termed congenital epidermoids (Figure 74.2-44). They may contain brightly reflective echoes within them from floating crystals. Pseudocysts, which contain no epithelial lining, are believed to be secondary to infection, trauma, or infarction and cannot be distinguished from true cysts radiologically. Some neoplasms, such as lymphangioma, hemangioma, and hamartoma, may appear largely cystic on US. Septations and rim calcifications may be present.[83] Hemorrhage or protein content can increase the attenuation on CT. MRI will also demonstrate true solid lesions (Figure 74.2-45).

Splenic involvement by lymphoma and leukemia presents in several ways. On US, homogeneous enlargement with normal echogenicity, solitary or multifocal hypoechoic lesions, or diffuse infiltration with heterogeneous echogenicity can be seen. CT and MRI demonstrate similar findings, although MRI may be less useful because lymphomatous tissue can have signal intensity similar to that of normal splenic tissue.[55]

Pancreatic Tumors

Pancreatic tumors are a very small percentage of pediatric intra-abdominal tumors and usually present with either a mass, abdominal distention, or a variety of endocrine abnormalities. Atypically, they present with the more usual adult presentation of bowel obstruction, weight loss, or jaundice.[84] These tumors can be classified into nonfunc-

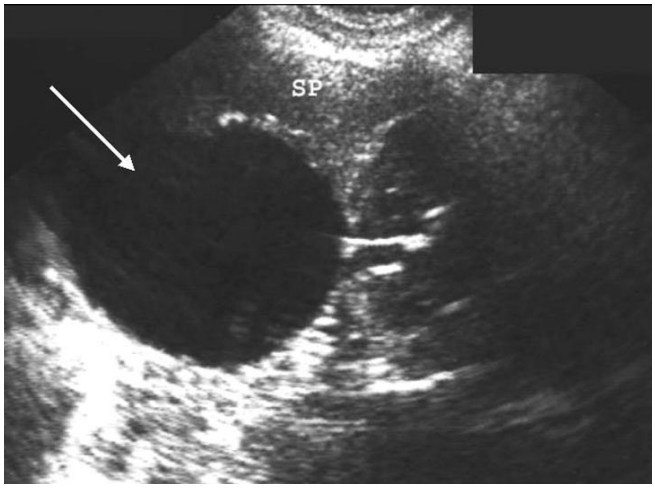

FIGURE 74.2-42 Sagittal sonogram of the left upper quadrant reveals a simple cyst (*arrow*) arising from the spleen (SP).

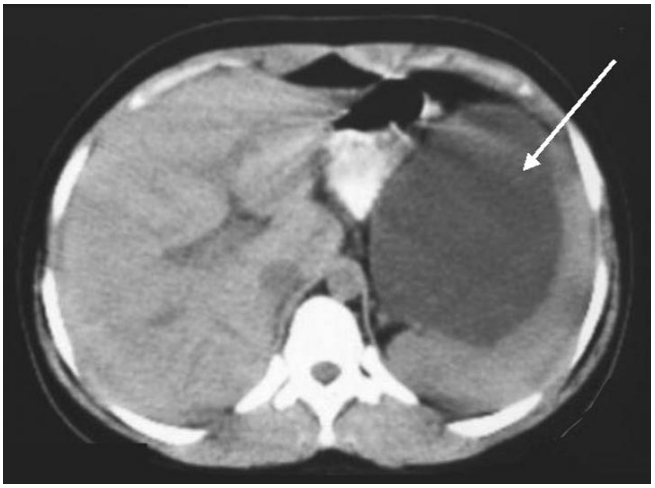

FIGURE 74.2-43 Non–contrast enhanced computed tomographic scan demonstrates a large cyst arising from the spleen (*arrow*).

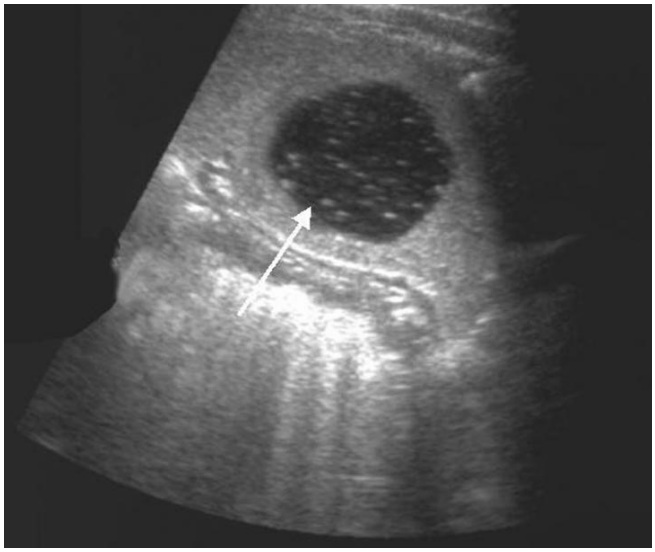

FIGURE 74.2-44 Sagittal sonogram of the spleen reveals a central cyst with particular echoes dispersed throughout. This is a typical appearance for an epidermoid cyst (*arrow*).

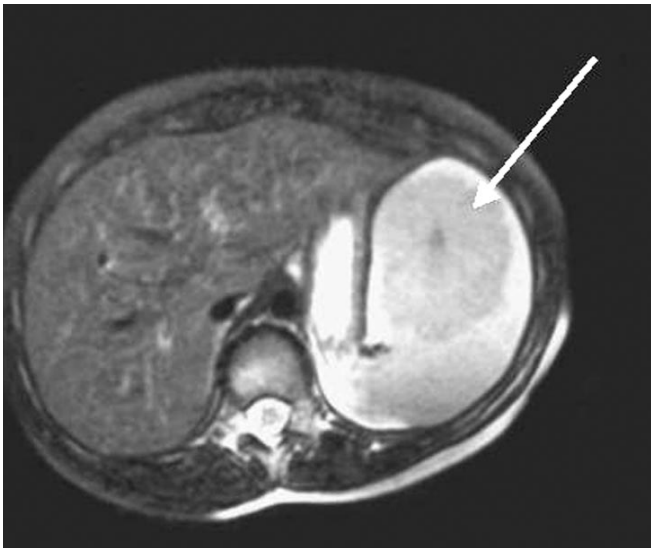

FIGURE 74.2-45 Axial T_2-weighted magnetic resonance image of the abdomen reveals a solid mass with a central scar arising from the spleen, which was subsequently proven to be a hamartoma (*arrow*).

tioning and functioning tumors, as well as malignant or benign tumors.[85] Pancreatoblastomas usually occur before 8 years of age as large, well-defined masses, with both cystic and solid components arising from the body or tail. Although pancreatoblastomas can be seen on US, CT is better at fully defining these tumors and demonstrating any associated calcifications.[86] Solid and papillary epithelial neoplasm is a rare, low-grade malignancy that typically occurs in young adult women, but a third of cases are described in adolescents. These tumors typically have cystic areas, as well as areas of hemorrhage and calcification, producing a very heterogeneous appearance on imaging studies (Figure 74.2-46).[87] Acute lymphocytic leukeumia is a rare cause of diffuse pancreatic infiltration and enlargement (Figure 74.2-47).[88] Thin-section contrast-enhanced CT is the best modality to image the pancreas because it is unaffected by overlying bowel gas, which can obscure the pancreas on US. Functioning pancreatic tumors, including insulinoma, gastrinoma, and VIPoma, are notoriously difficult to image, especially because these lesions may be very small. Gadolinium-enhanced MRI, which can depict subtle changes in tissue planes, may be of benefit in the detection of small tumors.[89]

Gastrointestinal Tumors

Primary malignancies such as adenocarcinoma, lymphoma, and sarcoma are rarely reported in childhood. Cross-sectional imaging is most useful for staging to determine the extent of tumor and the presence of metastatic disease. In general, intra-abdominal lymph nodes in excess of 1 cm should raise suspicion.[90] Primary lymphoma is rare, occurring most commonly in the ileocecal region, followed by gastric origin.[91] Polyps can sometimes be detected on US as incidental findings. The use of compression US in unprepared patients can be useful. Juvenile polyps may appear almost cystic on US but vascular on color flow

Doppler US.[92] Desmoid tumors, encountered in association with familial polyposis, are not malignant but can be quite aggressive (Figure 74.2-48).[93] PTLD can present in the immunosuppressed patient as nonspecific hepatosplenomegaly or focal masses within the bowel wall, organs, or lymph nodes. US and CT combined will detect up to three-quarters of cases of PTLD (Figure 74.2-49).[94]

INFECTIOUS PROCESSES

The most common infectious processes that affect the liver are abscesses and hepatitis. Fungal, bacterial, or other infectious abscesses are more commonly seen in immunocompromised children, those with human immunodeficiency virus (HIV), chronic granulomatous disease, or leukemia or on immunosuppression therapy following organ transplant or for treatment of diseases such as inflammatory bowel disease (IBD). US is an excellent initial study and a useful guide for diagnostic biopsy. Fungal abscesses are usually small and multiple and appear on US as "target" lesions, with a central echogenic focus surrounded by a hypochoic rim. On contrast-enhanced CT, the central area enhances within the low-attenuation lesions.[95] Bacterial abscesses tend to be singular, round, and located in the periphery. A hypoechoic halo may be seen on US.[96] Similarly on CT and MRI, pyogenic abscesses are well-defined, low-density lesions that demonstrate peripheral enhancement after contrast administration.[97] Staphylococcal abscesses can also present as target lesions. Amebic or hydatid abscesses can be difficult to diffentiate from pyogenic abscesses.[98] Echinococcosis is endemic in certain parts of the world, and two-thirds of cases will involve only the liver. US, CT, or MRI will show either single or a few very large cysts,

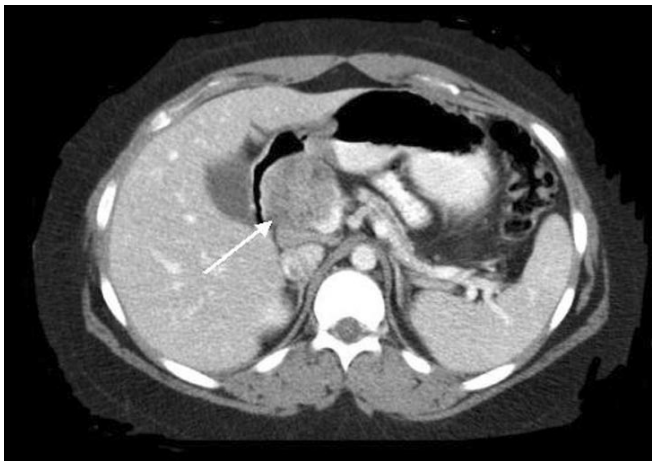

FIGURE 74.2-46 Contrast-enhanced axial computed tomographic scan of the abdomen of a 13-year-old girl with abdominal pain reveals a solid tumor in the head of the pancreas (*arrow*). The patient underwent a modified Whipple procedure for what proved to be a cystadenoma.

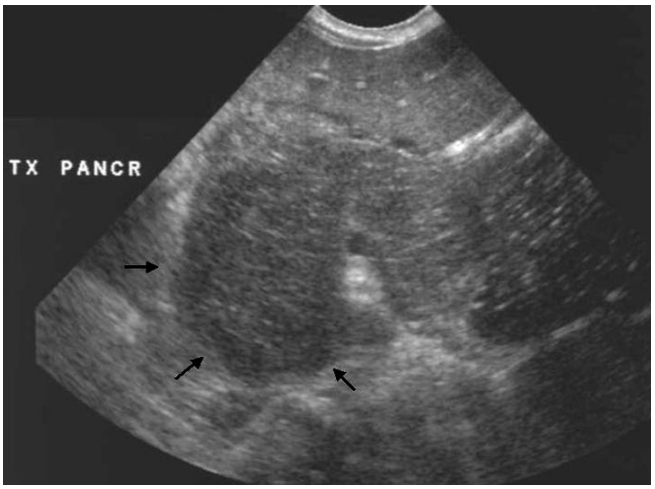

FIGURE 74.2-47 Transverse sonogram of a 3-week-old female infant showed marked enlargement of the pancreas. The echotexture appears decreased. The patient was diagnosed with acute lymphocytic leukemia. *Arrows* indicate the leukemic infiltration.

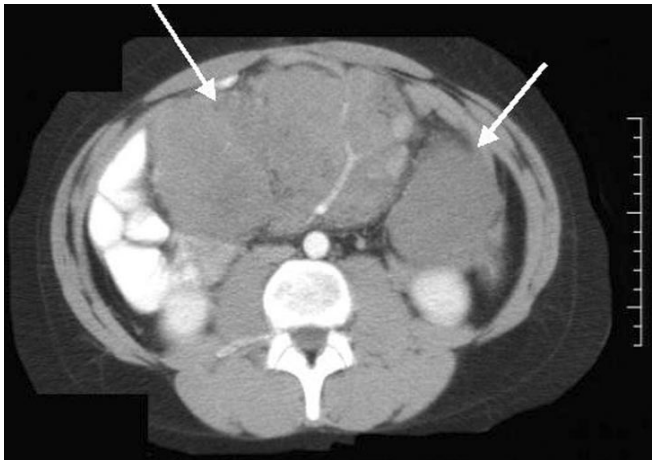

FIGURE 74.2-48 Axial contrast-enhanced computed tomographic scan of the lower abdomen of a 14-year-old boy who underwent a total colectomy for familial polyposis demonstrates bulky soft tissue masses (*arrows*). The patient was diagnosed with Gardner syndrome and desmoid tumors.

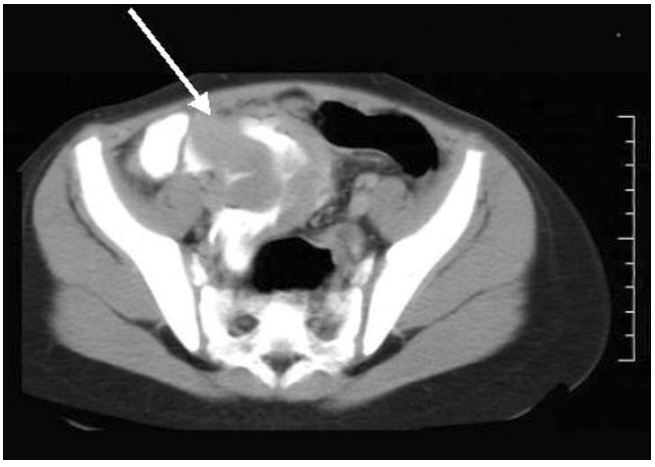

FIGURE 74.2-49 Axial contrast-enhanced computed tomographic scan of the pelvis of a 6-year-old female heart recipient on immunosuppression shows a large soft tissue mass encasing loops of bowel (*arrow*). Biopsy confirmed the diagnosis of monoclonal lymphoproliferative disease.

sometimes with calcifications (Figure 74.2-50). Perforation into the biliary tree has been reported.[99]

In general, most children with acute hepatitis have a normal US examination, although diffuse hepatomegaly may be present and echogenicity may be either increased or decreased.[31] Thickening of the gallbladder wall is sometimes seen in acute hepatitis as well as a contraction of the total volume of the gallbladder.[100] In certain settings, as with ingestion of a fatty meal, paradoxical dilatation of the gallbladder can also be seen on US.[101] CT and MRI will show similar nonspecific changes. Assorted neonatal infections, including cytomegalovirus, toxoplasmosis, and coxsackievirus, have been associated with parenchymal liver calcifications. Interestingly, infections in older children rarely result in calcifications.[102]

Splenic abscesses appear hypoechoic on US, with low attenuation on CT and with high fluid content on MRI. Peripheral enhancement after contrast administration is seen on CT and MRI. Fungal abscess may be very small, multiple, and occasionally calcified and is seen better on CT scan.[103,104] The spleen is subject to opportunistic infections, including *Pneumocystis* and *Mycobacterium*, especially in immunocompromised patients.[105]

Primary pancreatic abscesses do not occur, but there can be secondary infection of pseudocysts, usually occurring 4 to 6 weeks after acute pancreatitis and appearing as well-circumscribed complex cysts on imaging studies. This occurrence usually requires surgical débridement.[106]

Intra-abdominal abscesses occur with some frequency in children and are usually secondary to ruptured appendicitis. They can also be secondary to trauma and Crohn disease.[107] US has limited usefulness in searching for intra-abdominal abscess, and contrast-enhanced CT of the abdomen and pelvis with both oral and rectal contrast is the modality of choice.

Various bacterial enteritides, including *Campylobacter jejuni* and *Yersinia enterocolitica*, can also cause thickening and inflammation of the bowel wall, especially in the ileum, and may be associated with mesenteric adenitis, making them difficult to distinguish from Crohn disease.[108]

ACQUIRED CONDITIONS

Liver

Parenchymal disease from various processes, including metabolic diseases, drug and environmental toxicities, and congenital hepatic fibrosis, does not offer specific imaging findings. Some of these processes cause fatty infiltration, fibrosis, and even cirrhosis, all of which have further imaging findings, albeit nonspecific. Fatty infiltration with enlargement of the organ is a common finding in early liver disease, seen on US as diffuse increased echogenicity (Figure 74.2-51). On CT, the liver with fatty infiltration has lower attenuation to the spleen rather than the normally expected similar or increased attenuation (Figure 74.2-52). As liver parenchyma is destroyed and the organ becomes fibrotic, there is a commensurate decrease in size and a heterogeneous increase in echogenicity on US.

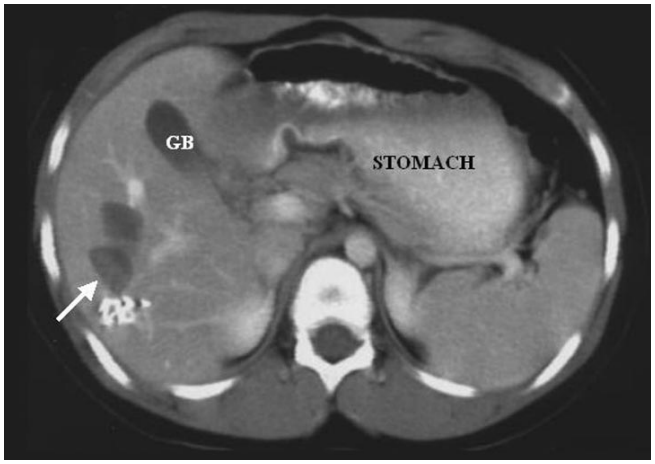

FIGURE 74.2-50 Contrast-enhanced computed tomographic scan of a 14-year-old boy reveals recurrent cystic masses (*arrow*) arising anterior to multiple clips, status postresection of ecchinococcal cysts.

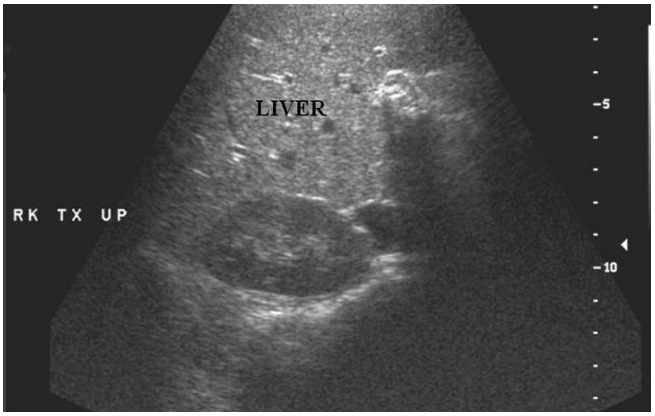

FIGURE 74.2-51 Transverse sonogram of the right upper quadrant shows the liver to be of increased echogenicity compared with the right kidney, suggesting fatty infiltration. Normally, the liver is of decreased attenuation compared with the kidney.

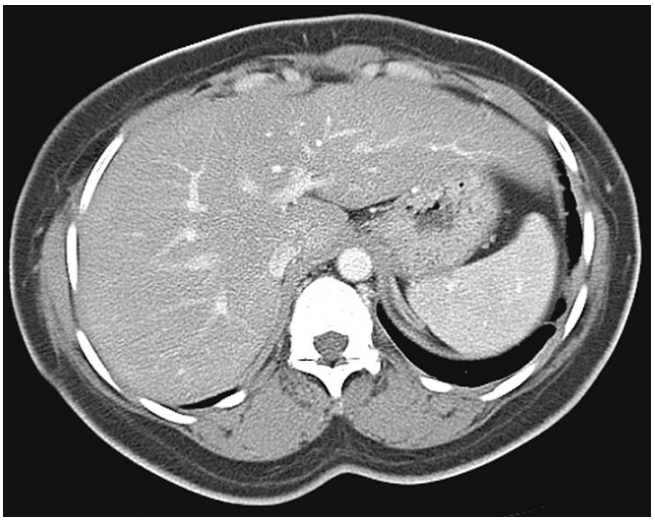

FIGURE 74.2-52 Axial contrast-enhanced computed tomographic scan of the abdomen of an 18 year old with cystic fibrosis reveals that the liver has lower attenuation to the spleen rather than the normally expected similar or increased attenuation, a finding suggestive of fatty infiltration. The spleen is mildly enlarged.

US, CT, or MRI can also demonstrate changes associated with chronic liver disease and cirrhosis. Such findings include macronodular contours, regenerating relative enlargement of the caudate and left lobes,[109] and sclerotic nodules (Figure 74.2-53), as well as the development of portal hypertension, with splenomegaly, varices (Figure 74.2-54), and ascites. Recannalization of the umbilical vein, hypertrophy of the hepatic artery signal, and biphasic and eventual reversal of flow in the portal vein are demonstrated by color Doppler US. Enlarged coronary veins and retroperitoneal varices can be seen with portal vein thrombosis and cavernous transformation. CT and MRI can show collaterals and clots extending into vessels.[110] Increased attenuation of parenchyma can be seen with iron deposition, with glycogen storage disease, and after the administration of cisplatinum for chemotherapy.[111] MRI may be more sensitive than CT for detecting early deposition. Because deposition can be preceded by the accumulation of fat, the liver may initially appear of decreased attenuation on CT.[112] Budd-Chiari syndrome is a relatively rare disorder caused by the acute or chronic obstruction of the hepatic veins, usually by membranous obstruction. It may also be caused by hypercoagulability associated with hematologic and systemic disease. The presentation of Budd-Chiari syndrome is similar to other liver diseases, with right upper quadrant pain, tender hepatomegaly, and possibly ascites. Astute clinical suspicion and proper imaging are essential for diagnosis. Angiography had been the principal diagnostic modality; however, noninvasive duplex Doppler US is now preferred to show the venous anomalies, with a sensitivity that approaches angiography.[113] Contrast-enhanced CT and MRI do not demonstrate venous obstruction as well but do detect the associated parenchymal disease, the relative preservation of the caudate lobe, and large regenerative nodules (Figures 74.2-55 and 74.2-56). [114]

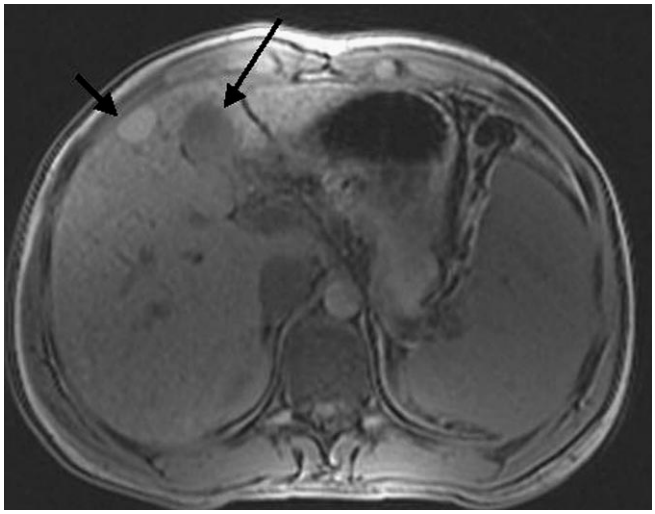

FIGURE 74.2-53 Spoiled gradiant axial magnetic resonance image of the liver in an 18-year-old male patient with chronic active hepatitis B demonstrates multiple nodules within the liver. Most were of low signal characteristic and did not enhance after gadolinium, similar to the nodule demarcated by the *thin arrow*. These were presumed to represent siderotic type nodules. One lesion was of bright signal on multiple sequences but also did not enhance (*short arrow*) and was thought to represent a regenerating nodule. All nodules remained stable over a 3-year follow-up.

Liver Transplant

The most significant and dramatic acquired condition of the liver is transplant. Accurate imaging is crucial for both pre- and post-transplant evaluations. Potential recipients are typically followed by serial US examinations to document progression of disease, the development of portal hypertension, and the patency of vessels essential for graft anastomosis. Congenital anomalies that might complicate the surgical procedure, such as a preduodenal portal vein, are documented. CT and MRI are reserved for

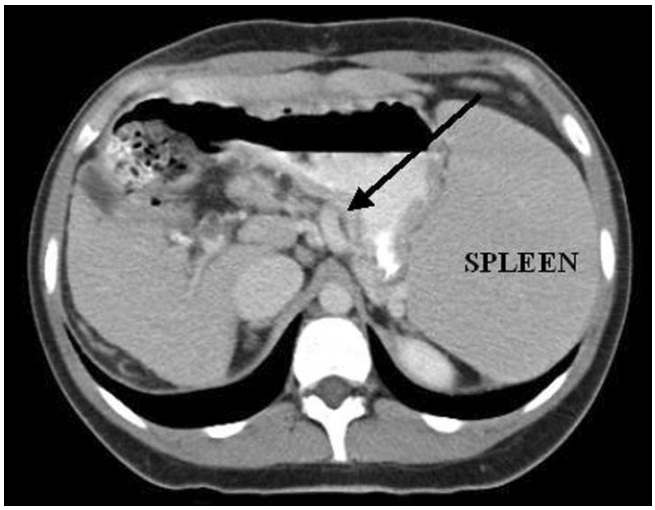

FIGURE 74.2-54 Axial contrast-enhanced computed tomographic scan of the abdomen of a 16-year-old female patient with autoimmune hepatitis reveals splenomegaly, varices (*arrow*), and the inferior portion of a small liver. Clinically, the patient had portal hypertension.

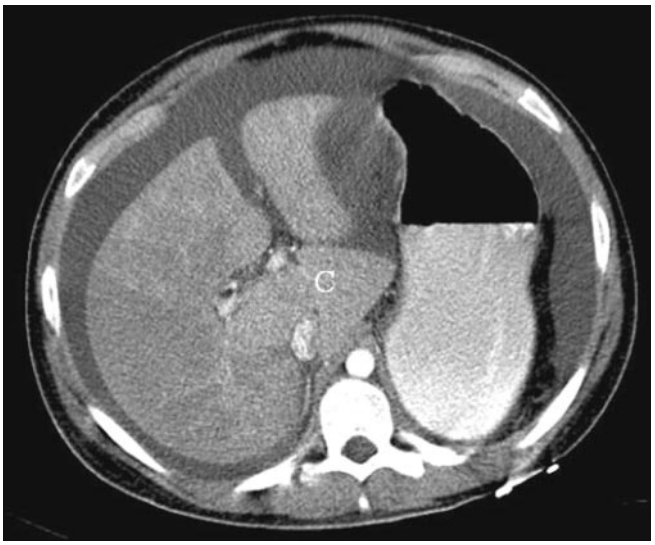

FIGURE 74.2-55 Budd-Chiari syndrome. Axial computed tomographic scan after the intravenous administration of contrast reveals heterogeneous enhancement of the liver, except for the caudate lobe (c), which appears preserved.

excluding the possibility of malignancy[115] and establishing accurate liver volume for surgical planning. MRCP, in conjunction with liver biopsy, is particularly important in excluding large duct primary sclerosing cholangitis as the cause of liver failure because this diagnosis requires that a Roux-en-Y portoenterostomy be performed rather than a duct-to-duct anastomosis.

Serial US examinations are crucial in the postoperative period to access for patency of the newly anastomosed vessels. Vascular complications of stenosis or occlusion, if diagnosed promptly and corrected in the immediate postoperative period, can significantly improve graft success and decrease the incidence of biliary compromise. Bile duct dilatation can be related to vascular compromise or anastomotic stricture. Dilatation or beading of the biliary system on a chronic basis may also be related to prolonged cold-ischemia at the time of transplant. Biliary dilatation will be seen on US or CT if moderate to severe[116] and on MRCP even if mild (Figure 74.2-57). Small focal areas of infarction are not uncommon in the immediate postoperative period and can be detected on US as hypoechoic, peripheral, wedge-shaped areas of decreased perfusion but may be more evident on CT scan, where they appear to be of decreased attenuation.[117] US-guided biopsies and interventional percutaneous transhepatic drainage of bilomas or abscesses[118] play an important role in the postsurgical management. Contrast-enhanced CT of the abdomen and pelvis remains the mainstay of evaluating for post-transplant abscess, collections, and PTLD.

Biliary Disease

The most common form of acquired biliary disease is cholelithiasis, and although the incidence is not as common as in adults, it does occur with some frequency in children, especially in the setting of sickle cell disease, hereditary spherocytosis, and related hematologic diseases (Figure 74.2-58). In children, both gallstones and sludge can be well seen on US (Figure 74.2-59), although the clinical significance of each is different. Sludge is more often associated with prior parenteral nutrition or antibi-

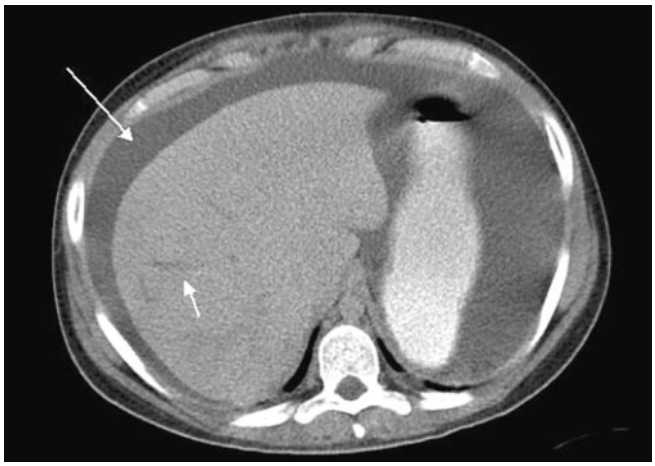

FIGURE 74.2-56 Delayed axial computed tomographic scan after the intravenous administration of contrast reveals filling defects in the liver corresponding to thrombosed hepatic veins (*short arrow*) in this 16-year-old patient with Budd-Chiari syndrome. The *long arrow* demonstrates ascitic fluid surrounding the liver.

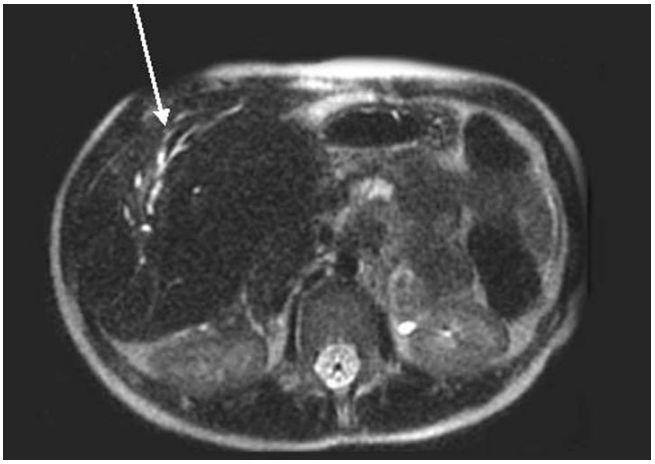

FIGURE 74.2-57 Axial magnetic resonance cholangiopancreatography performed on a 6-year-old girl with rising liver enzymes 5 years following a segmented liver transplant reveals mild intrahepatic biliary dilatation and beading (*arrow*).

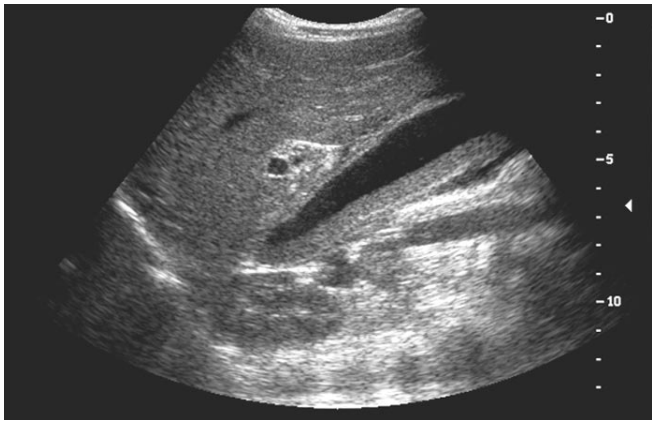

FIGURE 74.2-58 Acute cholecystitis. Sagittal sonogram of the gallbladder reveals a thick wall and a scant amount of pericholecystic fluid (*arrow*).

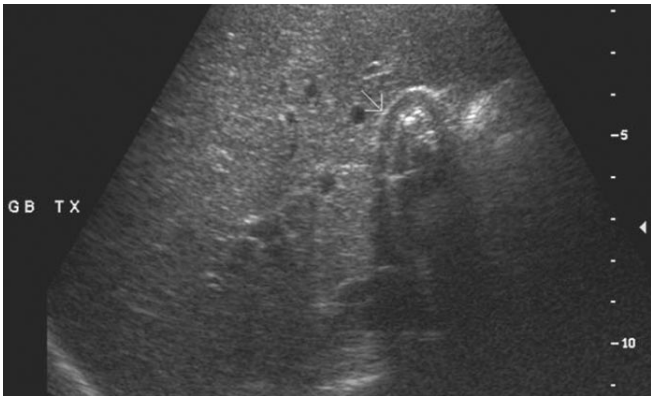

FIGURE 74.2-59 Transverse sonogram of the liver of a 16-year-old female patient with right upper quadrant pain reveals a thick-walled, contracted gallbladder (*arrow*) filled with shadowing stones. The patient was treated for acute cholecystitis.

otics and can appear and resolve in a matter of days. Most stones are related to hemolytic processes and, unlike in adults, do not necessarily require surgery.[119] Common duct stones are rare in children (Figure 74.2-60). Acalculous cholecystitis may show thickening of the gallbladder wall on US, although hypoalbuminemia and infections may cause similar findings.[32]

Primary sclerosing cholangitis, a chronic inflammatory process affecting the extrahepatic and/or medium to large bile ducts, is seen in children and has features suggesting an autoimmune pathogenesis. It is associated with IBD, histiocytosis, and, less commonly, lymphoma and infections related to immunodeficiencies.[120] MRCP is as sensitive as ERCP in demonstrating large duct primary sclerosing cholangitis in children by demonstrating beading of the ducts (Figure 74.2-61).[121]

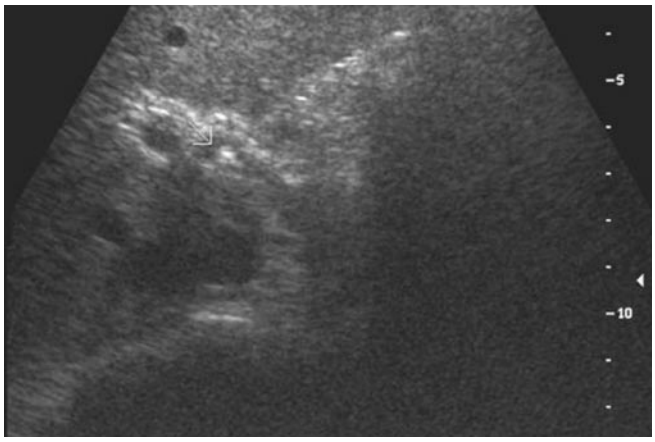

FIGURE 74.2-60 Sagittal sonogram of the porta hepatis reveals an echogenic focus within the common duct (*arrow*), consistent with a stone.

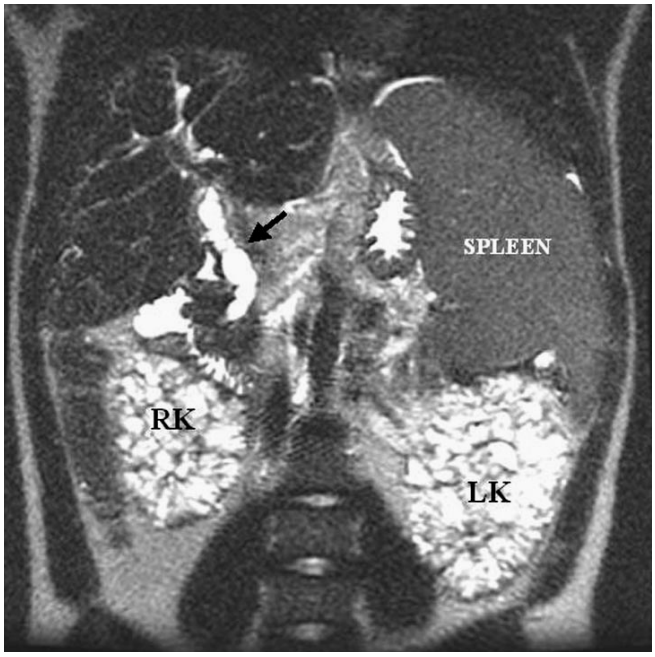

FIGURE 74.2-61 Coronal magnetic resonance cholangiopancreatogram of an 8-year-old boy with autosomal dominant polycystic kidney disease reveals dilatation and beading of the extrahepatic bile duct (*arrow*), as well as milder intrahepatic biliary beading, suggesting the appearance of primary sclerosing cholangitis. Note the multicystic enlarged kidneys and splenomegaly.

Spleen

The spleen is not usually the primary organ of disease processes, but it is frequently secondarily enlarged by neoplasia, infection, venous engorgement from portal hypertension, or infiltrative disease as in storage diseases. Gaucher disease is associated with some of the largest documented spleens (Figure 74.2-62). On all modalities, the parenchyma is usually uniform. Complicating splenomegaly, infarction appears as hypoperfused, wedge-shaped, round, or irregular areas on US and as low-attenuation areas on contrast-enhanced CT unless superimposed hemorrhage causes an increase in attenuation. When followed serially, infarcts tend to contract and progressively liquefy, and may calcify. In sickle cell disease, multiple, recurrent infarctions result in eventual destruction of the entire spleen.[122]

Pancreas

Of the identifiable causes of pancreatitis, trauma is the most common in childhood, and abuse should always be excluded. Nontraumatic pancreatitis may be caused by viral infection, hypercholesterolemia, and obstruction. Obstruction as an etiology is much less common in children than in adults. It may appear as focal or diffuse enlargement, which is sometimes difficult to define because there is great variability in the size of the normal pancreas. US is an excellent modality for the initial study; however, owing to obscuring bowel gas, demonstration of the entire pancreas can present challenges. Associated findings of acute pancreatitis include ductal enlargement, either decreased or increased echogenicity of the entire gland, or heterogeneous echogenicity within the same gland. Peripancreatic fluid can be detected loculated

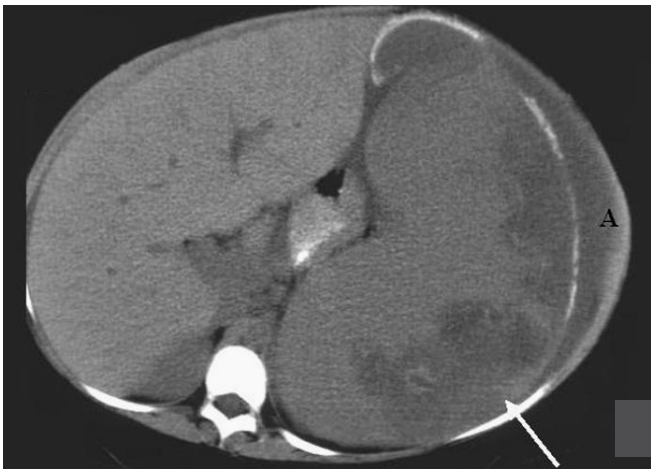

FIGURE 74.2-62 Axial non–contrast-enhanced computed tomographic scan of the abdomen of a 16-year-old female patient with Gaucher disease demonstrates marked splenomegaly with multiple peripheral low-attenuation areas consistent with infarcts (*arrow*). A thin calcified rim is present. Free ascites (A) is also present.

adjacent to the pancreas or free in the peritoneal space as ascites and pleural effusions (Figure 74.2-63). Phlegmons, fistulae, infarctions, and pseudocysts[123] can complicate pancreatitis. CT with contrast has been used to evaluate pancreatic morphology, detect pancreatic necrosis, and depict retroperitoneal complications, resulting in a CT staging severity index that has proven to be a reliable indicator of prognosis (Figure 74.2-64).[124] In children with recurrent episodes of idiopathic acute pancreatitis, MRCP allows visualization of the pancreatic duct anatomy, including side branches, ductal narrowing, endoluminal filling defects, irregular ductal contour, cavities, and pancreas divisum, thus increasing the ability to diagnose risk factors associated with chronic pancreatitis (Figure 74.2-65).[125]

Chronic pancreatitis, relatively rare in childhood, shows ductal dilatation, irregularity, calcification, pseudocyst formation, and, ultimately, a smaller gland. In cystic fibrosis, pancreatic atrophy and fatty infiltration may be dramatic (Figure 74.2-66).[126] Diffuse enlargement or focal alteration of the gland is seen in fibrosing pancreatitis and may result in biliary obstruction.[127]

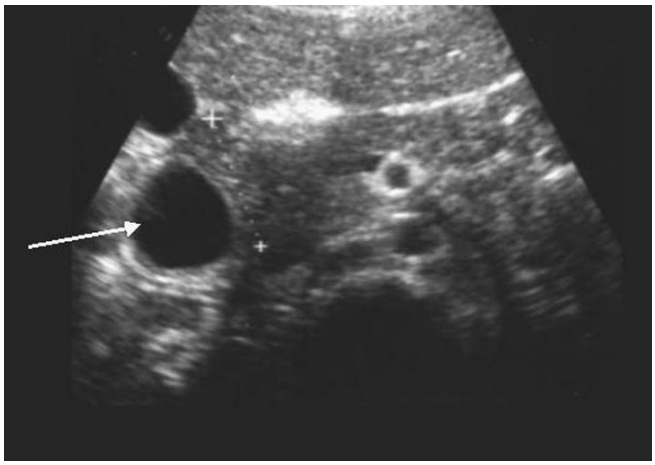

FIGURE 74.2-63 Transverse sonogram of the abdomen reveals an enlarged head of the pancreas with a central pseudocyst (*arrow*).

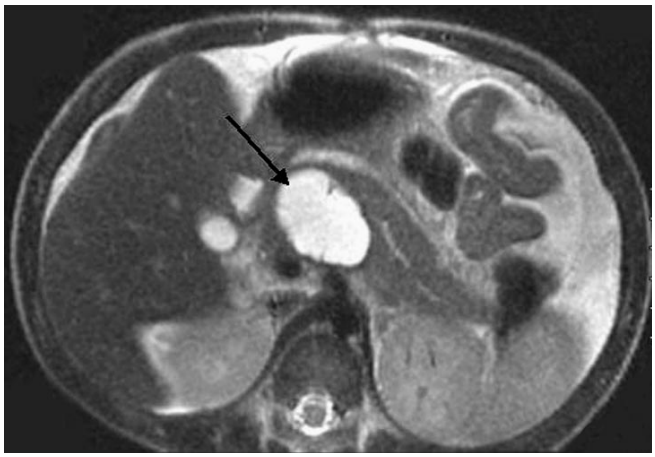

FIGURE 74.2-65 Axial magnetic resonance cholangiopancreatogram of a 5-year-old boy with a history of abuse demonstrates a large pseudocyst in the pancreas (*arrow*).

Gastrointestinal Tract

Cross-sectional imaging of the gastrointestinal tract has dramatically altered the standard of care in gastrointestinal disorders over the past several decades. Various imaging studies are routinely ordered for diagnosing entities such as appendicitis, intussusception, IBD, and even bowel obstruction, although plain films and contrast studies, coupled with clinical judgment, still play a very large role in the diagnosis and management of these disorders.

Up to 20% of small bowel obstruction will not be evident on plain films,[128] but it is unclear whether these undetected cases can be managed conservatively or indeed need surgical management. Contrast studies are at times not useful owing to delayed transit time and dilution of contrast. US examination of small bowel obstruction will demonstrate either hyperactive or hypoactive, atonic and dilated, fluid-filled bowel, but the same findings can be seen in gastroenteritis.[129] US may suggest malrotation based on demonstrating the superior mesenteric vein to the left of the superior mesenteric artery.[130] CT can reveal

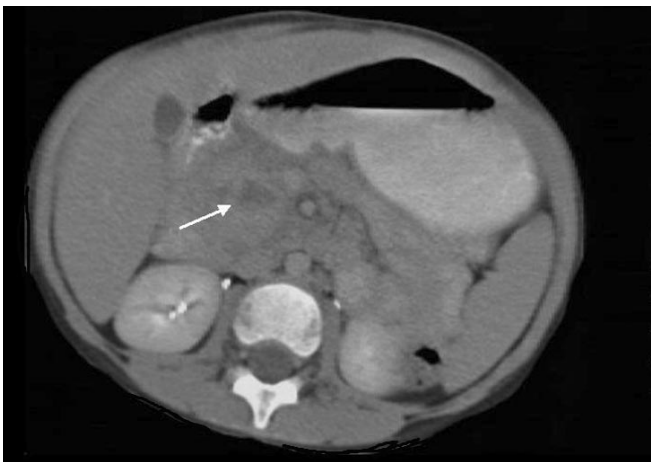

FIGURE 74.2-64 Axial contrast-enhanced computed tomographic scan of a 12-year-old boy with familial hypercholesterolemia and pancreatitis reveals an enlarged head of the pancreas and a central pseudocyst (*arrow*).

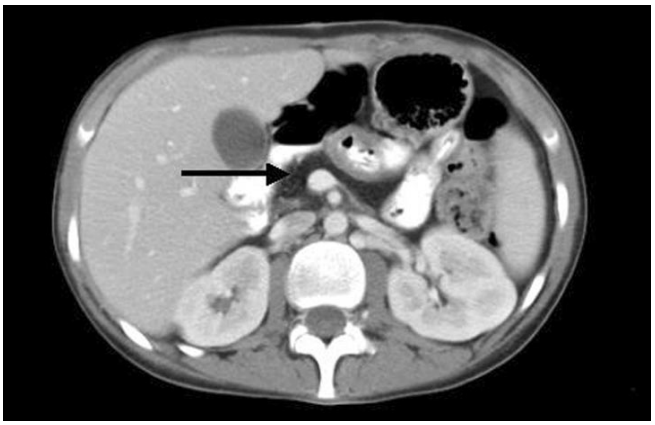

FIGURE 74.2-66 Axial contrast-enhanced computed tomographic scan of an 18-year-old female patient with cystic fibrosis reveals complete fatty replacement of the pancreas (*arrow*). The patient clinically had insulin-dependent diabetes.

high-grade obstruction with dilated loops of bowel and closed-loop strangulating obstruction, and some authors advocate CT as the investigative modality of choice. Specific causes of small bowel obstruction can be seen on cross-sectional imaging studies, although traditional SBFT remains helpful.[128]

Causes of obstruction include congenital duplication and mesenteric cysts. If the cyst contains gastric mucosa, it can ulcerate, bleed, or even perforate.[129] Another cause of intestinal obstruction is Meckel diverticulum, which can contain gastric mucosa and therefore can be detected by radionucleotide scan. Occasionally, the diverticulum can be imaged by US or CT[131] as the lead point for an intussusception, with a blind-ended, thick-walled bowel segment projecting beyond the apex of the intussusceptum.[132]

Appendicitis

The diagnosis of appendicitis,[133] once a clinical one with a large margin of error, has been considerably changed by advances in cross-sectional imaging. The US finding of an appendix greater than 6 mm in diameter, with a blind-ending lumen that is noncompressible and hyperemic, became the hallmark of acute appendicitis, with up to a 90% sensitivity and 95% specificity.[134] The presence of a appendicolith and periappendiceal fluid supports the diagnosis,[135] although documentation of an appendicolith may be associated with normal appendices in up to 14% of surgically explored cases.[136] US is useful to exclude ovarian pathology in female patients and can demonstrate abscess formation suggesting perforation. Unfortunately, as in physical examination, US is limited by overlying bowel gas and the fact that many appendices are not in a typical location. The inability to demonstrate an inflamed appendix does not exclude acute appendicitis on US. Because the technique uses graded compression, US is uncomfortable and can be difficult to perform in uncooperative children. Furthermore, US of the appendix is highly operator dependent.

Because of these limitations, CT of the abdomen and pelvis has largely replaced US for the diagnosis of acute appendicitis. Various techniques are advocated, including contrast- and noncontrast-enhanced techniques. Some radiologists prefer oral, rectal, and intravenous contrast, whereas others perform the examination with only rectal contrast. Despite these differences in technique, the objective is to visualize the enlarged and inflamed appendix (Figure 74.2-67) and an appendicolith if present (Figure 74.2-68) and to identify any associated findings of pericecal fluid and inflammation. CT is also used extensively to document suspected complications such as abscess formation both pre- and postoperatively (Figure 74.2-69). With the widespread use of US and CT, the negative appendectomy rate has dropped from 15 to 4%, whereas the rate of perforation has decreased from 35 to 16% in one reporting institution.[137]

Intussusception

The most common cause of intestinal obstruction from 6 months to 6 years of age is intussusception, which most commonly occurs before age 2 years, with a peak incidence between 3 and 9 months. Of the intussusceptions that

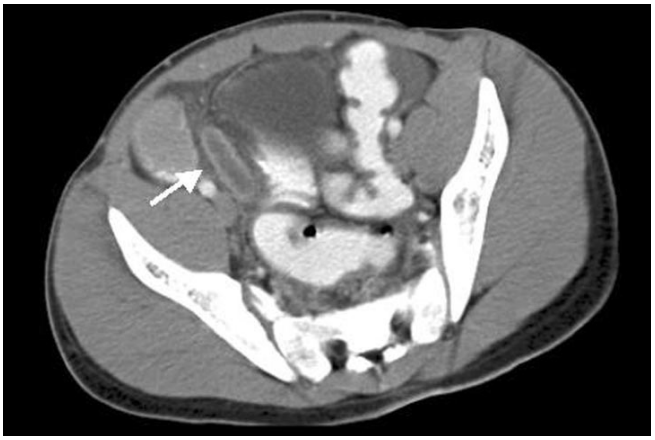

FIGURE 74.2-67 Axial contrast-enhanced computed tomographic scan with oral and rectal contrast reveals a fluid-filled, swollen appendix in the right lower quandrant (*arrow*). The inflammed appendix wall is enhancing.

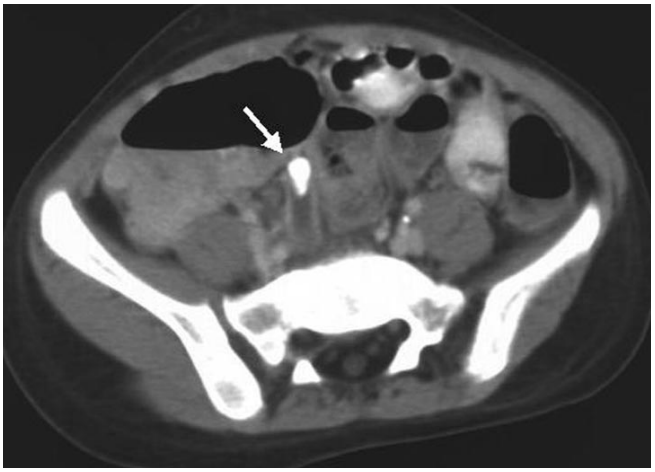

FIGURE 74.2-68 Axial computed tomographic scan of the pelvis reveals a thick tubular structure in the right lower quandrant consistent with a swollen appendix. A calcified appendicolith (*arrow*) is present.

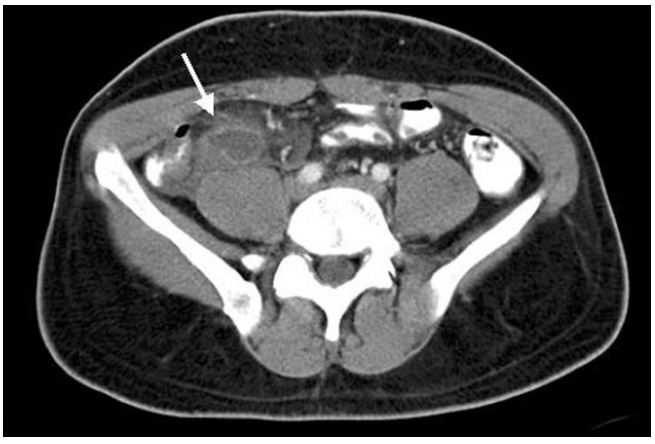

FIGURE 74.2-69 Axial contrast-enhanced computed tomographic scan of the pelvis reveals a fluid collection with a thin enhancing rim in the right lower quandrant (*arrow*) adjacent to thick-walled bowel. At surgery, a perforated appendix with a periappendiceal abscess was found.

occur in the typical age group, 90% are ileocolic and probably only 5% have a lead point, whereas the occurrence under the age of 6 months and over 3 years of age can be located in other segments of the bowel and may have a lead point.[138] Not all children will have the typical clinical presentation of episodic cramps, currant jelly stools, and abdominal pain, making it most important to maintain a high clinical suspicion and have sensitive imaging techniques to confirm. Findings of intussusception may be evident on plain film radiographs, but in 25% of cases, the bowel pattern is normal. Enema, using either barium or air, is the gold standard, both for diagnosis and treatment.

US is a reliable study for diagnosing intussusception, although it may not reach the high sensitivity and 100% negative predictive values that have been reported.[139] The characteristic finding is an intra-abdominal mass that demonstrates a hypoechoic ring of bowel wall mucosa surrounding an echogenic center of trapped mesenteric fat and vessels, the "doughnut sign" (Figure 74.2-70).[140] Multiple concentric circles may be seen when there are multiple layers of both intussusceptum and intussuscipiens. In the plane longitudinal to the mass, there are alternating hypoechogenic and echogenic layers, the "sandwich" or "pseudokidney" sign.[141] Thickening of the echolucent rim greater than 8 mm and fluid within the intussusceptum, especially if there is a dilated apex, have been suggested as indications of significant ischemia and irreducibility by enema reduction.[142] Free intraperitoneal fluid and bowel thickness did not alter outcome of reduction in another large series; however, the presence of small bowel obstruction on plain film reduced the success rate from 90 to 65%.[143]

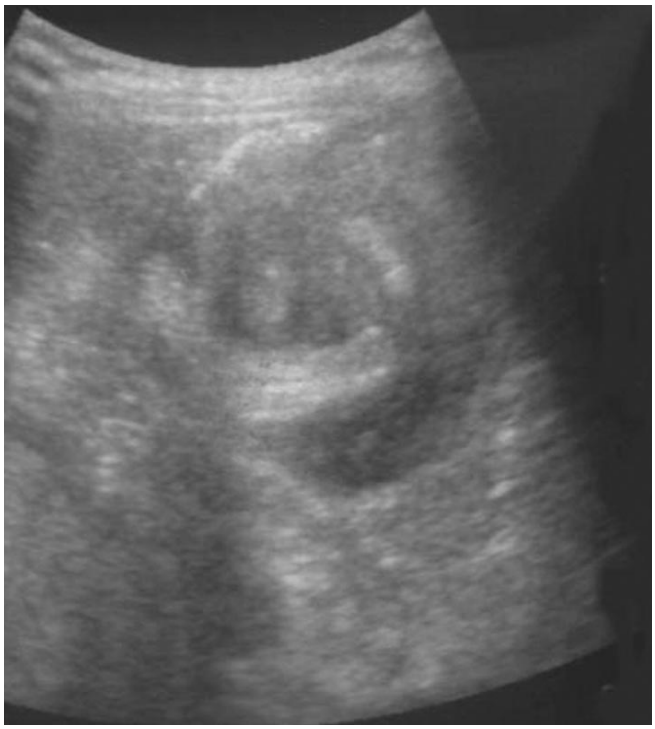

FIGURE 74.2-70 Sonogram of the abdomen in a 2 year old with crampy abdominal pain reveals a soft tissue mass in the right upper quadrant. The alternating echogenic and hypoechoic layers are typical for an intussusception.

Clinical peritonitis is a contraindication to hydrostatic or air reduction. An intussusception present for greater than 48 hours or one in an infant less than 6 months with bowel obstruction on plain films has a high likelihood of perforation.[144] Lead points have included most polyps, Meckel diverticulum, duplication cysts, and submucosal bleeds associated with Henoch-Schönlein purpura. Although the vast majority of intussusceptions are reduced under conventional fluoroscopic guidance, recent attempts at air reduction under US guidance have been met with some success.[145]

INFLAMMATORY CONDITIONS

Various inflammatory processes demonstrate bowel wall thickening on cross-sectional imaging. Although this is usually caused by an infectious process and is accompanied by mesenteric adenitis, IBD can cause similar findings. Using bowel wall thickness as a criterion for diagnosing IBD on US and comparing it with colonoscopic findings as the gold standard, US showed a sensitivity of 88% and a specificity of 93%.[146] In addition, CT can demonstrate transmesenteric disease associated with Crohn disease, such as creeping apposition of fat, mesenteric lymphadenopathy, and fistula formation helping to distinguish Crohn disease from ulcerative colitis (Figures 74.2-71 and 74.2-72). Intravenous contrast will enhance the margins of inflamed bowel wall or an inflammatory mass (Figure 74.2-73), whereas oral or rectal contrast will identify lumen. Another study, which looked at a combined group of IBD, infectious colitis, pseudomembranous colitis, Henoch-Schönlein purpura, and hemolytic syndrome, could not differentiate one condition from another.[147] Bowel thickening without transmesenteric disease is, however, more typical of colitis from causes other than Crohn disease (Figure 74.2-74).[148] Currently, MRI is less effective in demonstrating bowel pathology.[149]

In necrotizing enterocolitis, US has been demonstrated to be sensitive in detecting bowel wall thickening, pneumatosis intestinalis, portal venous gas, and perforation causing inflammatory mass[150]; however, serial plain films

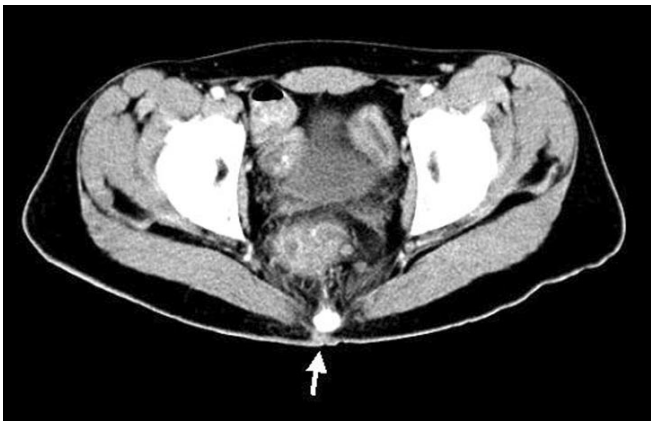

FIGURE 74.2-71 Contrast-enhanced computed tomographic scan of the pelvis in this 15-year-old male patient with Crohn colitis reveals contrast extending into a perirectal fistula (*arrow*).

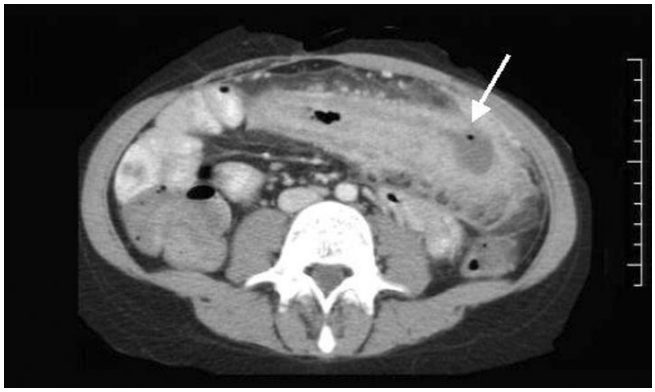

FIGURE 74.2-72 Axial contrast-enhanced computed tomographic scan of the abdomen in an adolescent female with Crohn disease reveals an abscess arising from the wall of the transverse colon (*arrow*).

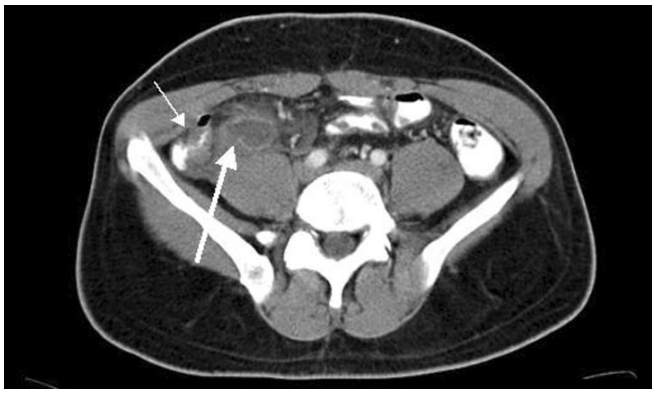

FIGURE 74.2-73 Axial contrast-enhanced computed tomographic scan of the pelvis reveals a collection with an enhancing rim abutting the psoas muscle (*long arrow*) in this adolescent male with Crohn disease and right lower quadrant pain. Note the thick-walled bowel adjacent to the abscess (*short arrow*).

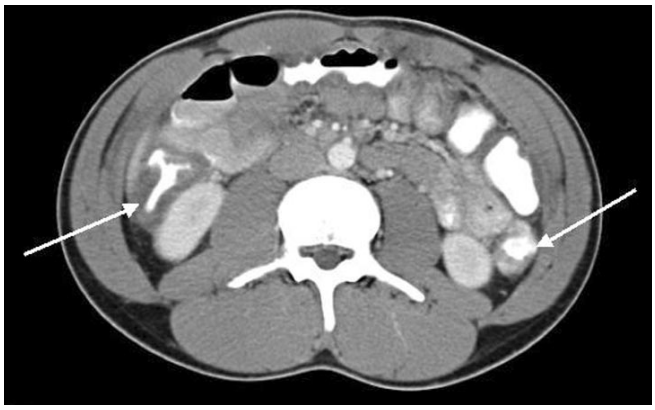

FIGURE 74.2-74 Contrast-enhanced computed tomographic scan of the lower abdomen reveals a thick-walled ascending colon (*up arrow*) and a descending colon (*down arrow*) in this teenager with known colitis.

remain the mainstay of diagnosis. In an investigative study, MRI showed good correlation with operative findings in a small group of premature infants determined to have necrotizing enterocolitis.[151] However, in view of the technical and practical considerations, cross-sectional imaging probably does not have a great deal to contribute to the care of these critically ill infants.

CONCLUSION

Imaging technology has markedly altered the traditional workup of gastrointestinal disorders. Ongoing advances in US, CT, and MRI have revolutionized the approach to many common diseases and greatly increased the accuracy of diagnosis. The risks of radiation exposure, contrast toxicity, and sedation should always be considerations, especially when a pediatric patient is concerned. As with any modality, an understanding of the risks, benefits, and limitations of a procedure is needed to effectively use these sophisticated technologies.

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3. Interventional Gastrointestinal Radiology

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Pediatric interventional radiology[1,2] has expanded in the last 5 to 10 years as a direct result of the improvement in cross-sectional imaging, including ultrasonography, computed tomography (CT), and magnetic resonance imaging (MRI), as well as rapid biotechnologic advancement in the development of catheter materials, balloons, wires, stents, filters, retrieval devices, and embolic and sclerosing agents.[3,4] The development of this subspecialty has been facilitated by the emergence of pediatric radiologists specifically trained in interventional procedures. To perform these procedures, a fully equipped, dedicated interventional facility must be established. This facility would include anesthetic equipment and monitoring for sedation, color Doppler ultrasonography with a variety of high-resolution interventional probes, a CT scanner, and, finally, a C-arm interventional fluoroscopic table with digital subtraction.

INTERVENTIONAL RADIOLOGY SERVICE

To provide a highly successful service that achieves excellence in patient care, a team approach is stressed.[3,5] Dedicated professionals should include a pediatric interventional radiologist; a fellow or resident in training; pediatric radiology nurses with training in patient assessment, sedation, and postsedation recovery; and technologists trained in angiography, interventional procedures, CT, and ultrasonography. It is important for the team members to liaise with referring physicians, other radiologists, the parent, and the patient to provide the best care possible.[5] The development of the interventional radiology service provides both inpatient and outpatient care and establishes interventional radiology as an important primary service.[4,6]

The following overview is based largely on the experience at The Hospital for Sick Children, Toronto, during the past 4 years. In this period, there has been significant growth and development of interventional procedures, with approximately 1,500 interventional procedures being performed per year. Gastrointestinal (GI) and biliary procedures represent almost half of these procedures. Gastrostomy tube (G tube) placement, follow-up studies, and G-tube changes represent the largest single technique performed at our hospital.

PREPROCEDURE PLANNING

Careful assessment of all patients prior to performing procedures is an essential prerequisite. This includes discussion with the referring physician with respect to the indications and specifics of the procedure required; assessment of the patient; and review of previous imaging studies and laboratory findings, including prothrombin time, partial thromboplastin time, platelets, and hemoglobin, the patient's medical history, drug allergies, and response to previous sedation. Where necessary, further imaging or laboratory studies are ordered and other services are consulted, particularly with regard to patient safety and the suitability of the procedure.[7,8] If sedation or anesthesia is required, intravenous access is placed prior to the procedure. According to the guidelines of the American Society of Pediatrics, presedation or anesthetic orders should include no solid food for 6 hours prior to the procedure, but clear fluids up to 2 hours are permissible.[9] We strictly enforce these guidelines and delay the procedure if necessary. At least 1 hour prior to the procedure, Emla cream (Astra, Mississauga, ON), a topical anesthetic, is applied to the area where percutaneous entry is to be performed. This significantly decreases the pain experienced when local anesthetic is administered.

SEDATION AND ANALGESIA

During the past year, 45% of procedures were performed with a local anesthetic alone, 50% with a local anesthetic plus sedation, and 5% with a general anesthetic. The need for a general anesthetic is determined by the status of the patient, the nature of the procedure, and, in some cases, the needs of the physician.

Patients are categorized according to the American Society of Anesthesiologists' classification[10]:

I. Normal healthy patient
II. Patient with mild systemic disease
III. Patient with severe systemic disease that limits activity but is not incapacitating
IV. Patient with incapacitating systemic disease that is a constant threat to life
V. Moribund patient not expected to survive 24 hours with or without intervention

Class I and II patients are candidates for conscious deep sedation. Patients in class III or IV require special considerations; they generally require general anesthesia but should be dealt with on an individual basis. Patients who have airway anomalies or who have experienced airway complications during past anesthesia or sedation should be thoroughly assessed prior to the administration of any sedative or anesthetic agent. In neonates with oropharyngeal or airway problems, the procedures may be performed with a local anesthetic alone. In older, cooperative children, a local anesthetic alone or combined with

sedation may be all that is needed. General anesthetic is also indicated for (1) lengthy procedures, (2) complete patient cooperation, (3) an area of interest close to vital structures, and (4) procedures requiring a high degree of technical expertise and accuracy.

Numerous drugs are available for sedation and pain control.[11–19] Individuals ordering and administering the drugs and those monitoring the patient should be comfortable with the drugs in use; their effective use is best accomplished by using a carefully described protocol. We use a combination of drugs for sedation and pain control (Table 74.3-1).

We do not use intramuscular sedation. If sedation is required, intravenous access is essential, whereas the response to intramuscular injection is variable. The pain experienced during the introduction of 1% lidocaine into subcutaneous tissues is thought to be due to its low pH. Premixing the lidocaine with bicarbonate prior to injection increases the pH and appears to effectively reduce the pain.[20] It is also helpful to inject the lidocaine with a 27- or 30-gauge needle at a slow rate. Patients are monitored with pulse oximetry, blood pressure, and electrocardiography (ECG) throughout the procedure and following the procedure until they are fully responsive.[21] Postprocedure vital signs are recorded every 5 minutes until the patient awakes and then every 15 minutes. The patient is discharged from the radiology department when cardiovascular and airway stability is ensured and the patient is alert and can talk, sit unattended, and ambulate with assistance.

PATIENT PREPARATION

It is important to predetermine the best position for the patient undergoing the procedure, whether it be supine, oblique, decubitus, or prone. The position of the head of the patient, the ultrasound machine, the intravenous (IV) pole, the anesthetist (if present), and the radiologist should all be predetermined prior to placing the patient on the table. The position of the patient's arms, monitors, and ECG wires is optimized for the procedure and for patient comfort, which is always a high priority. For smaller patients, a restraining device (Figure 74.3-1) is useful to reduce the need for sedation and improve patient control. The body temperature of the neonate or small child is maintained by either increasing the room temperature, placing the child on heating blankets, covering the child with blankets or plastic wrap, using radiant heat from a baby warmer, or blowing hot air over the infant. Most gastrointestinal procedures accessed percutaneously require antibiotic coverage. For upper gastrointestinal procedures, a first-generation cephalosporin (cefazolin 20–30 mg/kg) is used as a single dose prior to the procedure.[22] For small bowel and colorectal procedures, we use cefoxitin (25 mg/kg) and for biliary procedures cefazolin (20 mg/kg).

IMAGING GUIDING SYSTEMS

Interventional procedures may be performed under fluoroscopic, ultrasonographic, or CT guidance or a combination of these modalities. The relative merits of the various guidance systems are listed in Table 74.3-2. Pediatric patients are ideally suited to ultrasonography guidance because of the small size of the patient and the decreased subcutaneous and intraperitoneal fat.[23,24] Furthermore, ultrasonography uses no ionizing radiation, is portable, and provides a real-time image. It is not suitable for structures that are poorly visualized or obscured by overlying bone or air-filled structures. Therefore, structures that can be clearly visualized, such as abdominal solid organs and masses, are ideally suited for ultrasonography guidance. Fluoroscopy is generally used for guidance in areas in which there are differences in x-ray density, such as needle placement into a gas-filled stomach. When ultrasonography guidance is used to place a needle in the correct position, fluoroscopy is often used to allow further intervention, whether this is wire placement, stent deployment, or merely injection of contrast. CT is infrequently used in the pediatric setting. It is indicated primarily when lesions are small or not well visualized by ultrasonography or fluoroscopy.[25] The prime disadvantage of this modality is that it is not portable and fails to provide real-time images. Furthermore, it is time-consuming and therefore expensive and exposes the patient to significant radiation. It is, however, often used in the diagnostic evaluation to exclude other pathologies and to define anatomy and structural relationships, thereby allowing planning of a procedure. After prior imaging and laboratory studies have been adequately reviewed, the optimal guidance method is chosen to provide the safest, easiest, and least invasive approach. Intervening structures such as bowel loops, fluid-filled bladder, vessels, and other sterile spaces, particularly the pleura and the peritoneal cavity, are avoided to reduce complications.

CONTRAST MEDIA

Water-soluble contrast media are used for all interventional procedures performed under fluoroscopy or those requiring fluoroscopic guidance after ultrasonography or CT placement of a needle. High osmolar contrast media are hypertonic ionic triodinated fully substituted benzene derivatives with osmolalities of 1,200 to 2,000 mOsm/L (four to seven times the osmolality of blood). Low osmolar

TABLE 74.3-1 DRUGS AND DOSAGES FOR SEDATION AND PAIN CONTROL

0–5 KG	5–20 KG	> 20 KG
Chloral hydrate 50–80 mg/kg (oral) then meperidine (IV) 1 mg/kg or morphine (IV) 0.05 mg/kg	Pentobarbital 3 mg/kg (IV) then 5 min meperidine 1 mg/kg (IV) then repeat if necessary	Diazepam 0.1 mg/kg (IV) then meperidine 1 mg/kg (IV) then repeat if necessary

IV = intravenous.

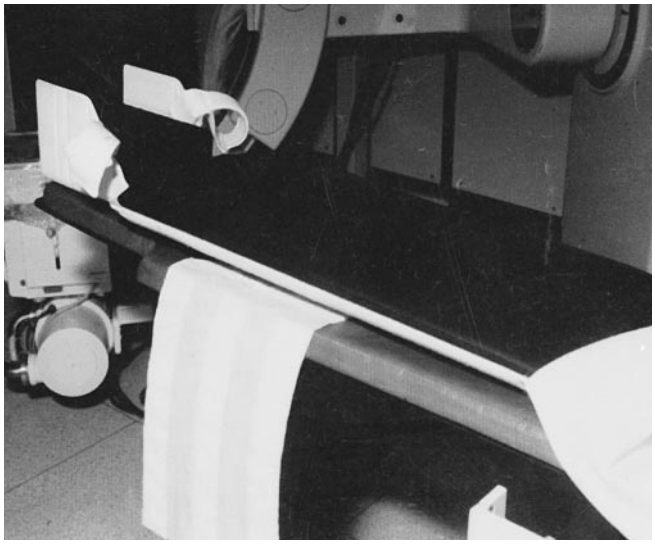


FIGURE 74.3-1 General Electric restraining device designed primarily for computed tomography.

contrast media (LOCM) are significantly more expensive, with significantly less osmolar composition,[7] with an osmolality of approximately 470 mOsm/L. Numerous studies have documented a reduction in overall adverse reactions with the use of LOCM in childhood.[8–12] LOCM also offer a reduction in side effects, especially nausea and vomiting. All water-soluble contrast media are absorbed from the GI tract and other tissues. The high osmolar agents cause significant pain if introduced into the subcutaneous tissue, may cause tissue necrosis,[26] and, obviously, produce an increased risk of pulmonary edema if aspirated. Because of these considerations, low-osmolar, nonionic contrast media are uniformly used for procedures in our institution. Barium sulfate and other varieties of barium suspensions are rarely used, and they are contraindicated if there is any risk of peritoneal spill,[27] extravasation into tissues, or aspiration.[28] Barium is used to do G- or gastrojejunostomy (GJ) tube checks to confirm positions provided that there is no risk of intraperitoneal spillage.[29]

SPECIFIC PROCEDURES

Percutaneous Gastrostomy and Gastroenterostomy

Percutaneous G tube and GJ tube are by far the most common procedures performed in our diagnostic imaging department. Before 1980, the only approach to G-tube placement was surgical.[30] In 1980, Gauderer and colleagues described G-tube placement by a percutaneous endoscopic gastrostomy (PEG) technique.[31] Most pediatric institutions now use this technique.[32–36] A radiologically guided retrograde technique was described a few years later,[37–44] followed by a radiologically guided antegrade technique similar to the PEG1 (Table 74.3-3[45–58]). More recently, laparoscopically performed gastrostomy has been described as a safe alternative to open surgical gastrostomy in patients who cannot undergo percutaneous gastrostomy.[59–64] All approaches to nonsurgical G-tube placement have proved to be cost-effective, with reduced morbidity and mortality.[32,65–67]

There has been a concomitant increase in the number and variety of replacement tubes (Figure 74.3-2) and types of enteral feeding formulas. This has allowed provision for nutrition support on an ambulatory basis using pump-regulated overnight enteral feeding. Currently, the Home Feeding Program at The Hospital for Sick Children, Toronto, monitors approximately 200 patients on overnight enteral feeds compared with only 8 to 10 patients on home total parenteral nutrition. Almost all G tubes are placed in diagnostic imaging; approximately 120 new G tubes are inserted annually. This large patient group requires specialized care, which is best approached by a medical team including interventional radiologists, nutritionists, gastroenterologists, dietitians, and trained enterostomy nursing staff.[68]

Informed consent is obtained either from the patient (if over 16 years and able to give consent) or from the parent or

TABLE 74.3-2 RELATIVE MERITS OF DIFFERENT IMAGING GUIDING SYSTEMS

ADVANTAGES		
FLUOROSCOPY	**ULTRASONOGRAPHY**	**COMPUTED TOMOGRAPHY**
Availability	Rapid localization	Small lesions shown
Rapid localization	Flexible imaging	Needle tip easily seen
Needle tip easy to identify	Flexible patient positioning	Precise anatomic relationship revealed
Diaphragm easily seen	No radiation	Precise target sampling
Modality of choice for further imaging and intervention after needle placement	Ideal for superficial structures or solid organs or masses	No interference because of overlying bowel or gas images, easy to comprehend in three dimensions
Real time	Portable	
	Real time	
DISADVANTAGES		
FLUOROSCOPY	**ULTRASONOGRAPHY**	**COMPUTED TOMOGRAPHY**
Poor target visibility	Needle difficult to see	Time-consuming
Radiation exposure	Limited anatomic information	Expensive
Not portable	Obscured by gas or bone	Radiation exposure
	More difficult technically with significant learning curve	Not portable
		Not real time

TABLE 74.3-3 RELATIVE MERITS OF ANTEGRADE AND RETROGRADE GASTROSTOMY TECHNIQUES

ADVANTAGES	
ANTEGRADE	**RETROGRADE**
Stable catheter	Seldom needs general anesthetic—can be performed on unstable patient with airway instability with local anesthetic alone[45] Catheters are smaller and less bulky Can be performed in patient with esophageal strictures or atresia No risk to upper airway or esophagus Change, removal, or manipulation of catheter is easily performed on an outpatient basis Versatile
DISADVANTAGES	
ANTEGRADE	**RETROGRADE**
May go through other loops of bowel (colon)[46] Potential esophageal damage[47] Not possible if esophageal stricture or atresia Pulls down bacteria and infection from the mouth, pharynx, and esophagus[47,48] Difficult to remove (may need repeat gastroscopy)[47–51] Requires nasogastric and orogastric tubes for radiologic placement[49,52–54] If catheter is cut, it may cause obstruction[55,56] Catheters are bulky[57,58]	Easier to dislodge Catheter smaller

guardian. The various risks of the procedure are explained, including gastric leak with associated cellulitis or peritonitis, which is the most significant complication. Other complications include the risk of hemorrhage and some discomfort and pain after the procedure.[69] Six hours before the procedure, a nasogastric tube is inserted and barium is administered so that the colon will be outlined. Prior to the procedure, antibiotics (cefazolin 30 mg/kg) and an analgesia (rectal acetaminophen 15 mg/kg) are administered and Emla cream is applied to the left upper quadrant to reduce the discomfort during introduction of a local anesthetic. The position of the lower edge of the liver and spleen is marked using ultrasonography (Figure 74.3-3). The patient is assessed to determine the need for sedation, a general anesthetic, or a local anesthetic alone. The choice of drugs and the doses used are outlined in the previous section on sedation.

Procedure

The patient is placed on a C-arm fluoroscopic table (Figure 74.3-4).[44] Fluoroscopy is used to identify the contrast-filled colon (Figure 74.3-5). If this is not adequately filled with contrast, a dilute barium single-contrast enema is performed. The patient is given IV glucagon (0.2–0.5 mg). Stomach contents are aspirated via the nasogastric tube. Air is then injected into the stomach under direct vision, and the chosen site for placement of the G tube (lateral to the left rectus muscle and below the costal margin) is marked with a metallic object. Safe access at this site is ascertained prior to skin cleansing and introduction of a local anesthetic. The C-arm table is tilted as necessary to ensure a safe route into the stomach. The stomach is then deflated. Using standard sterile technique, with operating room gowns and gloves, the anterior abdominal wall is prepared and draped.

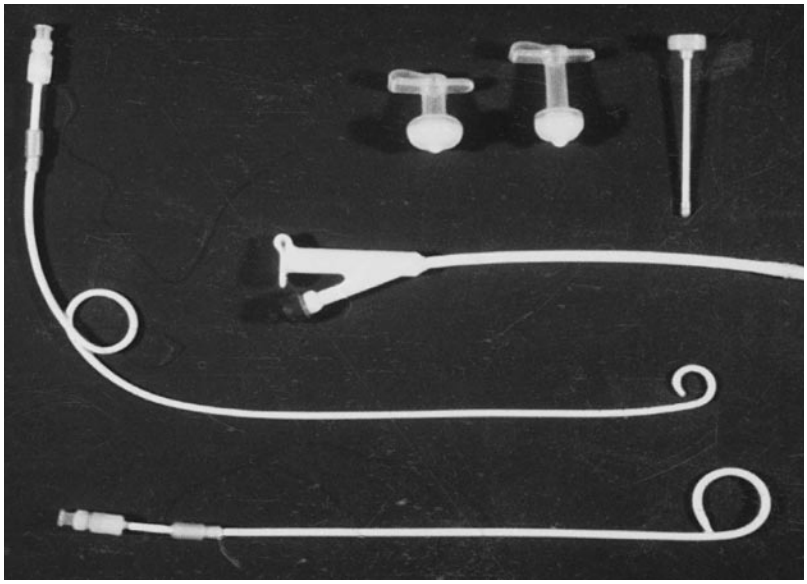


FIGURE 74.3-2 A variety of feeding tubes. *A*, Cope loop gastrostomy tube. *B*, Chait gastrojejunostomy tube. *C*, Balloon gastrostomy tube. *D*, Bard low-profile button gastrostomy tube.

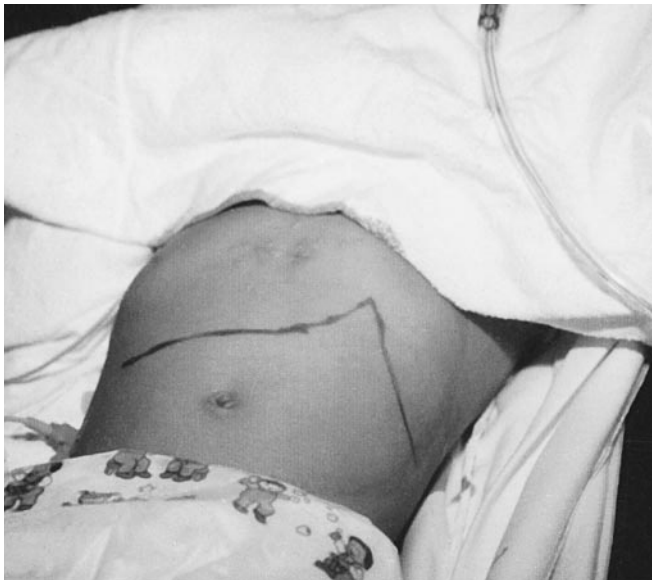


FIGURE 74.3-3 Ultrasonography is used to mark the lower limits of the liver and spleen on a patient prior to gastrostomy placement.

The chosen site for puncture is then infiltrated with 1% lidocaine (maximum dose of 0.5 cc/kg) using a 27-gauge needle. A spinal needle is used if the depth to the stomach is of significant distance. An incision is made with a number 11 scalpel blade to approximately equal the size of the catheter that is to be introduced. Usually, an 8.5 French catheter is used in the neonate; in infants and slightly older children, a 10 French catheter is used, whereas in children over 5 years of age, a 12 French catheter is introduced. The stomach is then reinflated with air, and the distended stomach is punctured percutaneously with a 19-gauge single-wall puncture needle (Cook, Bloomington, IN) using rapid entry. Contrast is then injected to confirm the position, and the retention suture, which is loaded within the access needle, is deposited in the stomach using an 0.25-inch guidewire (Figure 74.3-6).[70–72] The needle is then removed, and the retention suture is pulled up to maintain the stomach against the anterior abdominal wall. A second puncture is then performed with a 19-gauge Seldinger-type needle (Inrad, Kentwood, MI), through which a 0.35-inch straight guidewire (Cook; 70 cm) is introduced. A Coons dilator (Cook) is then used to dilate the tract (Figure 74.3-7).

Dilatation is assisted by using muco jelly on the tip of the dilator and by viewing the dilatation under fluoroscopy to reduce the risk of the retention suture breaking. The dilator is then removed, and the G tube (15 cm Dawson Mueller Mac-Loc, Cook) of chosen size is introduced. The locking loop is tied, and the loop is pulled up to the anterior abdominal wall. The position is checked with contrast to confirm placement and to check for leaks (Figure 74.3-8). If indicated, a GJ catheter can be placed as a primary procedure.[73] In this situation, once access has been obtained with the Seldinger needle, a 5 French dilator is then introduced, followed by a 5 French directional catheter (JB-1, Cook). A Benston wire (0.035 inch) is then introduced through the directional catheter, and the catheter and wire are manipulated into the pylorus and down the duodenum into the proximal jejunum. Once the wire is in position, the catheter is removed and the tract is dilated to the required size. An 8.5 or 10 French GJ catheter (Chait, GJ tube, Cook) (see Figure 74.3-7) is then introduced with a stiffener (Figure 74.3-9). The position of the GJ tube is confirmed prior to returning the patient to the ward.

Postprocedure Orders

Patients fast for 12 hours after the procedure. IV fluids are given to maintain hydration and to replace fluids lost via GI drainage. Nasogastric and G tubes are left to gravity drainage. Antibiotics are continued if there was any difficulty with the procedure or if there is an increased risk of infection. However, in 99% of cases, a single dose of antibiotics given at the time of the procedure is all that is needed. Analgesia in the form of morphine (0.05 mg/kg IV for the first 24 hours) and then acetaminophen (15 mg/kg) is given.

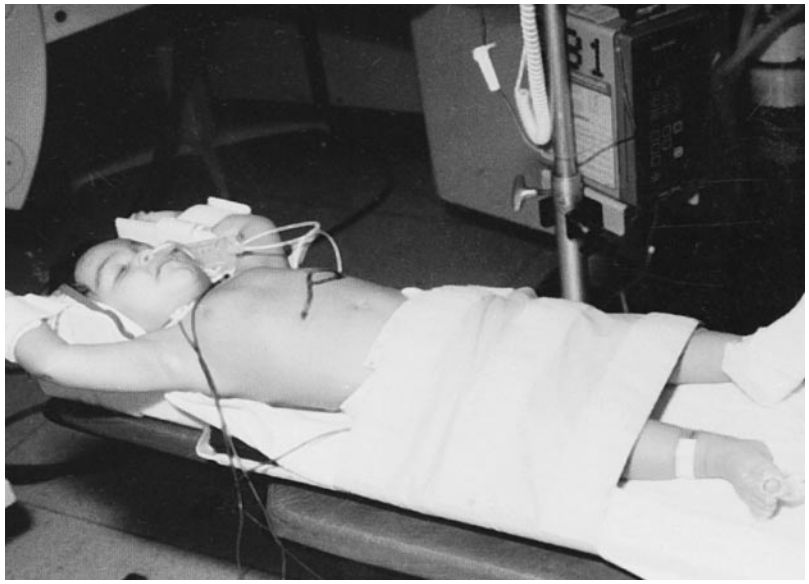


FIGURE 74.3-4 Patient is placed in the restraining device on the C-arm fluoroscopic table with a nasogastric tube, electrocardiograph, and pulse oximetry in position, as well as peripheral intravenous access in the left foot. The position of the liver has been outlined with ultrasonography and the site for gastrostomy placement outlined.

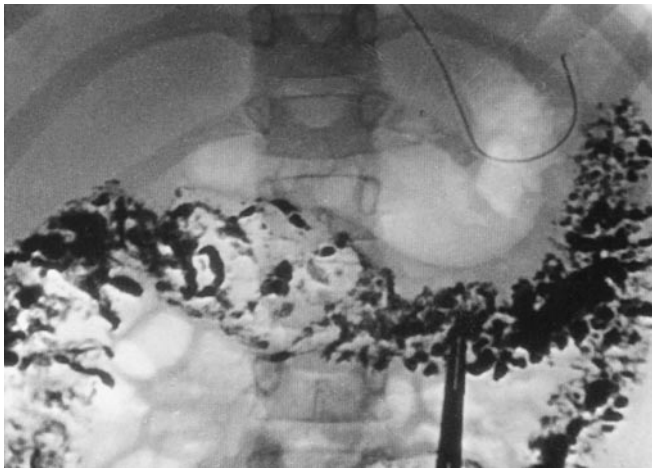


FIGURE 74.3-5 Contrast-filled transverse colon is seen in a patient with a nasogastric tube in position. The side position of a gastrostomy tube is marked with forceps, and the stomach is undistended.

At 12 hours, tube feeding is begun if the patient is clinically well and bowel sounds are present. Initially, clear fluids (Pedialyte) are given either intermittently or continuously, followed by half-strength feed and, finally, full-strength feed. Patients are usually kept in hospital for 72 hours and then discharged. Patients are assessed at 6 weeks, at which time long-term nutritional needs are assessed. At this stage, the Cope loop catheter is exchanged for a balloon type G catheter (see Figure 74.3-2) (MICC, Milpitas, CA). The balloon catheter permits the parent to replace it at home without the need to return to the hospital. Second, the size of the catheter can be increased over the ensuing 3 months to allow placement of a button G tube.

Some patients require gastric drainage and GJ feeding. This can be achieved by placing two catheters, one as a GJ tube and the other as a draining G tube. Alternatively, a balloon type G catheter containing a central jejunostomy feeding catheter can be placed, thereby allowing for feeding and gastric drainage through the same G tube site (MICC).

The indications for this procedure are to maintain the nutritional support of patients who are unable to maintain adequate nutrition by mouth or are unable to tolerate nasogastric tubes or for gastric decompression.[74–78] The vast majority of patients have neurologic deficits,[79] have swallowing disorders, or suffer from malignancies. We also use the G tube for gastric decompression in patients with small bowel obstruction or GI dysmotility.

Results and Follow-Up Studies

Our interventional service has placed an average of 160 new G and 25 primary GJ tubes per year since 1995. There were no major complications of bowel perforation or catheter placement through another loop of bowel. In one patient, the catheter was malpositioned, and peritonitis developed, requiring laparotomy. Initially, a small number of patients developed local cellulitis, but routine use of antibiotics before the procedure has almost completely eliminated this complication.[55]

Two types of low-profile silicon button type G catheters are available. These are not placed before 3 months.[80,81] In most cases, the procedure is performed without difficulty by the enterostomy nurse in the outpatient clinic, but if there is any difficulty or if the patient requires sedation, it is performed in the radiology department. The indwelling G tube is removed, and a wire is left in position in the stomach. Lidocaine jelly is applied to the G tube site. Dilatation of the tract is performed if necessary. The more popular Bard button catheter (see Figure 74.3-2) is placed in boiling water, and then with the stiffener in place, the button is introduced into the stomach under fluoroscopic control using the wire in the tract as a guide. Dilatation of the tract may be needed because this is an 18 French catheter. The position is then confirmed radiologically. The other balloon low-profile catheter (MICC) is used usually in smaller children when dilatation is thought to be a problem. This catheter has the advantage of being small (14 French) and can be placed over a wire because it has a central opening. This catheter is best used as a replacement catheter rather than as a primary catheter because it is not

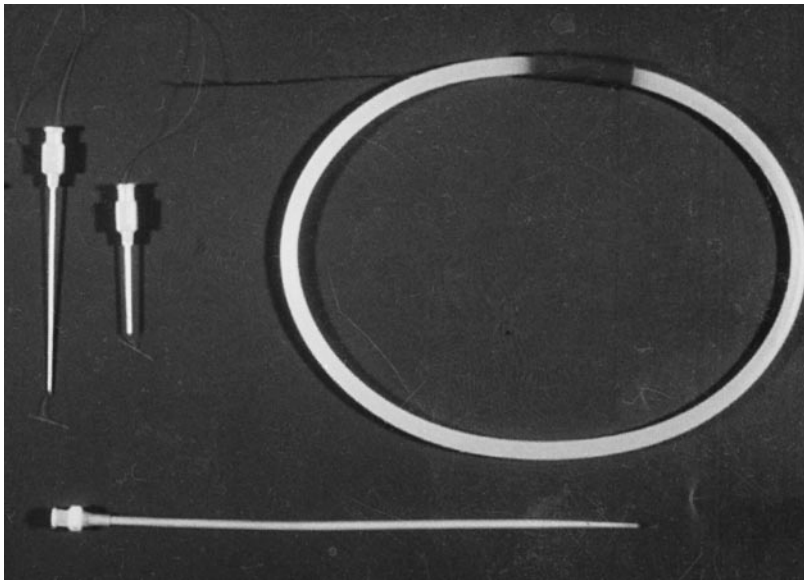


FIGURE 74.3-6 Retention suture is seen with the 19-gauge needle and wire introducer, as well as a Coons fascial dilator.

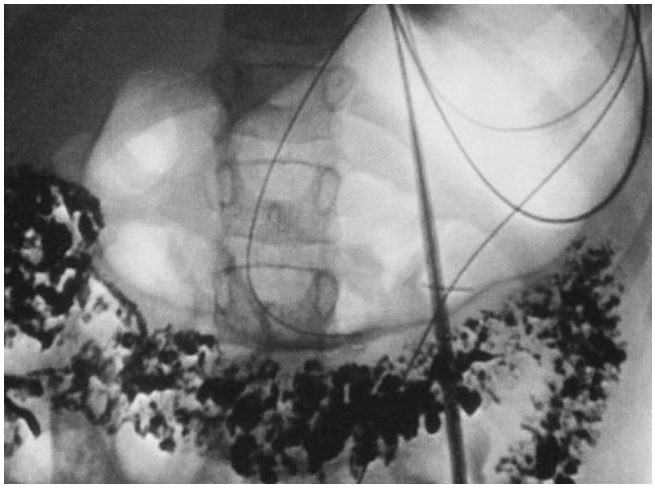


FIGURE 74.3-7 After a second puncture with a Seldinger needle, the tract was dilated with a Coons fascial dilator under fluoroscopic control.

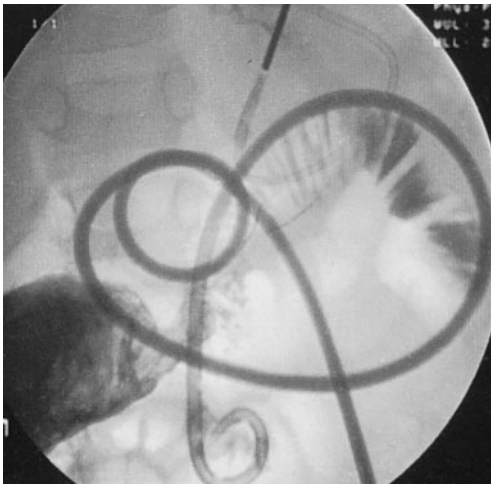


FIGURE 74.3-9 Example of the gastrojejunostomy catheter with the proximal loop in the stomach and the distal catheter in the proximal jejunum with a distal tight pigtail.

very well tapered. The MICC balloon catheter is not as durable as the Bard button catheter because it is more prone to rupture or leakage of the balloon. It is used in selected cases or because of parent preference.

In the small number of patients with proximal small bowel dysmotility, a gastrojejunal feeding tube and a gastric drainage tube are needed; in this situation, we favor the placement of two catheters at separate sites (Figure 74.3-10).

Percutaneous Cecostomy

Percutaneous cecostomy, commonly used in adults for decompression,[82–85] is less frequently performed in children. A surgically described technique of performing reversed appendicocecostomy for performing antegrade enemas has been described.[83] On occasion, a cecostomy is required in patients with severe intestinal pseudo-obstruction or in those with spina bifida and meningomyelocele who have severe constipation or fecal incontinence. The cecostomy permits introduction of a catheter to allow for direct enemas to the large bowel or to allow decompression. The percutaneous procedures can be performed under sedation. Before the procedure, the patient is kept on oral fluids for 2 days. Then, prior to the procedure, a balanced electrolyte lavage solution is infused via a nasogastric tube at a rate of 25 mL/kg/h until rectal effluent is clear of solid fecal material. Antibiotics are given prior to the procedure. A large Foley catheter is introduced into the rectum, and air is introduced to fill the cecum. The technique is similar to that described for placement of a G tube using a transperitoneal or retroperitoneal approach (Figure 74.3-11).[83] There has been a single report of abdominal wall cellulitis secondary to percutaneous cecostomy.[86]

Placement of Nasoduodenal Tubes

Catheters used primarily for duodenal manometry or pancreatic function tests are placed nasojejunally, but if a G tube is present, a gastrojejunal approach is feasible. The latter approach is less invasive and easier. Initially, a direc-

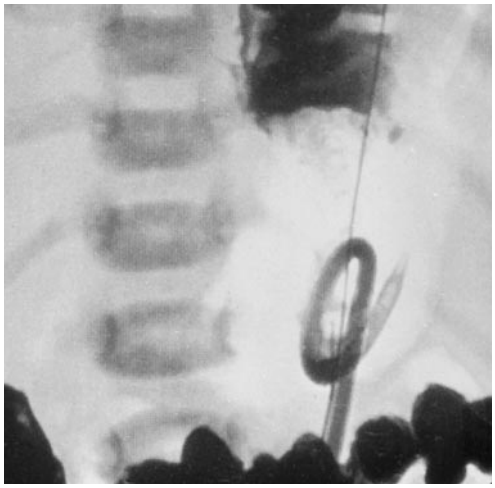


FIGURE 74.3-8 Cope loop gastrostomy tube is tightened and pulled up against the anterior abdominal wall and its position confirmed with contrast.

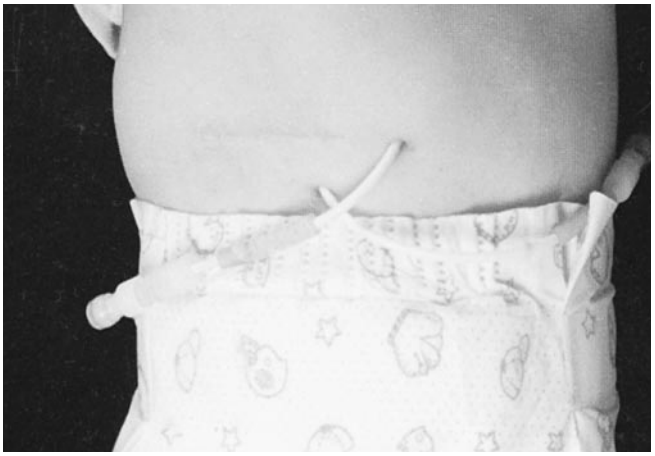


FIGURE 74.3-10 Gastrostomy and gastrojejunostomy tubes shown in a patient with separate entrance site, with the gastrostomy tube used for gastric aspiration and drainage and the gastrojejunostomy tube for feeding.

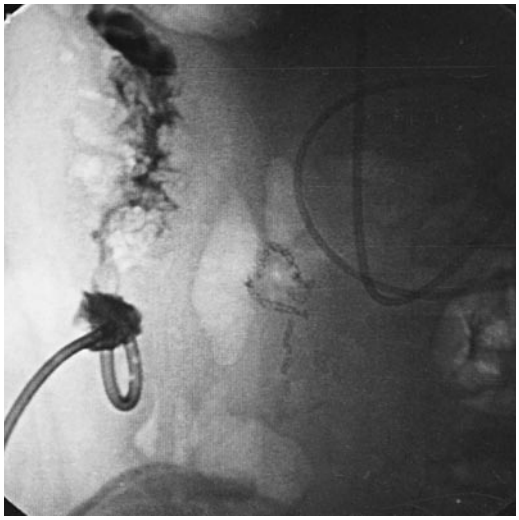


FIGURE 74.3-11 Cecostomy showing a catheter in the right iliac fossa with contrast filling the cecum.

tional catheter and wire are used to get into the proximal jejunum. The manometry catheter has multiple ports and a very small central lumen requiring an 0.018- or 0.025-inch wire over which the catheter is introduced.

Dilatation Procedures

Esophageal Dilatation. Traditionally, esophageal strictures are dilated blindly following the introduction of bougie dilators through the mouth. However, the introduction of balloon catheters for angioplasty has resulted in the development of catheters for dilatation of esophageal strictures.[87] Larger balloons have also been developed for use in the GI tract. Balloon dilatation appears to carry a reduced risk of complications in comparison with traditional bougie dilatation, particularly when used with fluoroscopic guidance. Over a 3-year period, we have done 70 esophageal dilatations in 30 patients carrying a variety of diagnoses, including achalasia,[88] strictures owing to caustic ingestion or radiation,[89] and gastroesophageal reflux. The choice of catheter size depends on the patient and the diameter of the esophagus above and below the stricture. In patients with achalasia, balloons of 25, 30, and 35 mm are routinely used, and a variety of balloon diameters from 4 mm up are used for other cases.

The procedure is performed under sedation or, if indicated, under general anesthetic. Local anesthetic is sprayed into the back of the throat. A nasogastric tube is placed to aspirate any residual gastric contents and to introduce contrast. A bite block is used to keep the teeth apart, and a directional catheter (JB-1) stabilized by a Benston 0.035-inch wire is directed down the esophagus. This procedure can be done through either the transoral or nasal approach. Occasionally, oblique or lateral fluoroscopy is required to direct the wire into the correct position. The length and diameter of the stricture and its relationship to the rest of the esophagus are assessed with contrast. The wire and catheter are placed through the stricture with little risk of damage or perforation. The correct-sized balloon catheter is introduced over the wire. Dilatations are performed by inflating the balloon for 30 seconds, and then, after deflation, the procedure is repeated three times at each level (Figure 74.3-12). Contrast is then injected to assess for any leaks, and the proximal esophagus is aspirated during withdrawal of the nasogastric tube. After the procedure, the patient is kept with nothing per mouth for 24 hours and is monitored for any complications. Occasionally, combined radiologic and endoscopic procedures are performed, and,

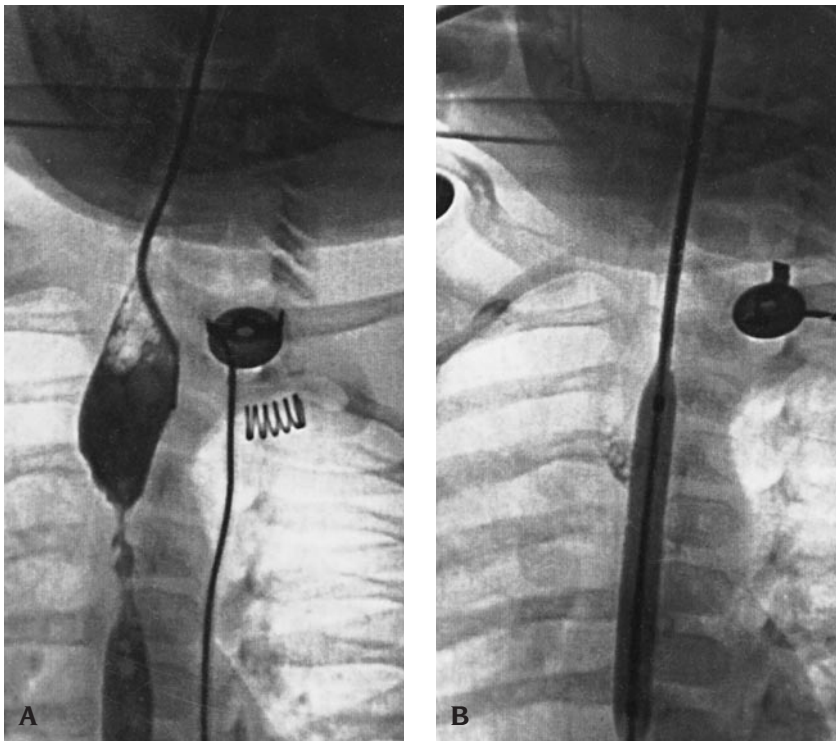


FIGURE 74.3-12 *A*, Esophageal stricture post tracheoesophageal fistula. *B*, Stricture dilatation using a balloon.

if indicated, a corticosteroid is injected directly into the wall of the esophagus to reduce the risk of scarring and inflammation. Repeat dilatation is performed as necessary. In patients with a G tube, esophageal strictures can be dilated via the G-tube site instead of the more traditional oral or nasal approach. Treatment of the underlying cause of the stricture must also be considered, which might include antireflux surgery for reflux esophagitis or chemotherapy or radiotherapy for malignant strictures. Recently, expandable metallic stents have been used for esophageal strictures, particularly in strictures caused by malignancies. We have not had any experience with this technique.

Using the aforementioned approach, no patients have developed mediastinitis or an esophageal leak.[90] However, we have treated several patients who experienced complications from bougie dilatation who required percutaneous drainage of abscesses and leaks.

Dilatation of Other Intestinal Strictures. Occasionally, prepyloric strictures develop following the ingestion of corrosive materials. These patients are usually treated with gastroenterostomy. However, in those patients deemed unsuitable for operative treatment, dilatation may be considered. This technique, which requires the use of a balloon catheter, is very similar to that used for esophageal dilatation. The correct size of the balloon may be difficult to determine because the pylorus is a dynamic channel. Similarly, the technique for the dilatation of the esophageal strictures may be applied to anastomotic strictures elsewhere in the intestinal tract provided that they are accessible.

Colonic strictures may be treated by balloon dilatation via the rectal approach or, if present, through a colostomy (Figure 74.3-13).[91,92] Owing to the higher risk of infection, antibiotic coverage is required. These procedures are not commonly performed in the pediatric setting.

Percutaneous Biopsy of Abdominal Masses

Percutaneous biopsy of abdominal masses is a commonly performed procedure using ultrasonography or CT guidance.[24,93–96] Increased availability and use of image-guided biopsy is a direct result of the development of various cross-sectional imaging modalities, including fluoroscopy, ultrasonography, CT, and MRI.[97,98] As well, thin-walled fine-gauge needles possessing a variety of tips have been developed, which allows for obtaining larger tissue specimens. At the same time, great advances in the histopathologic methods for identifying and grading tumor material determine the need for larger tissue specimens.[99–101] A high proportion of intra-abdominal tumors in the pediatric population are sarcomas, and aspiration biopsies or small-core biopsies fail to provide sufficient tissue for diagnosis. Furthermore, in patients with known primary malignancies, fine-needle biopsies are ideal for confirmation of malignancy, to document metastases, or to identify recurrent tumors.

Prior review of the patient's imaging studies is required, which, together with consultation with responsible physicians, surgeons, and pathologists, will predetermine the probable identity of the tumor, the amount of tissue required, and the potential risks and benefits of using a percutaneous approach. Patient preparation is similar to that with other interventional procedures, and choice of sedation versus a general anesthetic is made on an individual basis. The primary contraindications to percutaneous biopsy include a significant, uncorrectible coagulopathy and/or lack of a safe pathway for needle placement and guidance. The most accessible lesion is identified if they are multiple, and a skin entrance site is chosen. If it is a large lesion, the periphery of the lesion is biopsied preferentially to avoid the risk of biopsying a central necrotic area. Most intra-abdominal biopsies are performed under

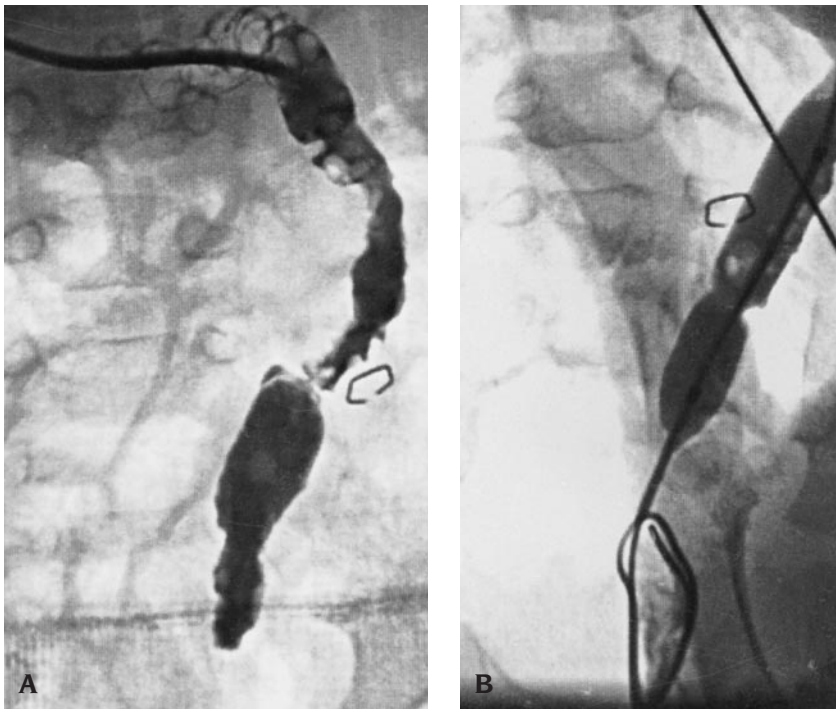


FIGURE 74.3-13 *A*, Colonic strictures following necrotizing enterocolitis. *B,* Stricture dilated via the colostomy with a 10 mm balloon.

ultrasonography guidance (Figure 74.3-14). CT is seldom used because it is time-consuming and expensive and requires patient cooperation or a general anesthetic. Ultrasonography is ideal for biopsies of solid organs such as the liver, accessible solid lesions arising from the pancreas or spleen, or other intra-abdominal masses. The chosen puncture site is marked prior to the procedure using a permanent marker. The patient is positioned for the procedure to allow optimum access. The overlying skin is then prepared and draped using sterile technique. After lidocaine is introduced in the subcutaneous tissues, a small nick is made in the skin with a scalpel blade.

A variety of needles are available for percutaneous biopsies. They differ in gauge, length, and mechanism of obtaining tissue. They can be categorized into aspiration or cutting mechanisms and automated or manual devices.

The Chiba and Turner needles are both aspiration biopsy needles (20–23 gauge). The Chiba needle has a beveled edge of 25° and the Turner needle of 45°. Aspiration needles can be used for obtaining cytologic and bacteriologic material. Occasionally, histologic material may be obtained, especially with the Turner needle, owing to the acutely beveled edge.

Cutting needles, categorized as end-cutting and side-cutting types, are designed to increase the probability of obtaining histologic material. The end-cutting needles either have an end-cutting bevel or a serrated edge, which allows for optimal cutting and retention of core material. The side-cutting needles include the Truecut, the automated, reusable Biopty (Bard, Murray Hill, NJ) (Figure 74.3-15), and minopty guns (Meditech, Westwood, MA) (Figure 74.3-16). The Autovac needle (Angiomed, Karlsruhe, Germany), an end-cutting needle, is automated to allow a variety of depths of tissues to be biopsied, ranging from 1 to 4 cm (see Figure 74.3-16). This needle, which permits the operator to guide the needle with ultrasonography, is ideally suited for most abdominal core biopsies. In general, fine needles (20–23 gauge) should be used when there is a risk of entering vital structures such as blood vessels or bowel loops.[102] Larger needles may be selected for percutaneous biopsy in certain situations in which there is no intervening vital structure, when a retroperitoneal or a posterior extraperitoneal approach is used, or when round cell tumors are suspected.

FIGURE 74.3-15 Bard resterilizable Biopty gun with disposable needles in (14, 16, and 18 gauge) and a 2.3 cm throw.

Percutaneous Liver Biopsy. Blind percutaneous biopsy of the liver has been performed for a number of years, with a high success rate. However, biopsies of focal hepatic lesions are best performed with image guidance using either ultrasonography or CT. It is possible to biopsy a lesion as small as a few millimeters in size under ultrasonography control (Figure 74.3-17). We routinely use an 18-gauge Surecut (TSK Laboratory, Tochigi, Japan) needle for biopsy of diffuse parenchymal lesions and a Surecut, Autovac (Angiomed), or Biopty gun for the biopsy of focal lesions.[103–105]

In patients with coagulopathies, transcutaneous biopsy of liver lesions can be performed coaxially with removal of the biopsy needle and then introduction of Gelfoam or coils to reduce the risk of bleeding.[106–108]

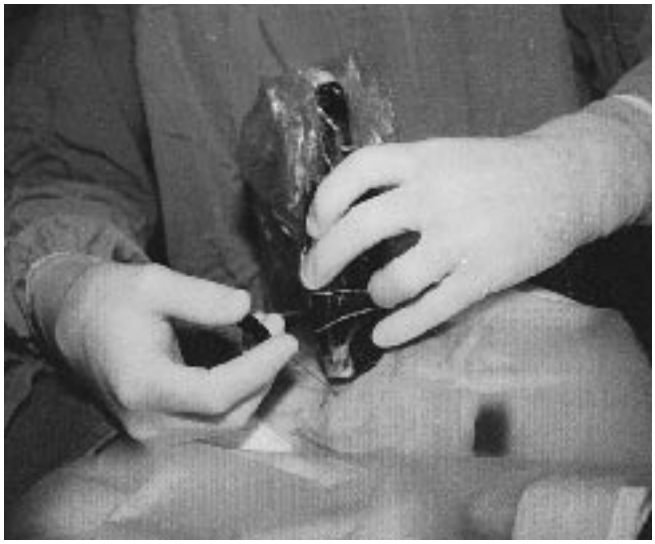


FIGURE 74.3-14 Ultrasonography guidance is shown using an ultrasound probe with a sterile cover and freehand guidance.

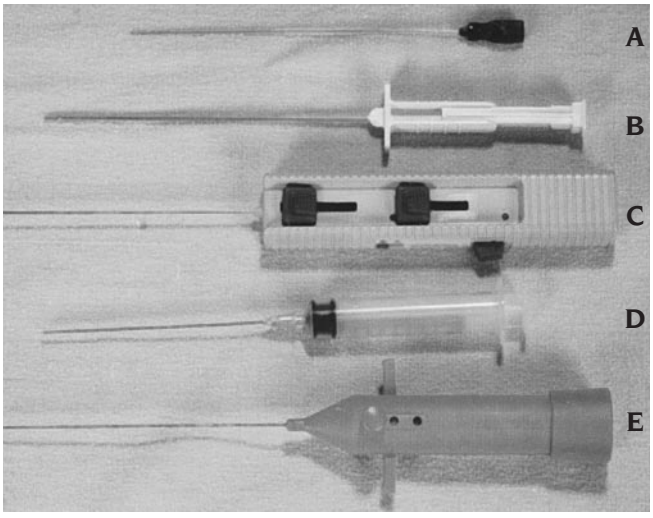


FIGURE 74.3-16 A variety of needles. *A*, Chiba needle. *B*, Truecut needle. *C*, Minopty gun (disposable). *D*, Surecut needle. *E*, Angiomed autovac needle.

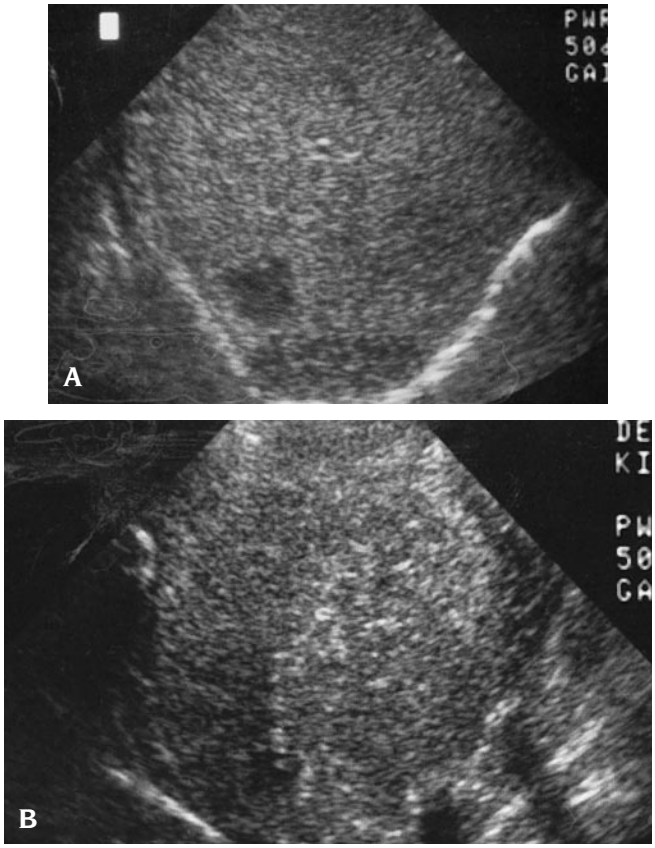


FIGURE 74.3-17 *A*, Deep 5 mm lesion is seen in the posterior aspect of the right lobe of the liver on ultrasonography. *B*, Biopsy under ultrasonography guidance is performed successfully with the needle seen traversing the lesion.

Transjugular Liver Biopsy. Transjugular biopsy is reserved for patients with severe coagulopathies that are uncorrectable.[109–111] This procedure is usually performed under a general anesthetic with the patient in the supine Trendelenberg position. The right internal jugular vein is accessed percutaneously, and a wire and sheath are introduced. Selective catheterization of the right hepatic vein is performed. Wedge pressures and free hepatic and inferior vena cava pressures are taken. A wedge of hepatic and free hepatic venogram is performed. The biopsy is performed with a Colapinto (Cook)-type biopsy needle with the tip of the needle directed posteriorly and a sheath placed within the right hepatic vein (Figure 74.3-18). The biopsy technique involves rapid puncture with aspiration. Saline is injected to release the core of liver tissues. Postprocedure venograms are performed to exclude perforation of the liver capsule (Figure 74.3-19). Transvenous biopsies can also be performed from a femoral approach using a claw-type biopsy forceps through a sheath and catheter.

Transcatheter biopsy of hepatic lesions is not commonly required in the pediatric setting because primary hilar lesions are rare. These are performed using a brush biopsy technique or by introducing a needle through the biliary drainage catheter, and biopsies are made at the site of the mass.[112] Fluoroscopy can also be used to guide a biopsy needle to a presumed tumor site shown with percutaneous transhepatic cholangiography (PTC) or as an area of stricture or mass.

Pancreatic Biopsy. Primary pancreatic tumors are relatively rare in pediatric patients. The principles for obtaining a biopsy are similar to those for other solid masses. The decision to perform a percutaneous biopsy depends on the extent of the primary lesion, the presence of secondary pathology, and the surgical alternatives. Direct visualization with ultrasonography or CT[111] is usually required to allow accurate placement of needles for small pancreatic lesions.[106,113,114]

Lymph Node Biopsy. Percutaneous biopsy of intra-abdominal lymph nodes can be accomplished with fluoroscopic guidance following opacification by lymphangiography. In the case of suspected lymphoma, percutaneous lymph node biopsy can be performed using ultrasonography or CT guidance. The results obtained from percutaneous lymph node biopsy are generally not as rewarding as those of biopsies of other organs.

Splenic Biopsy. Splenic aspiration and biopsy under ultrasonography or CT guidance are usually indicated for the histologic diagnosis of the etiology of diffuse splenomegaly[115] or focal masses. Fine-needle biopsy may be sufficient for the confirmation of known tumors or identifying an organism, but core biopsy with either 20- or 18-gauge needles provides a much better yield. In our experience in those patients with normal clotting, there is little risk of intraperitoneal hemorrhage, even with the larger needles.

Percutaneous Aspiration and Drainage

Percutaneous drainage of abdominal abscesses has proved to be one of the most successful and gratifying of all interventional procedures. In a relatively short time, it has achieved a remarkable degree of acceptance within the surgical community.[116] Intra-abdominal abscesses can now be

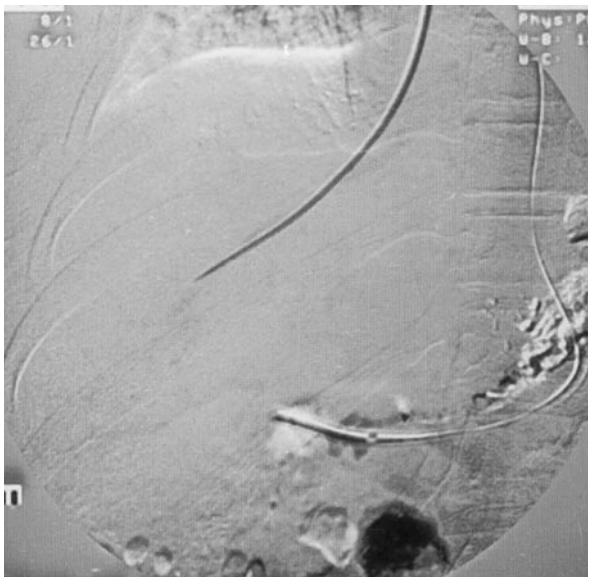


FIGURE 74.3-18 Subtracted image with a sheath seen in the right hepatic vein in the position ready for placement of the Colapinto biopsy needle.

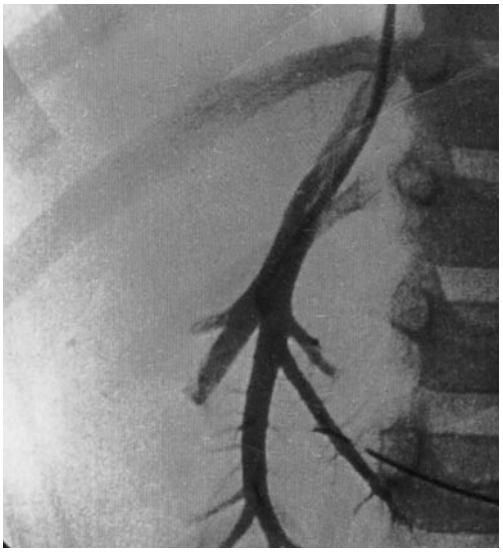


FIGURE 74.3-19 Postprocedure venogram is performed to exclude extravasation of contrast and perforation.

aspirated and drained percutaneously, with a "cure" rate approaching 85%.[117–121] A variety of other intra-abdominal fluid collections can be safely aspirated and drained percutaneously, including hematomas, seromas, lymphoceles, bilomas, pancreatic pseudocysts, and loculated ascites. In general, the technique is similar for all fluid collections. This involves guided introduction of a needle and aspiration of the material. If drainage is required, the needle is exchanged over a wire with a catheter. Contraindications to aspiration and drainage include the absence of a safe access route, which would include the transgression of major organs such as liver, spleen, or kidney; the presence of overlying blood vessels and nerves; or sterile spaces such as the peritoneum or pleura. The presence of coagulation abnormalities is a relative contraindication.

The procedure is performed under sterile conditions. The skin is prepared and draped, and 1% lidocaine is introduced. The technique usually involves placement of a 22-gauge Chiba needle into the collection under ultrasonography guidance. The collection is aspirated, and if drainage is required, a 0.018-inch wire is exchanged using a Neff introducer system (Cook) for a 0.035-inch wire. The tract is dilated, and a catheter is introduced. An 8.5 or 10 French all-purpose drainage catheter (Meditech) is usually sufficient for most fluid collections. A larger-caliber catheter such as a Sump drain or a Thalquick (Cook) abscess drainage catheter is used if necessary. The abscess or fluid collection is drained using a closed system drainage bag (Medics, Hilliard, OH) either by gravity drainage or suction using a Hemovac (Zimmer, Dover, OH). For large or superficial lesions, a trocar technique can be used with direct introduction of an all-purpose drain (Meditech), which is supplied with a sharp inner stylet.

Monitoring Drainage Procedures. Regular saline irrigation is used to help clear the cavity of pus or particulate debris. Antibiotics are continued postprocedure until the signs and symptoms of infection have resolved. The volume of drainage is reviewed daily, and if there is a sudden drop in volume, a block in the catheter should be suspected. If the volume increases dramatically, this might represent development of a fistula or leak. A sinogram, using dilute water-soluble contrast medium, should be performed 1 to 2 days after the initial drainage. This will define the extent of the abscess and its communication with other structures. Ultrasonography or CT should be repeated 3 to 4 days after the procedure and again immediately before the catheter is removed. Percutaneous drainage may not be successful because of a persistent fistula, necrotic material within the collection, viscous pus, multiloculated (or multiple) abscesses, or a large abscess cavity. To improve the success of drainage and to minimize complications, bowel loops should be avoided and sterile spaces should not be contaminated. Catheter manipulation should be minimized to reduce the spread of infection. Tissue trauma should also be kept to a minimum to reduce complications, and a large catheter should be used if the collection is viscous or contains cellular debris.

Abscesses. *Hepatic Abscess.* Primary hepatic abscesses are relatively uncommon in pediatrics. Pyogenic abscesses are most commonly seen in children with chronic granulomatous disease or those immunocompromised owing to treatment.[122] They are also seen as a complication of appendicitis with portal vein thrombophlebitis.

Amebic liver abscesses are seen in Southeast Asia, Africa, and South America and are typically single and loculated in the posterosuperior or anteroinferior aspect of the right lobe of the liver. These can be complicated by rupture intra-abdominally or even into the pleural space. Depending on the size of the abscess, they can be treated either by antibiotics (metronidazole) or by percutaneous drainage (Figure 74.3-20).

In the immunocompromised patient, microabscesses of fungal origin are often present. These are usually too small to require drainage but may be aspirated to make the diagnosis.[123]

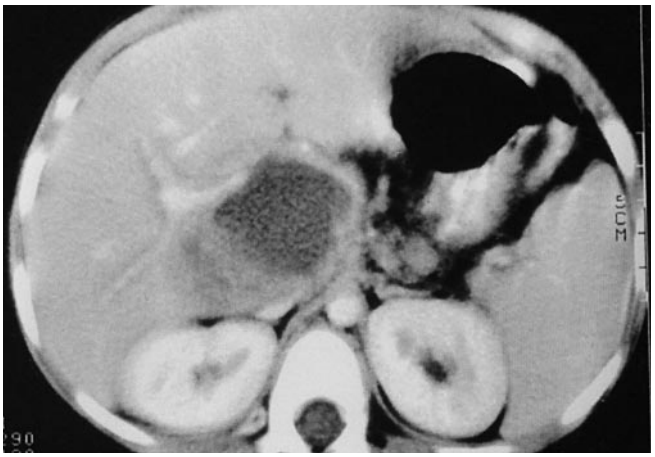


FIGURE 74.3-20 A hypodense collection is seen in the caudate lobe of the liver, which is well seen on computed tomography and is best drained under ultrasonography and fluoroscopic guidance.

Subphrenic Abscess. Subphrenic abscesses arise postoperatively or occur quite commonly in childhood following a ruptured appendix. Subphrenic abscesses are frequently difficult to access, and a transhepatic or intercostal route may be necessary (Figure 74.3-21). A combination of CT and ultrasonography guidance may be required.[124,125]

Splenic Abscesses. Splenic abscesses can be aspirated or drained successfully under ultrasonography control.[126] Most cases can be treated effectively with repeated aspiration or catheter drainage with a relatively low rate of complication (13%) (Figure 74.3-22).[127] Splenectomy should be performed only in splenic abscesses that are not accessible percutaneously and in those cases with percutaneous drainage failure.[127]

Pelvic Abscess. Pelvic abscesses are often deep and obscured by overlying bowel, vessels, urinary bladder, and bony pelvis (Figure 74.3-23). If they are superficial and easily visualized under ultrasonography or CT guidance, transabdominal access for drainage can be performed. If the lesions are deep and anterior to the rectum, a transrectal approach can be used.[128] The patient is placed in the decubitus position. Ultrasonography is performed transabdominally to identify the abscess and to place the needle (Figure 74.3-24). Guidance can also be obtained with a transrectal ultrasound probe.[129–131] An enema tip catheter is introduced into the rectum, and a trocar needle is advanced to the tip of the catheter. Under ultrasonography control, the needle and stylet are advanced through the anterior rectum into the abscess. This is followed by placement of a wire, dilatation, and catheter placement. We have performed this procedure in 30 patients and have shown that we can achieve a significant reduction in the time of recovery when compared with the traditional transabdominal or surgical approach (Figure 74.3-25). These deep abscesses can also be drained under CT guidance via the paracoccygeal route.[132]

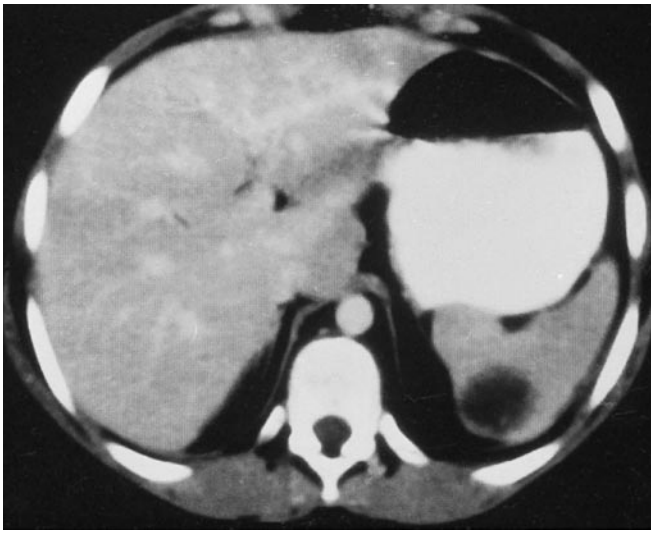
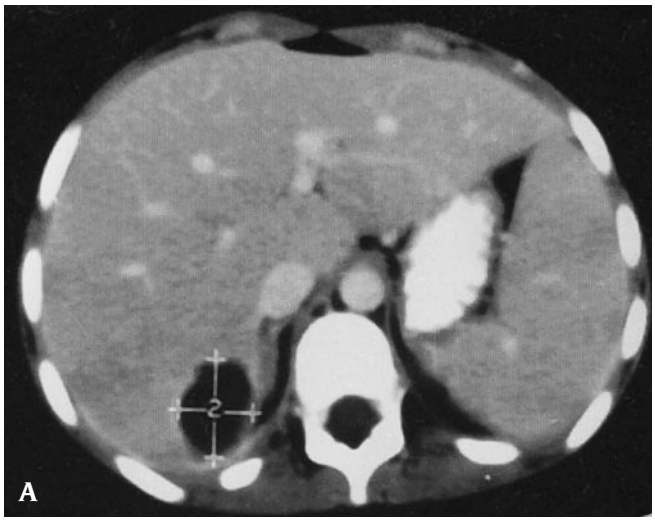


FIGURE 74.3-22 A posterior hypodense lesion seen in the spleen, which was aspirated successfully under ultrasonography guidance.



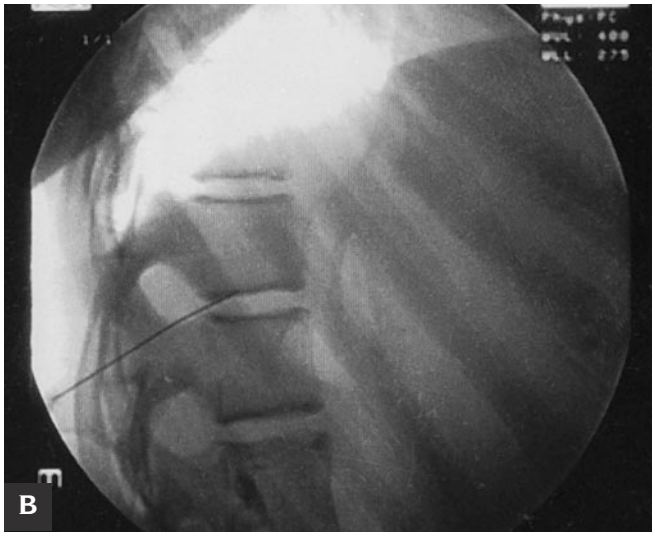


FIGURE 74.3-21 *A*, Subphrenic abscesses on computed tomography just below the right hemidiaphragm posterior to the liver. *B*, Lateral view of fluoroscopy seen with a needle entering the abscess. Guidance was performed under ultrasonography and further wire and catheter placement was performed under fluoroscopy.

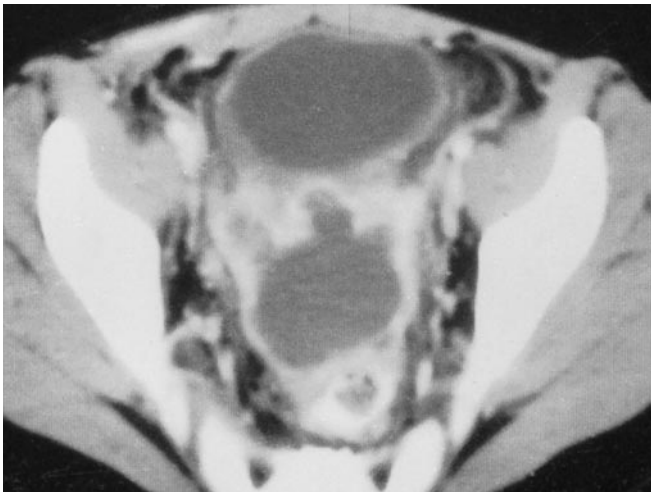


FIGURE 74.3-23 Deep pelvic abscess seen posterior to the bladder (*arrow*) with enhancing rim and the rectum seen posteriorly.

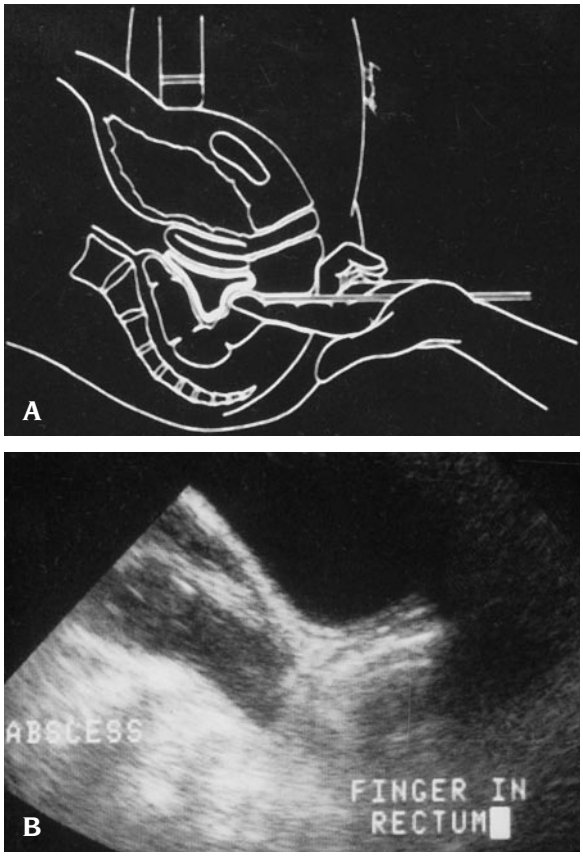


FIGURE 74.3-24 *A*, Diagram of transabdominal ultrasonography with rectal placement of the index finger with a trocar needle anterior to the index finger seen through the distended urinary bladder. *B*, Ultrasonographic view of the same patient with the finger seen in the rectum. The abscess posterior to the bladder is seen in the longitudinal section.

Interloop Abscess. Because of the position and the overlying bowel loops, these abscesses are difficult to access. It is best to assess interloop abscesses with CT to determine the precise location. Aspiration can be performed under CT or ultrasonography guidance, transgressing bowel with a 22-gauge Chiba needle.[133]

Pancreatic Pseudocyst. Pseudocysts develop following acute pancreatitis, which in the pediatric age group can be due to multiple etiologies.[134] They present as low-density collections on CT and hypoechoic masses on ultrasonography (Figure 74.3-26). Most pseudocysts resolve spontaneously over a period of weeks or months. If the patient is asymptomatic, a pseudocyst can be drained percutaneously or preferably transgastrically. Under ultrasonography guidance, the position of the pseudocyst is marked on the skin of the patient. The stomach is then inflated using a nasogastric tube, and a trocar needle (18 gauge) is introduced through the anterior and posterior stomach walls into the pseudocyst behind. After the pseudocyst has been drained, the needle is replaced with a wire, the tract is dilated, and a Cope loop catheter is introduced into the pseudocyst. The pseudocyst is left to drain for 4 to 6 weeks. A cystogram may be performed to assess communication with the pancreatic duct. Once the catheter is removed, residual drainage will continue through the fistulous tract into the stomach (Figure 74.3-27).

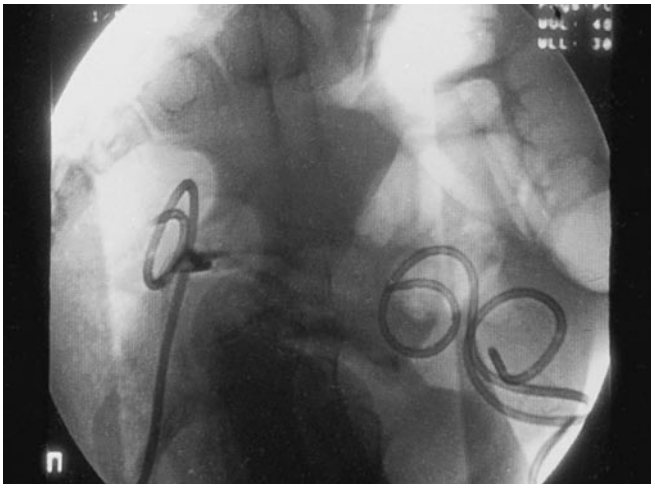


FIGURE 74.3-25 Lateral radiograph of a patient who has a transrectal drain in position and two transpercutaneous drains seen anteriorly for the treatment of a ruptured appendix.

Pancreatic Abscesses. Abscesses related to pancreatitis can be treated percutaneously if a safe access route is available. Percutaneous aspiration of these lesions can also be performed for diagnostic purposes. Phlegmonous pancreatitis does not respond to simple drainage and is not suited to simple percutaneous drainage because of the nature of the tissues (Figure 74.3-28).

Biliary Intervention

PTC, percutaneous transhepatic transcholecystic cholangiography (PTTC), percutaneous biliary drainage, dilatation of biliary strictures, and stone removal are established interventional techniques in the diagnosis and management of biliary tract disease.[135–140] Owing to the advent of liver transplant, there has been an increase in the need for

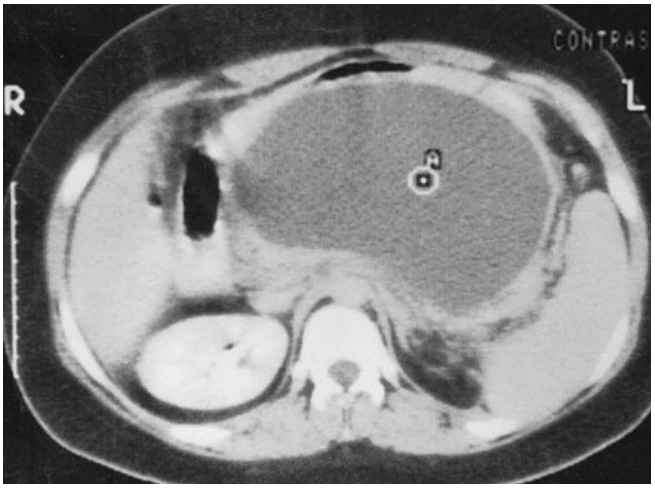


FIGURE 74.3-26 A computed tomographic scan of an abdomen showing a large, relatively hypodense collection in the midabdomen posterior to the stomach consistent with a large pseudocyst.

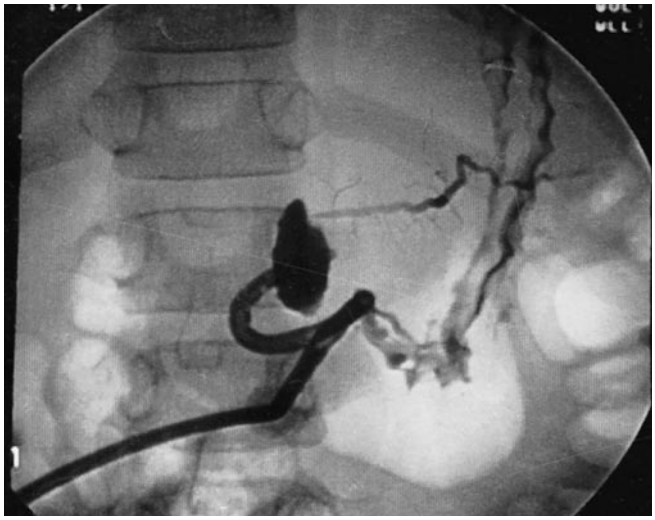


FIGURE 74.3-27 Transgastric drainage of a pseudocyst had been performed, with 6 weeks of drainage. A contrast study through the tube demonstrates filling of the small remaining cyst and the distal part of the pancreatic duct. Some contrast is draining into the stomach.

these procedures in the pediatric population.[141–143] Endoscopic retrograde cholangiographic procedures are indicated in those patients with intact common bile ducts and where access is possible.[144]

Percutaneous Transhepatic Cholangiography

The primary indications for PTC or PTTC include the differentiation of medically treatable from surgically treatable causes of cholestasis, demonstration of intrahepatic calculi, or extrahepatic choledocholithiasis. In addition, cholangiographic investigations are invaluable to diagnose a congenital abnormality such as biliary atresia or choledochal cyst and inflammatory conditions such as sclerosing or ascending cholangitis. They can also be used to evaluate surgical conditions such as biliary-enteric anastomoses and to assess if intrahepatic abscesses communicate with the biliary radical. Contraindications include uncorrectable bleeding disorders or a previous life-threatening reaction to iodinated contrast material. Vascular hepatic tumors and vascular malformations, as well as ascites, are relative contraindications.

General anesthesia is used for most children because the procedure requires significant patient control, especially in those with small biliary radicals. If there is a strong suggestion of cholangitis, triple antibiotics (ampicillin, gentamicin, and metronidazole) are started 6 hours before the procedure. If there is no obvious cholangitis, a single IV dose of cefazolin (30 mg/kg) is given prior to the procedure.

Cholangiograms are performed with a 22-gauge Chiba needle with ultrasonography guidance.[145] We make use of a hiliter needle (Inrad), which is better seen on ultrasonography. All procedures are performed under ultrasonography guidance (Figure 74.3-29). After prior imaging investigation has been reviewed, a choice is made between a right hepatic, left hepatic, or transcholecystic approach. The right hepatic approach at the midaxillary line is most commonly used (Figure 74.3-30). Fluoroscopy of the upper abdomen is performed to identify the position of the chest cavity. The right-sided approach has the disadvantages of transgressing pleura, increased pain owing to the intercostal approach, and the possibility of intervening bowel. The left-sided approach is more vertical and may allow easier access to the common bile duct via the common hepatic duct. This is a safer approach because the pleura is not being transgressed.

Transcholecystic studies are performed in patients who do not have dilated bile ducts or if repeated attempts at transhepatic cholangiography have been unsuccessful. This procedure is also performed using a 22-gauge needle under ultrasonography guidance, with a transhepatic route (Figure 74.3-31). Once the gallbladder is penetrated, a specimen is obtained, and dilute contrast is injected. After completion of the procedure, it is important to drain the gallbladder completely to reduce the risk of bowel peritonitis. In my own experience, the success rate with trans-

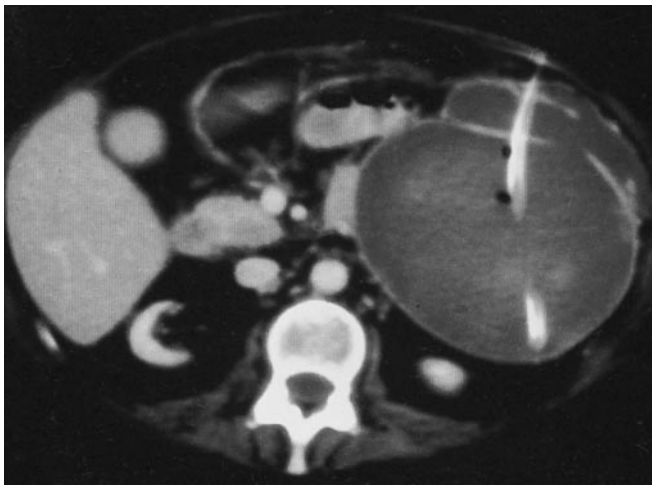


FIGURE 74.3-28 A complicated pancreatic pseudocyst with hemorrhage and infection. Percutaneous drainage was attempted but was unsuccessful owing to the thickness of the materials.

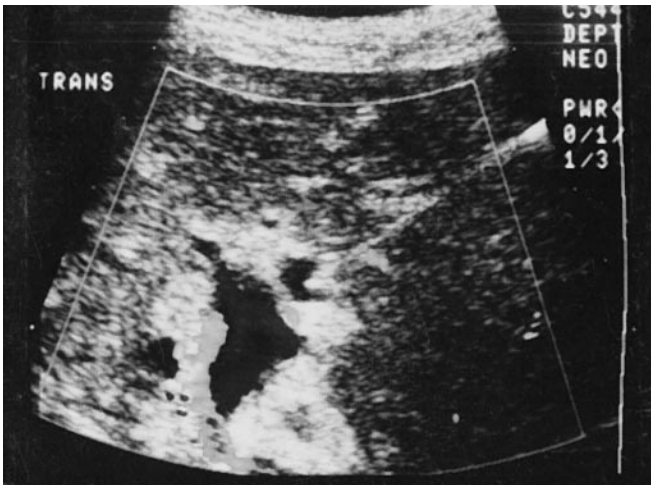


FIGURE 74.3-29 Ultrasonography guidance is used to place a 22-gauge Chiba needle into mildly dilated bile duct seen close to the portal vein and hepatic artery.

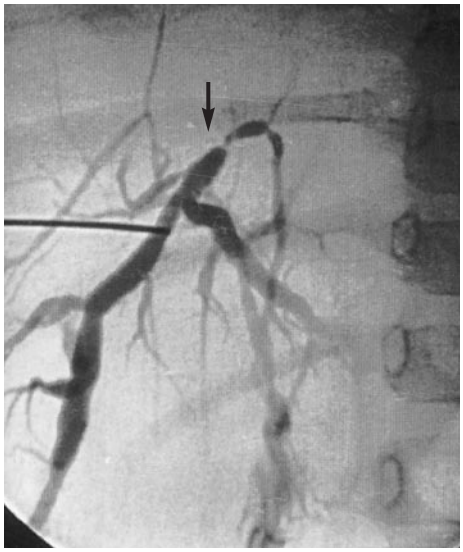


FIGURE 74.3-30 Right hepatic approach into transplanted reduced left hepatic lobe. A relatively normal-sized duct is seen with a focal biliary stricture just distal to the puncture site (*arrow*).

hepatic cholangiography in the patient with undilated intrahepatic ducts is approximately 70%, whereas the success rate of transcholecystic cholangiography approaches 100%. Our experience with 30 PTTCs has been very successful, with only one complication in our first patient, who developed a bile leak. In this case, the procedure was not performed under a general anesthetic.

Biliary Drainage

The decision to perform biliary drainage depends on the nature of the pathology. Indications include malignant obstruction with associated cholangitis, pruritus with liver dysfunction, biliary stricture owing to previous surgery, or sclerosing cholangitis. Drainage is also performed in patients with bile leaks, which is a relatively frequent postoperative complication following hepatic transplant.[146–148]

After cholangiography has been performed, it is important to carefully select the correct duct to drain. The duct should be entered peripherally, thus reducing the risk of damage to major vessels and increasing the purchase (ie, the length of catheter within the ductal system). The puncture site should be chosen so that there is a minimum of curvature, thus allowing for the forces during dilatation to be directed inferiorly. A 22-gauge Chiba needle is introduced into the selected duct, either under fluoroscopic guidance with a C-arm unit or with ultrasonography. Bile is aspirated, and a 0.018 Mandril (Cook) wire is introduced. The stricture is usually transgressed using selective wires and dilators. If there is evidence of cholangitis, manipulation should be kept to a minimum and drainage achieved as soon as possible. Further manipulation can occur once infection has resolved.

The ultimate goal of biliary drainage is to place a 10 French or larger catheter in position. We use a Cope loop catheter and hole punch to fashion the catheter according to the patient's size. After the stricture has been dilated or the obstruction has been transgressed, the tube should be allowed to drain externally until the bile is clear. Subsequent daily irrigations can be done with the catheter closed (Figure 74.3-32).

Biliary Dilatation

Dilatation of a biliary stricture is usually performed with a balloon catheter (Figure 74.3-33).[149] The size of the dilatation should be gauged from the size of the normal duct proximal and distal to the obstruction. The dilatation is performed, and a catheter with multiple side holes is inserted. Occasionally, an internal stent may be used. The polyethylene type of internal stent should be considered only in patients with a short life expectancy or irreversible hepatic dysfunction.[150] We have made use of a wall-expandable stainless steel stent in a patient who developed an anastomotic stricture after hepatic transplant. The patient has remained symptom free, with no evidence of obstruction for a period of 18 months (Figure 74.3-34).[151]

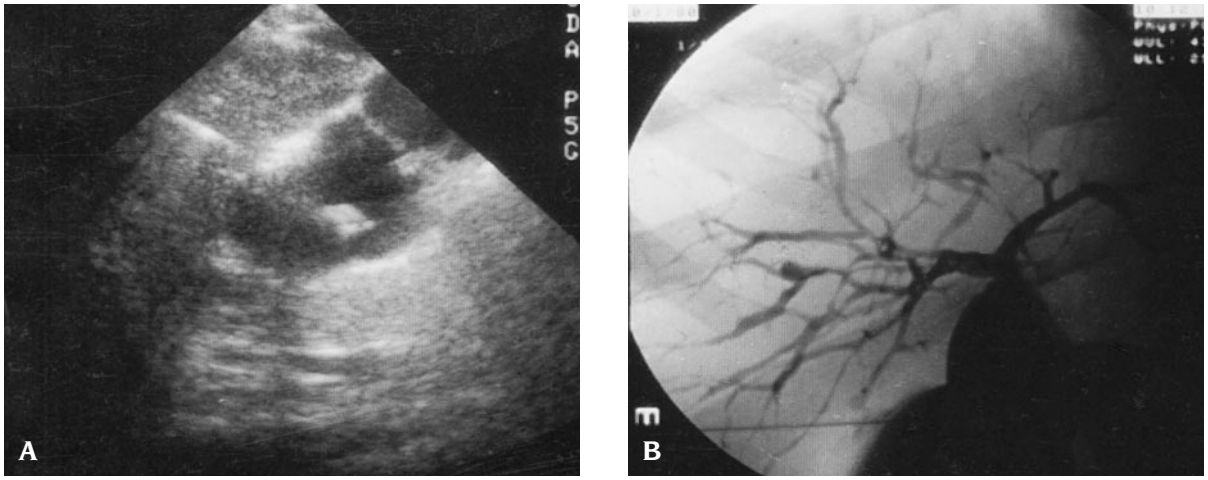


FIGURE 74.3-31 *A*, Sonogram of transhepatic placement of a Chiba needle into the gallbladder for the performance of a cholecystic cholangiogram. *B*, Contrast is injected through the catheter, and this demonstrates filling of the intrahepatic radicals, which demonstrate the features of sclerosing cholangitis with bleeding and irregularity of the bile ducts.

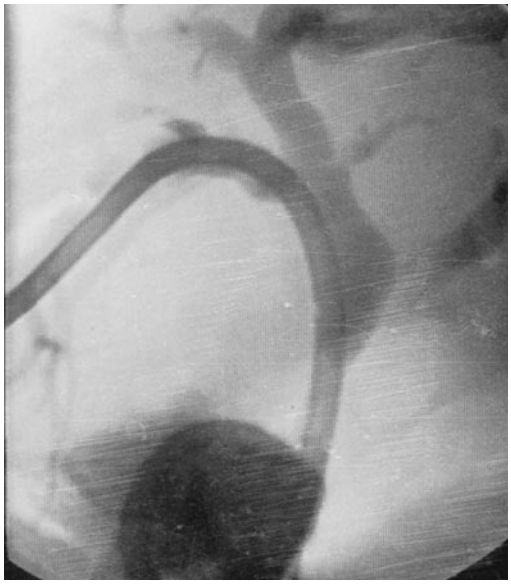


FIGURE 74.3-32 Internal and external biliary drainage has been performed in a patient with a focal stricture at the site of the choledochal jejunostomy anastomosis.

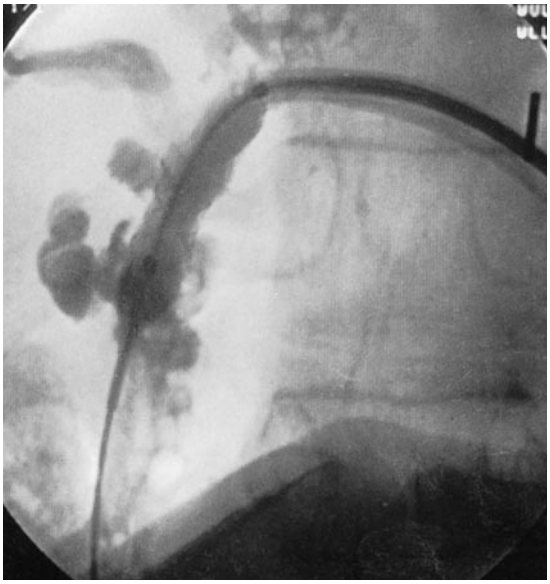


FIGURE 74.3-34 An internal metallic wall stent has been placed in a patient with a persistent choledochal jejunostomy stricture that was not responsive to repeated biliary dilatation.

T-Tube Cholangiography and Biliary Intervention

T-tube cholangiography is performed in patients who have a T tube following surgical removal of the gallbladder or in patients who have had a total liver transplant with a bile duct anastomosis. Retained stones or debris can be removed via the tract. The tube is usually left in place for 6 weeks to form a fistulous tract. The T tube is then removed over a wire, a catheter is introduced, and the calculi or debris is removed with a basket. This is best performed using a steerable Burhenne (Cook)-type catheter.

Biliary debris or calculi can be removed percutaneously. After the correct duct has been accessed, a sheath is introduced followed by a basket, which is then used to grab or crush the calculi prior to removal (Figure 74.3-35).[152] Debris can be removed in a similar manner.

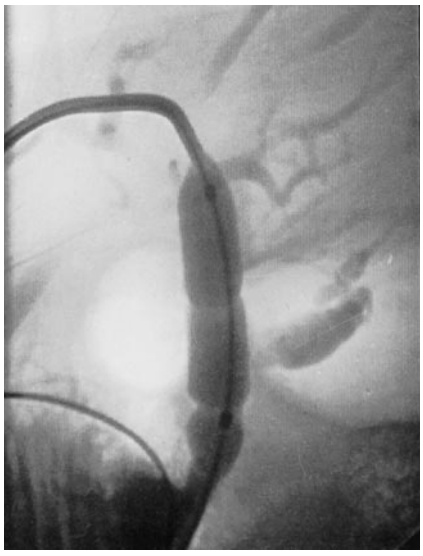


FIGURE 74.3-33 Dilatation of a choledochal jejunostomy stricture with a balloon dilator, with areas of narrowing seen in the balloon.

Transhepatic Portal Venous Intervention

The portal venous system can be accessed percutaneously quite easily using ultrasonography guidance. The technique is similar to that used for biliary cannulation using a 22-gauge Chiba needle followed by a 0.018 wire and then an exchange system. This allows for measurement of pressures, embolization of bleeding varices, and dilatation of portal venous anastomotic strictures. It can also be used to embolize portal venous to hepatic venous malformations. Dilatation of portal venous strictures after hepatic transplant can also be achieved (Figure 74.3-36).

Transjugular Intrahepatic Portosystemic Shunts

Transjugular intrahepatic portosystemic shunting (TIPSS) is a recently developed procedure for the treatment of GI bleeding from varices owing to portal hypertension. It involves the creation of a parenchymal tract from the hepatic to portal vein, which is given support by the insertion of a metallic stent.[153–155]

TIPSS is described as a safe, effective, short-term procedure for the reduction of portal pressure. The short-term success rate is reported to be over 90%. The long-term outlook for the procedure will ultimately depend on the treatment and prevention of intimal hyperplasia within the shunt and draining veins.

The procedure is highly effective in lowering portal pressure and controlling acute variceal bleeding. However, its long-term effectiveness in preventing recurrent bleeding and as a treatment for ascites has not been clearly established when compared with conventional therapy. In addition, acute and chronic complications of the procedure are not insubstantial.

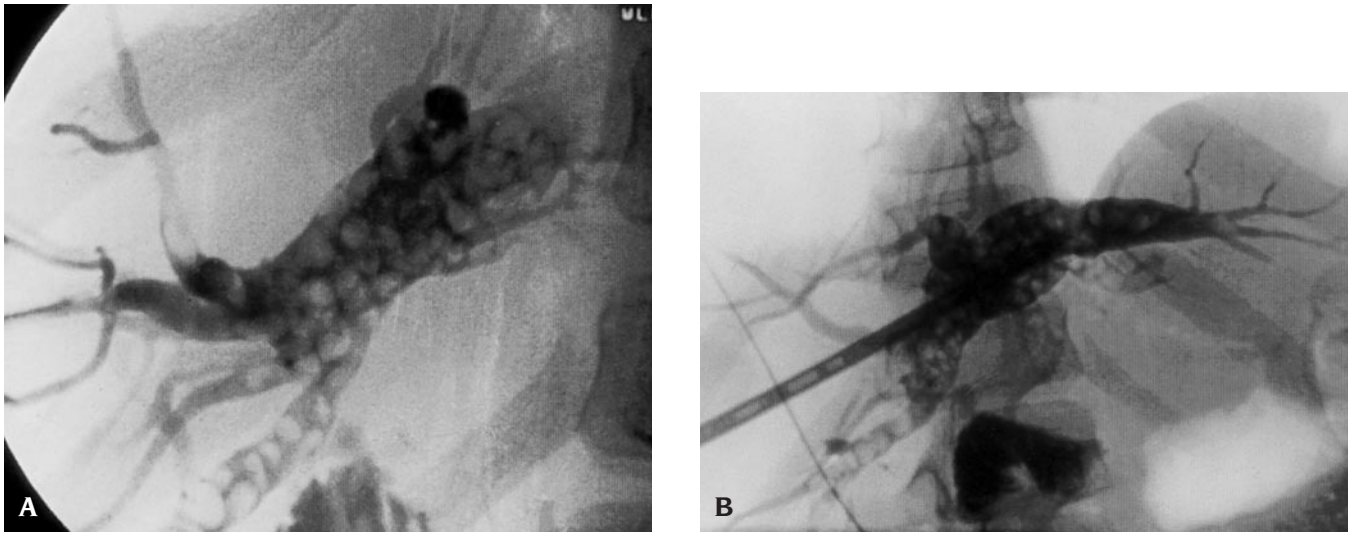


FIGURE 74.3-35 *A*, Cholangiogram demonstrating the biliary system of a patient with numerous calculi. *B*, Calculi were removed with the placement of a sheath and basket. This required repeated removal of calculi.

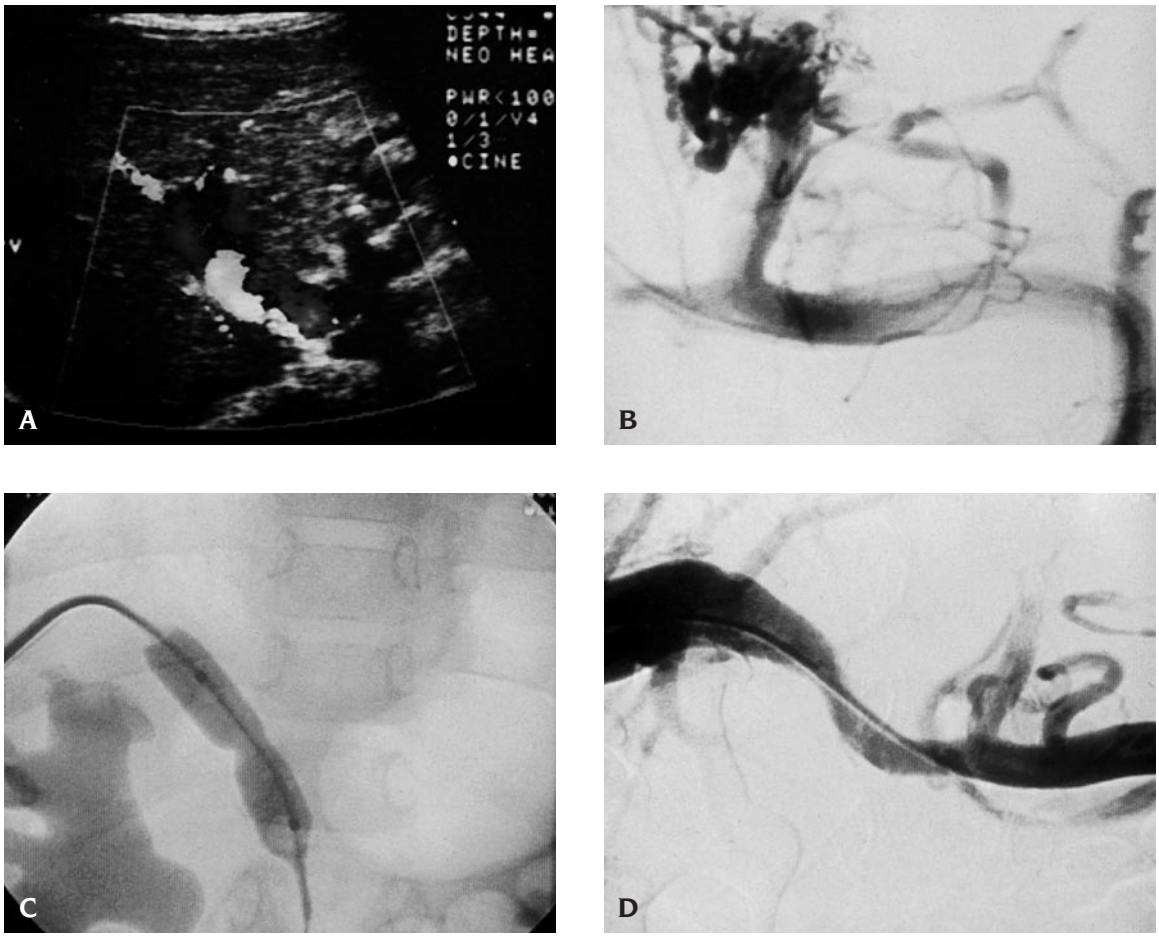


FIGURE 74.3-36 *A*, Ultrasonography of the portal venous system showed turbulence, and color Doppler ultrasonography showed changes consistent with a portal venous stricture. *B*, Transhepatic placement of a catheter in the portal venous system demonstrated gastric varices and prominence of the cardinal veins and esophageal varices. Portal hypertension was due to a portal venous anastomotic stricture. *C*, Balloon dilatation of the portal-venous stricture. *D*, Postdilatation venogram demonstrated resolution of the portal hypertension and pressure differential and antegrade flow into the liver.

The two main indications are (1) acute variceal bleeding uncontrolled by medical treatment, including sclerotherapy, and (2) recurrent variceal bleeding in patients who are refractory or intolerant to conventional management.[156] TIPSS is preferable to surgical shunting in Child's class C patients for both indications and in patients with refractory bleeding awaiting liver transplant.

Unproven indications are initial therapy of acute variceal hemorrhage, prophylactic therapy to prevent recurrent variceal hemorrhage, refractory ascites, and Budd-Chiari syndrome and to reduce intraoperative morbidity during liver transplant surgery. Contraindications include heart failure, polycystic liver disease, infection, severe hepatic encephalopathy, and fulminant liver failure.

Minor procedural complications occur in 10% of cases. Severe, life-threatening complications occur 1 to 2% of the time and include hemoperitoneum, hemobilia, acute hepatic ischemia, and pulmonary edema. Chronic complications include portal vein thrombosis, hemolysis, shunt stenosis, and hepatic encephalopathy. The latter may occur in 15 to 30% of cases and is associated with prior history of hepatic encephalopathy, severe liver disease, an older age group, large shunt diameters, and low final portosystemic gradient. Most respond to medical therapy, although the occasional shunt has to be occluded.[156]

The procedure is approached via puncture of the right internal jugular vein. A wire is then introduced, and the right hepatic vein is selectively catheterized. The catheter is wedged, and pressures are recorded. A wedged hepatic venogram may demonstrate the portal vein. The catheter is replaced by a sheath, and a Colapinto transjugular biopsy needle is introduced into the sheath and into the hepatic vein. The needle is directed anteromedially 2 to 3 cm into the parenchyma. Gentle aspiration is applied as the needle is slowly withdrawn, and when blood appears, contrast is injected to confirm entry into the portal vein or a branch. A wire is then introduced into the portal vein. The parenchymal tract is dilated with an 8 mm angioplasty balloon. The balloon is deflated, and the transjugular catheter and 9 French sheath are advanced into the portal vein. The transjugular catheter and balloon are removed, and a wall stent catheter, with a balloon attached, is introduced over the wire across from the hepatic vein to the portal vein. The balloon is inflated to distend the stent. Portal venous pressures are recorded, and venography is performed. This usually demonstrates the shunt and shows hepatopedal flow to the inferior vena cava with collapse of the varices. Rarely, the varices persist despite a well-functioning shunt, in which case, embolization is performed. If the shunt is felt to be open but the pressure remains above 12 mm Hg, the stent is dilated up to 10 mm.

Problems with accessing the portal vein do occur. In these circumstances, use of ultrasonography or the placement of a wire into the portal vein under ultrasonography control may help to direct the portal venous transjugular puncture. Some pain may be experienced during the procedure; for this reason, the procedure is best performed under a general anesthetic. If the stent is not long enough or does not cover the entire length of the parenchymal tract, an additional stent can be deployed to complete the shunt; parallel shunts may be used in patients with persistent portal hypertension. Doppler ultrasonography is used to monitor shunt patency approximately every 3 months, and venography is performed twice yearly. If shunt stenosis or occlusion is suspected, the patient is restudied. Stenosis is treated by angioplasty first and, in some cases, by additional stent placement. Thrombus within the shunt can be easily pushed into the portal vein using a soft occlusion balloon catheter. The clot presumably passes into the reopened varices and occludes them.[157–159]

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4. *Radionuclide Diagnosis*

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GENERAL ASPECTS OF PEDIATRIC NUCLEAR MEDICINE

Nuclear medicine is the branch of medicine and imaging that uses radionuclides (radioisotopes) for diagnostic and therapeutic purposes. The radioisotope is bound to a compound to form a radiopharmaceutical that is often organ specific. Images are produced by mapping the distribution of radioactivity of the administered radiopharmaceutical in the body using radioactivity detectors. The most commonly used detector is the gamma camera. In most studies, planar images are produced, but by moving the gamma camera around the patient, a three-dimensional data set is obtained and can be reconstructed and viewed in different planes, a technique known as single photon emission computed tomography (SPECT). This technique is used routinely in certain areas of nuclear medicine (eg, cardiac and brain imaging). In gastrointestinal radionuclide imaging, SPECT may be used to detect small amounts of isotope uptake or excreted isotope not visible on planar scans, perhaps owing to overlying structures.

Nuclear medicine studies provide information about organ function, and although they give some anatomic information, this is often limited, as is their ability to differentiate between pathologies, which ultimately have the same effect on organ function. There are few situations in pediatric gastroenterology in which nuclear medicine should be used as a first-line investigation, but the role of nuclear medicine in gastrointestinal diagnosis is often to provide the answer to a specific question, and the results must be interpreted in the context of other investigations.

The underlying principles of nuclear medicine are parallel in both adult and pediatric practice. However, certain aspects of pediatric nuclear medicine are unique. Indications for some investigations in pediatrics are different because of the different spectrum of pathology in children compared with adults (Table 74.4-1), and there is frequently a requirement for modification of technique.

In all cases, obtaining a high-quality diagnostic nuclear medicine study is dependent on the cooperation of the child, parent, or other caregiver and staff in the nuclear medicine department. In nuclear medicine departments dealing primarily with adult patients, it is essential that staff carrying out procedures on children are familiar with handling and communicating with children and understand their special needs. A full explanation of the procedure to both the child and parent or carer is mandatory and will often allay any fears and misconceptions early. Many departments supply information leaflets with the appointment letter or on arrival in the department, which can answer a number of frequently asked questions. The patient and their parent should be aware of what the investigation entails, including the anticipated length of time that they will need to be in the hospital. This is often several hours for a nuclear medicine study, and advice should be given to bring toys or books to keep a young child amused. The unpleasant aspects of the study, such as the intravenous injection of isotope, should also be fully explained. This is usually acceptable to both child and parent when a local anesthetic cream is applied to the skin before the injection, rendering it a relatively painless procedure. When venipuncture is required, it must be done by trained staff, skilled in obtaining intravenous access in children to minimize discomfort and stress to the child.[1]

During the nuclear medicine study, parents should be allowed to remain with the child at all times. Immobiliza-

TABLE 74.4-1 CURRENT INDICATIONS FOR RADIONUCLIDE IMAGING IN PEDIATRIC GASTROENTEROLOGY

^{99m}Tc SULFUR COLLOID OR PHYTATE COLLOIDS
Assessment of esophageal dysmotility and gastric emptying
Evaluation of gastroesophageal reflux
Investigation of suspected pulmonary aspiration
^{99m}Tc IDA COMPOUNDS
Investigation of neonatal jaundice
Confirmation of biliary origin of choledochal cysts
Assessment of hepatobiliary function in acquired liver disease
Postoperative and post-trauma assessment of bile duct integrity
Heat-denatured red blood cells
Detection of functioning splenic tissue (heterotopic or ectopic)
Labeled white cell scans
Diagnosis of inflammatory bowel disease, detection of active disease
^{99m}Tc HUMAN SERUM ALBUMIN
Diagnosis of protein-losing enteropathy
^{99m}Tc-LABELED RED BLOOD CELLS AND ^{99m}Tc PERTECHNETATE
Investigation of gastrointestinal bleeding
Labeled somatostatin analogues
Detection of somatostatin receptor positive tumors
Labeled bile acid analogues
Diagnosis of bile acid malabsorption in cases of unexplained chronic diarrhea

IDA = iminodiacetic acid; ^{99m}Tc = technetium 99m.

tion is an essential requirement for obtaining high-quality images, and this can be a particular challenge, given the length of time taken to obtain views for some nuclear medicine studies. For neonates and children up to the age of 2 years, it is usually sufficient to hold the child in place or use sandbags and padding to aid immobilization. Sleep deprivation prior to the procedure and feeding the child while lying on the gamma camera table may also be useful. In many departments, music and television are used to help prevent the child from becoming bored and restless. Provided that the procedure is carried out in a calm environment and the child is accompanied and kept amused throughout, sedation is seldom required. During the scan, whenever possible, the gamma camera should be positioned underneath the child on the table because positioning the camera above children tends to frighten them. It is also customary to obtain the most important images first.[2,3]

Most pediatric nuclear medicine studies use the isotope technetium 99m (^{99m}Tc), which emits gamma rays and has a half-life of 6 hours. ^{99m}Tc can be bound to several substances, which are taken up by specific organs. The dose of the radiopharmaceutical should be scaled down according to the child's body surface area or weight (Table 74.4-2). Age is not used because of the wide variation in body surface area and weight, which would result in a variation in dose that may not necessarily be appropriate. There is also a minimum dose of radiopharmaceutical for each examination, below which the injected activity is too low to obtain adequate images no matter how small the child (Table 74.4-3). Absorbed radiation doses from gastrointestinal radionuclide examinations are usually lower than from radiographic procedures such as fluoroscopy or computed tomography (CT) of the abdomen. After the injection, where possible, patients should be encouraged to void frequently for those examinations in which the radiopharmaceutical is excreted by the kidneys so as to reduce the radiation burden to the pelvic organs.

TABLE 74.4-2 FRACTION OF ADULT ADMINISTERED ACTIVITY FOR CHILDREN BASED ON BODY WEIGHT*

KG	FRACTION OF ADULT-ADMINISTERED ACTIVITY	KG	FRACTION OF ADULT-ADMINISTERED ACTIVITY
3	0.1	30	0.62
4	0.14	32	0.65
6	0.19	34	0.68
8	0.23	36	0.71
10	0.27	38	0.73
12	0.32	40	0.76
14	0.36	42	0.78
16	0.40	44	0.80
18	0.44	46	0.82
20	0.46	48	0.85
22	0.50	50	0.88
24	0.53	52–54	0.90
26	0.56	56–58	0.95
28	0.58	60–62	1.00

*Recommended by the Pediatric Task Group of the European Association of Nuclear Medicine.[90]

TABLE 74.4-3 MINIMUM AMOUNTS OF ADMINISTERED ACTIVITIES FOR CHILDREN FOR GASTROINTESTINAL SCINTIGRAPHY*

RADIOPHARMACEUTICAL	MINIMUM ADMINISTERED ACTIVITY FOR CHILDREN (MBq)
^{99m}Tc colloid (liver and spleen)	15
^{99m}Tc colloid (gastric reflux)	10
^{99m}Tc spleen (denatured RBCs)	20
^{99m}Tc HIDA (biliary)	20
^{99m}Tc HMPAO (WBC)	40
^{99m}Tc pertechnetate (Meckel diverticulum)	20
^{99m}Tc RBCs (blood pool)	80

HIDA = hepatobiliary iminodiacetic acid; HMPAO = hexamethylpropyleneamine oxime; RBCs = red blood cells; ^{99m}Tc = technetium 99m.
*Recommended by the Pediatric Task Group of the European Association of Nuclear Medicine.[90]

SCINTIGRAPHIC ASSESSMENT OF GASTROESOPHAGEAL DYSFUNCTION AND REFLUX

Many techniques are used for the diagnosis of esophageal disorders, gastroesophageal reflux, and gastric emptying in children. These include pH studies, barium radiology, manometry, ultrasonography, impedance studies, endoscopy with biopsy, and scintigraphy. Each of these techniques has its own merits and limitations, and each may be informative about different aspects of esophagogastric physiology and pathophysiology. There is little recent published work comparing these methodologies and their relative contributions.

In this field, the main symptoms of relevance to pediatric practice are related to gastroesophageal reflux. Esophageal dysfunction and delay in gastric emptying are thought to contribute to reflux, as well as representing disease entities in their own right. The assessment of each is difficult for several reasons. There is no consensus as to what represents the reference or "gold standard." Additionally, the presence of gastroesophageal reflux in itself does not necessarily equate with a disease state. Thus, a distinction should be made between gastroesophageal reflux and gastroesophageal reflux disease. This is further complicated in pediatric practice by marked clinical and physiologic differences between age groups and between those children who seem to have reflux disease as an isolated problem and those in whom it is associated with another disorder, such as cerebral palsy or chronic lung disease. Because gastroesophageal reflux is mostly intermittent, methods of investigation must be longitudinal. Such limitations must be borne in mind when considering the role of scintigraphy.

Oral ^{99m}Tc sulfur colloid or phytate colloids are used in gastroesophageal scintigraphy because they are not absorbed from the gastrointestinal tract. Also, if aspirated, they remain localized, are readily detectable, and are eventually cleared without sequel.[4] The labeled colloid is mixed with the feed to be administered, residual activity is cleared from the mouth and esophagus with an unlabeled liquid or feed, and the patient is imaged for 1 hour. Ideally, milk

should be used because this does not enhance gastric emptying. Dynamic images are taken at regular intervals (eg, every 10 seconds with the child lying comfortably on the scanning table). The stomach activity is masked by the use of lead shields to maximize esophageal visualization. Both images and time-activity curves are recorded. This enables the identification of the frequency of reflux episodes together with their proximal extent (Figure 74.4-1).

Using this method, the results obtained can be considered to represent only the postprandial situation. This

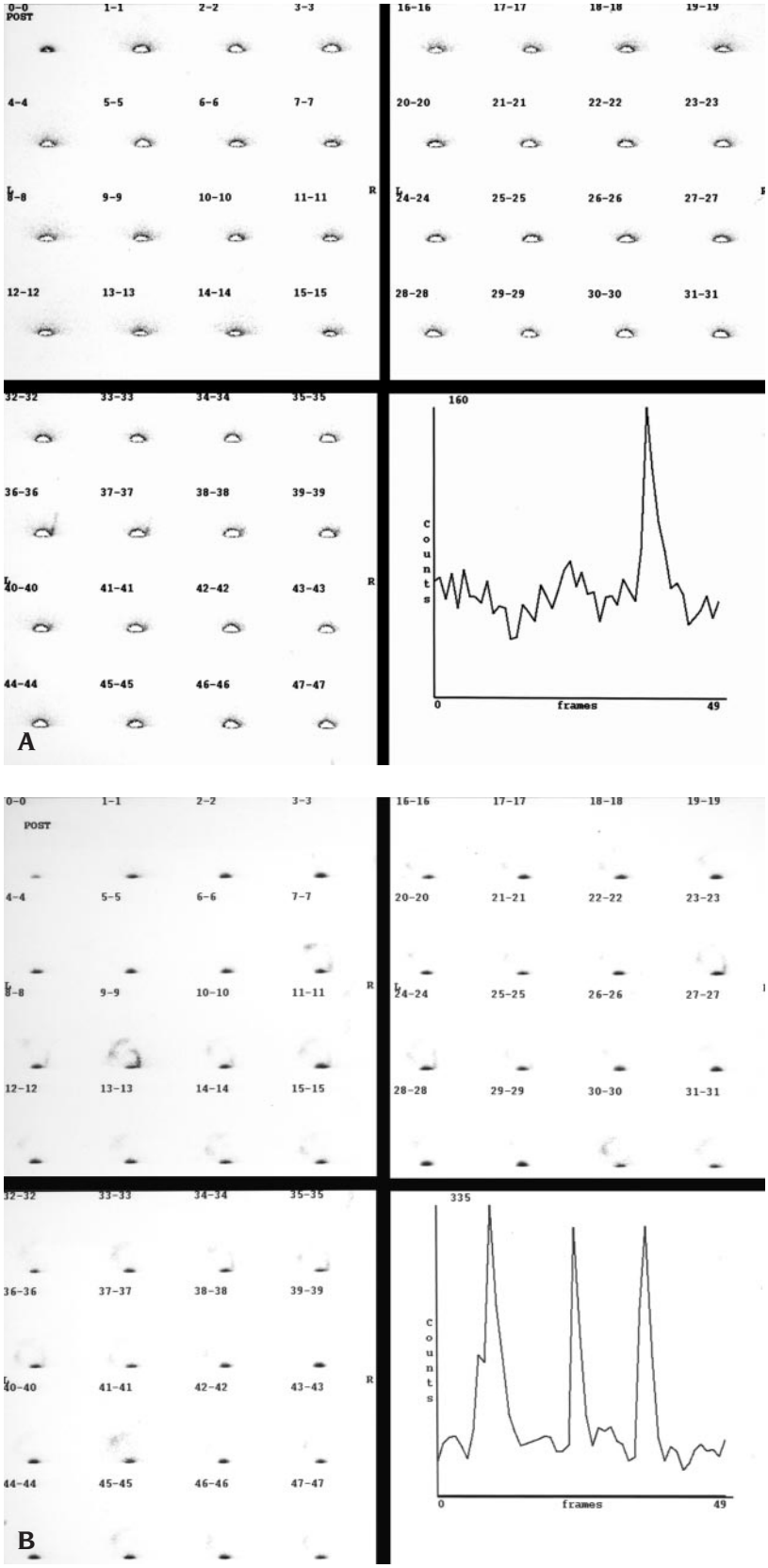

FIGURE 74.4-1 Gastroesophageal reflux studies using labeled sulfur colloid mixed with milk feed. Episodes of gastroesophageal reflux are indicated by the peaks on the activity curves. These represent individual reflux episodes, one in *A* and three in *B*. The proximal extent of the reflux is assessed from the accompanying images.

offers certain advantages over pH studies because gastric acidity may be neutralized by food, especially milk, in the immediate postprandial period, making pH studies unreliable.[5,6] Although scintigraphy is unable to delineate anatomic features, such as hiatus hernia, the radiation exposure is considerably less than that with barium studies. In addition, it has the advantage of allowing longer periods of observation to detect reflux. Piepsz and colleagues performed gastroesophageal scintigraphy in 35 children of varying ages who were felt clinically to have gastroesophageal reflux.[7] Compared with barium studies and findings on endoscopy with esophageal histology, gastroesophageal scintigraphy is more likely to detect reflux episodes than barium studies. However, there was no consistent correlation of scintigraphic results with findings at endoscopy. A second study compared several different methodologies in the diagnosis of 30 infants and children with symptoms of gastroesophageal reflux.[8] Gastroesophageal scintigraphy was positive in only 17 (56.6%) patients. The authors suggest that barium studies and scintigraphy are complementary evaluations and that a combination of the two increases sensitivity.

Images of the thorax taken 1 and 4 hours after the reflux study occasionally detect pulmonary aspiration, but this method of detecting aspiration is generally unreliable.[9] A salivagram has been developed in which a small amount of labeled colloid is placed on the tongue and is allowed to mix with saliva. Subsequent images of the tracheobronchial tree collected up to an hour afterward may indicate aspiration of saliva.[10] However, the accuracy of this method has not been assessed, and it is specific for aspiration of saliva as opposed to refluxate.

SCINTIGRAPHIC ASSESSMENT OF GASTRIC EMPTYING

The concept of abnormal gastric emptying as a disease entity and its possible relationship to gastroesophageal reflux disease has not been well validated. It has been suggested that delayed gastric emptying predisposes infants to reflux and to aspiration,[11] but this point was not confirmed in other studies.[12] Much of the uncertainty is due to the number of variables that can affect gastric emptying, such as age, feed type, and the lack of standardized normal values. Therefore, although scintigraphic assessment of gastric emptying in association with the assessment of reflux is feasible, its value must be considered questionable.

Gastric emptying studies may be useful in selected patients, for example, when considering gastrostomy placement or evaluation of patients with dumping syndrome. The patient's usual volume of milk or food is labeled with ^{99m}Tc sulfur colloid and is administered either orally or by nasogastric or gastric tube. Patients are imaged supine for 1 hour. If gastric emptying is roughly 50% at 1 hour, the study can be terminated. If there is delayed emptying at 1 hour, the patient is allowed to sit upright or walk around before delayed images are acquired at 90 minutes and 2 hours. If emptying is graphically displayed, then certain patterns may be recognized. These include plateau phase seen in pylorospasm or antral web, increasing gastric counts owing to overlapping duodenum, and a rapid fall in activity seen in dumping syndrome.[13]

SCINTIGRAPHIC ASSESSMENT OF THE ESOPHAGUS

Scintigraphy can be used to assess esophageal motility and transit time. It is a dynamic method and so allows appropriate physiologic analysis. The technique can be performed only in cooperative children. A labeled colloid is used, as in the assessment of reflux, and is delivered as small oral boluses, which can be observed individually and summated to allow activity in any particular segment. Generalized dysmotility can be distinguished from focal areas of abnormality, such as a stricture.[14] As with many scintigraphic techniques, this approach has not been well validated compared with other methods, and its place in the routine investigation of children is not established. Esophageal scintigraphy may be considered in children with persistent symptoms of dysphagia in whom contrast studies are normal.

HEPATOBILIARY SCINTIGRAPHY

Hepatobiliary scintigraphy is used to assess hepatocyte function and the excretion of bile into the small intestine. There are many uses of hepatobiliary scintigraphy, but in pediatric practice, the most frequent one is to help determine the etiology of jaundice in the neonatal period.

The radiopharmaceuticals employed in hepatobiliary scintigraphy are those of the ^{99m}Tc-labeled iminodiacetic acid (IDA) group. The agent is given intravenously and is then transported to the liver bound to albumin, where it is taken up by the hepatocytes via an active process. Excretion into the bile ducts is by a combination of active and passive transport mechanisms. The kidneys excrete a proportion of the agent, but with increasing hepatocellular dysfunction, a higher percentage is renally excreted.[15] ^{99m}Tc diisopropryl-IDA (DISIDA) is the radiopharmaceutical most frequently used in hepatobiliary scintigraphy. In the normal patient, ^{99m}Tc DISIDA has a hepatic extraction of 88% and urinary excretion of 11%. ^{99m}Tc trimethylbromo-IDA (TBIDA; mebrofenin) has a hepatic extraction of 98% and urinary excretion of 1.5% and is currently the best agent for use in patients with high bilirubin levels because it has a higher resistance to displacement by bilirubin, which is also carried in the blood bound to albumin.[16] ^{99m}Tc TBIDA has a greater than 70% hepatic extraction with bilirubin levels over 20 mg/dL, whereas ^{99m}Tc DISIDA has a lower hepatic extraction at 36% with bilirubin levels of 10 mg/dL.[15]

INVESTIGATION OF NEONATAL JAUNDICE

Hyperbilirubinemia is common in the neonatal period and in the majority of cases is due to benign physiologic jaundice. Prolonged neonatal jaundice is defined as jaundice lasting more than 14 to 21 days. The immediate priority in prolonged neonatal jaundice is to differentiate between

pathologic neonatal cholestasis (conjugated hyperbilirubinemia) and unconjugated hyperbilirubinemia, which is usually benign. More than 15% conjugated hyperbilirubinemia is considered to be significant rather than the absolute bilirubin level.[17] Cholestasis can be due to multiple intrahepatic causes such as infection, metabolic or genetic conditions, or extrahepatic abnormalities causing mechanical obstruction to bile flow. Early recognition of extrahepatic causes is vital because early surgical intervention in extrahepatic biliary atresia (EHBA) is associated with a better outcome.[18,19] In EHBA, all or part of the extrahepatic bile ducts are obliterated, leading to complete biliary obstruction. In approximately 5% of cases, the gallbladder or parts of the common bile duct are patent. Treatment is by the formation of a Kasai portoenterostomy in which the atretic extrahepatic biliary tissue is removed and a Roux-en-Y jejunal loop is anastomosed to the hepatic hilum to try to establish biliary drainage into the intestine. The likelihood of this surgery being successful is reduced after 60 days of age[20]; therefore, early diagnosis of EHBA is vital.

The complete workup of an infant with cholestasis will include laboratory tests of liver function, serologic tests for infection, metabolic screening tests, and imaging investigations, which are all correlated with clinical information. Ultrasonography is the primary imaging modality used to visualize the hepatobiliary anatomy and exclude congenital abnormalities of the liver and biliary system and other abdominal organs. Congenital bile duct dilatation, or choledochal cyst, is the disorder most commonly diagnosed by ultrasonography. Gallstones or sludge in the biliary system may be detected, sometimes in association with proximal biliary dilatation. Biliary dilatation is not a feature of EHBA. Ultrasonography is unable to diagnose EHBA, although many series report certain ultrasound findings that increase or decrease the likelihood of EHBA. These include the "triangular cord sign," which is a triangular- or tubular-shaped echogenic structure seen ultrasonographically in the vicinity of the portal vein at the porta hepatis and is said to represent the fibrous cone of atretic extrahepatic biliary tissue in patients with EHBA.[21] Although this is a helpful sign, it is not considered to be a reliable method of detecting or diagnosing EHBA.[22] A small or absent gallbladder in a fasting infant is suggestive of EHBA. Visualization of a normal-sized gallbladder makes the diagnosis of EHBA less likely, but appearances of the gallbladder at ultrasonography cannot be accurately relied on.[23]

The role of hepatobiliary scintigraphy in the investigation of neonatal cholestasis is primarily to exclude EHBA, but it cannot differentiate between other forms of cholestasis. Hepatobiliary scintigraphy demonstrates the ability of the hepatocytes to take up isotope, as a measure of hepatocyte function, and demonstrates patency of the biliary tree by the visualization of excretion of isotope into the gut. In neonates being investigated to differentiate between intrahepatic and extrahepatic causes of cholestasis, premedication with phenobarbital at a dose of 5 mg/kg/d orally is used for at least 3 days to induce hepatocyte microsomal enzymes, thereby increasing bilirubin conjugation and excretion. This approach increases the specificity of the test in distinguishing EHBA from other causes of neonatal cholestasis by ensuring the best possible excretion of hepatobiliary agents. If the test is performed urgently without premedication, it may have to be repeated following phenobarbital premedication if excretion of isotope into the bowel is not seen. Patients should be fasted for 3 to 4 hours prior to the study. Following injection of a radiopharmaceutical, dynamic imaging is performed for the first 5 minutes to visualize the distribution of tracer in the blood pool and to assess hepatic uptake. The gamma camera is placed anteriorly over the abdomen and positioned to include the heart, liver, and bowel. Thereafter, static images are acquired at intervals up to 1 hour after the injection. A suggested regimen is to obtain static images at 5, 10, 15, 30, and 45 minutes and 1 hour. If there is no excretion into the bowel at 1 hour, then further imaging is performed at later intervals, for example, at 3 to 4 hours until activity is seen in the bowel. If bowel activity remains undetectable, imaging at 24 hours is undertaken with anterior and lateral images to detect activity in the bowel or rectum. If the gallbladder has been visualized but fails to empty significantly during the first 60 minutes, an additional series of images should be taken following a normal feed to stimulate gallbladder contraction. Cholecystokinin can also be used to stimulate gallbladder emptying.[24]

Images can be interpreted to assess the following parameters: blood flow and extraction of isotope by the liver, time of excretion and visualization of the biliary tree, time of visualization of isotope in the duodenum, parenchymal clearance of isotope, gallbladder contractility, duodenogastric reflux, delayed images of bowel or rectal activity, and position of the small bowel. However, there are two main phases: hepatic extraction (uptake) and excretion of isotope into the bowel. In normal neonates, hepatic extraction of isotope is prompt, with a uniform distribution. Maximum hepatic accumulation is usually seen within 5 minutes. The gallbladder may be visualized as early as 10 minutes into the study but is not always seen. Bowel activity is usually observed by 30 to 40 minutes. The hepatic, cystic, and common bile ducts are not normally visualized in the neonatal period but become more obvious after 12 months of age. Over the age of 8 years and in adulthood, the left hepatic bile ducts become prominent on hepatobiliary scintigraphy (Figure 74.4-2).[25] The hepatic extraction function (HEF) is defined by the extraction of isotope by the liver and reflects hepatocyte function. It can be assessed visually and quantitatively by analysis of the initial hepatic uptake phase of the study. The normal pediatric HEF is over 92%.[26] Hepatic half clearance times ($t_{1/2}$) are defined as hepatic parenchymal clearance divided by excretion and can be quantitatively assessed. The hepatic $t_{1/2}$ is normally less than 37 minutes.[25] However, there is a large overlap in the neonatal period, and determination of the hepatic $t_{1/2}$ has not been found helpful in differentiating between EHBA and other forms of cholestasis.

Typically, patients with EHBA presenting in the first 2 months of life show prompt hepatic extraction with a normal HEF (over 92%), nonvisualization of the gallbladder, prolonged retention of isotope in the liver, and absent

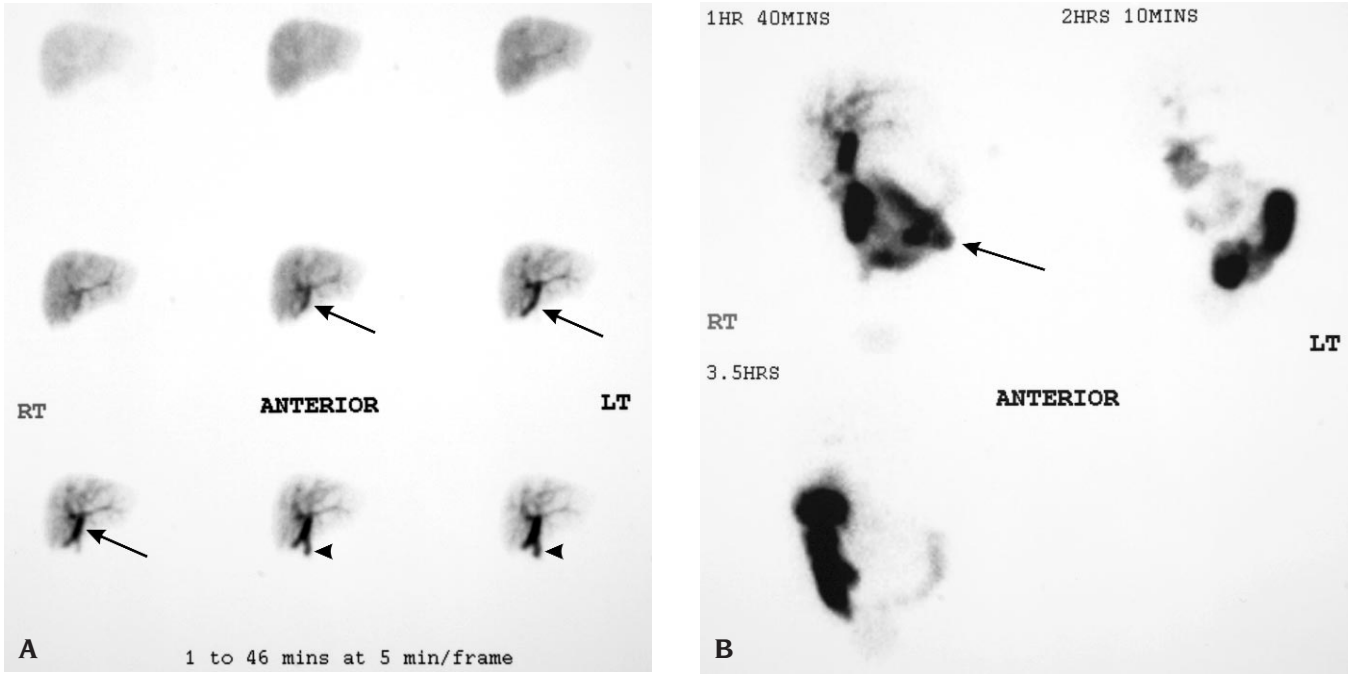

FIGURE 74.4-2 Normal hepatobiliary scintigraphy in a 9-year-old child. *A*, Initial images taken over the first hour of the study demonstrate normal uptake of isotope with good visualization of the intrahepatic bile ducts. The second and third rows of three images also demonstrate gallbladder filling (*arrows*) and the common bile duct (*arrowheads*). *B*, By 1 hour and 40 minutes, isotope has been excreted into the bowel (*arrow*). Subsequent images demonstrate gallbladder emptying and increased isotope excretion into the bowel.

excretion into the bowel even at 24 hours. Patients presenting after 3 months of age usually have compromised hepatocyte function and show reduced hepatic extraction, with reduced HEF and no biliary excretion. With this picture, differentiation from severe neonatal hepatitis or cholestasis may prove difficult. There are reports of patients with EHBA in whom excretion of isotope into the bowel was seen on scintigraphy in the early neonatal period, but repeat studies showed no excretion.[27] This may be due to progressive obliteration of the extrahepatic ducts continuing after birth. Therefore, it is appropriate to carefully monitor infants who show good extraction of isotope but only minimal excretion into the bowel.

Three patterns of hepatobiliary scintigraphic findings are described in neonatal hepatitis.[28] There may be normal hepatic extraction with visualization of isotope in the bowel, which excludes biliary atresia. Reduced hepatic extraction with absent excretion indicates severe parenchymal liver damage but would be inconsistent with EHBA in the first 3 months of life. Normal or near-normal hepatic uptake with no excretion is consistent with EHBA, but this pattern has been described in cases of severe neonatal hepatitis, cystic fibrosis (CF), α_1-antitrypsin deficiency, and syndromes with paucity of intrahepatic bile ducts.

In patients with neonatal hepatitis, the HEF is generally reduced, reflecting reduced hepatocyte function. There is persistent blood pool activity with delayed clearance because of reduced hepatocyte extraction. If there is severe hepatocyte dysfunction, there is reduced or absent excretion into the bowel and concurrent increased renal excretion of isotope (Figure 74.4-3). Any excretion of isotope into the bowel excludes EHBA.

The single most informative investigation in neonatal cholestasis is a liver biopsy. In experienced hands, liver biopsy has over 90% diagnostic accuracy, but it must be performed in a specialist pediatric center because it is not without risk, and the histopathology must be interpreted in the context of clinical information and other laboratory investigations.[29] The diagnosis of EHBA is ultimately confirmed at laparotomy and intraoperative cholangiography.

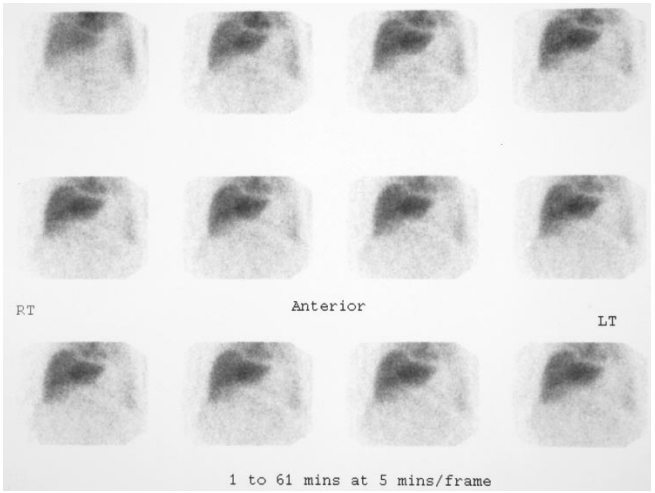

FIGURE 74.4-3 Hepatobiliary scintigraphy demonstrating generalized poor hepatic uptake of isotope, with persistent high background activity. The gallbladder is not visualized, and there is no excretion of isotope into the bowel. These findings reflect severe hepatocyte dysfunction in a patient with neonatal hepatitis, but extrahepatic biliary atresia cannot be excluded on the basis of this scan.

Spontaneous rupture of the common bile duct usually occurs in the first 1 to 2 weeks of life, and the cause is unknown. Infants present with jaundice and abdominal distention owing to ascites, which is bile stained on aspiration. Hepatobiliary ultrasonography may be normal in these children but often demonstrates a pseudocyst at the porta hepatis, usually without biliary dilatation. Occasionally, sludge or stones are seen in the common bile duct distal to the site of perforation, which is at the junction of the cystic duct and common bile duct. Hepatobiliary scintigraphy may show a photopenic area owing to the pseudocyst or extravasation of isotope into the peritoneal cavity. Intraoperative cholangiography confirms the diagnosis at the time of surgery.[25]

USE OF HEPATOBILIARY SCINTIGRAPHY BEYOND THE NEONATAL PERIOD

Patients with congenital bile duct dilatation (choledochal cyst) may present with cholestatic jaundice and acholic stools in the first few months of life. Most cases of choledochal cyst present under the age of 10 years, with 30% under 1 year of age. Presentation can even be delayed into adulthood, although this is uncommon. The diagnosis is usually made with ultrasonography at all ages. Following ultrasound examination, ^{99m}Tc IDA scintigraphy can be used to confirm the biliary origin of the cyst if thought clinically necessary. There is a known association of choledochal cysts with EHBA. Scintigraphy may help to determine the type of cystic dilatation and whether the cystic structure communicates with the biliary system. The scintigraphic appearance will depend partly on the degree of biliary obstruction caused by the cyst because extremely large cysts may obstruct biliary flow completely. There may be normal hepatic extraction and excretion of isotope, or, alternatively, isotope may accumulate in dilated ducts or cysts. A photopenic defect may be seen in the region of the porta hepatis depending on the size of the cyst. Complete obstruction with negligible biliary flow and nonfilling of the mass may occur. Rarely, activity is seen in the peritoneal cavity after cyst rupture. Like the gallbladder, choledochal cysts may contract after a fatty meal or cholecystokinin. Caroli disease (type 5 choledochal cyst) may present in infancy and shows an irregular pattern of cystic dilatation, with accumulation of isotope in the intrahepatic bile ducts on delayed images.[25] Although these findings are well described, with the advent of high-resolution ultrasonography and magnetic resonance cholangiography, radionuclides are currently seldom used in these clinical situations.

Patients with increased bile viscosity are at risk of developing bile plugs (inspissated bile syndrome), which can cause biliary obstruction. These include patients with CF, patients on total parenteral nutrition, and following extensive ileal resection. Appearances on ^{99m}Tc IDA scintigraphy are variable because there may be secondary liver damage, but there is typically good hepatic extraction of isotope and poor excretion into the gut. Occasionally, absent excretion is found in the neonatal period or in infancy, and the study is unable to exclude EHBA.[30] In Alagille syndrome, which is characterized by a paucity of interlobular bile ducts in association with certain phenotypic features and congenital cardiac and vertebral abnormalities, patients develop cholestasis, and ^{99m}Tc IDA scintigraphy demonstrates good extraction of isotope by the liver with marked retention of isotope in the hepatic parenchyma and usually minimal excretion into the gut. Occasionally, absent excretion is shown, and EHBA cannot be excluded.

HEPATOBILIARY SCINTIGRAPHY IN CF

^{99m}Tc IDA scintigraphy may be incorporated in the assessment and follow-up of patients who develop CF-related liver disease and is complementary to ultrasonography. Deficiency of the CF transmembrane regulator in the bile duct epithelial cells of these patients causes increased viscosity of bile that becomes inspissated, leading to plugging of intrahepatic bile ducts. Impairment of both intra- and extrahepatic biliary drainage contributes to the development of liver disease in these patients, with the development of fatty liver, chronic cholestasis, and, eventually, cirrhosis. Peak onset is during adolescence. ^{99m}Tc IDA scintigraphy allows assessment of hepatic extraction, clearance of isotope from the hepatic parenchyma, and biliary drainage and may have a role in monitoring disease progression and the success of treatment.[31]

POSTOPERATIVE AND POST-TRAUMATIC HEPATOBILIARY SCINTIGRAPHY

Postoperative evaluation of biliary drainage using ^{99m}Tc IDA scintigraphy is useful in patients who have undergone portoenterostomy (Kasai procedure) and also following liver transplant. The main indications for performing scintigraphy are to assess hepatic perfusion, parenchymal function, detect bile leaks, and assess transit of radiopharmaceutical from the liver into the intestine.[32]

In cases of liver trauma, CT scanning is the best method of assessing liver injury acutely. ^{99m}Tc TBIDA scintigraphy is useful in the early detection of bile duct injuries and biliary leaks.[33] The technique demonstrates active leakage of isotope into the peritoneal cavity or focal abnormal areas of activity owing to bile collections (Figure 74.4-4).

RADIONUCLIDE IMAGING OF THE LIVER AND SPLEEN

Radionuclide imaging of the liver and spleen can be performed using ^{99m}Tc sulfur colloid by virtue of the reticuloendothelial activity of these organs. This technique was used to provide structural information and to evaluate focal or diffuse disorders affecting the liver or spleen, but it has now been superseded by ultrasonography, CT, and magnetic resonance imaging (MRI), which provide excellent anatomic information and better resolution. Injected ^{99m}Tc sulfur colloid particles are phagocytosed by the reticuloendothelial cells of the liver, spleen, and bone marrow. The bone marrow of normal patients is not visualized owing to greater concentration of reticuloendothelial activity in the

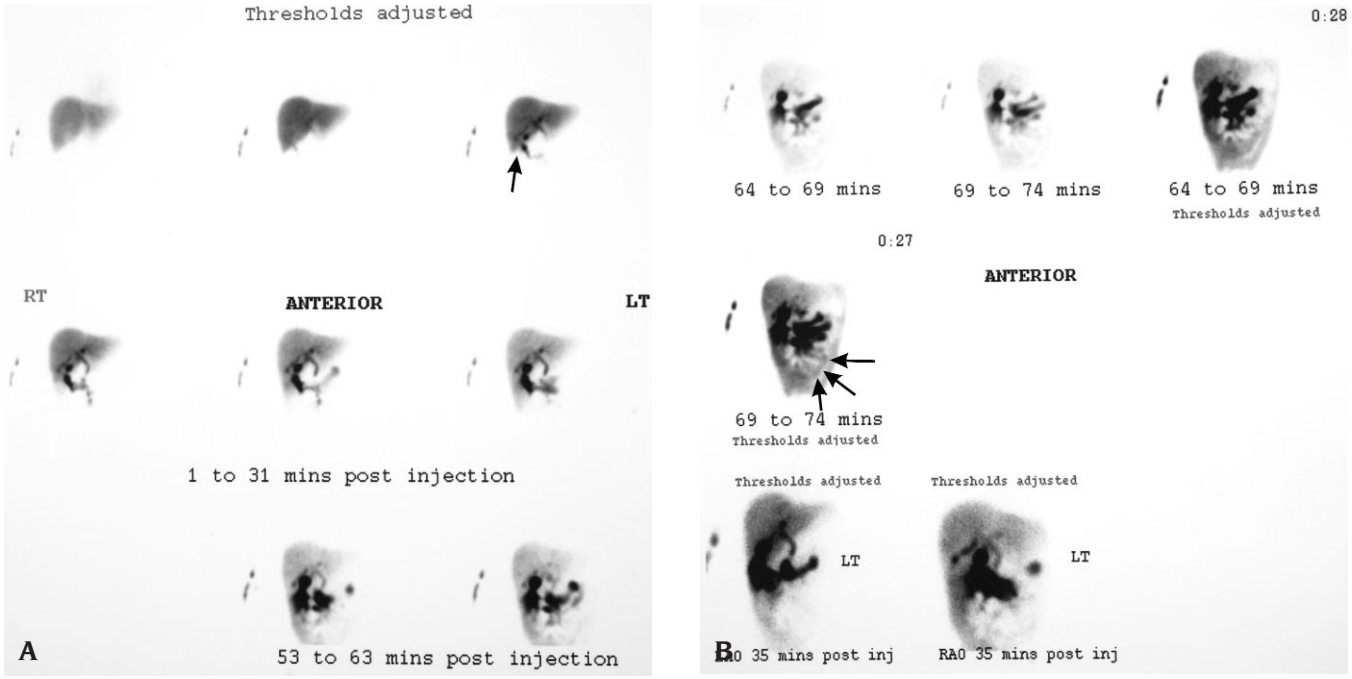

FIGURE 74.4-4 An 8-year-old boy who sustained liver lacerations in a road traffic accident. *A*, Hepatobiliary scintigraphy demonstrates normal hepatic uptake of isotope with visualization of main intrahepatic ducts. Focal collections of isotope around the porta hepatis on the far right image of the top row (*arrow*), and subsequent images are due to focal bile collections as a result of biliary leakage. There is excretion of isotope into the bowel but on images taken at 53 to 63 minutes (*bottom row*) and subsequently (*B*), peritoneal reflections are outlined by free isotope within the peritoneal cavity (*arrows*). This is better demonstrated following adjustment of the gamma camera parameters (threshold adjustment). Biliary leakage was confirmed at surgery.

liver and spleen. ^{99m}Tc sulfur colloid particles are cleared rapidly from the bloodstream following intravenous injection (typical $t_{1/2}$ of less than 3 minutes). Rapid sequence (dynamic) imaging is performed to show blood flow to the organs. There is earlier and more rapid uptake in the spleen and kidneys because the majority of the hepatic blood flow is via the portal vein. Static views are then obtained in multiple projections to visualize the organs and separate overlapping structures. SPECT images may improve resolution.

Imaging of primary and secondary hepatic tumors is now primarily with CT and MRI, and radionuclide imaging is not indicated. When performed, these studies show increased blood flow to the tumor on dynamic imaging, with photopenic defects on delayed static imaging using ^{99m}Tc sulfur colloid or ^{99m}Tc IDA compounds.[13] Vascular lesions, such as hepatic hemangiomas or hemangioendotheliomas, can be differentiated from hypervascular tumors because although both demonstrate increased perfusion during dynamic imaging using ^{99m}Tc sulfur colloid, there is prolonged retention of activity in the vascular spaces of hepatic vascular lesions (which does not occur in tumors). Both vascular lesions and tumors show photopenic defects on delayed static images owing to the absence of reticuloendothelial cells. The use of ^{99m}Tc-labeled red blood cells (RBCs) has been advocated in the case of suspected hepatic vascular lesions because of the overlap in scintigraphic findings with hypervascular tumors. However, three-phase CT and MRI have been shown to have greater sensitivity and accuracy and have therefore replaced scintigraphy in the evaluation of such lesions.

Hepatic infection, either diffuse hepatitis or focal infection with abscess formation, may show hepatomegaly with heterogeneous distribution of isotope. However, radionuclide imaging is not used routinely or as the primary method of imaging in these conditions. ^{99m}Tc sulfur colloid has been used as a screening or monitoring tool in patients with diffuse liver disease such as cirrhosis, α_1-antitrypsin deficiency, CF, storage diseases, or congenital hepatic fibrosis. In these disorders, the primary findings are diminished or heterogeneous uptake by the liver, with relatively increased uptake in the spleen and bone marrow in the setting of progressive disease. Hepatomegaly or reduction in liver size will depend on the stage of the disease.[32] In hepatic venous occlusion (Budd-Chiari syndrome), there is typically caudate lobe hypertrophy with increased uptake of ^{99m}Tc sulfur colloid and reduced uptake in the remainder of the liver, although other patterns are described.[34] Again, ultrasonography, CT, and MRI have replaced radionuclide imaging in screening for veno-occlusive disorders.

RADIONUCLIDE IMAGING OF THE SPLEEN

The spleen alone can be anatomically and physiologically imaged using ^{99m}Tc-labeled heat-denatured RBCs. The main indication for performing splenic scintigraphy is to identify functioning splenic tissue in patients with visceral heterotaxy syndromes and disorders that affect splenic function, such as sickle cell disease. Demonstration of splenic tissue on ultrasonography or cross-sectional imaging does not indicate splenic function, and this is particu-

larly true for patients with visceral heterotaxy or abnormalities of isomerism associated with congenital cardiac disease. Functional asplenia may be suspected from the detection of Howell-Jolly bodies in the circulating blood. It is important to identify patients with asplenia or functional asplenia because they are susceptible to overwhelming bacterial sepsis and require vaccination and antibiotic prophylaxis. However, when functional asplenia is suspected, the necessary precautions against sepsis are usually instituted, without risk and without resorting to splenic scintigraphy for confirmation.

Splenic scintigraphy may be employed in the detection of abnormally sited splenic tissue, which can be implanted in the chest, abdomen, or pelvis following trauma to the native spleen (splenosis). The extrasplenic tissue accumulates ^{99m}Tc-labeled RBCs in the same manner as the spleen, and this finding is useful in identifying apparently abnormally sited masses, which are, in fact, implanted splenic tissue. A further related use of splenic scintigraphy is in the identification of accessory spleens or splenunculi. These are considered to be a normal variant, but their identification is important in patients undergoing splenectomy for hematologic reasons.

Heat-denatured RBCs are injected intravenously and are avidly sequestered by the normal spleen, thereby demonstrating splenic function in addition to showing the position and size of the organ. This is accompanied by only faint uptake in the liver. If no functioning splenic tissue is present, there will be hepatic uptake owing to its reticuloendothelial activity and some excretion of free pertechnetate by the kidneys.

Between 3 and 8 mL of blood are taken from the patient depending on chronologic age and body weight. Under sterile conditions, the cells are labeled with ^{99m}Tc pertechnetate and then heated in a water bath at 49.5°C for 12 to 15 minutes.[32] The ^{99m}Tc-labeled RBCs are then reinjected intravenously, and static scans are performed in a number of planes after at least 30 minutes. SPECT scanning can be performed to improve localization, particularly if only small volumes of splenic tissue are present.

LEUKOCYTE SCINTIGRAPHY IN INFLAMMATORY BOWEL DISEASE

Leukocyte scintigraphy is a well-established technique that has a role in the detection of inflammation and sepsis. Its initial use in the investigation of inflammatory bowel diseases (IBDs) was reported in 1981.[35,36] Since that time, the place of labeled white cell scans (WCSs) has not been well defined in existing guidelines for the investigation of IBD, and use seems to reflect preferences in personal practice.[37] The technique has been evaluated as part of the routine investigation of suspected IBD, as a method for assessing both disease location and disease activity, and as a tool for following disease progression.

White cell scanning offers several advantages over other techniques commonly used to investigate IBD. The technique is noninvasive, does not require any patient preparation, and is well tolerated. It also allows whole-bowel examination with low radiation exposure,[38] and patients prefer WCSs to barium follow-through studies or enteroclysis.[39] These features suggest that white cell scanning is a technique that could be applied more frequently than either barium imaging or endoscopy and, more specifically, that it might be a more appropriate technique for children.

As the migration of leukocytes into tissues is a direct manifestation of a pathophysiologic process, it has been suggested that WCSs represent a direct measure of disease activity. Assessment of mucosal inflammation is otherwise possible only for areas of bowel that can be directly visualized and biopsied.

Several different indices of scintigraphic activity have been published. Broadly, these encompass visual grading systems requiring comparison of bowel activity with that of bone marrow, liver, or spleen[40,41] and computer-based methods with or without background subtraction.[42] Visual assessment has been demonstrated to have a high degree of interobserver variability.[43] Currently, there is no apparent consensus between studies as to how various areas of the bowel should be divided for an assessment of disease activity. Commonly, segmental analysis is reported according to uptake in the small bowel; ileum; cecum; right, transverse, and left colon; sigmoid colon; and rectum. A lack of universally accepted measures likely contributes to the variability of reported results and the lack of a generally agreed on role for use of WCSs in the assessment of disease activity in IBD.

There are two main isotopes used in leukocyte imaging. White cells may be labeled with ^{111}indium (^{111}In) oxime or ^{99m}Tc hexamethylpropyleneamine oxime (^{99m}Tc HMPAO). ^{111}In is cyclotron produced, whereas ^{99m}Tc is generator produced and is therefore more readily available. ^{111}In also gives a relatively higher bone marrow dose to children compared with adults owing to the greater proportion of red marrow in children.[44] HMPAO has the advantage of having a longer shelf life of 5.5 months and is more specific for granulocytes in a mixed cell population. ^{99m}Tc HMPAO also generates a superior image quality. Therefore, this compound has become the most widely used radioisotope in current practice.[45,46] Using ^{111}In or ^{99m}Tc HMPAO, the labeling process is relatively simple, but the leukocytes must first be separated from whole blood using sedimentation, centrifugation, and washing. The leukocyte pellet is then resuspended in sterile saline. When using ^{99m}Tc HMPAO, the preparation is simply added to the leukocyte suspension and is gently mixed. Following a short period of incubation at room temperature, washing, and resuspension in a small quantity of plasma, the labeled leukocytes are ready for reinjection. The labeling yield is usually about 50%.[47]

After injection, the labeled granulocytes migrate into the inflamed area. Using ^{99m}Tc, the most reliable images are generated soon after injection of labeled cells, generally at 30 to 60 minutes, although delayed scans, at 2 to 4 hours, may also be helpful (Figure 74.4-5).[48] Careful interpretation of the images is important because activity occurring in the bowel on later images can represent normal "physiologic" bowel activity. The process underlying this is uncertain, although there is some suggestion that it may represent cells being shed into the bowel lumen.

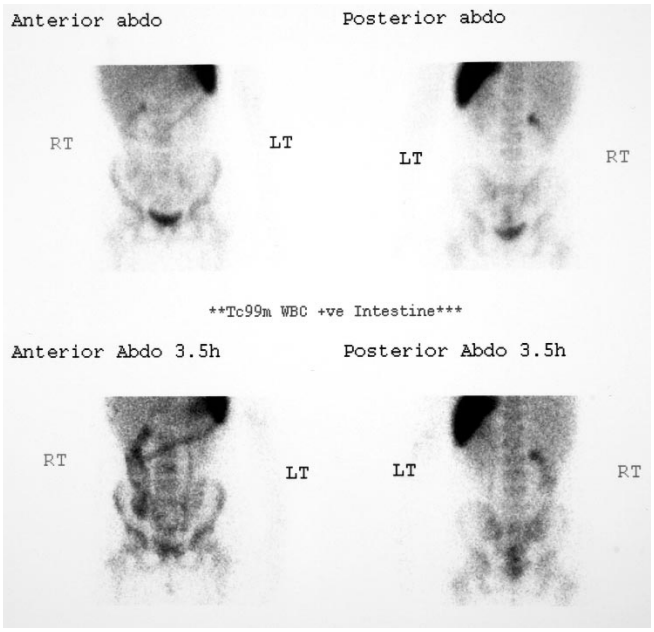

FIGURE 74.4-5 Labeled white cell scan demonstrating increased uptake in the cecum, ascending colon, and transverse colon in a 12-year-old boy with Crohn disease.

WCSs have obvious limitations. They cannot define anatomic features such as stricture, fistula, or prestenotic dilatation, although they may assist in distinguishing an inflammatory from a noninflammatory stricture. In interpretation of the images, potential causes of a false-positive WCS also must be considered. These include the presence of gastrointestinal bleeding, stoma sites, wounds, hematomas, deep vein thrombosis, and ischemic and infective bowel disease.[49] Other causes of bowel inflammation, such as infection or chronic granulomatous disease, may also generate positive studies.[50] Interpretation of anatomic distribution may be limited by bone, liver, spleen, and renal uptake, which can impair accurate assessment of small bowel involvement, and by urinary excretion because isotope within the bladder can affect assessment of uptake by rectal mucosa.

Several studies in children have compared the results of WCSs with histologic analysis of mucosal biopsies taken at colonoscopy. Such studies allow an assessment of the accuracy of the technique compared with the accepted diagnostic gold standard. A prospective investigation of 39 cases demonstrated that white cell scanning was able to diagnose IBD with a sensitivity of 90% and a specificity of 75% compared with colonic histology.[51] A similar prospective study compared scintigraphy in 137 children in whom colonoscopy and biopsy were also performed within 30 days and reported a sensitivity of 90%, a specificity of 97%, a positive predictive value of 97%, and a negative predictive value of 93% for WCSs.[39] However, endoscopic findings and histology remain the cornerstones for the diagnosis of pediatric IBD. It is not envisaged that these techniques would be replaced by scintigraphy.

Studies comparing WCSs and barium radiology demonstrate that scintigraphy has a higher accuracy for identifying IBD.[51,52] This suggests that the role of barium studies needs re-evaluation, particularly in screening for IBD. However, studies report less enthusiastic results for the scintigraphic diagnosis of IBD.[53,54] Reported differences in reliability likely reflect several factors, including different patient populations and differences in the severity of disease activity.

Bowel scintigraphy examines the whole of the bowel during one procedure. However, disease location is not always accurate when assessed by WCSs. Charron and colleagues assessed the accuracy of scintigraphy compared with endoscopy and biopsy for identifying colonic or ileal disease and found that scintigraphic location of disease activity limited to the colon yielded sensitivity and a positive predictive value of 97% and 97%, respectively.[55] For locating disease activity to the ileum, scintigraphy was less sensitive and specific. Inflammation within the terminal ileum is frequently difficult to distinguish from lymphoid nodular hyperplasia seen in up to 24% of barium studies when investigating IBD.[56] Furthermore, direct endoscopic visualization of the terminal ileum is not always possible for technical reasons. Studies in patients being investigated for IBD or for nonspecific gastrointestinal symptoms and other unrelated medical reasons have not reported terminal ileal uptake in any children without a final diagnosis of IBD, even in the presence of lymphoid nodular hyperplasia.[54,55]

Very few studies comment on the ability of scintigraphy to distinguish Crohn disease from ulcerative colitis on the appearance of activity within the colon. With continuous colonic activity, a distinction between Crohn disease and ulcerative colitis is not possible. Demonstration of discontinuous activity does not indicate Crohn disease with absolute certainty because discontinuous uptake has also been found in patients with ulcerative colitis, perhaps related to the attenuation of already minimal disease activity.[53,57] In this respect, WCSs are not sufficiently accurate and cannot be considered an alternative to endoscopic evaluation.

The accuracy of scintigraphy in the evaluation of small bowel disease is uncertain. Activity in overlying liver, spleen, transverse colon, and skeletal structures makes WCSs less sensitive in detecting upper gastrointestinal disease, and localization of proximal small bowel disease is poor. Reduced sensitivity of WCSs may also be related to the absence of active disease, with only the sequel of previous inflammation evident on barium studies.[58] The timing of scans is also important to avoid errors of interpretation owing to the normal hepatobiliary excretion of ^{99m}Tc.[59] Scintigraphy cannot be as informative as barium radiology in the definition of anatomic detail such as stricture formation. However, WCSs may provide information, allowing a distinction to be made between an inflammatory and a fixed stricture.

Currently, the role of scintigraphy in the routine investigation and monitoring of IBD is not well established. However, an understanding of the principles underlying these investigations and knowledge of the advantages and disadvantages of the technique allow the practitioner to decide whether scintigraphy could contribute to the optimal management of individual patients. Demonstration of florid disease activity, for example, would reinforce a decision to use more aggressive medical and surgical treatment.[60]

WCSs might have a role in the detection of postoperative disease activity, thus avoiding invasive tests in such patients. However, there are only limited reports in the pediatric literature, many of which are anecdotal. In a study of adults, Biancone and colleagues reported using leukocyte scintigraphy for the early detection of postoperative recurrence in Crohn disease.[61] Anastomosis-related activity was controlled for by comparison with patients who had undergone ileocecal resection for cecal carcinoma, and the findings were compared with those noted at endoscopy. There was good correlation between the endoscopic and the 30-minute scintigraphy scores at both 6 and 12 months postoperatively, suggesting that white cell scanning may prove to be a sensitive technique for this purpose.

The relatively noninvasive nature of leukocyte scintigraphy recommends its use for the screening of IBD. As with other indications for these investigations, there is uncertainty about its exact role and the advantages it has over endoscopy, biopsy, and barium radiology. Several studies in adults have looked at the role of WCSs in the screening of patients with suspected irritable bowel syndrome. Some authors express concerns over the high rates of false-positive results.[62] Shah and colleagues evaluated the accuracy of WCSs in screening children with suspected IBD and found false-positive scans in patients with postenteritis syndrome, infectious colitis, and anorexia nervosa, but there were no false-negative WCSs.[49] In clinical practice, if the clinical suspicion of IBD is high, it is likely that even with a negative WCS, further investigation should be recommended. Concerns over the possible inadequacies of WCSs in identifying small bowel disease also mitigate against the use of WCSs as a screening tool.

Leukocyte scintigraphy has also been used for identifying bowel inflammation associated with other disease entities. Hoare and colleagues reported a positive scan in a child with colitis owing to chronic granulomatous disease.[50] WCSs have been used following bone marrow transplant and cord cell infusion as treatment for chronic granulomatous disease to assess whether there is recurrence of colonic inflammation, without recourse to more invasive diagnostic tests.

Overall, it appears that there is currently no generally accepted role for leukocyte scintigraphy in pediatric IBDs. Nevertheless, WCSs are of value in certain situations, and the clinician should be aware of their potential. Wider use may well lead to a better understanding of their place in the diagnostic armamentarium.

ROLE OF SCINTIGRAPHY IN PROTEIN-LOSING ENTEROPATHY

Protein-losing enteropathy (PLE) is defined as a condition in which excess protein loss into the gastrointestinal lumen is severe enough to produce hypoproteinemia. PLE can occur in conditions such as ulcerative colitis, Ménétrier disease, celiac disease, and intestinal lymphangiectasia.[63] Most proteins lost into the bowel undergo proteolysis and are thus unreliable as a generalized measurement of PLE. α_1-Antitrypsin is resistant to intestinal proteolysis and thus its fecal level is indicative of the degree of loss of serum protein into the gastrointestinal tract.[64] Although this test provides a quantitative measure of intestinal protein losses, it does not allow localization of the site where this loss is occurring. Studies with ^{99m}Tc-labeled human serum albumin suggest that it is stable in the gut and that it is impermeable to normal bowel, making it an appropriate nucleotide for scintigraphic detection of PLE.[65] After intravenous injection of freshly prepared ^{99m}Tc human serum albumin, serial images of the abdomen are obtained from 10 minutes up to 24 hours after injection. A ^{99m}Tc human serum albumin scan is considered positive for PLE if there is visible tracer exudation in the gut (Figure 74.4-6).

Lan and colleagues presented two cases of PLE in children in which the loss was localized to the stomach in one child with transient Ménétrier disease and the small bowel in another child with primary oxalosis.[66] The diagnosis was confirmed in both cases by elevated 72-hour fecal α_1-antitrypsin levels. Halaby and colleagues reported a retrospective review of ^{99m}Tc human serum albumin scans in 18 children.[67] Scans were positive in 12 children, of whom 10 had primary intestinal lymphangiectasia, 1 had active *Salmonella* enterocolitis, and 1 had giardiasis. Scans were normal in the remaining 6 children, of whom 5 were subsequently demonstrated to have a primary intestinal lymphangiectasia. The authors noted that serum indices of PLE were less decreased than in those with positive scans, suggesting that a lower rate of protein loss might explain the false-negative scans. Chiu and colleagues stressed the importance of obtaining images up to 24 hours following the administration of the labeled albumin.[63] In summary, labeled albumin scintigraphy may be a useful tool in establishing a PLE. However, owing to concerns about accuracy, it is most appropriately used in association with other investigations.

NUCLEAR MEDICINE IN THE INVESTIGATION OF GASTROINTESTINAL TRACT BLEEDING

The causes of gastrointestinal bleeding in children are numerous, and radiology has a role in detecting the origin of the blood loss, defining associated pathology, and, in certain cases, intervening to control hemorrhage. There are two main techniques in nuclear medicine used to localize the site of gastrointestinal bleeding. Administration of ^{99m}Tc sulfur colloid or radiolabeled RBCs during active bleeding can localize the source of hemorrhage. It is said that RBC scans have detection rates higher than angiography at lower rates of blood loss—even as little as 0.1 mL per minute.[68] RBC and sulfur colloid scans have the advantage of being less invasive than angiography. The second technique, the so-called Meckel scan, involves administration of radiolabeled pertechnetate, which localizes in gastric mucosa and can identify ectopic sites of gastric mucosa as the source of blood loss. This type of scan is more frequently used when bleeding is intermittent and more occult.

GASTROINTESTINAL BLEEDING SCANS

There is an important difference between the two agents used to perform gastrointestinal bleeding studies. ^{99m}Tc sulfur colloid is a non–blood pool agent, which is cleared rapidly from the blood following injection. ^{99m}Tc-labeled RBCs are a blood pool agent that circulate in the blood for hours. When ^{99m}Tc sulfur colloid is injected intravenously, the particles circulate through the mesenteric vascular supply before being cleared by the liver and reticuloendothelial system. Any site of bleeding, vascular leak, or tear will allow the ^{99m}Tc sulfur colloid particles to extravasate, and this is detected using the gamma camera as a site of focal accumulation of isotope. Very small amounts of hemorrhage can be detected, but the patient must be actively bleeding between the time of injection and clearance of tracer, a window of approximately 15 to 20 minutes. For this reason, there is a high incidence of false-negative scans.[69] Bleeding near the liver or spleen may be obscured by activity in these organs, with a false-negative test result.

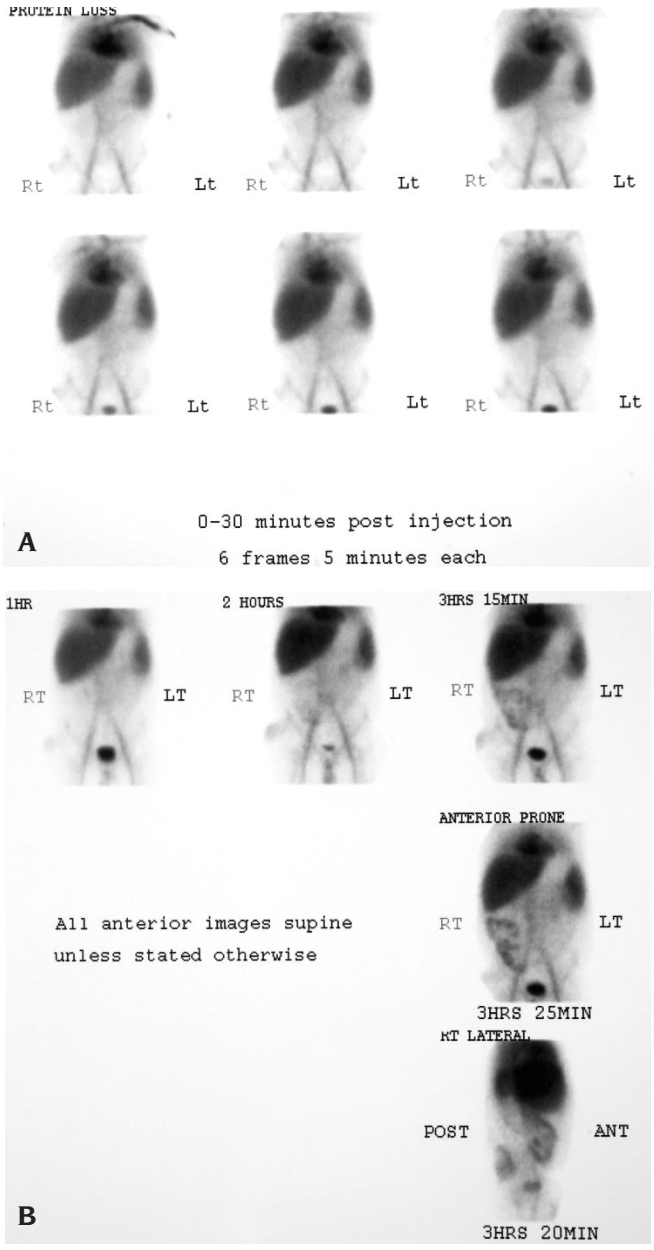

FIGURE 74.4-6 Protein-losing enteropathy. *A* demonstrates normal isotope uptake in the liver and spleen, normal activity in the vascular system, and renal excretion of isotope with activity in the bladder. *B*, By 2 hours, activity is seen within the gut in the right side of the abdomen and becomes more prominent on later images. This is due to loss of labeled albumin into the gut lumen.

The use of ^{99m}Tc-labeled RBCs is the preferred method for investigating acute gastrointestinal bleeding in children using radioisotope. It is more suitable for patients who are bleeding slowly or intermittently. However, this method requires more preparation time for labeling the RBCs and careful handling of the patient's blood. The labeling process can occur in vivo or in vitro. With in vivo labeling, an initial intravenous injection of nonradioactive stannous (tin) ion is given, followed 10 to 20 minutes later by an injection of ^{99m}Tc pertechnetate. The RBCs react first with the stannous ion and then the pertechnetate. Pertechnetate is reduced by the intracellular stannous ion, forming a complex with intracellular hemoglobin. Images can be acquired for several hours after the injection. However, high background activity reduces the sensitivity of in vivo labeled RBCs. Therefore, an in vitro labeling method is more commonly employed with a sample of approximately 5 mL blood taken into a syringe containing acid citrate dextrose to which stannous chloride is added.[70] The blood is then re-injected, and the patient is imaged immediately to demonstrate initial blood flow, followed by imaging for up to 2 hours. A normal ^{99m}Tc RBC study (both in vivo or in vitro labeled RBCs) will demonstrate tracer within the blood pools of the aorta, inferior vena cava, iliac vessels, and portal, renal, and mesenteric vessels. Tracer activity also persists in the kidneys and penis. Free pertechnetate can be seen in the kidneys, ureters, and urinary bladder. These are normal scintigraphic features and, most importantly, do not change over time. Sites of active bleeding are seen as focal, increasing accumulation of tracer that does move over time owing to gut peristalsis. Peristaltic movement may lead to erroneous impression of the exact site of bleeding. Computerized acquisition with dynamic cinescintigraphic display of sequential images is reported to improve the localization of bleeding sites.[71]

MECKEL SCAN

A Meckel diverticulum is a remnant of the omphalomesenteric (vitelline) duct, which is normally obliterated between the fifth and ninth week of gestation. It is present in approximately 2% of the population on the antimesenteric border of the small intestine, approximately 2 feet proximal to the ileocecal valve. Approximately half of Meckel diverticula contain ectopic mucosa, 80% of which contain gastric mucosa and therefore may become symptomatic owing to mucosal ulceration and intestinal bleeding. Ectopic pancreatic mucosa is present in approximately 5%, and this can occur concomitantly with gastric mucosa.[72]

^{99m}Tc pertechnetate is used to evaluate patients with lower gastrointestinal bleeding and abdominal pain and in whom a Meckel diverticulum is suspected. ^{99m}Tc pertech-

netate is taken up by the gastric mucin-producing cells and is then secreted into the gut lumen. Excretion of ^{99m}Tc pertechnetate is not dependent on the presence of parietal (acid producing) cells. It therefore detects the presence of gastric mucosa contained within a Meckel diverticulum rather than the diverticulum itself. In large case series, the test has a surgically proven sensitivity up to 85% and specificities as high as 95% for the detection of ectopic gastric mucosa.[73,74]

Ectopic gastric mucosa is visible as a focal area of isotope uptake simultaneous with uptake in eutopic gastric mucosa, usually within 10 minutes of injection of ^{99m}Tc pertechnetate (Figure 74.4-7). The intensity of the tracer accumulation may be less than that within the stomach, depending on the amount of mucosa present within the diverticulum and its secretory activity. Meckel diverticula are typically located in the right lower quadrant but may be found in different quadrants of the abdomen and can even move during the examination (Figure 74.4-8).[75] Normal excretion of ^{99m}Tc pertechnetate in the kidneys, ureters, and bladder should not be confused with pathology. Hydronephrosis, hydroureter, a pelvic kidney, and communicating urachal cysts can be mistaken for a Meckel diverticulum.[76] The bladder should be included on the abdominal images and postvoid images should be taken to ensure that a focal area of uptake is not obscured by activity within a full bladder.

Drugs that increase uptake or decrease the secretory activity of gastric mucosa can enhance the detection of a Meckel diverticulum. Pentagastrin given subcutaneously prior to the injection of ^{99m}Tc pertechnetate stimulates gastric mucosal uptake, whereas histamine$_2$-receptor antagonists inhibit the secretion of ^{99m}Tc pertechnetate from the mucin cells into the gut lumen. Glucagon acts to decrease gut peristalsis, thereby increasing retention of isotope within a diverticulum. In some institutions, pharmacologic enhancement is reserved for patients with a negative scan and a persisting high index of clinical suspicion.[13]

False-negative scans do occur and may be due to a number of factors, including suboptimal examination technique, impaired blood supply to the bowel, or insufficient mass of gastric tissue in the Meckel diverticulum to take up isotope. Continued bleeding or excessive secretions may cause washout of the isotope, leading to a false-negative test result. Repeat scanning, up to three times, is justified in patients with lower intestinal tract bleeding that is highly suspicious for originating from ectopic gastric mucosa and an initial negative ^{99m}Tc pertechnetate scan.[77] The use of SPECT has been advocated to increase the diagnostic accuracy of Meckel scans, particularly if the scan result is equivocal or if there is a strong clinical suspicion together with a negative Meckel scan.[78] Barium and contrast can interfere with visualization on scintigraphic examination. Therefore, Meckel scans or ^{99m}Tc RBC studies should be performed before barium studies or angiography. If both Meckel scan and ^{99m}Tc RBC imaging are to be performed, then Meckel scan should be performed first because stannous-containing agents affect the distribution of ^{99m}Tc pertechnetate within the body.[79]

False-positive scans may be seen with intestinal duplications that contain gastric mucosa. The presence of ectopic mucosa predisposes the patient to peptic ulceration, bleeding, and, occasionally, perforation as observed with Meckel diverticula. If a communication between the

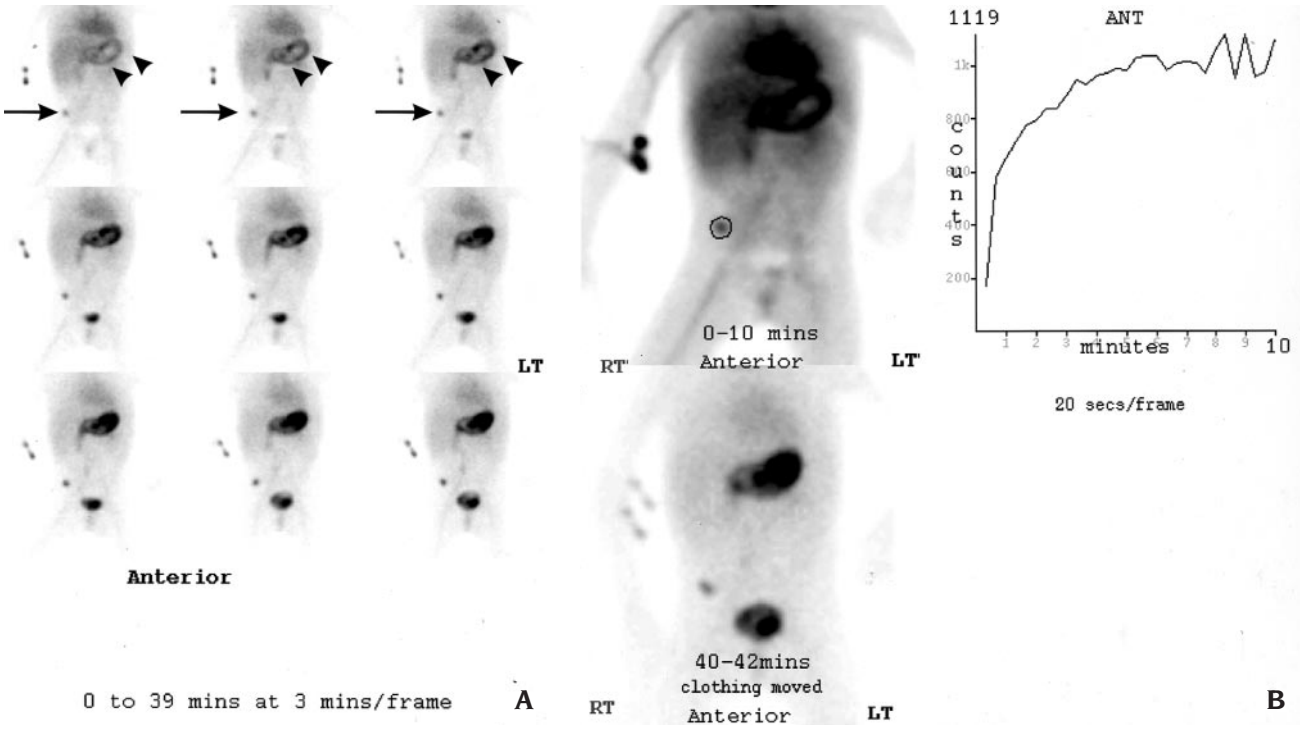

FIGURE 74.4-7 Positive Meckel scan. Uptake in the ectopic gastric mucosa of the Meckel diverticulum situated in the right lower quadrant (*arrow*) is simultaneous with uptake in gastric mucosa of the stomach (*arrowheads*). By drawing a region of interest around the ectopic gastric mucosa and plotting the count rate, maximum uptake is seen at approximately 5 minutes (*B*).

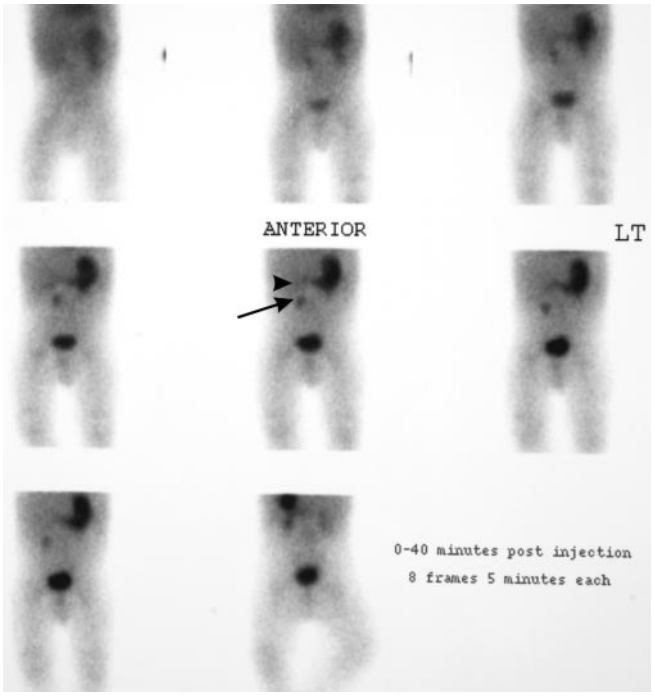

FIGURE 74.4-8 Positive Meckel scan. The Meckel diverticulum in this patient is located in the right upper quadrant (*arrow*), inferior to secreted activity in the first part of the duodenum (*arrowhead*).

cyst and the bowel exists, then bleeding will be intraluminal. If there is no communication, blood can accumulate within the cyst, and the patients present with a painful, rapidly enlarging abdominal mass.[72] Barrett esophagus, peptic ulcers, IBD, intussusception, vascular malformations, and neoplasms can also cause false-positive scans owing to the nonspecific accumulation of isotope.[13,72]

SOMATOSTATIN RECEPTOR SCINTIGRAPHY

Although neuroendocrine tumors affecting the gastrointestinal tract are uncommon in children, the gastrinoma (Zollinger-Ellison) syndrome and the VIPoma (Verner-Morrison) syndrome are well described (see Chapter 47, "Secretory Tumors Affecting the Gut"). These neuroendocrine tumors predominantly overexpress the somatostatin receptor SSTR2, which has a high affinity for synthetic analogues of somatostatin, such as octreotide.[80] This property has been exploited to allow accurate localization of the tumors. Radiolabeled peptides, such as ^{111}In pentreotide, bind to the SSTR expressed on the tumor, thereby allowing identification and localization.[81] Compared with other imaging techniques, including ultrasonography, CT, MRI, and angiography, somatostatin receptor scintigraphy is the most sensitive.[82,83] The ability of scintigraphy to image the whole body allows identification of both primary lesions and sites of metastatic spread. The technique has been further developed to allow tumor localization at surgery by use of a probe that detects preoperatively injected labeled octreotide.[84] Consideration has also been given to using receptor expression to target radiotherapy.

SCINTIGRAPHY IN BILE ACID MALABSORPTION

Bile acids are reabsorbed by an active transport mechanism in the terminal ileum as part of the enterohepatic circulation. Bile acid malabsorption is described as a rare cause of chronic diarrhea in infants owing to the effect of the bile acids on the colonic ability to reabsorb sodium.[85] Malabsorption of bile acids can also arise as a result of ileal resection (eg, in Crohn disease) and has been observed in association with cholecystectomy, chronic pancreatitis, celiac disease, and diabetes mellitus.[86] ^{75}Selenium (Se)-labeled homocholic acid conjugated with taurine (^{75}SeHCAT) can be used to measure bile acid losses from the gut. Tauroselcholic acid does not occur naturally but is an analogue of the naturally occurring bile acid conjugate taurocholic acid. ^{75}SeHCAT is administered intravenously and, through hepatobiliary excretion, becomes mixed with the endogenous bile acid pool. Malabsorption is assessed either by regular fecal collections or by assessing the 7-day retention, which is measured as a whole-body gamma camera count. The effective dose for this study in adults is 0.3 mSv, but there are no equivalent pediatric data. For comparison, the effective dose of a posteroanterior chest radiograph is 0.02 mSv and for a CT scan of the abdomen, 10 mSv. The average effective dose from natural background radiation per year is 3 mSv.[87]

In adults, normal retention at 7 days is over 10%.[88] Unfortunately, no lower limit has been determined for use in pediatric practice. However, this has not prevented its use in the diagnosis of primary bile acid malabsorption as a cause of protracted diarrhea in infants.[89] In this report, two sisters with chronic diarrhea are described in whom the ^{75}SeHCAT retention over 7 days was less than 0.1%. Subsequent investigations confirmed the absence of the bile acid transporter in the apical membrane of ileal enterocytes. Thus, although experience is still quite limited in children, the use of ^{75} SeHCAT can be justified in the investigation of children with unexplained chronic diarrhea and impaired growth.

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CHAPTER 75

1. Fluid and Dietary Therapy of Diarrhea

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Because diarrheal diseases have long been a major cause of morbidity and mortality,[1] the development of oral rehydration therapy (ORT) solutions has been hailed as one of the most important medical advances of the twentieth century.[2] This therapy is a rare but important example of the reverse transfer of technology from developing countries to developed countries. The initial trials of ORT were carried out primarily in less developed populations, where its effectiveness was established in children and adults with acute diarrhea from many etiologies.[3–6] The impact of ORT was established early in its development when it was used with dramatic effect during a cholera epidemic in West Bengal refugee camps in 1971.[7] ORT has been estimated to save about 1 million lives each year in the developing world.[8]

Although many of the basic science studies that provided the foundation of ORT were carried out in industrialized countries, these countries were slow to use ORT despite its successful use in the developing world.[9,10] The low rate of use of ORT occurred despite the endorsement of organizations including the American Academy of Pediatrics (AAP), the US Centers for Disease Control and Prevention (CDC), and the European Society for Pediatrics, Gastroenterology, Hepatology, and Nutrition.[11–13]

Optimal oral therapy has continued to evolve and now includes appropriate feeding during and after diarrhea in addition to the use of fluid and electrolyte solutions.[1,8] The emphasis on early feeding has helped to address the problem of malnutrition, which is often a major accompanying problem of diarrheal illness in developing countries.

This chapter discusses the continuing evolution of oral therapy, with special emphasis on dietary management of diarrhea. The principles of care established in developing countries now provide the basis for treatment in all parts of the world.

PHYSIOLOGIC BASIS OF ORAL THERAPY

To understand the concepts involved in effective oral therapy, it is helpful to review the physiology of intestinal fluid movement. Each day, about 9 L of fluid are processed by the adult intestine.[8,10] These fluids dilute food and are essential to the digestive process.

Reabsorption of that large volume of fluid occurs through the villous cells of the small intestine and the epithelial cells of the colon. The main driving force for water absorption is the osmotic response to the absorption of electrolytes and solutes, especially sodium and glucose. The bulk of sodium and water absorption from the small intestine results from coupled transport, in which the absorption of sodium molecules is linked to glucose or other small organic molecules such as amino acids, short-chain polypeptides, bile acids, or water-soluble vitamins by a variety of sodium-solute cotransporters expressed in the enterocyte apical membrane.[8] Sodium uptake in the small intestine can also occur passively down a concentration gradient created by active pumping of the ion out of the enterocyte by several ion-coupled mechanisms, facilitated by sodium/potassium-exchanging adenosine triphosphatase.[14] In addition to coupled transport, sodium transport occurs by solvent drag through the paracellular pathway.[14,15]

Diarrhea results when these secretion or reabsorption mechanisms are disrupted, causing dehydration by depletion of intravascular fluid and electrolytes. Diarrhea-causing organisms can increase the chloride-secreting activity of the crypt cells (toxigenic), can disrupt the absorption of sodium by villous cells (invasion or adherence), or can affect both mechanisms (see Chapter 2, "Microbial Interactions with Gut Epithelium"). Coupled transport of sodium and glucose or other organic solutes is not affected in toxigenic diarrhea and is at least partially competent in other types of diarrhea, such as that caused by *Rotavirus*.[8] The key physiologic foundation for successful ORT is a functioning sodium-glucose transporter.[2]

In 1964, Phillips was the first to show that glucose-linked sodium absorption is retained during severe diarrhea owing to cholera.[3] The initial success of ORT for adult cholera patients was soon tested under controlled conditions in the treatment of children with cholera, *Rotavirus*,

enterotoxigenic *Escherichia coli*, and other etiologic agents.[6,16–18] ORT emerged as a powerful therapeutic tool to correct dehydration resulting from acute diarrhea in all but the most severe cases and in all ages, irrespective of etiologic agents.[1,8] In severe cases, such as *Rotavirus* enteritis in infants, insufficient glucose-sodium absorptive capacity may be present to support ORT, perhaps because the infection involves all or most of the small intestine.

In addition to sodium and glucose, ORT also contains potassium, chloride, bicarbonate or citrate, and water (Table 75.1-1). Clinical balance studies have provided guidelines for the optimal concentration of the five components of ORT solutions and resulted in the formulation recommended by the World Health Organization/United Nations International Children's Emergency Fund (WHO/UNICEF) for over 30 years (see Table 75.1-1).[8] Maximal cotransport occurs when the molar ratio of glucose to sodium approaches 1.[8,10] Potassium is required because diarrheal dehydration can cause substantial loss of the ion, especially in infants and small children.[10] Chloride losses also occur during diarrhea, and the ion is required to balance the positive sodium and potassium ions. Varying degrees of base-deficit acidosis occur in diarrheal dehydration, and base replacement can speed the therapeutic response.[10] These other ions follow passively the coupled transport of sodium and glucose.

In North America, several commercial sugar-electrolyte solutions similar to the WHO/UNICEF formulation are widely available (see Table 75.1-1). They differ primarily in having slightly higher glucose and lower sodium concentrations than the WHO/UNICEF solution and are supplied in the more costly reconstituted form. In controlled trials in the United States, sugar-electrolyte solutions with sodium concentrations of 45 to 75 µmol/L have proven to be effective in the treatment of well-nourished children with mild to severe dehydration.[18–21] However, such solutions with sodium concentrations < 75 µmol/L are not suitable for use in adults and older children with cholera and similar secretory diarrheas.[10,11]

CONTINUING EVOLUTION OF ORAL THERAPY FOR DIARRHEA

Since 1971, WHO has recommended a single formulation of oral rehydration salts (ORS) to treat dehydration from diarrhea of any cause, including cholera, in all age groups.[1,8] Although ORS has been immensely successful,[1,8] it has not been used as widely as anticipated, primarily because it has no effect on shortening the duration or volume of diarrhea[9]; in fact, stool volume may increase on ORS. To help address this important limitation, a variety of strategies have been tried to add antidiarrheal properties to oral therapy.

Polymers and Added Solutes

Efforts to replace glucose with more effective cotransport molecules have included trials of amino acids, including glycine, glycyl-glycine, alanine, and glutamine.[22] These solutions have performed no better than ORS, especially in noncholera diarrhea, and have some important limitations, including the limited availability and high cost of amino acids and peptides, the development of metabolic abnormalities, and instability during storage.[22] The use of glucose polymers, including maltodextrins, permits the inclusion of additional cotransport molecules without incurring an osmotic penalty.[23] However, the reduced-osmolarity maltodextrin solutions resulted in no appreciable improvement over ORS.[22]

Cereal-Based Oral Therapy

A more effective approach to improving absorption of solutes and water proved to be the use of cereal-based solutions that use the complex carbohydrates and simple proteins of cereals as cotransport molecules.[23] Many of the studies of cereal-based therapy have used cooked rice and salts added in concentrations identical to the standard ORS.[24–26] Other cereals that have been used effectively include wheat, corn, sorghum, and millet.[26,27] A meta-analysis of randomized clinical trials of cereal-based ORT, especially rice-based ORT, has demonstrated that this form of ORT is superior to glucose ORT in adults and children with cholera and other high-purging diarrheas.[28] These solutions produced a mean reduction in stool output in the first 24 hours of therapy of ~ 30 to 40%. For children with acute noncholera diarrhea, the reduction in stool output was ~ 18%.[28] Despite these impressive results, cereal-based ORT has not been widely used, probably for a combination of reasons. One important constraint relates to the ease of preparation. Most studies of cereal-based ORT used cooked cereals, which require time and fuel to prepare, making these solutions less attractive than the easy-to-prepare packets of ORS. However, commercially prepackaged cereal-based ORT

TABLE 75.1-1 COMPOSITION OF GLUCOSE ELECTROLYTE SOLUTIONS

	CONCENTRATION (mmol/L)				
SOLUTION	CHO	NA	K	BASE	OSMOLALITY
KaoLectrolyte (Pharmacia/Pfizer, New York, NY, USA)	112	48	28	20	240
Pediatric Electrolyte (PharmaScience, Montreal, QC, Canada)	140	45	20	30	250
Pedialyte (Ross, Columbus, OH, USA)	140	45	20	30	250
Infalyte (Mead Johnson Evansville, ID, USA)	70	50	25	30	200
Rehydralyte (Ross Columbus, OH, USA)	140	75	20	30	301
Cera-lyte (Cera Products, Jessup, MD, USA)	80	70	20	30	235
Reduced osmolar ORS (WHO/UNICEF)	75	75	20	30	245
Previously recommended WHO/UNICEF ORS	111	90	20	30	310

CHO = carbohydrate; ORS = oral rehydration solution.

solutions that dissolve easily in water are now available (see Table 75.1-1). Perhaps more importantly, the use of these solutions was affected by the nearly simultaneous introduction of early appropriate feeding along with ORS. The interest in feeding as an integral part of oral therapy arose from the same potential advantages of enhanced solute uptake from easily digested foods that stimulated the development of cereal-based ORT and is discussed below.

Hypo-osmolar Solutions

The renewed interest in oral hypo-osmolar solutions for the treatment of diarrhea stems from perfusion studies that demonstrate a significant inverse correlation between ORT osmolality and water absorption in animals and humans.[29] Recent studies in children have demonstrated that hypo-osmolar ORT formulations can result in lower stool output (~ 20%), less vomiting (~ 30%), and less need for intravenous therapy for failures of ORT (~ 33%) compared with standard ORS.[30–34] A meta-analysis of 15 randomized controlled studies has helped to confirm the superiority of the reduced-osmolarity ORS over the standard WHO/UNICEF ORS.[35] However, concern exists for the development of hyponatremia when the reduced-osmolarity ORS is used in patients with high purging rates such as those caused by cholera because of the reduced sodium concentration. In the clinical trials, asymptomatic hyponatremia was reported in some patients, and WHO/UNICEF recommends that careful monitoring of electrolytes be done for patients with high purging rates who receive the new reduced-osmolarity solution.[36] Because of less need for intravenous therapy, decreased vomiting, and lower stool output, WHO/UNICEF now recommends the use of the reduced-osmolarity solution (see Table 75.1-1) as the single ORS for use in persons with diarrhea.[36]

Early Appropriate Feeding

Theoretical Considerations. The inclusion of early appropriate feeding along with ORT required the overturning of the common practice, used in many countries and cultures, of withholding feedings during diarrhea.[37] The rationale for withholding food during diarrhea was based primarily on the concern for malabsorption, which can occur because of an altered intestinal mucosa, decreased brush border enzymes, and a more rapid intestinal transit time.[38] The theoretical risk of increased macromolecular uptake across the damaged mucosa leading to intestinal allergy has been postulated but has never been shown to be of practical importance in diarrhea.[39] In contrast, the several important potential benefits of early feeding became a strong stimulus to study its safety and efficacy. These benefits include the chance to provide nutritional therapy to malnourished patients with diarrhea, especially in developing countries.[37,38] In addition, nutrient uptake plays an important role in intestinal repair.[40]

Nutrient Absorption during Diarrhea. Although some element of malabsorption is often associated with diarrhea, it is rarely complete, and substantial amounts of nutrients can be absorbed.[37,38,41,42] This is especially true for the digestion and absorption of complex carbohydrates in staple foods because the luminal concentrations of amylases and pancreatic enzymes appear to be largely unaffected during diarrhea.[37,38] Balance studies of nutrient absorption of staple foods have demonstrated that as much as 90% of complex carbohydrates, 70% of protein, and 60% of fat can be absorbed during acute diarrheal episodes.[41–43] Factors that influence the success of feeding include the age of the child, the etiology of the diarrhea, the severity of the stooling, and the composition of the diet.

Impact of Feeding on Diarrhea. Feeding is usually well tolerated in acute infectious diarrhea and can have a direct beneficial effect on diarrhea when combined with ORT.[38] Controlled clinical trials have shown that appropriate early feeding combined with ORT can reduce stool output[44–49] and the duration of diarrhea[46,48–53] compared with ORT or intravenous therapy alone.

Effective Feeding Regimens. Cereal-based staple-food diets have been among the most effective diets studied, but if cereals or legumes provide the sole source of protein, an amino acid profile deficient in essential amino acids is likely to result.[54] Also, a greater proportion of protein may be required in cereal- or legume-based diets because of their digestibility.[54] A solution to this problem is to include milk, a more complete protein source, along with cereals to improve the amino acid profile and digestibility. Ideally, milk can provide these essential amino acids, but because brush border lactase levels are often reduced during diarrhea, milk has often been avoided or diluted because of concerns about possible lactose intolerance.[37] The amount of lactose that can be tolerated by children with diarrhea is still a subject of controversy, but several principles have become clear.[54] Breastfed infants, who receive a higher concentration of lactose than children receiving cow's milk or cow's milk formula, can be fed safely through diarrhea.[37,38] Full-strength animal milk or animal milk formula is usually well tolerated by children who have mild, self-limited diarrhea, which is very common in the United States.[50] A meta-analysis on the use of lactose in children with acute diarrhea found that most children can safely tolerate full-strength animal milk, especially when combined with staple foods.[55] These mixed diets are better tolerated than milk alone[43] and are thought to be successful in part because of the smaller total lactose load and because solid foods help delay gastric emptying and thus slow transit time.[56]

Banana, Rice, Applesauce, and Toast Diet. This diet, commonly referred to as the BRAT diet, has long been used in North America to begin refeeding children who have had diarrhea. However, this diet is no longer recommended because it is low in energy density, protein, and fat and because the banana and applesauce may add an excessive load of sugars to the gut.[11] In addition, diets high in simple sugars (including sweet tea, juices, and soft drinks) (Table 75.1-2)[57] or fats should be avoided.[12] Optimal diets should provide a nutritionally acceptable balance of carbohydrate, protein, and fat.

TABLE 75.1-2 COMPOSITION OF REPRESENTATIVE CLEAR LIQUIDS NOT APPROPRIATE FOR ORAL REHYDRATION THERAPY*

LIQUID	CHO, mmol/L	Na, mmol/L	K, mol/L	BASE, mmol/L	OSMOLALITY, mmol/L
Cola	700 (F, G)	2	0	13	750
Apple juice	690 (F, G, S)	3	32	0	730
Chicken broth	0	250	8	0	500
Sports beverage	255 (S, G)	20	3	3	330

*Adapted from Snyder JD.[69]
CHO = carbohydrate; F = fructose; G = glucose; K = potassium; Na = sodium; S = sucrose.

Soy Formulas. Many practitioners in North America have switched their pediatric patients with diarrhea to soy formulas because of concerns for malabsorption of lactose in cow's milk and cow's milk formulas. However, data from controlled clinical trials demonstrate no benefit in soy formulas compared with cow's milk, especially when used with staple-food diets.[55] In addition, recent studies show that soy formulas were not as well tolerated in children with acute diarrhea as mixed staple-food diets[47] or soy formula with fiber.[58]

MANAGEMENT GUIDELINES

ORT can be administered to infants with a cup and spoon, a cup alone, or a feeding bottle. For weak small babies, a dropper or a syringe can be used to put small volumes of solution into the mouth. A nasogastric tube can be used to administer the ORT in babies or children who cannot drink because of fatigue or drowsiness but who are not in shock. In the vomiting child, small volumes, often 5 mL at a time, can be given frequently, as often as every few minutes (see below).

Early appropriate feeding, which has become an integral component of oral therapy, is the component that has the potential for the greatest impact on stool volume and duration.[11] Successful feeding trials have been carried out using breast milk, dilute or full-strength animal milk or animal milk formulas, dilute and full-strength lactose-free formulas, and mixed diets of staple foods with milk.[44–56] Recent studies indicate that diets of naturally occurring, culturally acceptable, inexpensive foods can be effective in diarrhea.[54] The implications of these findings are enormous from a health policy standpoint because they provide hope that the important ingredients of successful feeding therapy are already present, even in developing countries.

The specific recommendations for treatment depend on the severity of the diarrhea (Table 75.1-3).

No Dehydration

Intake of ORT is often low, in part because of the potentially salty taste of ORT.[9,11] Fortunately, if the stool output remains modest, ORT may not be required. If no dehydration develops, which is the case for the great majority of diarrhea patients in the United States, continued age-appropriate feeding is the only therapy required. Controlled clinical trials have demonstrated a number of foods that can be used safely and effectively in most children with diarrhea (Table 75.1-4). Unweaned infants should receive breast milk or continue to receive the regular formula. The formula does not require dilution if the diarrhea remains mild. If a diluted formula is used, the concentration should be increased rapidly if the diarrhea does not worsen. Weaned infants and children should have their regular nutritionally balanced diet continued, emphasizing complex carbohydrates (such as rice, wheat, and potatoes), meats (especially chicken), and the child's regular milk or formula. Diets high in simple sugars and fats should be avoided.[11,12]

Mild, Moderate, or Severe Dehydration

After dehydration is corrected, appropriate feeding is begun, using the guidelines above (see Table 75.1-3). Children with severe dehydration, which is a shock or a shock-like condition,[11,12] should be treated as an emergency. A large-bore catheter should be used for the infusion of Ringer lactate, normal saline, or similar solution, and boluses of 20 to 40 mL/kg should be administered until signs of shock resolve. Fluid and electrolyte resuscitation may require more than one intravenous site, and alternate access sites (including venous cutdown, femoral vein, or interosseous locations) may be needed.[11,12] As the patient's level of consciousness improves, ORT can be instituted. The hydration status must be frequently reassessed to monitor the effectiveness of the therapy. When rehydration is complete, feeding is continued as directed above.

TABLE 75.1-3 EFFECTIVE FOODS FOR USE DURING DIARRHEA

FOOD	OUTCOME
Breast milk[44] + ORT	↓ Stool volume compared with ORT alone
Cow's milk + staples[43] + ORT	No difference compared with ORT alone
Rice-based diet[26,28,42] + ORT	↓ Stool volume and duration compared with ORT alone
Wheat-based diet[27] + ORT	↓ Stool volume and duration compared with ORT alone
Wheat-pea diet[45] + ORT	↓ Stool volume and duration compared with ORT alone
Potato diet[43] + ORT	↓ Stool volume and duration compared with ORT alone
Chicken[51] + ORT	↓ Stool volume and duration compared with ORT alone
Egg[53] + ORT	↓ Stool volume and duration compared with ORT alone

ORT = oral rehydration therapy.

TABLE 75.1-4 MANAGEMENT OF ACUTE DIARRHEA

TREATMENT	MILD DIARRHEA, NO DEHYDRATION	MILD DEHYDRATION (< 5%)	MODERATE DEHYDRATION (5–9%)	SEVERE DEHYDRATION (> 10%)
ORT	Rehydration: may not be required	Rehydration: 50 mL/kg over 4 h	Rehydration: 100 mL/kg over 4 h	Rehydration: IV therapy with solution like normal saline or Ringer lactate, 20–40 mL/kg/h until rehydrated; then may begin ORT
	Continuing losses: if required: 10 mL/kg/each stool	Continuing losses: 10 mL/kg for each stool and estimated emesis volume	Continuing losses: same as for mild dehydration	
Early appropriate feeding	Continue age-appropriate diet (see Table 75.1-3)	Begin age-appropriate diet when dehydration corrected	Begin age-appropriate diet when dehydration corrected	Begin age-appropriate diet when dehydration corrected and patient stabilized

Adapted from American Academy of Pediatrics, Subcommittee on Acute Gastroenteritis.[11]
ORT = oral rehydration therapy.

Special Considerations

Vomiting. Vomiting, commonly associated with acute diarrhea, can make ORT more challenging, but almost all children with vomiting can be treated successfully with ORT.[11,12] When small volumes (eg, 5 mL) are given frequently (eg, every 1 to 2 minutes), an effective volume of ORT can be administered to a vomiting child.[11,12] Although this technique is labor intensive, as much as 150 to 300 mL/h of fluid can be administered.

Correction of fluid and electrolyte deficits by balanced-electrolyte ORT can help speed recovery from vomiting.[11,12] As vomiting decreases, ORT can be given in larger volumes, and when rehydration is complete, feeding can begin.

Nasogastric tubes can sometimes be of benefit in administering ORT to vomiting children. Continuous infusion of ORT can result in improved absorption of fluid and electrolytes and may permit more aggressive therapy in a child with poor intravenous access. Nasogastric infusion cannot be considered in children who are comatose or who have ileus or evidence of intestinal obstruction.

Zinc Supplementation. Several recent studies have identified zinc supplementation to have a significant impact on the duration and severity of acute and persistent diarrhea in children in nonindustrialized countries.[59–61] The mechanisms for this effect are not yet known but may include the effect of zinc on the absorption of water and electrolytes, improvement of intestinal function and enzyme activities, or enhanced immunocompetence.[62]

Hypernatremia. A long-standing concern, especially in developed countries, is the perceived risk of hypernatremia from ORT, especially with a relatively high sodium concentration in rehydration solutions.[8,10,11] However, a large body of data has clearly shown that oral rehydration solutions are an effective treatment for hypernatremia, given normal renal function.[63,64] These studies demonstrate that ORT can result in better outcomes than the best results reported with intravenous therapy.

Refusal to Take ORT. Refusal of pediatric patients to take ORT is a common complaint of practitioners in North America,[11] but children who are dehydrated rarely refuse ORT because they usually crave salt and water. The problem is usually found in children who have little or no dehydration. Most children do not require ORT if their diarrhea is mild, if they remain stable, and if they show no evidence of dehydration.[11]

Methods for increasing ORT intake include the use of flavored ORT, which does not alter the composition of fluid and electrolytes but which improves taste.[11] These flavored solutions are now the most popular forms of ORT sold in North America. Another effective way to increase intake is to freeze the ORT solution in an ice-pop form.

Management at Home. The principles for the effective household treatment of diarrhea, which have been developed primarily from extensive experience in developing countries, are the same for all settings.[9,11,12] As mentioned above, the most essential principles are the use of ORT to replace fluid and electrolyte losses and the continuation of appropriate feeding as soon as dehydration has been corrected. This is true both for developing and developed countries, but greater educational efforts are needed in all settings to help parents and caretakers provide effective therapy and to recognize the development of serious conditions.[65] The need for better education is underscored by the fact that approximately 10% of preventable infant deaths in the United States are caused by improper recognition and inappropriate treatment of acute diarrhea.[66]

Severe Malnutrition and ORT. Diarrhea is a serious and often fatal event in children with severe malnutrition. Although treatment and prevention of dehydration are essential, care must also focus on careful management of malnutrition and on treatment of other infections. Dehydration owing to acute diarrhea in children with severe protein-energy malnutrition (eg, marasmus, kwashiorkor) can be managed with ORT as described above.[1,12] In children with kwashiorkor, ORT must be closely supervised

because of the increased risk of edema and congestive heart failure, especially with intravenous therapy.

Use of Antibiotics or Nonspecific Antidiarrheal Agents. The message that neither antibiotics nor nonspecific antidiarrheal agents are indicated for most diarrheal episodes also requires emphasis.[11,12,67] However, antibiotics are indicated for a few specific infections, including cholera *Shigella* with systemic spread, *Clostridium difficile*, *Salmonella* in children < 3 months old, *Giardia*, and *Entamoeba histolytica*.[68]

Drugs that alter intestinal motility, secretion, adsorption of fluid and toxins, or intestinal microflora have little or no data to support their use in children.[11,12,68] The AAP, CDC, and WHO all recommend that such drugs not be used to treat acute diarrhea.[11,12,68]

Low Use Rates for ORT. Low use rates continue to be an important problem in both the developing and the developed world. Despite its unquestioned success in saving lives, only about 40% of diarrheal episodes are treated with ORT in the developing world,[67] and the estimates for use in North America are far lower.[9,65,69] The efforts to improve the antidiarrheal properties of ORT may have an important impact on use. In the United States, the cost of ORT is often 20 to 50 times higher than the $0.10 to $0.15 per-packet cost for ORS in the developing world, limiting its use in the most disadvantaged portions of the population, who are at the greatest risk for morbidity and mortality from diarrhea.[66] In countries without state-sponsored health care, many third-party payers do not reimburse clinicians and hospitals for oral fluid therapy despite its proven ability to reduce unnecessary medical visits and hospitalization.[65]

Persistent Diarrhea. Persistent diarrhea, defined operationally as episodes that continue for 14 days or longer, is responsible for a large proportion of deaths in young children with diarrhea.[70–73] General treatment guidelines include the use of appropriate fluids to prevent or treat dehydration, a nutritionally effective diet that does not worsen the diarrhea, and supplementary vitamins, minerals, and antimicrobial agents to treat concurrent infections such as pneumonia, sepsis, and urinary tract infections.

A spectrum of illness exists for persistent diarrhea, but a common pattern is for affected children to pass several liquid stools in a day, without much dehydration. Growth failure and adverse nutritional consequences may result. In other cases, children may have severe and persistent watery diarrhea with dehydration. When more severe diarrhea is present, the principles of ORT are the same as for acute diarrhea except for the few children who will exhibit evidence of temporary glucose malabsorption. These children may require intravenous fluids until their glucose malabsorption resolves.

The feeding principles described earlier also apply to children with persistent diarrhea. A multicenter study that evaluated a diet algorithm for the treatment of persistent diarrhea in young children in developing countries provided several practical guidelines.[73] In this study, the initial diet used locally available staple foods (rice or corn), milk or yogurt (< 4 g lactose/150 kcal), vegetable oil, and sugar. The children who did not improve were given a lactose- and sucrose-free cereal-based diet. Most of the children had resolution of or improvement in their diarrhea while on these nutritional therapies even though this was a population of very compromised children, including many who had extraintestinal infections. Influenced by this study, the current guidelines on nutritional therapy for persistent diarrhea include (1) breastfeeding whenever possible, (2) animal milk (limited to 50 mL/kg/d) mixed with cereal for non-breastfed children, (3) a daily intake of at least 110 calories/kg, and (4) supplemental vitamins and minerals.[73]

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2. *Feeding Difficulties*

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Although primary nutrient deficiencies are now rare in developed countries,[1] feeding difficulties are a continuing challenge for those who care for infants and children. It is estimated that up to 54% of infants and toddlers have problematic feeding behavior and that in 25% of children, feeding problems prompt parents to seek professional advice.[2,3] Feeding difficulties may persist and in 1 to 2% of infants will lead to retarded growth.[4–6] A survey of children with cerebral palsy found that in the first 12 months of life, 38% had problems with swallowing, 57% had problems with sucking, and more than 90% had significant oral-motor dysfunction.[7]

Disorders of the gastrointestinal tract will interfere with adequate intake of nutrients and may compromise the nutritional status of the infant at several levels. Poor intake owing to anorexia, fatigue, or dysphagia may add to the dilemma of increased losses from vomiting or malabsorption. The goals of nutritional therapy are to restore the patient to normal nutritional status, to enhance recovery from the underlying disease process, and, ultimately, to attain nutritionally adequate and developmentally appropriate feedings. This chapter examines the basis of feeding disorders in infants and children and current approaches to their evaluation and treatment.

NORMAL DEVELOPMENT AND MECHANICS

Feeding problems represent a deviation from normal feeding skill development and mechanics.[8–10] Maturation of deglutition, which begins in utero and is completed by 3 years of age, depends on integration of oral-motor, fine-motor, gross-motor, sensory, and behavioral skills. An outline of feeding development in normal infants is seen in Table 75.2-1. Whereas sucking movements can be seen at 15 to 18 weeks gestation, adequate coordination of suck, swallow, and breathing to permit significant nutrient intake is not present until 34 to 35 weeks gestation. Reflex behaviors present at term may disappear later in infancy (rooting, phasic bite) or may persist (gag, swallow).[11]

For the infant, successful feeding depends on coordination of suck, swallow, and breathing. Sucking itself has a developmental progression, with the earliest tongue movements described as "suckling" and characterized by an in-out horizonmental movement. "Sucking" appears later in infancy, indicates an up-down tongue movement with firm lip approximation, and is used in a general sense in this discussion. Swallowing, or deglutition, occurs in at least three phases, as shown in Figure 75.2-1.[11,12] The oral phase includes bolus formation of solid food or liquid by the

TABLE 75.2-1 NORMAL FEEDING DEVELOPMENT

AGE	REFLEXES	ORAL-MOTOR SKILLS	SELF-FEEDING
15–18 wk gestation		Sucking movement	
34–35 wk gestation		Adequate suck-swallow coordination	
Term	Rooting, gag, phasic bite	Jaw and tongue move up and down; air swallowing common	
3–4 mo	Phasic bite disappearing	Tongue protrudes in anticipation of feeding	Visual recognition of bottle/nipple
5–6 mo	Rooting diminishes	Munching begins, smacks lips together, strained foods begin	Puts hands on bottle, begins finger-feeding
7 mo	Mature gag		May insert spoon in mouth
9 mo		Lip closure, lateral tongue movement	
12 mo		Rotary chewing, controlled, sustained bite	Finger-feeds independently, brings spoon to mouth
18 mo		Swallows without food loss, tongue elevates intermittently or consistently	Cup drinking with two hands, spoon-feeds messily
24 months		Lips contain food/saliva within mouth, tongue transfers food one side to other side	Fills spoon with finger or spoon to mouth without inversion

Adapted from Glass RP and Wolf LS[8] and Cloud H.[9]

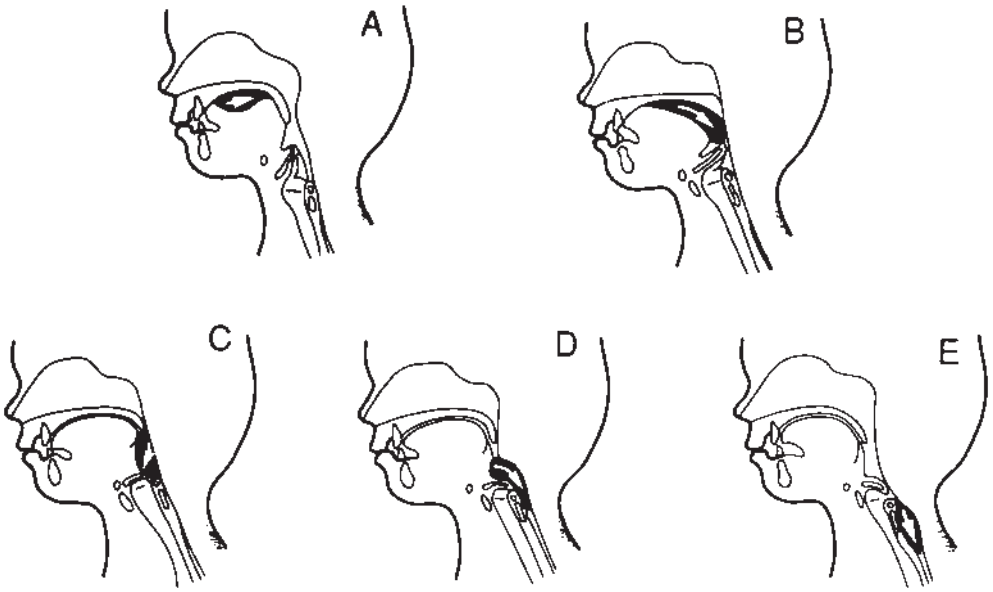

FIGURE 75.2-1 Schematic drawing to show oral, pharyngeal, and esophageal phases of normal swallow in a young child. *A*, Oral phase showing formed bolus moving posteriorly through the oral cavity. *B*, Beginning of pharyngeal phase. *C*, Bolus moving through the pharynx with adequate airway protection. *D*, End of pharyngeal phase as cricopharyngeus opens. *E*, Esophageal phase with bolus in the cervical esophagus. Reproduced with permission from Arvedson J and Brodsky L.[11]

tongue, with the soft palate lowering to prevent food from entering the pharynx and initiating a swallow. The pharyngeal phase is both voluntary and involuntary and begins as a swallow is initiated. As food enters the pharynx, the soft palate elevates to protect the nasopharynx and the larynx elevates and closes to protect the airway. The final or esophageal phase begins with relaxation of the upper esophageal sphincter (cricopharyngeus muscle) and carries the bolus to the stomach under involuntary control. The return of the upper esophageal sphincter to its tonically contracted state prevents laryngeal penetration or aspiration of esophageal contents or reflux of the bolus back into the pharynx.

ETIOLOGY

Infants with feeding problems are usually referred for evaluation when inadequate intake has led to growth faltering or eventually failure to thrive, or perhaps less frequently when a caregiver perceives significant difficulty in feeding the infant even if weight has not dropped off. The feeding problem may be the primary cause of poor growth, secondary to other disease processes, or both. Understanding which patients are at risk for feeding disorders will promote early intervention.[13]

Table 75.2-2 presents selected etiologies for feeding difficulties. Appetite will be altered if the timing or volume of feedings is inappropriate, an example being the "grazing" behavior of toddlers.[14] A common iatrogenic alteration of appetite occurs with the use of supplemental tube feedings. Although these may be necessary for adequate weight gain, they eventually interfere with normal hunger-satiety cycles. Anorexia is common in disorders of the urea cycle and organic acid metabolism, whereas a major cause of weight loss in Crohn disease is decreased intake owing to poor appetite.[15,16]

A child's diet can lead to feeding difficulties, and food allergy is one cause of vomiting or irritability associated with feeds.[17] Age-appropriate foods are essential to acquiring normal feeding behaviors.[18]

Dysphagia specifically refers to difficulty with swallowing and implies an organic basis.[19] Illingworth distinguished between anatomic, neuromuscular, and inflammatory factors for dysphagia.[20] Anatomic lesions range from the cleft lip and palate that present at birth to the more subtle anomalies such as submucous cleft palate or vascular ring. The neurologic immaturity of the premature infant results in predictable feeding concerns based on the gestational age and general medical conditions.[21] Other neuromuscular disorders may affect coordination of suck and swallow or esophageal peristalsis (pseudo-obstruction, repaired tracheoesophageal fistula).[22] Although not specifically related to difficulty in producing a swallow, as with neuromuscular and anatomic causes, the term dysphagia is also applied when the act of eating or swallowing produces pain or other discomfort. Acute inflammatory disorders are usually infectious, and chronic conditions are likely acid related (peptic esophagitis). A review of 600 infants with gastroesophageal reflux found that 4% had severe feeding resistance sufficient to warrant tube feedings, and this feeding resistance was often the initial or only symptom.[23] Dysphagia may be the first complaint in older children with gastroesophageal reflux disease (GERD).[24]

Systemic conditions, such as cardiovascular or pulmonary disease, may require extra work of breathing and stress the feeding infant. Such illnesses place greater caloric demand on the patient while also interfering with intake.[25–27]

Successful feeding results from appropriate infant responses to food and nurturing caregivers in a dynamic interaction. Not surprisingly, many infant feeding problems involve primarily nonorganic or behavioral factors.[13] These nonorganic feeding problems have been categorized or described by several authors. One categorization is tripartite: young infants (2–8 months) with an attachment disorder owing to poor parent–infant interaction, the older infant or toddler (6–36 months) with "infantile anorexia" and struggles around autonomy, and the less affected "picky eater" who is not yet malnourished.[28] Others describe early nonorganic failure to thrive as "failure to

TABLE 75.2-2 CAUSES OF FEEDING DIFFICULTIES

DECREASED APPETITE
Abnormal feeding patterns
Supplemental feedings
Metabolic disorders
Inflammatory bowel disease
DIET
Inappropriate foods
Food allergy
DYSPHAGIA
Anatomic
Macroglossia
Cleft lip and palate
Submucous cleft palate
Pierre Robin sequence
Laryngeal cleft
Tracheoesophageal fistula
Vascular ring
Foreign body
Neuromuscular
Prematurity
Cerebral palsy
Bulbar palsy
Infant botulism
Muscular dystrophy
Pseudo-obstruction
Connective tissue disease
Repaired tracheoesophageal fistula
Inflammatory
Viral stomatitis
Candida stomatitis, pharyngitis
Peptic esophagitis
Mucositis (graft-versus-host disease)
Systemic
Cardiac disease
Pulmonary disease
BEHAVIORAL
Attachment disorder
Infantile anorexia
Picky eater
Failure to imbibe
Delayed introduction of solids
Oral aversion
Conditioned dysphagia
Post-traumatic eating disorder
Vulnerable child
Parental responses

Adapted from Rudolph CD,[10] Kedesdy JH and Budd KS,[14] and Illingworth RS.[20]

imbibe" in young infants with poor intake and no apparent problems with mother–infant interactions.[29–31]

When the introduction of feedings is delayed beyond a sensitive or critical period, later acquisition of feeding skills may be difficult.[32] Medical or surgical disorders early in life may necessitate the use of nasogastric or gastrostomy feedings or parenteral nutrition to maintain adequate nutrition. If supplemental feeding is required on a short-term basis, growth is maintained, and generally the infant quickly resumes normal feeding behavior. However, if long-term tube feeding or intravenous alimentation is required, especially if the infant has had little previous positive experience with normal feeding, the child is at risk for the development of an aversion to oral feedings. Intrusive oral procedures such as nasogastric tube placement, endotracheal intubation, and oropharyngeal suctioning have been associated with aversion to oral feedings.[33] The long-term pairing of eating and pain, as occurs in GERD, may establish a negative feeding experience and a "conditioned dysphagia."[23,34,35] Older children who experience an episode of choking have developed an acute food refusal, described as a post-traumatic eating disorder.[36]

Even in the case in which medical conditions impact nutrition, the parental reaction to feeding can play a pivotal role. Parents may be faced with innumerable negative feeding interactions because of the need to focus on the child's nutrient intake. These experiences can change feeding from a satisfying parent–child interaction to a grim, anxiety-producing chore. Further, infants with early medical problems may be perceived as "vulnerable" by their parents, who consequently may have difficulty setting age-appropriate limits, in this case around eating.[3,37] Therefore, even after an organic disorder is resolved, the feeding interaction remains stressed.

Multiple etiologies are often present in the child being evaluated for poor feeding. In 50 infants with feeding disorders, Budd and colleagues found that 64% had problems with both organic and nonorganic symptoms, contributing to their insufficient food intake.[38] Burklow and colleagues reviewed 103 children with complex feeding problems, identifying 80% with a significant behavioral component and 85% with multiple conditions leading to the feeding disorder.[39] Careful study of infants with a diagnosis of nonorganic failure to thrive has revealed that subtle oral-motor dysfunction may be responsible for inadequate feeding skills, contributing to their poor weight gain.[40,41] In children with multiple risk factors, these problems may coexist or evolve separately in time, with the nonorganic component remaining after the organic problem is resolved.

EVALUATION

The presence of feeding difficulties may be first noted by the infant's caregiver, primary care physician, early intervention specialist, or other health care personnel. The type and severity of the feeding problem will usually lead to the involvement of several allied professionals (Figure 75.2-2). Often an interdisciplinary team approach facilitates an efficient evaluation.[14,42]

The first step in evaluating feeding difficulties is a complete history and physical examination. The prenatal history, gestational age, birth weight, and neonatal course may suggest particular risk factors. The answers to questions regarding general psychomotor developmental progress will inform an understanding of feeding progress and why it might be delayed. The review of systems can identify those infants with chronic lung disease, cardiac malformations, or neurologic conditions that are likely to affect feeding (see Table 75.2-2). Symptoms of GERD should be sought because feeding resistance may be one of the early manifestations.[23] The nutritionist has a major role in obtaining the diet history, which will include the type, amount, and nutritional value of what is offered to the infant or child, as well as what is actually ingested. A 24-hour diet recall or a more formal 3-day diet record assists in calculating fluid, calorie,

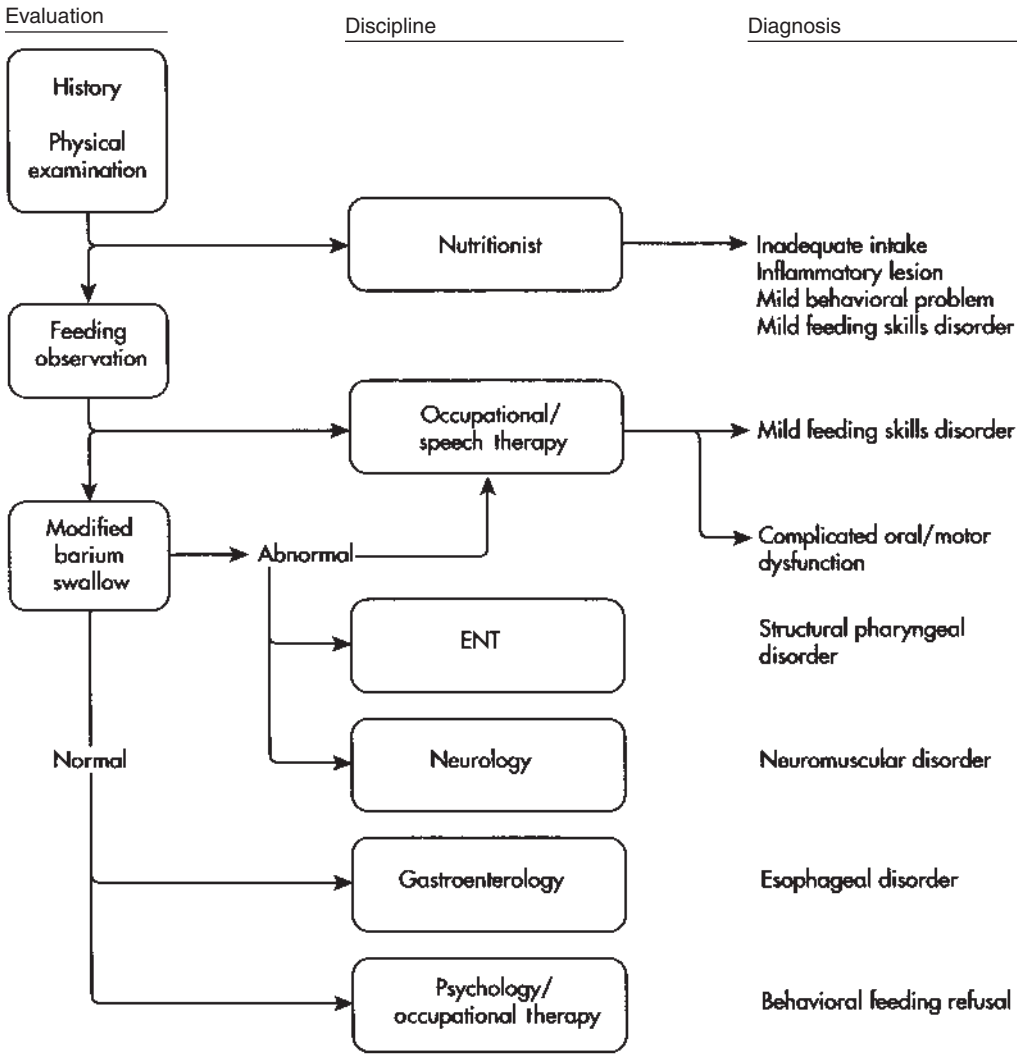

FIGURE 75.2-2 Steps in the evaluation of feeding difficulties. ENT = otorhinolaryngology.

mineral, and vitamin intake. This information, together with a review of feeding methods, will often suggest inadequate calorie intake or developmentally inappropriate food choices indicative of a feeding skill disorder.

Physical examination begins with careful anthropometric measurement, which can be plotted on the appropriate growth charts.[43] Progressive movement away from previous growth percentiles may indicate inadequate intake and the need for further investigation. Weight-for-length ratio is a sensitive measure of acute malnutrition.[44]

Oral examination should evaluate lip closure, tongue position and movement, and palatal movement. Nonnutritive sucking can be evaluated by allowing the infant to suck on the examiner's gloved finger. Infants with oral-motor problems may have poor coordination of suck, swallow, and breathing; poor mouth closure; decreased tongue movement; decreased jaw stability; or sustained or prolonged jaw closure. Oral hypersensitivity with increased gag reflex is common after mechanical ventilation or tube feeding.[45] Further neurologic examination may reveal cranial nerve deficits associated with dysphagia, as well as problems with posture, head and neck control, muscle tone, and persistence of primitive reflexes.[46]

The feeding observation is the central element of the clinical evaluation. The occupational therapist or speech-language pathologist can assist in assessing nutritive sucking and feeding by the primary caregiver, which may detect subtle disorders of feeding skills.[40,47] A prospective study of neonates found that those with disorganized or dysfunctional sucking patterns were more likely to have feeding problems at 6 and 12 months of age.[48] When observing parent–child interactions around feeding, the clinician should notice how the food is presented, which foods an infant prefers, and the parental responses to good and inappropriate feeding behaviors.

Several imaging methods are available to evaluate more complex oral-motor dysfunction. The primary technique is the modified barium swallow, also referred to as a videofluoroscopic swallow study.[49] It requires involvement of a feeding specialist (occupational therapist or speech-language pathologist) and radiologist. Unlike standard upper gastrointestinal barium contrast radiographs, in this study, the infant is placed in a standard feeding position and presented with a variety of textures (see Chapter 23, "Disorders of Deglutition"). This study is the best method of evaluating the pharyngeal phase of swallowing. Clini-

cally inapparent difficulties with suck and swallow, nasopharyngeal reflux, ineffective pharyngeal closure, and aspiration can be seen and taped for later review. Aspiration is often subclinical. It is present in up to 40% of adults without previously identified aspiration undergoing modified barium swallow.[50] Knowledge of aspiration risk and the ability to handle food of different consistencies will assist in developing a safe feeding program.

Ultrasonography is ideal for evaluating the oral preparatory phase of swallow.[51,52] The infant and feeder can be studied in a more physiologic state, with repeated observations of swallow possible. No contrast material is needed, and there is no radiation exposure. Ultrasound study of the pharyngeal phase is difficult owing to air-filled spaces, and aspiration will not be detected.

Flexible endoscopic evaluation of swallowing is another technique that allows for evaluation of swallowing, particularly the pharyngeal phase.[53] After topical anesthesia of nasal tissues, a nasopharyngoscope is passed transnasally for observation of the anatomy and function of the palate, pharynx, and larynx. Next, swallowing function is evaluated with administration of food. The ability to handle liquids and risk of aspiration can be directly assessed. The study can be repeated as often as necessary because there is no preparation needed and no radiation exposure.[54]

Manometry is used to assess the esophageal phase of swallowing. It will detect abnormalities of upper esophageal sphincter relaxation or esophageal peristalsis but may be impractical during early infancy or childhood.

For the infant with behavioral refusal to feed, the presence of a psychologist on the treatment team can be crucial to understanding the development of the problem and the best way to approach the family. Behavioral assessment includes a thorough feeding history, including details of both the infant's feeding and the parental responses, intake history over several days to demonstrate the pattern of feedings, and feeding observation live or on videotape.[55]

TREATMENT

Treatment of feeding problems, which are often complex, should emanate from the interdisciplinary assessment. Generally, not all aspects of the plan are begun simultaneously. Medical conditions causing pain or discomfort during eating should be resolved. Initial attention should be directed to providing sufficient intake for maintenance and catch-up growth. As nutritional status improves, the occupational therapist, speech therapist, and psychologist can help develop a program to establish normal feeding skills and parent–child interactions.

Whenever possible, oral feeding should be maintained even if alternative feeding is also used. This preserves the sensory function of taste and smell and the neuromotor skills of suck and swallow. Oral feeding promotes speech development and provides what should be a pleasurable social experience for the infant. Sucking appears to increase the concentration of lingual lipase, which, together with breast milk lipase, is important for digestion of lipid in the neonatal period.[56,57] Salivary amylase, along with small intestinal glucoamylase, is primarily responsible for starch digestion in early infancy because pancreatic amylase activity is low until 4 months of age.[58] Swallowing initiates primary peristalsis in the esophagus, enhancing esophageal acid clearance.

If nutritional support beyond oral supplementation is required, the route for provision of supplements can be chosen and therapy initiated (Figure 75.2-3). No advantage is achieved in delaying nutritional therapy in children with feeding problems because malnutrition is the primary cause of growth retardation and altered body composition in chronic illness.[59] Details of enteral and parenteral feeding are described in Chapter 75.4, "Nutrition Support."

Nutrient administration is based on patient needs and limitations. Nasogastric or orogastric tubes are easy to place, with feedings given on an intermittent (bolus) or continuous basis. Bolus feeds are more physiologic in preserving hunger-satiety cycles and allow greater mobility for the patient and family. Continuous feedings may be preferable when there is delayed gastric emptying or decreased gut absorptive capacity.[60,61]

Although gastric feeding is preferable, transpyloric tube placement is indicated when there is a high risk of aspiration.[62,63] The type of gastric tube used should be based on the length of time the tube is required. Polyvinylchloride tubes are relatively easy to pass but must be changed every 3 to 4 days. For many clinical situations, a flexible silicone

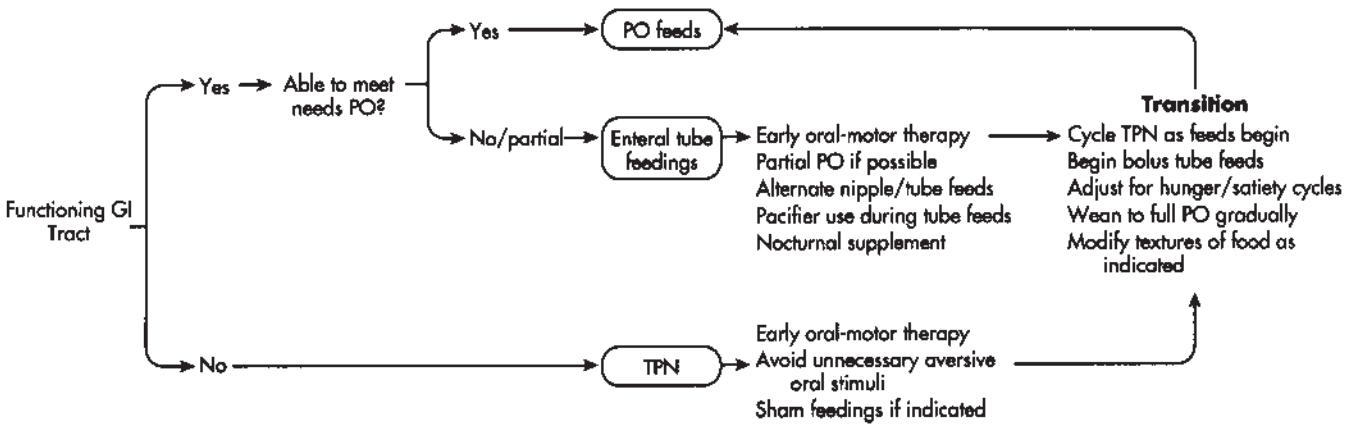

FIGURE 75.2-3 Treatment of feeding difficulties. GI = gastrointestinal; PO = per os; TPN = total parenteral nutrition.

elastic tube is more comfortable for the patient. These tubes produce less sensation when the child swallows, interfere less with feeding, and can remain in place for up to several weeks.[64] Fewer tube changes decrease noxious oral-facial stimuli for the child, which may help reduce the likelihood of future negative feeding behaviors.

Nocturnal feedings have been shown to provide excellent nutritional support while not interfering with daytime activities.[65–68] Percutaneous endoscopic gastrostomy and use of low-profile gastrostomy buttons facilitate the care of patients on long-term enteral support.[69] Families have often had significant distress regarding feedings by the time a gastrostomy is placed and need support and understanding when the decision is finally made.[70]

Various approaches to advancing feedings are available.[62,71] Tolerance can be assessed by gastric residuals and the absence of vomiting, diarrhea, or abdominal pain. Infants with chronic diarrhea and malnutrition can be successfully managed with enteral therapy, but the volume of the infusion should be increased gradually.[72] Attention to adequate water intake will prevent problems of dehydration owing to formulas with a high renal solute load.[73]

Gastrostomy tube feedings should not be considered an obstacle to overcoming feeding problems. A review of 100 infants fed by nasogastric tube indicated that earlier placement of a gastrostomy tube for the poor feeders was associated with improved success in transition to oral feeding.[74] In a study of 19 patients with severe feeding refusal, those gastrostomy-fed infants took slightly longer than the nasogastric tube–fed infants to achieve oral feeding, but use of gastrostomy feedings did not adversely affect eventual transition to oral feeds.[75]

Careful attention to appropriate techniques for the provision of proper diets can be of great benefit to children with major feeding difficulties. Two examples of such clinical problems are discussed below. Other dietary issues encountered in a range of gastrointestinal disorders are discussed in Chapter 75.5, "Special Dietary Therapy."

Oral-Facial Anomalies

The newborn with cleft lip or palate has an immediate feeding problem. With a minor cleft lip or palate, the feeding problem is usually related to the inability to generate adequate negative pressure for suction. Apart from its nutritional advantages, breastfeeding may best facilitate intake because the soft tissue of the breast can help seal the cleft during feedings. When there is cleft lip and palate, the breastfed infant may be unable to generate sufficient negative pressure to hold the nipple and areola in place for compression, and the bottle-fed infant may not develop efficient suction on a nipple. Breastfeeding may be assisted with use of the Supplemental Nursing System (Medela, Inc., McHenry, IL); feeding devices for bottle-fed cleft lip and palate infants should deliver an adequate volume of milk into the mouth while allowing time for the infant to swallow (Table 75.2-3).[76,77]

Oral feeding of the infant with cleft lip or palate may be facilitated by upright positioning, small frequent feedings, careful burping, and applying pressure to the infant's cheek and beneath the jaw to assist lip closure and sucking.[78] Nonoral feedings become necessary if significant risk of aspiration is present or if the infant fails to thrive because of either inadequate volume intake or prolonged feeding sessions. When micrognathia and glossoptosis occur with cleft palate (the Robin sequence), respiratory distress may preclude oral feeding. These infants have feeding difficulties caused by inadequate suction, swallowing difficulties, and compromised breathing owing to airway obstruction by the tongue.[79] Some infants will require surgical tongue-lip adhesion, use of a nasopharyngeal airway, or even tracheostomy.[80,81] These operative procedures are reserved for patients with the inability to maintain a stable airway using conversative measures. The incidence of failure to thrive was reduced when the families of infants with Robin sequence received assistance from a feeding-support nurse and supervised airway management.[82]

Cerebral Palsy

Cerebral palsy is a chronic, nonprogressive disorder of the nervous system, with an estimated incidence of 1 in 500 infants.[83] Cerebral palsy is a disorder of movement and posture, and most patients demonstrate spasticity of the extremities. Swallowing difficulties result from supranuclear bulbar palsies, and more severely affected children may have language and intellectual deficits and seizures. In a large survey of families with children with neurologic impairment, prolonged feeding times of 3 or more hours per day were common.[84] Early on, affected infants may have decreased sucking reflex and weak or uncoordinated sucking behavior; later, they can have tonic bite, tongue thrust or lip retraction, and lip pursing.[85,86]

Before oral feeds are begun, non-nutritive sucking can be encouraged at the breast (emptied by pumping) or with a pacifier. Non-nutritive sucking during gavage feeds has been shown to help the maturation of the sucking reflex in premature infants and may improve integration of oral and pharyngeal reflexes in the infant with an immature nervous system.[87]

Treatment techniques are available to enhance oral feeding at the breast and with bottle-feeding.[77,86,88] For the older

TABLE 75.2-3 SPECIAL NIPPLES AND FEEDING DEVICES

NIPPLE/DEVICE	CHARACTERISTICS
Supplemental Nursing System	For breastfeeding infants; allows control of volume of milk from reservoir
Ross red premature nipple	Soft, durable
Mead-Johnson cleft palate feeder	Squeeze bottle helps with control of milk delivery; cross-cut nipple
Haberman feeder	Easy to use, with nipple the feeder can compress to help express milk
Any broad-based nipple	May conform to a cleft lip and help attain suction
Feeding obturator (prosthesis)	To stabilize wide cleft

Adapted from Case-Smith J and Humphry R.[86]

infant, the texture of foods can be adjusted, and with thicker liquids passing more slowly from mouth to throat, they often will be better tolerated. The use of a spoon requires that the infant open the mouth wide enough so that lateral tongue movements are adequate to remove the food and that jaw control is adequate. Spoons are designed with narrow, shallow bowls and are made of durable, plastic material; they may have specialized handles to help with the grasp. Cups are available with cut-out rims (to allow easier lifting), covers to decrease spillage, and wide bases to prevent tipping. The feeder can use techniques to provide external jaw and cheek support and to stabilize head position. Adaptive equipment is available to improve postural alignment during feedings. The feeder may hold an infant in the lap or use a foam-filled Tumble-Forms (Sammons Preston Rolyan, Bolingbrook, IL) seat. The older infant or toddler with fair sitting stability can use the Rifton chair (Rifton Equipment, Chester, NY) or other adaptive seating device for upright positioning with foot rests, arm supports, and pelvic strapping.[86] As noted above, some children with an inability to meet nutritional needs orally will require a feeding gastrostomy. The safety of oral feedings and risk of aspiration can be evaluated with videofluoroscopy of swallowing. Early attention to treatment of feeding disorders in these patients can result in significantly improved nutritional status.[89,90]

Behavioral Approaches to Feeding

Principles of learning are used to understand the development of feeding disorders and to design treatment programs for children with feeding problems when there is no apparent organic disease or when correction of an organic disorder or oral-motor deficit does not result in normal feeding.[14,91–99] Respondent learning or classic conditioning posits that the occurrence of some behaviors is increased or decreased based on their association with normal physiologic reactions, in this case hunger or pain. Specifically, eating may have been associated with pain in the development of the disorder. Further, in children with feeding disorders, the positive association between hunger, eating, and satiety is inconsistent. Often interventions for feeding disorders manipulate hunger to prompt eating behaviors. The rationale is that children are more likely to emit eating behaviors (show interest in food, accept food put into their mouth, put food into the mouth, swallow food) when they are hungry.

Hunger may be manipulated in several ways.[91] For the child fed by the mouth, the timing of meals should be regulated, and between-meal eating and drinking should be minimized or eliminated so that the child is hungry at mealtime. The length of meals is also regulated, so that meals last only 20 to 30 minutes, regardless of the amount of food ingested. After 20 to 30 minutes, it is assumed that rising blood sugar from the food that has been consumed leads to diminished sensation of hunger. From the respondent learning perspective, keeping meals short continues to reinforce the association of hunger-eating-satiety.

For children who are tube-fed, the timing and amount of the feeding should be regulated to maximize hunger. Bolus, rather than continuous, feeding and daytime, rather than nighttime, feeding will more closely mimic meals and thus move the child toward normal eating patterns. Tube feeding after, rather than before, an oral feeding session is generally recommended. Modestly decreasing the calories received by tube will, in many patients, increase appetite and better motivate the child to eat by mouth.

One caveat is necessary with respect to the manipulation of hunger. Although a modest amount of calorie restriction is typically helpful to encouraging eating behavior, severe calorie restriction is not. Many naive interveners, both lay and professional, have found that the statement "He'll eat when he gets hungry enough" is not true for those feeding disorders usually seen in a tertiary care center.

Operant learning principles posit that behaviors are increased or decreased based on the responses to the behaviors. Operant techniques are used to increase desired behaviors and decrease undesirable behaviors.[92] In the treatment of feeding problems, the most commonly used and effective operant treatment technique is differential attention.[93–98] During the meal, the child receives positive reinforcement or attention for specific behaviors related to feeding and no attention for inappropriate or off-task behaviors. Positive attention is often social interaction and praise from the caregiver, but depending on the child, it can be music or access to a preferred food. Inappropriate and off-task behaviors include tantrums, not approaching the food, and playing with the food. The target behaviors (behaviors to be increased) are changed as the treatment progresses. For example, the child may initially be reinforced for allowing food to be put in his mouth, then for swallowing the food, then for self-feeding, and, finally, for self-feeding a sufficient quantity in a specified time. Depending on the child's feeding problem, physical quidance of appropriate feeding behaviors may be used along with differential attention.[100–105] The physical guidance is decreased and then stopped as the child progresses through the program.

Extinction is the operant principle that suggests that a behavior will eventually cease if it is never reinforced. Extinction-based techniques have been used in the treatment of severe feeding refusal. Most typically, these are children who, for medical reasons and from a very early age, have not been fed by mouth and/or have experienced very aversive oral procedures, such as intubation. When faced with oral feeding these children produce strong rejecting behaviors such as screaming, spitting food, and physically resisting placement of food in the mouth. When the caregiver stops the feeding in response to these rejecting behaviors, the child's behaviors have been reinforced (by escaping the negative situation). Extinction techniques dictate that feeding should not stop in response to rejecting behaviors. Thus, spoonfuls of food (sometimes very little food) are put into the child's mouth repeatedly despite the child's protest. Eventually, when the child's protest behavior does not stop the feeding (ie, the protest behavior is not reinforced), the protest behavior stops. Although these extinction techniques have been successful in cases of severe feeding refusal,[75,105–109] they must be carefully planned and executed in an inpatient setting.

The role of parents in carrying out behavioral treatment programs for feeding must be fully appreciated and sup-

ported.[14] As discussed earlier in this chapter, parental anxiety about nutrition is an important factor in the development and maintenance of many feeding problems. For parents to decrease unhelpful behavior that is motivated by their own anxiety, their concerns and anxiety must by addressed specifically. The entire treatment team should know and agree on the treatment plan for the child's nutritional and behavioral feeding problem, and this consensus plan should be presented to the family. Most parents need help to understand that the multiple components of their child's feeding problem cannot all be addressed at once. They need to understand that the team has a plan to resolve all of the concerns in time and that the child's physical health will not be in danger during this process. Parental lack of understanding of this process can severely impede the progress of treatment. For example, parents will not follow guidelines to stop meals after 30 minutes and may give in to inappropriate feeding rituals if they fear that the child's nutritional status will be irretrievably harmed.

For the child with mild to moderate feeding problems, most parents can be taught behavioral treatment techniques. The behavioral feeding plan needs to be carried out consistently, that is, at every meal, to change the child's negative feeding behaviors. Thus, training the parent to carry out the treatment at each meal is far superior to an "expert feeder" who treats the child weekly or even several times per week. Parents will need to understand the behavioral principles and the importance of consistency in applying these techniques and that the target behaviors will more closely approximate normal eating as the child progresses. For children with very severe feeding refusal, especially if extinction procedures are used, inpatient treatment with alternative feeders may be most appropriate. In these cases, parents must initially understand and support the treatment and then must be taught to carry out the procedures themselves as feeding is transferred to the more normal parent–child interaction and home environment.

TRANSITION TO ORAL FEEDS

When medical or surgical disorders necessitate nonoral feedings or parenteral nutrition, planning for transition to oral feedings should begin early. Oral-motor skills should be assessed and swallowing ability documented. Oral feedings, even in small amounts, should continue, if at all possible, to maintain oral-motor skills. Total parenteral nutrition can be cycled while enteral feedings are advanced, even in the young infant.[110] Sham feedings for children with esophageal atresia and esophagostomies facilitate the return to normal feedings.[111] Other useful techniques include non-nutritive sucking during tube feeding of premature infants and giving nipple feeds during the daytime, with the balance of the feeding volume delivered by tube at night.[112] Attention to eating-related behaviors, the feeding environment, and hunger-satiety cycles will help normalize feedings.[113] Ongoing communication between the family and involved professionals will enhance the transition to normal eating behavior.

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3. Nutritional Assessment and Requirements

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Children with gastrointestinal diseases are uniquely susceptible to many forms of malnutrition (Table 75.3-1), including acute and chronic protein-energy malnutrition,[1,2] micronutrient deficiencies,[3,4] and hypoalbuminemic malnutrition.[5] Patients who are force-fed (either enterally or parenterally) are even liable to overnutrition or obesity.[6] Although nutritional management of children with gastrointestinal disease is often considered a secondary therapeutic goal behind primary medical or surgical care, there is an increasing appreciation that nutritional therapy can and should be the prime focus. Gastrointestinal diseases for which nutritional management assumes primary importance include inflammatory bowel disease, cow's milk protein and other allergic enteropathies, celiac disease, short-bowel syndrome (and any condition in which parenteral nutrition is employed), and a wide variety of metabolic diseases of the liver. Further, many studies have shown that malnourished patients are more prone to both infectious and noninfectious complications of their disease or therapy[7] and require longer lengths of stay in hospital and more resources.[8,9] It is therefore essential that pediatric practitioners understand the principles of nutritional assessment, as well as the scientific basis of nutritional requirements, for effective medical and nutritional care of their patients.

TABLE 75.3-1 SUSCEPTIBILITY OF THE PEDIATRIC GASTROINTESTINAL PATIENT TO MALNUTRITION

DECREASED INTAKE/ANOREXIA
Primary anorexia nervosa
Secondary anorexia owing to chronic disease, inflammation, infection, micronutrient deficiencies, abdominal pain
Dysphagia
MALABSORPTION/MALDIGESTION
Mucosal, hepatobiliary, or pancreatic disease
Drug–nutrient interactions
Short-gut syndrome
Congenital transport defects
ALTERED NUTRIENT REQUIREMENTS
Fever, stress, malabsorption, recovery from malnutrition, prematurity
Immobility, decreased energy expenditure, hypothyroidism
Congenital metabolic defects or conditions requiring pharmacologic doses of some nutrients and/or dietary avoidance of others
DEPENDENCE ON CARETAKERS FOR NUTRIENT INTAKE
Parenteral/enteral nutrition is "force-feeding"
Neglect/maltreatment

NUTRITIONAL ASSESSMENT TECHNIQUES

The medical history, physical examination, and selective laboratory testing form the basis of nutritional assessment. Increasingly sophisticated biologic methodologies are also available for nutritional assessment,[10] especially in the area of body composition analysis. This chapter reviews those aspects of nutritional assessment that are most germane to the care of children with gastrointestinal diseases.

History

A detailed medical history is essential to the nutritional evaluation of a patient. In addition to recording the type and onset of gastrointestinal symptoms, the physician should document the following:

- Current symptoms and their effect on nutrient intake, absorption, and retention
- Past history, including neonatal history, duration of breastfeeding versus formula-feeding, past growth data, gastrointestinal surgery
- Chronic illnesses with known risk factors for malnutrition
- Developmental status, with special attention to milestones of swallowing function
- Known or perceived food allergies
- Medications, with special attention to those with known drug–nutrient interactions (eg, sulfasalazine and folate, corticosteroids and calcium)
- Family history, parental heights, and sibling growth patterns
- Social history, food preferences/beliefs, and food availability

A careful history, in conjunction with a physical examination, is an effective method to diagnose and evaluate patients with idiopathic growth failure,[11] but in patients with chronic gastrointestinal diseases, more detailed assessment techniques are often indicated.

Dietary Intake Methods

Techniques of dietary assessment range from the straightforward recall of recent intake to the detailed and methodical measuring and weighing of all intake during a 7- to 14-day period.[12] Semiquantitative food frequency questionnaires have been developed and validated in adults[13] and, more recently, in children.[14] From a population perspective, food

disappearance and agricultural data may be used, but, of course, they offer no insight into individual nutrient intake. A comprehensive discussion of dietary assessment techniques is provided elsewhere.[15]

In the clinical setting, a 24-hour dietary recall, which is the most rapid dietary intake method, sheds light on family eating patterns and food availability; it can be easily incorporated into the general history and physical examination. Problems of recall bias and under- or overreporting of intake[16] limit the validity of this technique.

A prospective food diary, in which a patient measures and writes down all intake for 3 to 5 days, is probably the most reliable and valid clinical tool. Proper interpretation of these diet records requires consultation with a qualified dietitian. The nutritional composition of these foods is determined by using one of the common nutritional databases, often with the use of proprietary software.[17] An average daily intake of energy, macronutrients, and micronutrients can then be calculated and compared with published reference data. Prospective diet records should generally be performed while the patient is feeling well, free of the effects of acute illness, and should include at least one weekend day in school-age children.

Physical Examination and Anthropometric Data

The examination of all patients with growth failure should be thorough (Table 75.3-2). Careful inspection and palpation of tissues such as hair, skin, oral mucosa, and subcutaneous fat and muscle stores are particularly important in the physical examination. Formal measures of arm anthropometrics are possible, but encircling the patient's upper arm with the examiner's hand can provide informal assessment of arm muscle and fat stores.

Basic anthropometric data include body weight and height (or length in those children less than age 2 years). When compared with age- and sex-appropriate standards, these data can provide objective measures of nutritional status. The weight of infants should be recorded to the nearest gram with the child wearing no clothes or a diaper. In older children, shoes and heavy clothing should not be worn, and the scale should be accurate to the nearest 0.1 kg. Length should be measured on a length board with a tape measure attached and a moveable foot board. Children older than age 2 years should be measured with a stadiometer while standing erect. The average of three readings should be obtained for accurate height/length measurements and should be recorded to the nearest 0.1 cm.

TABLE 75.3-2 CLINICAL SIGNS ASSOCIATED WITH NUTRITIONAL DEFICIENCIES.

ORGAN	CLINICAL SIGN(S)	NUTRIENT DEFICIENCY
Hair	Thin, sparse, easily pluckable	Protein energy, zinc
Face	Diffuse pigmentation	Protein energy
	Moon face	Protein
	Nasolabial seborrhea	Riboflavin, niacin, or pyridoxine
Eyes	Pale conjunctivae	Iron, folate, or vitamin B_{12}
	Bitôt spots, conjunctival or corneal xerosis, or keratomalacia	Vitamin A
	Angular palpebritis	Riboflavin or niacin
Lips	Angular stomatitis or cheilosis	Riboflavin, niacin, iron, or pyridoxine
Mouth	Ageusia, dysgeusia	Zinc
Tongue	Magenta tongue	Riboflavin
	Atrophic filiform papillae	Folate, niacin, riboflavin, iron, or vitamin B_{12}
	Glossitis	Niacin, folate, riboflavin, iron, vitamin B_{12}, pyridoxine, tryptophan
Teeth	Caries	Fluoride
Gums	Swollen, bleeding	Vitamin C
Glands	Thyromegaly	Iodine
	Parotid enlargement	Protein energy
Skin	Xerosis, follicular keratosis	Vitamin A or essential fatty acids
	Perifolliculosis with blood or pigment	Vitamin C
	Petechiae, ecchymoses	Vitamin C or K
	Pellagrous dermatosis	Niacin, tryptophan
	Scrotal or vulval dermatosis	Riboflavin
Nails	Koilonychia	Iron
Subcutaneous tissues	Edema	Protein, thiamine
	Decreased subcutaneous fat	Protein energy
Musculoskeletal system	Muscle wasting	Protein energy
	Craniotabes, frontal bossing, rachitic rosary, epiphyseal enlargement	Vitamin D
	Epiphyseal enlargement, subperiosteal hemorrhage	Vitamin C
Hepatobiliary system	Hepatomegaly	Protein energy
Nervous system	Psychomotor changes, confusion, irritability	Protein
	Sensory loss, motor weakness, calf tenderness	Thiamine
	Loss of vibratory sense, decreased deep tendon reflexes	Vitamin B_{12} or E
Cardiovascular system	Cardiomegaly, tachycardia	Thiamine

Adapted from Suskind R andVarma R.[96]

Detailed methodologies are given elsewhere[18,19]; recent studies have confirmed that body length is often poorly measured in practice.[20]

Raw data are then plotted on reference curves for age and sex, and the corresponding percentile is determined. The 2000 release of updated growth curves was a welcome advance in the field of pediatric nutrition assessment. The previously used curves were criticized for the homogeneous genetic, geographic, and socioeconomic background of the participants less than age 2 years.[21] In addition, these infants were generally fed infant formulas, whereas breastfed infants show significantly different patterns of weight and length gain.[22,23]

The major differences between the 2000 US Centers for Disease Control and Prevention (CDC) growth charts and the 1977 National Center for Health Statistics (NCHS) charts were (1) inclusion of breastfed infants proportional to their distribution in the US population during the past 30 years; (2) wider representation of a cross-section of children living in the United States between 1971 and 1994 (versus primarily white, middle-class infants); (3) expansion to include up to 20 years of age; (4) body mass index (BMI) percentile curves for 2 to 20 years; and (5) using one data source to decrease the disjunction between recumbent length and stature when changing from the infant (0–36 months) to the older child growth chart.[24] The NCHS has prepared curves for the visual display of the data that are widely available in general pediatric texts, wards, and clinics.

Although deficits in weight for age were originally proposed as criteria for malnutrition by Gomez in the 1950s, the currently accepted standard for anthropometric screening is the criteria of Waterlow (Table 75.3-3).[25] These criteria recognize that children who are underweight may be either short and well proportioned (so-called "stunted") or truly underweight for their height (so-called "wasted"). Wasted children have an acute deficit of body mass that is more amenable to immediate nutritional therapy, whereas stunted children are more chronically undernourished. Acutely wasted patients suffer a variety of functional deficits (eg, decreased muscle strength, impaired immune function, and decreased organ mass, among others); the physiologic impairment of stunting is less obvious and is controversial.[26] Aggressive feeding in either case can actually lead to accumulation of excess body fat.

TABLE 75.3-3 WATERLOW CRITERIA FOR CATEGORIZING TYPE AND CHRONICITY OF MALNUTRITION

	ACUTE MALNUTRITION (WEIGHT FOR HEIGHT; % OF MEDIAN)	CHRONIC MALNUTRITION (HEIGHT FOR AGE) (% OF MEDIAN)
Normal	> 90	> 95
Mild	80–90	90–95
Moderate	70–80	85–90
Severe	< 70	< 85

Adapted from Waterlow J.[25]
Deficits of weight for height are termed "wasting" and those of height for age are called "stunting."

The statistical analysis of groups of patients, especially those whose anthropometric data place them at less than the 5th or greater than the 95th percentile, has been improved by the use of standard deviation scores (also termed z-scores).[27] By definition, a z-score of +2 represents a value 2 SD above the mean, +3 is 3 SD above the mean, etc. The calculation of a z-score is as follows:

$$\text{z-score} = \frac{(\text{actual value} - \text{median value for age and sex})}{\text{standard deviation of value for age and sex}}$$

Because the mean weight and standard deviation of boys aged 12 months are 10.1 kg and 1.0, respectively, a boy weighing 7 kg at this age has a z-score of $(7 - 10.1)/1.0 = -3.1$. His weight is therefore more than 3 SD below the mean. Z-scores can be calculated for patients with the use of software distributed free of charge by the CDC (<http://www.cdc.gov/epo/epi/epiinfo.htm>).

An increasingly used anthropometric index is the BMI.[28,29] The value, calculated by dividing weight in kilograms by height in meters squared, was shown by Quetelet in the nineteenth century to be a good measure of body mass but only minimally correlated with height, and therefore was a good candidate variable to measure body fatness. As the incidence of obesity has reached epidemic proportions in the United States and other industrialized countries,[30] assessment of BMI has been recommended as a valid and reliable screening tool for obesity. Children with BMIs greater than the 85th percentile for age and sex are termed overweight, and those with BMIs greater than the 95th percentile are obese. Reference values for BMIs are available, and the new growth curves include a visual display of BMI reference curves instead of weight-for-height curves of preadolescents. Because of the dramatic increase in obesity in childhood, the more recent survey data (Third National Health and Nutrition Examination Survey [NHANES III]) were not included in the weight-for-age and BMI-for-age curves for children age 6 years and older.

More sensitive than attained weight and height in diagnosing malnutrition are weight gain and growth velocity data. Serial growth data are very helpful in establishing a patient's previous growth pattern and interpreting its change with medical or nutritional interventions. For example, a substantial number of children with Crohn disease demonstrate a reduction in height velocity in the months preceding their diagnosis,[1] suggesting that malnutrition can be a presenting feature of this disease. Graphs are available for displaying height velocity data. Table 75.3-4 provides data on average rates of weight and height gain for a US sample of healthy children.[31] Determining an infant's or toddler's growth velocity between outpatient visits or in response to a nutritional intervention can help confirm or refute subjective clinical impressions of improved well-being.

Body Composition Methodologies

Perhaps the most significant limitation of the anthropometric techniques summarized above is their inability to precisely measure body composition, namely the amount of body weight that is either fat mass or fat-free mass

TABLE 75.3-4 MEAN INCREMENTS IN WEIGHT AND LENGTH FOR HEALTH US CHILDREN

	WEIGHT (g/d)		LENGTH (mm/d)	
AGE (MO)	BOYS	GIRLS	BOYS	GIRLS
Up to 3	31	26	1.07	0.99
1–4	27	24	1.00	0.95
2–5	21	20	0.84	0.80
3–6	18	17	0.69	0.67
4-7	16	15	0.62	0.60
5–8	14	14	0.56	0.56
6–9	13	13	0.52	0.52
7–10	12	12	0.48	0.48
8–11	11	11	0.45	0.46
9–12	11	11	0.43	0.44
10–13	10	10	0.41	0.42
11–14	10	9	0.39	0.40
12–15	9	9	0.37	0.38
13–16	9	9	0.36	0.37
14–17	8	8	0.35	0.36
15–18	8	8	0.33	0.34
16–19	8	8	0.32	0.33
17–20	8	8	0.31	0.32
18–21	7	8	0.30	0.32
19–21	7	7	0.30	0.31
20–23	7	7	0.29	0.30
21–24	7	7	0.28	0.29

Adapted from Guo SM et al.[31]

(FFM). The accepted standard test for body composition is hydrodensitometry (so-called underwater weighing). This technique relies on the principle that body composition can be known from measurements of body density, using assumptions about the average density of fat mass (0.9 g/cm^3) and FFM (1.1 g/cm^3). Weighing the subject in air and then while underwater allows calculations of body density. Obviously, this method is not feasible for infants and young children. Among the available clinical techniques for children are skinfold measurements, bioelectrical impedance analysis (BIA), and dual-energy x-ray absorptiometry (DXA).

Skinfold measurements, which offer the advantage of directly measuring body fat, have been widely used in clinical and epidemiologic studies. They are commonly obtained at four sites: the triceps, biceps, subscapular, and suprailiac. In addition to comparing individual skinfold measurements with published data for age and sex, some investigators have proposed the arithmetic sum of four skinfold measurements as a valid measure of body fatness.[32,33] Triceps skinfold measurement can be used in conjunction with mid–upper arm circumference measures to estimate measures of fat and lean body mass via nomograms[34] or equations:

$$\text{MAMA} = \frac{(\text{MUAC} - \pi * \text{TSF})^2}{4 * \pi}$$

where MAMA = midarm muscle area (cm^2), MUAC = mid–upper arm circumference (cm), TSF = triceps skinfold (cm), and π = 3.1416. This equation assumes that the midarm muscle is circular, that the triceps skinfold gives a good measure of the total rim of midarm fat, and that bone area is negligible. Because these are all questionable assumptions, others have made modifications in the equation, using either computed tomography[35] or magnetic resonance imaging[36] as gold standards.

Skinfold measurements are prone to significant inter- and intraobserver variation. As a measure of total body fat, they do not measure intra-abdominal fat, and it is therefore not surprising to note the relatively low correlation between skinfold measures and total body fat. Skinfold thickness, as a measure of peripheral versus truncal obesity, may be helpful in the evaluation of some patients. The optimal use of skinfold measurements continues to be debated.[37]

BIA is a noninvasive, nonradioactive measure of body composition analysis. The technique relies on the principle that FFM, being composed of water and ions, conducts an electrical charge better than fat mass.[38] Lean body mass therefore has a lower resistance to current. Reactance, a measure of cell membrane capacitance, is also measured by BIA and, together with resistance, can be used to measure total body water, both intra- and extracellular. Using assumptions about the water content of FFM, FFM is calculated. In patients with human immunodeficiency virus (HIV) infection, BIA has been widely used as a measure of fat and FFM.[39,40] BIA has also been performed in children and compared with anthropometric measures of body fat,[41,42] as well as deuterium dilution[43] and underwater weighing.[44] Because the total body water content of infants and young children is not stable, there are inherent difficulties with using BIA in this age group. Nonetheless, this technique holds promise as a bedside measure of lean body mass. More recent validation of BIA has been performed using larger datasets and a multicomponent body composition model.[45]

The primary role of DXA scanning is to measure bone mineral content, bone mineral density, and total body bone mineral content. Some instruments also measure total body bone mineral content, nonbone lean tissue, and fat, thereby providing body composition information using a three-compartment model. A number of studies have compared measurements of fat mass with DXA versus results obtained from hydrodensitometry,[46] and the correlation between the methods has generally been high. Unlike hydrodensitometry, DXA also measures the composition of particular body parts, thereby allowing one to compare visceral and subcutaneous adiposity. Adults with inflammatory bowel disease have been reported to have lower fat mass and altered ratios of intracellular water to FFM than age-matched healthy controls.[47] DXA scanning has also been used to document the high prevalence of osteopenia in children with inflammatory bowel disease[48] and cerebral palsy.[49] The ultimate applicability of DXA scanning to human body composition is still evolving.[50,51]

Laboratory Assessment of Nutritional Status

Laboratory tests are useful in confirming the assessment made initially by history and physical examination, as well as in diagnosing subclinical nutritional deficiencies. As with any assessment technique, knowledge of the normal

range of parameters and confidence in the reliability and validity of the technique used are crucial.

General Laboratory Measures

A complete blood count with differential is perhaps the most useful and least expensive laboratory measure of nutritional status. Lymphopenia is a well-known feature of protein-energy malnutrition owing to a reduction in circulating T lymphocytes. Total lymphocyte count (TLC) can be calculated as follows:

$$\text{TLC (cells/mm}^3\text{)} = \text{white blood cell count} \times \text{percentage of lymphocytes}$$

With mild malnutrition, TLC is < 1,500/mm^3; with moderate malnutrition, TLC is 800 to 1,200/mm^3; and with severe malnutrition, TLC is < 800/mm^3. TLC is both a nonspecific and an insensitive measure of nutritional status, however.

Another common functional test of immunocompetence and, therefore, adequate nutritional status is delayed-type hypersensitivity testing. Cutaneous anergy, a delayed or absent response to intradermal injection of antigens, is a consistent finding in moderate to severe malnutrition and has been associated with an increased risk of complications of surgery. Anergy is also a nonspecific measure of nutritional status; other factors, such as the use of immunosuppressive agents, radiotherapy, and critical illness, may be associated with anergy as well.

Nitrogen (N) balance is one of the oldest and best-known methods of assessing nutritional status,[52] having been described as early as the 1830s. It has generally been used as a technique to measure the adequacy of dietary protein. Because negative N balance will ensue if an essential amino acid is ingested in inadequate amounts, the method has also been used to define requirements for specific amino acids and to define which amino acids are essential or nonessential.[53] The N balance technique has also been used to evaluate the response of amino acid supplements to the diet and to determine how exercise or other interventions impact on protein metabolism. Children and adults who are actively gaining lean body mass should be in positive N balance, whereas healthy adults may be said to be in nitrogen equilibrium if N loss is within 5% of N intake.

The concept of the N balance technique is straightforward: N intake is compared to N output to calculate net N balance. The difficulties of the test reside in the full and complete collection of all intake and output. These efforts require cooperative subjects and intensive monitoring. In addition, a stabilization phase of several days to weeks is needed to truly measure the effect of diet on N balance owing to the body's adaptation to the altered intake. Thus, a full N balance is best considered a research tool.

Clinical application of a N balance technique, measuring urine urea N only, has been proposed as an easy method to estimate protein nutriture and the adequacy of nutritional support in hospitalized patients.[54] Urea is the main excretory product of N metabolism, and approximately 85% of the body's N is lost in the urine, the majority via urine urea N. Other sources of N loss include fecal losses, integumental losses (eg, desquamating skin, sweat, hair and nail growth), and miscellaneous losses (eg, saliva, vomitus, blood drawing and menstrual losses, etc). Some of these losses can be high in patients with wounds, burn injuries, or exudative gastrointestinal disease such as ulcerative colitis. Healthy adults excrete 7 to 10 g of urinary urea N per day. Because most proteins contain 16% N, dietary protein intake is customarily divided by a factor of 6.25 to estimate N intake. The equation for calculating N balance is therefore:

$$\begin{aligned}\text{N balance} &= \text{N intake} - \text{N output}\\ &= (\text{24-hour dietary protein intake in g}/6.25)\\ &\quad - \text{24-hour UUN} - \text{factor}\end{aligned}$$

where UUN = urine urea nitrogen (in grams) and factor = allowance made for uncollected N loss in stool, skin, and miscellaneous sources. In adults, this factor is 2 to 4 g/d, and in children, an estimate of 10 mg/kg/d may be used.

Negative N balance can result from inadequate energy intake, inadequate protein intake, or catabolic stress and lean body mass breakdown. Positive N balance implies adequate energy and/or protein intake and, generally, an anabolic state. Nonetheless, the conceptual and methodologic problems in N balance studies should be recognized. The mere demonstration of positive N balance does not disclose information about N distribution throughout the body or about accumulation of lean body mass. Moreover, a positive N balance can occur despite the presence of other important nutrient deficiencies (eg, some micronutrients).

Visceral Protein Levels

The blood concentrations of visceral proteins synthesized by the liver are often used to assess nutritional status because decreased levels presumably reflect a reduced supply of amino acid precursors and/or decreased hepatic (and other visceral) mass. The blood levels of these proteins, however, depend on their rates of synthesis, degradation, and escape from the circulatory system. Stable isotopes have been used to measure the rate of visceral protein synthesis, which is generally of greatest interest.[55,56]

Serum proteins are also affected by infectious or catabolic processes (Table 75.3-5). The concentrations of positive acute-phase proteins are increased in infectious or

TABLE 75.3-5 SERUM PROTEINS AND ACUTE ILLNESS

POSITIVE ACUTE-PHASE PROTEINS	NEGATIVE ACUTE-PHASE PROTEINS
C-reactive protein	Albumin
Fibrinogen	Prealbumin
Ferritin	Retinol binding protein
Ceruloplasmin	Transferrin
α_1-Antitrypsin	
α_1-Glycoprotein	

The blood concentrations of positive acute-phase proteins increase with fever, infection, or other catabolic stresses, whereas those of the negative acute-phase proteins generally decline.

other catabolic illnesses, whereas negative acute-phase proteins are decreased in these circumstances.

Albumin is the most abundant serum protein, making up nearly 5 of the 10 g/dL of total protein in the serum. It is the least expensive and easiest protein to measure and therefore is the most commonly used biochemical marker to assess protein status. Because more than half of body albumin is extravascular (primarily in skin and muscle), maintenance of normal serum levels can occur from mobilization of these stores despite prolonged energy or protein inadequacy. Combined with its long half-life of 20 days, these factors make serum albumin a relatively insensitive marker of nutritional status or a marker to follow nutritional interventions. Nonetheless, hypoalbuminemia is quite common among hospitalized pediatric patients[57] and is a surprisingly good predictor of mortality in hospitalized adults. Healthy adults with low normal concentrations of serum albumin have an increased risk of death than do those with higher levels,[58] although this may be a reflection of acute-phase protein shifts in visceral protein synthesis.[59]

Hypoalbuminemia is not necessarily diagnostic of malnutrition; it can occur in situations of decreased synthesis (eg, liver disease, age over 70 years, malignancy), increased losses (eg, nephrosis, protein-losing enteropathy, burn injuries), or redistribution between intra- and extravascular spaces (eg, acute catabolic stress with capillary leak syndrome). Fluid overload can also dilute albumin concentrations, and bed rest can decrease levels 0.5 g/dL.

Prealbumin is another visceral protein, named because of its proximity to albumin on an electrophoretic strip. It functions as a transport molecule for thyroxine—hence its alternative name, transthyretin. Prealbumin circulates in plasma in a 1:1 ratio with retinol binding protein (RBP). Its short half-life (2 days) and high ratio of essential to nonessential amino acids make it a good measure of visceral protein status, more sensitive than albumin as a measure of nutritional recovery. Studies have shown prealbumin to correlate well with N balance,[60,61] and it is likely the best available serum marker of nutritional status. Like albumin, concentrations fall with an acute-phase protein response or liver disease. Levels increase with renal failure.

Like prealbumin, RBP has a small body pool and a rapid response to protein-energy depletion and repletion. Its half-life is 12 hours. Because RBP is metabolized in the kidneys, levels will be artificially high in renal failure. RBP concentrations are lowered in vitamin A deficiency and, as with albumin and prealbumin, with infectious or other catabolic stresses.

Transferrin is another serum protein sometimes used to assess visceral protein status. It is synthesized primarily in the liver and has a half-life of 8 days. Transferrin concentrations are decreased in all situations that depress serum albumin (see above), as well as with steroid therapy, iron overload, and anemia of chronic disease. Increased concentrations are seen in pregnancy, oral contraceptive use, and iron deficiency anemia.

Other serum proteins of possible use in assessing nutritional status include insulin-like growth factor I (IGF-I), which is the mediator for the anabolic effects of growth hormone. Although IGF-I levels vary with liver and kidney disease, the levels seem to correlate with N balance reasonably well. Fibronectin, a plasma protein with a half-life of 15 hours, has also been used as a marker for nutritional repletion in some studies.

Indirect Calorimetry and Energy Requirements

When considering energy requirements in the pediatric patient, it is helpful to review the components of total energy expenditure (TEE):

$$TEE = BMR + SDA + E_{activity} + E_{growth} + E_{losses}$$

where BMR = basal metabolic rate (the amount of energy required by the body at rest and while fasted), SDA = the specific dynamic action of food or thermic effect of food (the energy produced as heat during digestion and metabolism of food), $E_{activity}$ = energy required for physical activity, E_{growth} = energy needed for somatic growth, and E_{losses} = obligatory energy lost in urine and stool owing to inefficiencies of absorption and metabolism.

BMR is the largest component of TEE, and several equations have been published to calculate BMR from readily available anthropometric data, age and sex. The oldest and best known of these are the Harris Benedict equations for adults (Table 75.3-6). In children, it has been reported that the correlation between measured and predicted BMR is highest for the equations of Schofield (Table 75.3-7).[62,63]

The technique of indirect calorimetry to measure a patient's resting energy expenditure (REE) has recently shed much light on the subject of the energy requirements in health and disease. As the name implies (*calor* is the Latin word for heat), indirect calorimetry is the determination of heat production of a biochemical reaction by measuring uptake of oxygen and liberation of carbon dioxide. With direct calorimetry, the heat produced by the body at rest is measured. Oxygen consumption and carbon dioxide production measured by the calorimeter are entered into the Weir equation to calculate REE:

$$REE = (3.94 \times VO_2) + (1.06 \times VCO_2) - (2.17 \times UUN)$$

where UUN = urinary urea N excretion (g), used as a correction factor for protein oxidation, VO_2 = oxygen consumption (mL/min), and VCO_2 = carbon dioxide production (mL/min).

Note that although REE measurements are usually taken to approximate BMR, REE actually includes BMR, as well as nonshivering thermogenesis and stress hyper-

TABLE 75.3-6 HARRIS BENEDICT EQUATIONS FOR CALCULATING BMR IN ADULTS

Males:
BMR = 66 + (13.7 × weight [kg]) + (5 × height [cm]) – (6.9 × age [yr])

Females:
BMR = 665 + (9.6 × weight [kg]) + (1.8 × height [cm]) – (4.7 × age [yr])

Adapted from Harris JA and Benedict FG. A biometric study of basal metabolism. Washington (DC): Carnegie Institution of Washington; 1919. Publication No.: 279.
BMR = basal metabolic rate.

TABLE 75.3-7 SCHOFIELD EQUATIONS FOR CALCULATING BASAL METABOLIC RATE IN CHILDREN

GROUP (AGE IN YR)	EQUATION
Males	
0–3	REE = 0.167W + 151.74H – 617.6
3–10	REE = 19.59W + 13.03H + 414.9
10–18	REE = 16.25W + 13.72H + 515.5
> 18	REE = 15.057W + 1.004H + 705.8
Females	
0–3	REE = 16.252W + 10.232H – 413.5
3–10	REE = 16.969W + 1.618H + 371.2
10–18	REE = 8.365W + 4.65H + 200
> 18	REE = 13.623W + 23.8H + 98.2

Adapted from Schofield W.[63]
REE = kcal/day, W = weight (kg), and H = height (cm).

metabolism. The difference between REE and BMR is estimated to be 10% or less.

The utility of indirect calorimetry in assessing energy needs has been evaluated by many investigators in adult[64] and pediatric patients[62,65]; these and other studies have generally confirmed that predictive equations have limited validity in hospitalized or sick patients. Among children with neurodevelopmental delay, studies of REE have shown that their basal energy needs are significantly lower than those predicted by a variety of methods.[6,66] These data have serious implications for a patient population often fed by gastrostomy tube and may explain their tendency to become overweight. A recent study of energy expenditure in children undergoing stem cell transplant has also suggested that these patients are not as hypermetabolic as previously thought.[67] Another study of patients with inflammatory bowel disease reported an increased REE in Crohn disease patients as opposed to healthy controls and those with ulcerative colitis.[68] More studies will be needed to determine whether patients with inflammatory bowel disease truly have an increased energy expenditure and, if so, whether this contributes to their undernutrition.

Indirect calorimetry can also help determine whether a patient is being overfed. The ratio of VCO_2 to VO_2 is termed the respiratory quotient (RQ), which is used to estimate substrate oxidation. For example, in the case of pure glucose oxidation, one mole of carbohydrate reacts with 6 moles of oxygen to create 6 moles each of water and carbon dioxide:

$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$$

The RQ would then be 6/6 = 1.0. When long-chain fat such as palmitic acid is oxidized,

$$CH_3(CH_2CH_2)_7COOH + 23\ O_2 \rightarrow 16\ CO_2 + 16\ H_2O$$

The RQ = 16/23 = 0.695. Thus, the RQ in a fasted state is normally 0.70 to 1.00, and the RQ in this range usually represents a mixed substrate oxidation. The lower RQ noted for lipid oxidation has been used as a rationale for feeding patients with advanced lung disease a diet higher in fat than in glucose to avoid an increased carbon dioxide load to excrete, although this strategy remains controversial.[69] When excess energy is provided and lipogenesis results,

$$9\ C_6H_{12}0_6 + 8\ O_2 \rightarrow 2\ CH_3(CH_2CH_2)_7COOH + 22\ CO_2 + 22\ H_2O$$

The resulting RQ = 22/8 = 2.75. Therefore, the finding of an RQ significantly greater than 1.0 is consistent with energy intake in excess of energy requirements. Other reasons would include hyperventilation (wherein CO_2 is excreted at high rates) or failure to achieve a steady state in gas measurement.

Stable Isotopes and Energy Requirements

The advent in the 1950s of using doubly labeled water (two stable isotopes of water: ^2H_2O and $H_2{}^{18}O$) to measure TEE was a breakthrough in nutritional science. For the first time, TEE in free-living individuals could be validly and reliably measured, and the technique was applied widely in the 1980s and continues to be an important tool for determining energy requirements and validating epidemiologic questionnaires.[70] The method relies on the fact that ^2H_2O is excreted solely in body water, but $H_2{}^{18}O$ is excreted via water losses but also via the carbonic anhydrase system, so that measuring the differential decay rates of the two isotopes provides a measure of CO_2 production from which TEE can be calculated.

The advantages of this technique include the fact that it is noninvasive and safe, requires only serial urine collection, and provides data over a 7- to 14-day period. It has been widely used in pediatric studies.[71–73]

An alternative isotopic approach is the use of labeled bicarbonate (eg, $NaH^{13}CO_3$), which measures energy expenditure in an analogous fashion to doubly labeled water but over a shorter time frame. Studies in neonates and other pediatric subjects have confirmed the validity of this technique,[74] and it is generally less costly than doubly labeled water.

NUTRITIONAL REQUIREMENTS

Definitions of Essential and Nonessential Nutrients

Perhaps the most revolutionary concept in modern nutritional science has been the change in our appreciation of what makes a nutrient "essential."[75] As noted above, the classic experiments of Rose and colleagues defined an essential amino acid as one that is required in the diet of healthy human adults to maintain N equilibrium. Largely using this definition, the 20 amino acids were defined as either essential or nonessential. Indeed, the first half of the twentieth century was marked by a tremendous outpouring of scientific knowledge in the field of nutrition, with the essential nature of most vitamins (Table 75.3-8) and minerals (Table 75.3-9) being determined.

However, the practical and theoretical limitations of balance experiments, as well as their inapplicability to sick or pediatric patients, have been noted. A variety of techniques have therefore been applied to augment how nutrients have come to be defined as essential and what the precise

TABLE 75.3-8 VITAMINS: FUNCTION, DEFICIENCY STATES, AND LABORATORY ASSESSMENT TECHNIQUES

VITAMIN	FUNCTION	CLINICAL DEFICIENCY STATE	LABORATORY ASSESSMENT
Vitamin A (retinol [β-carotene is dietary precursor])	Retinal in rhodopsin and iodopsin Carbohydrate transfer to glycoprotein Maintains epithelial integrity Required for cell proliferation	Night blindness Xerophthalmia Bitot spots Keratomalacia	Plasma retinol (HPLC) Plasma retinol binding protein Relative dose response Dark adaptation test Liver biopsy concentration
Vitamin D (cholecalciferol D_3 [endogenous], ergocalciferol D_2 [synthetic])	Regulates calcium and phosphate Gut absorption, excretion by kidney, and bone resorption	Rickets/osteomalacia Dental caries Hypocalcemia/hypophosphatemia Increased alkaline phosphatase Phosphaturia, aminoaciduria	Plasma 25-hydroxyvitamin D (HPLC) Serum alkaline phosphatase, calcium, and phosphate Radiography Bone densitometry
Vitamin E (α-tocopherol)	Cell membrane antioxidant	Anemia/hemolysis	Plasma tocopherol (HPLC) (corrected for total or LDL cholesterol)
	Inhibits polyunsaturated fatty acid oxidation	Neurologic deficit (ocular palsy, wide-based gait, decreased DTRs) Altered prostaglandin synthesis	Hydrogen peroxide hemolysis
Vitamin K (phylloquinone, menadione [synthetic])	Carboxylation of clotting factors Affects bone formation	Coagulopathy/prolonged PT Abnormal bone matrix synthesis	PT (prolonged) Plasma phylloquinone Clotting factor levels Proteins induced by vitamin K absence or antagonists II
Vitamin B_1 (thiamine)	Oxidative phosphorylation Pentose phosphate shunt Aldehyde transferase Triosephosphate isomerase	Beriberi ("wet" or "dry") Cardiac failure/neuropathy Korsakoff syndrome Wernicke encephalopathy Lactic acidosis	Red cell transketolase activity Whole blood level (HPLC) Urine thiamine-to-creatinine ratio
Vitamin B_2 (riboflavin)	Oxidation/reduction reactions	Seborrheic dermatitis/cheilosis/glossitis Decreased fatty acid oxidation Altered vitamin B_6 activation to coenzyme Decreased tryptophan to niacin conversion	Red cell glutathione reductase activity Red cell flavine adenine dinucleotide Urine riboflavin-to-creatinine ratio
Vitamin B_6 (pyridoxine)	Aminotransferase reactions	Dermatitis/cheilosis/glossitis Microcytic anemia/weight loss Decreased serum transaminases Peripheral neuritis/irritability/convulsions	Red cell aminotransferase activity Plasma pyridoxal phosphate (HPLC) Tryptophan loading test Urine 4-pyridoxic acid
	Irritability/convulsions Decreased tryptophan to niacin conversion		
Vitamin B_{12} (cyanocobalamin)	Methyl group donor Sulfur amino acid conversion Branched-chain amino acid catabolism	Megaloblastic anemia Hypersegmented neutrophils Demyelination/posterior spinal column changes Methylmalonicacidemia Hyperhomocysteinemia	Plasma level (RIA or microbiologic) Schilling test Plasma homocysteine Deoxyuridine suppression test
Vitamin C (ascorbate)	Reducing agent (regenerates vitamin E) Cofactor for hydroxylators Noradrenaline/carnitine synthesis? Cholesterol synthesis? Leukocyte function	Scurvy Perifollicular/petechial hemorrhages Hematologic abnormalities Poor wound healing Impaired collagen synthesis Psychological disturbances	Plasma level (enzyme assay/HPLC) Leukocyte concentration (longer term) Whole blood concentration Urine concentration
Folic acid	Methyl group donor DNA/RNA synthesis Amino acid metabolism	Megaloblastic anemia, neutropenia Altered amino acid metabolism Impaired growth Diarrhea	Plasma level (RIA/microbiologic) Red cell level
Biotin	Coenzyme for carboxylases, decarboxylases, and transcarboxylases	Multiple carboxylase deficiency Organic acidemia/acidosis Dermatitis/alopecia CNS: seizures/ataxia/depression	Plasma (microbiologic assay) Plasma lactate Urine organic acids Lymphocyte carboxylase

(*continues*)

TABLE 75.3-8 Continued

VITAMIN	FUNCTION	CLINICAL DEFICIENCY STATE	LABORATORY ASSESSMENT
Niacin	Dehydrogenase activity	Pellagra: diarrhea/dermatitis/dementia Glossitis/stomatitis/vaginitis Impaired absorption of fat, carbohydrate, and vitamin B_{12} Achlorhydria	Urine ratio of metabolites (*N*-methylnicotinamide: 2-pyridone) Tryptophan load Red cell NAD or NAD:NADP ratio
Pantothenic acid	Pyruvate dehydrogenase cofactor Carrier of acyl groups Acetylation of alcohol/amines	Postural hypotension Anorexia and vomiting Reduced acetylation Neuromuscular defects/hyperreflexia	Urine excretion Whole blood level (RIA/microbiologic)

Adapted from Loughrey C and Duggan C.[97]
CNS = central nervous system; DNA = deoxyribonucleic acid; DTR = deep tendon reflex; HPLC = high-performance liquid chromatography; LDL = low-density lipoprotein; NAD = nicotinamide adenine dinucleotide; NADP = nicotinamide adenine dinucleotide phosphate; PT = prothrombin time; RIA = radioimmunoassay; RNA = ribonucleic acid.

requirements are in health and disease. In addition to balance studies, investigators have used the "factorial approach" to estimate requirements. This method, which was pioneered in estimating protein requirements in children,[76] is a theoretical approach that relies on the following factors: the body content of a nutrient needed for normal body growth; the amount lost in skin, urine, and other secretions; and the efficiency of gastrointestinal absorption.

Another approach to define optimal nutrient intakes, especially well suited for infants, has been the "analogy to breast milk" approach. This approach starts with the assumption that the types and amounts of nutrients commonly found in human breast milk are reasonable starting points in estimating human nutrient requirements. Although for many nutrients, and perhaps more significantly non-nutrients, this concept has considerable merit, the very low vitamin D content of human milk points out the limitations of this approach alone.[77]

Among the more recent and attractive options are studies in which the metabolic pathways of nutrients are measured using stable isotopes.[78] These experiments employ nonradioactive probes to quantify rates of nutrient synthesis, absorption, excretion, and flux into the bloodstream and have allowed better and more precise estimates of nutrient requirements. Important examples of this work include the use of $^{2}H_2{}^{18}O$ (so-called doubly labeled water) to measure TEE[79]; ^{13}C-leucine to measure oxidation rates of a large number of amino acids[80]; ^{67}Zn, ^{68}Zn, and ^{70}Zn for studies of zinc nutriture[81,82]; ^{44}Ca, ^{46}Ca, and other calcium isotopes for the assessment of calcium absorption[83]; and many others.

Well-conducted clinical trials of nutrient supplementation have also been used to more fully define nutrient requirements, especially in a variety of disease states. The finding of an improved clinical or metabolic outcome with supplementation of a nutrient normally considered nonessential has led to the introduction of the term "conditionally essential." Conditionally essential nutrients have been defined as those nutrients that normally are synthesized endogenously by the body but whose synthetic rates may be inadequate to meet needs in times of disease, stress, or developmental stages. Examples of nutrients thought to be conditionally essential include several amino acids (cysteine,[84] taurine, and glutamine[85]), inositol, nucleotides,[86] and others.[75] Other nutrients well known to be essential but increasingly appreciated in optimizing health include folate (which prevents neural tube defects[87] and lowers plasma homocysteine levels[88]), vitamin A (which reduces mortality rates in communities at risk of deficiency[89]), and zinc (which reduces rates of diarrheal diseases and respiratory infections[90,91]).

Finally, the use of nutrients and other biologic substances in high, perhaps pharmacologic, doses has spawned the terms "nutritional pharmacology" or "nutraceuticals" to describe their use.[92] Among nutrients used in this fashion are precursors of nutrients (eg, organic phosphates, dipeptides), probiotics (eg, lactobacilli), prebiotics (eg, fructose oligosaccharides), and various growth factors (eg, growth hormone, IGF, epidermal growth factor).

New Definitions

In concert with the increasing appreciation of essential and conditionally essential nutrients has been a change in how nutrient requirements are set. Previous levels of requirements had largely been derived from the observations of the metabolic derangements and/or clinical signs of deficiency states that would ensue when otherwise healthy subjects consumed a diet with inadequate amounts of the nutrient in question. Indeed, the genesis of the Food and Nutrition Board in the 1940s was in part due to the finding of a high frequency of malnutrition among draftees for the US armed forces,[93] and vitamin and mineral enrichment of wheat flour soon followed. With the growing realization of the importance of diet in influencing long-term health outcomes as well, published nutrient requirements have been rethought and redesigned. In the 1990s, nutrition scientists in the United States and Canada embarked on a thorough revision of the Recommended Dietary Allowances (RDAs; United States) and Recommended Nutrient Intakes (Canada) and published new definitions more suitable to optimizing health. These efforts were spearheaded by the Institute of Medicine of the National Academy of Science, a private, nongovernmental organization chartered by the US federal government to provide advice on scientific matters.

TABLE 75.3-9 MINERALS AND TRACE ELEMENTS: FUNCTION, DEFICIENCY STATES, AND LABORATORY ASSESSMENT TECHNIQUES

MINERAL/ TRACE ELEMENT	FUNCTION	CLINICAL DEFICIENCY STATE	LABORATORY ASSESSMENT
Calcium	Bone structure	Bone demineralization	Plasma total calcium
	Cell metabolic regulator	Tetany/seizures	Plasma free calcium in altered protein binding (eg, hypoalbuminemia, acidosis)
	Nerve excitation threshold	Cardiac arrhythmias	Radiographs
			CT and photon densitometry
Chromium	Glucose tolerance factor	Glucose intolerance	Plasma chromium
	Metabolism of nucleic acids	Neuropathy/encephalopathy	Glucose tolerance
	? Iodine/thyroid function	Altered nitrogen metabolism	
		Increased free fatty acids	
Copper	Cofactor for several enzymes including superoxide dismutase, tyrosinase, ferrochelatase, cytochrome *c* oxidase	Hypochromic anemia, neutropenia	Plasma copper
		Skin depigmentation	Plasma ceruloplasmin (ferrochelatase)
		Dyslipidemia	
		CNS problems	Liver biopsy concentration
			Superoxide dismutase activity
Iodide	Component of thyroid hormones	Goiter	Thyroid hormones, TSH
		Cretinism	Urinary iodide-to-creatinine ratio
Iron	Heme synthesis	Hypochromic microcytic anemia	Plasma iron and ferritin
	Component of cytochromes	Altered oxidative phosphorylation	Total iron-binding capacity
			Hemoglobin/hematocrit, red cell indices
		Diminished concentrative ability	RBC zinc protoporphyrin-to-heme ratio
		Decreased exercise tolerance	Bone marrow aspirate stain
Magnesium	Cofactor for hexokinase and phosphokinase	Cardiac dysrhythmias	Plasma total or free magnesium
		Neuromuscular excitability	Magnesium loading test
	Alters ribosomal aggregation in protein synthesis	Decreased PTH level/activity	
		Hypocalcemia/hypokalemia	
	Increases nerve excitation threshold	Convulsions	
Manganese	Mucopolysaccharide synthesis	Dermatitis	Plasma level
	Cholesterol synthesis	Decreased clotting factors	Whole blood level
	Cartilage/bone formation	Decreased nail/hair growth	Mitochondrial superoxide dismutase
	Pyruvate carboxylase cofactor	?Hair color change	
	Superoxide dismutase cofactor		
Phosphorus	Bone structure	Tissue hypoxia	Serum/plasma levels
	Cell membrane structure	Respiratory failure (ventilatory dependence)	Alkaline phosphatase activity
	Energy use		Radiography
	Glycogen deposition	Hemolytic anemia	Densitometry
	Acid-base balance: buffering	Rickets	Renal tubular excretion threshold
	Oxygen release (2,3-DPG)	CNS abnormalities	
Selenium	Glutathione peroxidase constituent	Myositis	Plasma concentration
	Thyroid hormone metabolism	Cardiomyopathy	Glutathione peroxidase acitivity
		Nail bed changes	Nail/hair selenium
		Macrocytic anemia?	
Zinc	Cofactor for > 70 enzymes	Skin lesions/poor wound healing	Plasma concentration
	Immune function	Immune dysfunction (especially T cell)	Alkaline phosphatase activity
	Cell replication	Anorexia/dysgeusia	Urinary excretion
	Vision	Growth failure/nitrogen wasting	Leukocyte concentration
		Hypogonadism/delayed puberty	
		Diarrhea	

Adapted from Loughrey C and Duggan C.[97]
CNS = central nervous system; CT = computed tomography; DPG = 2,3-diphosphoglycerate; PTH = parathyroid hormone; RBC = red blood cell; TSH = thyroid-stimulating hormone.

The mere prevention of a deficiency state is no longer the gold standard in setting nutrient requirement levels; instead, a broader concept of nutrition health has been taken. Nutritional requirements are those levels of intakes that are most consistent with optimal physiologic functioning and well-being at all life stages. Examples include using bone mineral accretion data in setting dietary calcium intake levels and dental caries protection data in setting reference intakes of fluoride.[94] Table 75.3-10 outlines the definitions now used to describe nutrient requirements since the publication of these new standards in 1994. Figure 75.3-1 shows the relationship between the three Dietary Reference Intakes (DRIs) and the risks of dietary inadequacy and adverse effects at various levels of dietary intake.

TABLE 75.3-10 DEFINITIONS OF NUTRITIONAL REQUIREMENTS TO SET STANDARDS OF INTAKE (FNB/IOM/NAS)

TERM	ABBREVIATION	YEAR INTRODUCED	DEFINITION/USE
Old			
Recommended Dietary Allowances	RDAs	1943	Average daily dietary intake value sufficient to meet the requirement of nearly all (97–98%) healthy individuals in a group; more recently, the term has been calculated as EAR plus 2 SD of the EAR (see below)
New			
Dietary Reference Intakes	DRIs	1994	Umbrella term including RDA, EAR, AI, and UL (see below)
Estimated Average Requirement	EAR	1994	Nutrient intake value that is estimated to meet the requirement of half the individuals in a group
Adequate Intake	AI	1994	Used when no EAR is available and therefore no RDA can be calculated; nutrient intake value based on observed or experimentally determined approximations of nutrient intakes by a group or groups of healthy people
Tolerable Upper Intake Level	UI	1994	Highest level of a daily nutrient intake that is likely to pose no risks of adverse health effects to almost all individuals in the general population

FNB/IOM/NAS = Food and Nutrition Board of the Institute of Medicine/National Academy of Sciences.

Of the new DRIs, RDAs are still the most appropriate measure to use when reviewing an individual's dietary intake (because, by definition, dietary intake at or above the RDA is likely to be adequate for healthy persons). Conversely, because an individual's specific requirement for each nutrient cannot be easily determined, the fact that his/her intake does not meet the RDA is not a sufficient cause to conclude that the diet is deficient. Dietary intake patterns in healthy individuals follow a distribution curve showing a range of intake levels associated with normal health. The risks of dietary inadequacy increase significantly as intake falls to less than 2 SD below the Estimated Average Requirement (EAR). In contrast to the RDAs, Adequate Intakes (AIs) and EARs are better suited to evaluating dietary patterns of groups of subjects rather than individuals alone.

Tables 75.3-11 through 75.3-14 present the most recently published data from the Food and Nutrition Board for a variety of micronutrients, protein, and energy. In cases for which no EAR has been set, the AI is used instead.

Concurrent with the new terminology proposed by the Food and Nutrition Board, new definitions from the US Food and Drug Administration (FDA) used for food labeling have been published (Table 75.3-15). The similarity between these sets of abbreviations may lead to some confusion, but their functions are distinct. The DRIs of the Food and Nutrition Board are published as proposed reference dietary intakes for healthy US and Canadian persons by a nongovernmental institute. In contrast, the Daily Reference Values (DRVs) and Reference Daily Intakes (RDIs) are published by the FDA and are designed to help consumers use food information to plan a healthy diet. Using an estimated energy intake of 2,000 or 2,500 calories per day, the DRVs express nutrient content as a proportion of generally recommended guidelines for a healthy diet (eg, less than 30% of calories as fat, less than 10% of calories as saturated fat, 11.5 g of fiber per 1,000 calories, etc). A second role of these new terms is to allow objective assessment and regulation of foods marketed as "low fat" or "high fiber." For example,

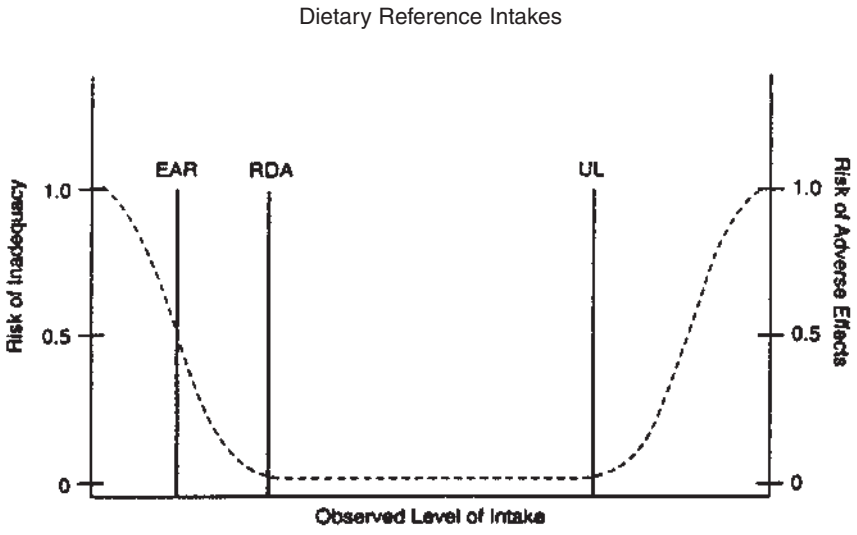

FIGURE 75.3-1 The relationship between Estimated Average Requirement (EAR), Recommended Dietary Allowance (RDA), and Tolerable Upper Intake Level (UL). The risk of inadequate intake is 50% at the EAR, 2 to 3% at the RDA, and close to 0% at the UL. Risks of adverse effects are close to 0% at the UL but increase with increasing intake. Reproduced with permission from Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine.[94]

TABLE 75.3-11 CRITERIA AND DIETARY REFERENCE INTAKE VALUES FOR PROTEIN BY LIFE STAGE GROUP

LIFE STAGE GROUP	CRITERION	AI OR RDA FOR REFERENCE INDIVIDUAL (g/d)		EAR (g/kg/d)		RDA (g/kg/d)		AI (g/kg/d)
		MALES	FEMALES	MALES	FEMALES	MALES	FEMALES	
0–6 mo	Average consumption of protein from human milk	9.1 (AI)	9.1 (AI)					1.52
7–12 mo	Nitrogen equilibrium + protein deposition	13.5	13.5	1.1	1.1	1.5	1.5	
1–3 yr	Nitrogen equilibrium + protein deposition	13	13	0.88	0.88	1.10	1.10	
4–8 yr	Nitrogen equilibrium + protein deposition	19	19	0.76	0.76	0.95	0.95	
9–13 yr	Nitrogen equilibrium + protein deposition	34	34	0.76	0.76	0.95	0.95	
14–18 yr	Nitrogen equilibrium + protein deposition	52	46	0.73	0.71	0.85	0.85	
>18 yr	Nitrogen equilibrium	56	46	0.66	0.66	0.80	0.80	

Adapted from Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine.[98]
EAR = Estimated Average Requirement: the intake that meets the estimated nutrient needs of half of the individuals in a group.
RDA = Recommended Dietary Allowance: the intake that meets the nutrient need of almost all (97–98%) of individuals in a group.
AI = Adequate Intake: the observed average or experimentally determined intake by a defined population or subgroup that appears to sustain a defined nutritional status, such as growth rate, normal circulating nutrient values, or other functional indicators of health. The AI is used if sufficient scientific evidence is not available to derive an EAR. For healthy infants receiving human milk, the AI is the mean intake. The AI is not equivalent to an RDA.

TABLE 75.3-12 CRITERIA AND DIETARY REFERENCE INTAKE VALUES FOR ENERGY BY ACTIVE INDIVIDUALS BY LIFE STAGE GROUP (FOR HEALTHY MODERATELY ACTIVE AMERICANS AND CANADIANS)

LIFE STAGE GROUP	CRITERION	ACTIVE PAL EER (kcal/d)	
		MALE	FEMALE
0–6 mo	Average consumption of protein from human milk	570	520 (3 mo)
7–12 mo	Nitrogen equilibrium + protein deposition	743	676 (9 mo)
1–2 yr	Nitrogen equilibrium + protein deposition	1,046	992 (24 mo)
3–8 yr	Nitrogen equilibrium + protein deposition	1,742	1,642 (6 yr)
9–13 yr	Nitrogen equilibrium + protein deposition	2,279	2,071 (11 yr)
14–18 yr	Nitrogen equilibrium + protein deposition	3,152	2,368 (16 yr)
> 18 yr	Nitrogen equilibrium	3,067*	2,403* (19 yr)
Pregnancy			
14–18 yr	Adolescent female EER plus change in TEE plus pregnancy energy deposition		
	1st trimester		2,368 (16 yr)
	2nd trimester		2,708 (16 yr)
	3rd trimester		2,820 (16 yr)
19–50 yr	Adult female EER plus change in TEE plus pregnancy energy deposition		
	1st trimester		2,403† (19 yr)
	2nd trimester		2,743† (19 yr)
	3rd trimester		2,855† (19 yr)
Lactation			
14–18 yr	Adolescent female EER plus milk energy output minus weight loss		
	1st 6 mo		2,698 (16 yr)
	2nd 6 mo		2,768 (16 yr)
19–50 yr	Adult female EER plus milk energy output minus weight loss		
	1st 6 mo		2,733‡ (19 yr)
	2nd 6 mo		2,803‡ (19 yr)

Adapted from Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine.[98]
EER = estimated energy requirement; PAL = physical activity level; TEE = total energy expenditure. The intake that meets the average energy expenditure of individuals at the reference height, weight, and age.
*Subtract 10 kcal/d for males and 7 kcal/d for females for each year of age above 19 years.
†The Estimated Average Requirement (EAR) and Recommended Dietary Allowance (RDA) for pregnancy are only for the second half of pregnancy. For the first half of pregnancy, the protein requirements are the same as those of the nonpregnant woman.
‡In addition to the EAR and RDA of the nonlactating adolescent or woman.

TABLE 75.3-13 DIETARY REFERENCE INTAKES FOR MINERALS

LIFE STAGE GROUP	CALCIUM (mg/d)	CHROMIUM (mg/d)	COPPER (mg/d)	FLUORIDE (mg/d)	IODINE (mg/d)	IRON (mg/d)	MAGNESIUM (mg/d)	MANGANESE (mg/d)	MOLYBDENUM (mg/d)	PHOSPHORUS (mg/d)	SELENIUM (mg/d)	ZINC (mg/d)
Infants												
0–6 mo	210*	0.2*	200*	0.01*	110*	0.27*	30*	0.003*	2*	100*	15*	2*
7–12 mo	270*	5.5*	220*	0.5*	130*	11*	75*	0.6*	3*	275*	20*	3
Children												
1–3 yr	500*	11*	340	0.7*	90	7	80	1.2*	17	460	20	3
4–8 y	800*	15*	440	1*	90	10	130	1.5*	22	500	30	5
Males												
9–13 yr	1,300*	25*	700	2*	120	8	240	1.9*	34	1,250	40	8
14–18 yr	1,300*	35*	890	3*	150	11	410	2.2*	43	1,250	55	11
19–30 yr	1,000*	35*	900	4*	150	8	400	2.3*	45	700	55	11
31–50 yr	1,000*	35*	900	4*	150	8	420	2.3*	45	700	55	11
51–70 yr	1,200*	30*	900	4*	150	8	420	2.3*	45	700	55	11
> 70 yr	1,200*	30*	900	4*	150	8	420	2.3*	45	700	55	11
Females												
9–13 yr	1,300*	21*	700	2*	120	8	240	1.6*	34	1,250	40	8
14–18 yr	1,300*	24*	890	3*	150	15	360	1.6*	43	1,250	55	9
19–30 yr	1,000*	25*	900	3*	150	18	310	1.8*	45	700	55	8
31–50 yr	1,000*	25*	900	3*	150	18	320	1.8*	45	700	5	8
51–70 yr	1,200*	20*	900	3*	150	8	320	1.8*	45	700	55	8
> 70 yr	1,200*	20*	900	3*	150	8	320	1.8*	45	700	55	8
Pregnancy												
≤ 18 yr	1,300*	29*	1,000	3*	220	27	400	2.0*	50	1,250	60	13
19–30 yr	1,000*	30*	1,000	3*	220	27	350	2.0*	50	700	60	11
31–50 yr	1,000*	30*	1,000	3*	220	27	360	2.0*	50	700	60	11
Lactation												
≤ 18 yr	1,300*	44*	1,300	3*	290	10	360	2.6*	50	1,250	70	14
19–30 yr	1,000*	45*	1,300	3*	290	9	310	2.6*	50	700	70	12
31–50 yr	1,000*	45*	1,300	3*	290	9	320	2.6*	50	700	70	12

Adapted from Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine.[94,99–101]

This table (taken from the Dietary Reference Intake reports (see <www.nap.edu>) presents Recommended Dietary Allowances (RDAs) in bold type and Adequate Intakes (AIs) in ordinary type followed by an asterisk. RDAs and AIs may both be used as goals for individual intake. RDAs are set to meet the needs of almost all (97–98%) individuals in a group. For healthy breastfed infants, the AI is the mean intake. The AI for other life stage and gender groups is believed to cover the needs of all individuals in the group, but lack of data or uncertainty in the data prevents specifying with confidence the percentage of individuals covered by this intake.

†As retinol activity equivalents (RAEs). 1 RAE = 1 mg retinol, 12 mg β-carotene, 24 mg α-carotene, or 24 mg β-cryptoxanthin. The RAE for dietary provitamin A carotenoids is twofold greater than retinol equivalent (RE), whereas the RAE for preformed vitamin A is the same as RE.

‡Cholecalciferol. 1 mg cholecalciferol = 40 IU vitamin D.

§In the absence of adequate exposure to sunlight.

||As α-tocopherol. α-Tocopherol includes RRR-α-tocopherol, the only form of α-tocopherol that occurs naturally in foods, and the 2R-stereoisomeric forms of α-tocopherol (RRR-, RSR-, RRS-, and RRS-α-tocopherol) that occur in fortified foods and supplements. It does not include the 2S-stereoisomeric forms of α-tocopherol (SRR-, SSR, SRS-, and SSS-α-tocopherol), also found in fortified foods and supplements.

#As niacin equivalents (NE). 1 mg of niacin = 60 mg of tryptophan; 0–6 mo = preformed niacin (not NE).

**As dietary folate equivalents (DFE). 1 DFE = 1 mg food folate = 0.6 mg of folic acid from fortified food or as a supplement consumed with food = 0.5 mg of a supplement taken on an empty stomach.

††Although AIs have been set for choline, there are few data to assess whether a dietary supply of choline is needed at all stages of the life cycle, and it may be that the choline requirement can be met by endogenous synthesis at some of these stages.

‡‡Because 10 to 30% of older people may malabsorb food-bound vitamin B_{12}, it is advisable for those older than 50 years to meet their RDA mainly by consuming foods fortified with vitamin B_{12} or a supplement containing vitamin B_{12}.

§§In view of evidence linking folate intake with neural tube defects in the fetus, it is recommended that all women capable of becoming pregnant consume 400 mg from supplements or fortified foods in addition to intake of food folate from a varied diet.

TABLE 75.3-14 DIETARY REFERENCE INTAKES FOR VITAMINS

LIFE STAGE GROUP	VITAMIN A (mg/d)[†]	VITAMIN C (mg/d)	VITAMIN D (mg/d)[†,§]	VITAMIN E (mg/d)[‖]	VITAMIN K (mg/d)	THIAMINE (mg/d)	RIBOFLAVIN (mg/d)	NIACIN (mg/d)[#]	VITAMIN B_6 (mg/d)	FOLATE (mg/d)[**]	PANTOTHENIC VITAMIN B_{12} (mg/d)	ACID (mg/d)	BIOTIN (mg/d)	CHOLINE[††] (mg/d)
Infants														
0–6 mo	400*	40*	5*	4*	2.0*	0.2*	0.3*	2*	0.1*	65*	0.4*	1.7*	5*	125*
7–12 mo	500*	50*	5*	5*	2.5*	0.3*	0.4*	4*	0.3*	80*	0.5*	1.8*	6*	150*
Children														
1–3 yr	**300**	**15**	5*	**6**	30*	**0.5**	**0.5**	**6**	**0.5**	**150**	**0.9**	2*	8*	200*
4-8 yr	**400**	**25**	5*	**7**	55*	**0.6**	**0.6**	**8**	**0.6**	**200**	**1.2**	3*	12*	250*
Males														
9–13 yr	**600**	**45**	5*	**11**	60*	**0.9**	**0.9**	**12**	**1.0**	**300**	**1.8**	4*	20*	375*
14–18 yr	**900**	**75**	5*	**15**	75*	**1.2**	**1.3**	**16**	**1.3**	**400**	**2.4**	5*	25*	550*
19–30 yr	**900**	**90**	5*	**15**	120*	**1.2**	**1.3**	**16**	**1.3**	**400**	**2.4**	5*	30*	550*
31–50 yr	**900**	**90**	5*	**15**	120*	**1.2**	**1.3**	**16**	**1.3**	**400**	**2.4**	5*	30*	550*
51–70 yr	**900**	**90**	10*	**15**	120*	**1.2**	**1.3**	**16**	**1.3**	**400**	**2.4**[‡‡]	5*	30*	550*
> 70 yr	**900**	**90**	15*	**15**	120*	**1.2**	**1.3**	**16**	**1.3**	**400**	**2.4**[‡‡]	5*	30*	550*
Females														
9–13 yr	**600**	**45**	5*	**11**	60*	**0.9**	**0.9**	**12**	**1.0**	**300**	**1.8**	4*	20*	375*
14–18 yr	**700**	**65**	5*	**15**	75*	**1.0**	**1.0**	**14**	**1.2**	**400**	**2.4**	5*	25*	400*
19–30 yr	**700**	**75**	5*	**15**	90*	**1.1**	**1.1**	**14**	**1.3**	**400**	**2.4**	5*	30*	425*
31–50 yr	**700**	**75**	5*	**15**	90*	**1.1**	**1.1**	**14**	**1.3**	**400**	**2.4**	5*	30*	425*
51–70 yr	**700**	**75**	10*	**15**	90*	**1.1**	**1.1**	**14**	**1.5**	**400**[§§]	**2.4**	5*	30*	425*
> 70 yr	**700**	**75**	15*	**15**	90*	**1.1**	**1.1**	**14**	**1.5**	**400**[§§]	**2.4**	5*	30*	425*
Pregnancy														
≤ 18 yr	**750**	**80**	5*	**15**	75*	**1.4**	**1.4**	**18**	**1.9**	**600**[***]	**2.6**	6*	30*	450*
19–30 yr	**770**	**85**	5*	**15**	90*	**1.4**	**1.4**	**18**	**1.9**	**600**[***]	**2.6**	6*	30*	450*
31–50 yr	**750**	**85**	5*	**15**	90*	**1.4**	**1.4**	**18**	**1.9**	**600**[***]	**2.6**	6*	30*	450*
Lactation														
≤ 18 yr	**1,200**	**115**	5*	**19**	75*	**1.4**	**1.6**	**17**	**2.0**	**500**	**2.8**	7*	35*	550*
19–30 yr	**1,300**	**120**	5*	**19**	90*	**1.4**	**1.6**	**17**	**2.0**	**500**	**2.8**	7*	35*	550*
31–50 yr	**1,300**	**120**	5*	**19**	90*	**1.4**	**1.6**	**17**	**2.0**	**500**	**2.8**	7*	35*	550*

Adapted from Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine.[94,99–101]

This table presents Recommended Dietary Allowances (RDAs) in bold type and Adequate Intakes (AIs) in ordinary type followed by an asterisk. RDAs and AIs may both be used as goals for individual intake. RDAs are set to meet the needs of almost all (97–98%) individuals in a group. For healthy breastfed infants, the AI is the mean intake. The AI for other life stage and gender groups is believed to cover the needs of all individuals in the group, but lack of data or uncertainty in the data prevents specifying with confidence the percentage of individuals covered by this intake.

Adapted from Dietary Reference Intakes for calcium, phosphorous, magnesium, vitamin D, and fluoride (1997); Dietary Reference Intakes for thiamine, riboflavin, niacin, vitamin B_6, folate, vitamin B_{12}, pantothenic acid, biotin, and choline (1998); Dietary Reference Intakes for vitamin C, vitamin E, selenium, and carotenoids (2000); and Dietary Reference Intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc (2001). These reports may be accessed at <www.nap.edu>.

TABLE 75.3-15 DEFINITIONS OF NUTRITIONAL REQUIREMENT USED FOR FOOD LABELING (FDA)

TERM	ABBREVIATION	YEAR INTRODUCED	DEFINITION/USE
Old			
US Recommended Daily Allowances	USRDAs	1973	Reference values for vitamins, minerals, and protein used in food labeling; based on RDAs (see Table 75.3-10)
New			
Reference Daily Intakes	RDIs	1994	Replaces the term "USRDAs"; values are generally comparable to them
Daily Reference Values	DRVs	1994	Dietary references applying to fat, saturated fat, cholesterol, carbohydrate, protein, fiber, sodium, and potassium; a 2,000- or 2,500-calorie reference diet is assumed

FDA = US Food and Drug Administration.

foods labeled as "high fiber" must contain at least 20% of the DRV for fiber per serving.

CONCLUSION

The precise nutrient needs of healthy persons is still a matter of intense scientific inquiry and debate, so it is not surprising that those of children with gastrointestinal disease are not well known. Despite the uncertainties of the field, clinicians must still use appropriate tools to accurately assess the dietary intake, anthropometric status, and biochemical profile of their patients. Significant malnutrition with functional and biochemical deficits can exist in patients with gastrointestinal disease even in the absence of overt symptoms or signs of active disease.[95] Advances in the technology of body composition analysis hold promise to further evaluate strategies to preserve and augment lean body mass. A heightened awareness of the susceptibility of the pediatric gastrointestinal patient to malnutrition and constant efforts to improve nutritional status are of paramount importance in patient care.

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4A. *Parenteral* Nutrition

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In 1968, Dudrick and colleagues reported that exclusive intravenous feeding via a central vein could support the long-term survival and growth of puppies.[1] This breakthrough in technology was quickly applied to adult humans[2] and then children.[3] Subsequently, parenteral nutrition (PN) solutions have been widely used. However, knowledge to support this technology dates to the 1600s, when the circulation of blood was discovered and the first infusion of a liquid, wine, was accomplished.[4] Approximately 200 years later, the practice of starving ill patients began to change. In the twentieth century, safe infusates of fats, carbohydrates, and proteins were synthesized, production processes for the manufacture of components arose, and nutritional scientists began to develop an understanding of nutritional requirements. Knowledge of the requirements for micronutrients and metabolic changes associated with infection, injury, or surgery permitted the tailoring of infusions for specific situations. The identification of nutrients that may be essential under specific situations, the compatibility of PN solutions with medications, and the use of pharmacologic substances to promote growth and healing, scavenge oxidants, and prevent or ameliorate metabolic changes associated with inflammation have permitted the treatment of patients who cannot fully support their nutritional requirements through their gastrointestinal (GI) tract. The design of backpacks, subcutaneously accessible central lines, and cycled nutrient delivery permits patients, especially children, to receive nutrition support while they attend school and engage in other age-appropriate activities. For patients who are critically ill or are not candidates for a small bowel transplant, PN can be lifesaving. Still, the technique is not free of complications. It was initially assumed that these complications could be overcome with further fine-tuning. Subsequent experience has shown this to be only partially true. For instance, there have been vast improvements in line technology that resulted in a decrease in line-related complications.[5] Amino acid solutions have been improved, and special solutions for special conditions have been developed.[6,7] The problem of fat administration and essential fatty acid deficiency (EFAD) has been largely solved.[8] Some complications have proven difficult to resolve, bone disease and cholestasis being among these. Because of evidence for the benefits of enteral feeds[9,10] and the serious complications associated with PN,[11–14] parenterally administered nutrition should be reserved only for specific indications when enteral nutrition is not possible or adequate. Table 75.4A-1 lists situations in which the GI tract is nonfunctional or partially functional. Figure 75.4A-1 is an algorithm for initiation of pediatric PN. PN can be partial, used as an adjunct when enteral nutrition cannot meet all nutrient needs, or total, supplying all nutritional needs. Total PN must supply all of the nutrients needed by an individual in amounts necessary to replete malnourished states, prevent muscle breakdown during metabolic stress, and support normal growth and development over the long term.

INDICATIONS FOR PN IN THE PEDIATRIC PATIENT

There are few absolute indications for PN. In some circumstances, such as prematurely born infants, severe malnutrition that is refractory to enteral feedings, pseudo-obstruction, short small bowel, and children who undergo surgery of the GI tract and are not able to be fed, PN is beneficial. In the premature infant, especially the very low birth weight infant, the GI tract may not be capable of processing the amount of nutrients needed for the infant to survive and grow. Over the past three decades, the use of PN in the pediatric population has shifted. In a recent review of 30 years experience with PN in a single institution for pediatric patients, the number of parenterally fed patients did not change; however, the

TABLE 75.4A-1 GASTROINTESTINAL FUNCTION

PARTIALLY FUNCTIONAL OR INADEQUATE FUNCTION
Cannot meet nutrient requirements after maximizing enteral support
Burns
Multiorgan failure
Malabsorption
Short bowel, intractable diarrhea, villous atrophy, dysmotility syndromes
Risk of aspiration when small bowel feeds are not possible
Malnutrition with hypoproteinemia
NONFUNCTIONAL
Paralytic ileus
Intractable vomiting when small bowel feedings are not possible
Small bowel ischemia
Necrotizing enterocolitis
Severe acute pancreatitis
Gastrointestinal surgery
Gastroschisis, omphalocele, multiple intestinal atresias, etc. until enteral route is accessible
Severe inflammatory bowel disease with possible impending surgery

Adapted from Baker SS.[123]

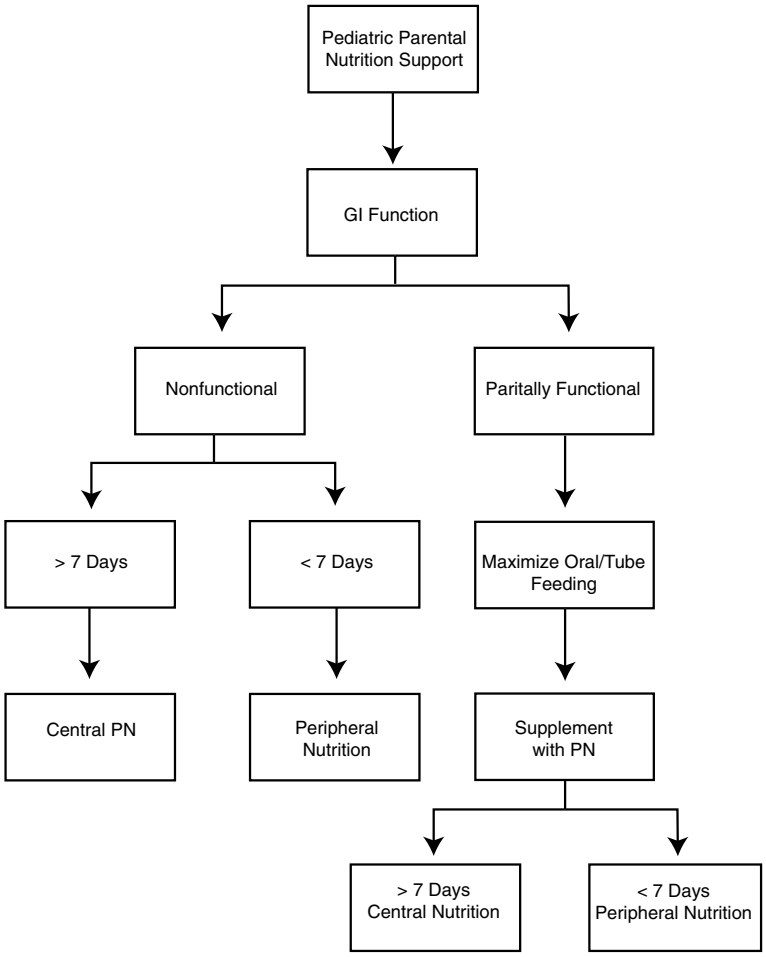

FIGURE 75.4A-1 Algorithm for initiating pediatric parenteral nutrition (PN) support. Adapted from Baker SS.[123] GI = gastrointestinal.

number of premature babies fed parenterally increased significantly, whereas the number of all other patients receiving PN decreased.[15] Severe malnutrition is associated with poor GI function, which is likely multifactorial in origin. Malnutrition causes atrophy of the GI tract in animals.[16] This phenomenon has not been demonstrated in humans, but "functional" atrophy is said to occur. Edema of the intestinal wall, radiographically visualized as separated loops of bowel, may impede nutrient absorption, as occurs in intestinal lymphangiectasia.[17] If of sufficient severity, pseudo-obstruction may require PN. However, intestinal pseudo-obstruction is a rare disease, and some cases of presumed pseudo-obstruction were shown to be Munchausen syndrome by proxy.[18] There is little evidence that nutrition support by itself is beneficial in pediatric oncologic disease. However, children in good nutritional status are better able to tolerate antitumor therapy. For a variety of reasons, children with cancer do not or cannot voluntarily consume adequate nutrition. Assisted enteral feeding in such cases should be considered before PN. PN, either pre- or postsurgery, has not been definitively demonstrated to be beneficial.[19] When surgery involves the GI tract and the child will not be able to be fed, PN is required.

SOLUTION COMPOSITION

The essential nutrients are water, protein, carbohydrate, fat, minerals, trace elements, and vitamins. In general, PN should not be used to correct acute fluid or electrolyte abnormalities. Parenteral solutions are compounded once a day. Over- or underestimates of electrolyte requirements that result in the need to change solutions more frequently than once every 24 hours could result in the patient receiving no nutrition support during part of the day and waste of an expensive therapeutic agent. PN solutions can be used to replace nutritional deficits over the long term. For example, PN solutions can be used to replete muscle mass, providing the extra protein, minerals, electrolytes, and calories that this process requires in addition to the needs for normal growth.

Fluid requirements for infants and children are based on seminal studies performed by Darrow.[20] The objective of fluid therapy is to provide water to meet the requirements for physiologic processes, including insensible and urinary losses. These requirements are based on healthy infants and children and do not take into account the increased losses that may occur with diarrhea, vomiting, fistula, burns, and fever; decreased losses that might occur with the use of mist tents; or altered patient states, as occurs with heart failure or renal disease. Fluid requirements roughly parallel energy metabolism and do not parallel body mass.[21] Although there are several methods to estimate fluid requirements (Table 75.4A-2), the most commonly used is that of Holliday, volume/weight.[21]

During fetal life, there is a gradual decrease in total-body water and extracellular fluid.[22] In the first trimester,

TABLE 75.4A-2 FLUID REQUIREMENTS PER DAY IN PEDIATRIC PATIENTS AS DETERMINED BY VARIOUS METHODS

METHOD	BODY WEIGHT (KG)	AMOUNT/D
Volume/weight	0–10	100 mL/kg
	10–20	1,000 + 50 mL/kg over 10 kg
	> 20	1,500 + 20 mL/kg over 20 kg
Volume/surface area	1–70	1,500–1,700 mL/m^2
Volume/kcal	0–70	100 mL/100 kcal metabolized

body water accounts for 95% of body weight. Body water decreases to 80% by 32 weeks and 78% at term. This shift is interrupted when an infant is born prematurely. Care must be taken to provide adequate water for the increased insensible skin losses yet permit the normal diuresis that occurs after birth. Too much water is associated with an increased incidence of patent ductus arteriosus, bronchopulmonary dysplasia, intraventricular hemorrhage, and necrotizing enterocolitis.[23] Anderson recommends 60 to 140 mL/kg/d for the first day of life, the higher amount for infants nursed under a radiant warmer or dry incubator and the lower amount for infants nursed in a humidified incubator or under a radiant warmer with a plastic blanket.[24] As the infant matures, the fluid requirement increases to 120 to 150 mL/kg/d to allow for increases in renal solute load, stool water output, and infant growth.[25]

Electrolytes are critically important in PN for the short term but also long term because normal muscle accretion cannot occur in the presence of insufficient electrolytes and minerals.[25] Essentially, all electrolyte loss occurs via the urine unless there is an ongoing source of additional losses such as diarrhea, fistula, or stoma. Table 75.4A-3 lists parenteral electrolyte requirements. When GI fluid losses occur, electrolytes and fluids must be replaced. Table 75.4A-4 lists the approximate electrolyte content of GI fluids.

PROTEIN

Proteins are essential for all metabolic functions in the body and serve as the main structural element of the body, as biochemical catalysts, and as regulators of gene expression. Proteins are complex, and their function is influenced by the availability of energy and nutrients such as minerals, vitamins, and trace elements. The average content of nitrogen in dietary protein is 16% by weight, and nitrogen metabolism is often considered synonymous with protein metabolism. Proteins consist of amino acids that have been categorized as essential, or indispensable, and nonessential, or dispensable. The nine essential amino acids—histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine—are those that cannot be synthesized from precursors and hence must be provided. However, as more information on protein and intermediary metabolism becomes available, the definition of essential becomes blurred. Laidlaw and Kopple propose adding a third category of amino acid, conditionally indispensable.[26] Conditionally indispensable amino acids are those amino acids that are synthesized from other amino acids or their synthesis is limited under special physiologic conditions.[26–28] These amino acids can be especially important in the neonate, for whom it is likely that alanine, aspartate, glutamate, serine, and perhaps asparagine are conditionally indispensable.[29]

Unlike energy and other nutrients, the body has little in the way of protein that can be mobilized during times of insufficient intake. In a 70 kg adult, the "reservoir" of labile protein is estimated at about 1% of total-body protein. More than half of the body protein is present as skeletal muscle, skin, and blood. The liver and kidney are metabolically active tissues that contain about 10% of total-body protein. Brain, heart, lung, and bone account for about 15% of whole-body protein. The distribution of protein among these organs varies with age because the newborn has proportionately more brain and visceral tissue and less muscle. Thus, when exogenous protein is inadequate, functional body proteins are used. The body can adapt to a wide range of protein intakes; however, patho-

TABLE 75.4A-3 PARENTERAL ELECTROLYTE AND MINERAL REQUIREMENTS

NUTRIENT	PRETERM INFANTS*	TERM INFANT*	CHILDREN > 1 YR
Sodium (mEq/kg/d)	3	3	3
Potassium (mEq/kg/d)	2	2	2
Chloride (mEq/kg/d)	5	5	5
Calcium† (mg/kg/d)	80–100‡	60–90	24–60
Phosphorus† (mg/kg/d)	43–62	48–68	18–45
Magnesium† (mg/kg/d)	3–6	6–10.5	2.4–6.0

*Preterm infants have the same requirements as term infants.[24,65] For preterm infants, electrolytes are generally not administered on the first day of life.

†Adapted from Greene HL et al.[65] American Society for Parenteral and Enteral Nutrition (ASPEN) based its recommendations[39] on the National Academy of Science Dietary Reference Intake.[178]—210 mg/d for infants 0 to 6 months, 270 mg/d for ages 6 months to 1 year, 500 mg/d for ages 1 to 3 years, 800 mg/d for ages 4 to 8 years, and 1,300 mg/d for ages 9 to 18 years—but notes that these levels may be difficult to achieve because administration is often limited by its solubility. ASPEN clinical guidelines make no recommendations for phosphorus or magnesium.

‡Adapted from American Academy of Pediatrics, Committee on Nutrition.[47]

TABLE 75.4A-4 ESTIMATES OF GASTROINTESTINAL FLUID AND ELECTROLYTES

FLUID	SODIUM (MEQ/L)	POTASSIUM (MEQ/L)	CHLORIDE (MEQ/L)
Gastric	20–80	5–20	100–150
Pancreatic	120–140	5–15	40–80
Small bowel	100–140	5–15	90–130
Bile	120–140	5–15	80–120
Ileostomy*	45–135	3–15	20–115
Secretory diarrhea	120	40	94
Osmotic diarrhea	54	33	94

Adapted from Institute of Medicine, Food and Nutrition Board,[178] and Johnson KB.[179]
*Decreases in volume occur with time after resection. Also dependent on length of small bowel removed.

logic conditions such as infection or trauma can cause substantial protein loss as the demand for either amino acids increases or as amino acid carbon skeletons are used to meet energy demands. If these extra needs are not met, a serious depletion of body protein mass occurs. Skeletal muscle is the largest single contributor to protein loss.

Protein requirements are based on the assumptions that adequate energy is provided so that the carbon skeletons of amino acids are not needed as an energy source and the protein quality is high. Table 75.4A-5 lists estimates of protein requirements by age. Protein quality is determined by digestibility (not a factor in PN) and the indispensable amino acid composition of the protein. If the content of a single indispensable amino acid is less than the requirement, that amino acid limits the use of other amino acids, preventing normal rates of protein synthesis even when the total nitrogen is adequate. Proposed amino acid requirements of the nine indispensable amino acids for infants (based on the amino acid composition of human milk), children, and adults have been reviewed.[30] Data exist, however, to suggest that histidine is conditionally essential for infants to 6 months of age[31] and cysteine is conditionally essential in low birth weight infants.[32] Table 75.4A-6 lists examples of the amino acid composition of some PN solutions.

The most reliable method to assess the adequacy of dietary protein is nitrogen balance, the difference between nitrogen intake and the amount excreted in urine, feces, skin, and sweat. This measurement is not practical clinically, especially for children. There is no reliable clinical measure of protein nutritional status. In infants and children, failure to gain weight or length can be used to assess the overall nutritional adequacy of a diet, and failure to gain length occurs with borderline inadequate protein intake.[33] Other anthropometrics are less sensitive. The most commonly used clinical tools to assess protein status are albumin and prealbumin.

Most individuals can tolerate a wide range of protein intakes. Foman reviewed adverse outcomes with increased protein intakes in healthy formula- and breastfed infants.[34] Diets high in protein were associated with an increase in renal solute load, a potential safety concern for water balance in the infant. Transient tyrosinemia as a consequence of delay in maturation of *p*-hydroxyphenylpyruvic acid oxidase can occur. The elevated levels can last as long as 6 weeks, raising a concern for long-term sequelae.

High-protein diets can alter the serum amino acid profile. The significance of this in infants and children who are already ill and may have an abnormal profile secondary to

TABLE 75.4A-5 ESTIMATES OF PROTEIN REQUIREMENTS BY AGE

AGE	IOM RECOMMENDATIONS (G/KG/D)*	RDA (G/KG/D)†	ESTIMATE FOR HEALTHY PATIENTS (G/KG/D)‡
Low birth weight	None	None	3–4
Full term to 6 mo	1.52 (AI)		2–3
7–12 mo	1.1 (EAR)	1.5	2–3
1–3 yr	0.88	1.1	1–1.2
4–8 yr	0.76	0.95	1–1.2
Adolescence			
Boys	0.76	0.76	0.9
Girls	0.76	0.76	0.8
Critically ill	None	None	1.5

AI = adequate intake, a recommended average daily nutrient intake level based on observed or experimentally determined approximations or estimates of nutrient intake by a group (or groups) of apparently healthy people who are assumed to be adequate; used when an RDA cannot be determined[181]; EAR = estimated average requirement, the average daily nutrient intake level estimated to meet the requirement of half the healthy individuals in a particular life stage and gender group[181]; IOM = Institute of Medicine; RDA = Recommended Dietary Allowance.
*Adapted from Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine.[30]
†Adapted from Subcommittee on the Tenth Edition of the RDAs, Food and Nutrition Board.[181]
‡Adapted from American Society for Parenteral and Enteral Nutrition Board of Directors and the Clinical Guidelines Task Force.[39]

TABLE 75.4A-6 AMINO ACID COMPOSITION OF COMMONLY AVAILABLE PARENTERAL SOLUTIONS

	PRODUCT (VENDOR)					
	FOR INFANTS LESS THAN 1 YR		FOR CHILDREN OLDER THAN 1 YR THROUGH ADULTHOOD			
	TROPHAMINE (B. BRAUN, MELSUNGEN, GERMANY)	AMINOSYN-PF (ABBOTT, CHICAGO, IL, USA)	AMINOSYN-HBC (ABBOTT)	AMINOSYN-II (ABBOTT)	TRAVASOL (BAXTER, DEERFIELD, IL, USA)	NOVAMINE (BAXTER)
Total nitrogen (g/L of a 10% solution)	15.5	15.2	16	15.3	16.5	23.9
Protein equivalent (approximate g/L of a 10% solution)	97	100	100	100	100	
pH (range)	5.5 (5.0–6.0)	5.0 (5.0–6.5)	5.2 (4.5–6.0)	5.8 (5.0–6.5)	6.0 (5.0–7.0)	5.6 (5.2–6.0)
Essential amino acids, mg/100 mL of a 10% solution						
Isoleucine	820	760	1127	660	600	500
Leucine	1,400	1,200	2251	1,000	730	693
Lysine	820	677	378	1,050	580	787
Methionine	340	180	294	172	400	500
Phenylalanine	480	427	325	298	580	693
Threonine	420	512	388	400	420	500
Tryptophan	200	180	126	200	180	167
Valine	780	673	1,127	500	580	640
Nonessential amino acids, mg/100 mL of a 10% solution						
Alanine	540	698	943	993	2,070	1,447
Arginine	1,200	1,227	717	1,018	1,150	980
Aspartic acid	320	527	—	700	—	289
Glutamic acid	500	820	—	738	—	500
Glycine	360	385	943	500	1030	693
Histidine	480	312	220	300	560	596
Proline	680	812	640	722	680	596
Serine	380	495	316	530	500	667
Taurine	25	70	—	—	—	—
Tyrosine	240	44	47	270	40	26

Manufacturers' product information, February 2003.

the primary disease is not known. In an assessment of the tolerable upper limits in healthy populations for individual amino acids recently published by the National Academy of Sciences, no adverse effects were noted for branched-chain amino acids prescribed for PN.[30] The report cautions, however, that the studies may not have carefully monitored for adverse outcomes. Similarly, there are few, if any, reports of adverse effects of glutamine in the doses generally prescribed in PN solutions.

Specialized amino acid solutions have been proposed for a variety of clinical situations, including trauma, liver failure, metabolic stress, and to improve immune function. Branched chain–enriched preparations, glutamine-enhanced solutions, and a solution specially designed for infants are most commonly used.

The term "immunonutrition" refers to the use of specific nutrients, arginine, glutamine, nucleotides, and ω-3 fatty acids, alone or in combination to influence nutritional, immunologic, and inflammatory parameters in laboratory and clinical studies. The use of immunonutrition was systematically reviewed.[35] The primary outcomes of interest were mortality and the number of patients with new infectious complications. In the 22 articles that met the inclusion criteria, the reviewers found no consistently used standard definition of what constitutes immunonutriton. Pneumonia, intra-abdominal abscess, sepsis, line sepsis, wound infection, and urinary tract infections were identified as primary adverse outcomes. Secondary outcomes included length of hospital and intensive care unit stays and duration of mechanical ventilation. From this review, the authors concluded that immunonutrition may decrease infectious complication rates, but the treatment effect varies depending on the patient population, the intervention, and the quality of the study. In surgical patients, immunonutrition was associated with a reduction in infectious complication rates and shorter length of hospital stay without adverse effects on mortality. In critically ill patients, immunonutrition was not associated with any apparent clinical benefit and may be harmful in some subgroups of patients. Thus, immunonutrition is not recommended for all critically ill patients.

The use of glutamine was systematically reviewed.[36] Fourteen randomized trials compared the use of glutamine supplementation in surgical and critically ill patients. The review concluded that in surgical patients, glutamine supplementation (doses > 0.20 g/kg/d) may be associated with a reduction in infectious complication rates and shorter hospital stay. In critically ill patients, glutamine supplementation may be associated with a reduction in complication and mortality rates. The authors caution, however, that further separate studies of the surgical and critically ill groups need to be performed and powered large enough to detect clinically important differences using parenterally delivered glutamine. There was no evidence of harm with glutamine supplementation.

Protein-energy malnutrition is common in patients with liver disease. As the disease progresses and encephalopathy ensues, branched chain–enriched PN solutions may offer benefits.[37] However, rigorous clinical studies are not available to support the use of branched chain–enriched PN solutions in all patients with liver disease.[38] Branched chain–enriched amino acid solutions can offer benefit for patients who have hepatic encephalopathy, and these solutions are recommended for such patients.[39–41]

Two amino acid solutions are specifically designed for infants less than 1 year of age. Use of these solutions results in plasma amino acid concentrations that approximate the profile seen in breastfed infants. The differences between these two solutions and others is that they contain taurine, glutamate, and aspartate; reduced amounts of methionine, glycine, and alanine; and increased arginine and leucine. Although there are differences between the two solutions, both have shown benefits in term and preterm infants.[42–44] There appears to be no difference in occurrence or magnitude of cholestasis between children who receive either of the two solutions.[45] Use of these solutions is recommended for infants less than 1 year of age. In addition to the differing amino acid compositions, these solutions are more acidic and permit the solubilization of a higher concentration of calcium and phosphorus than the amino acid mixtures prepared for adults.

CARBOHYDRATES

Glucose is the major energy source in PN because it is safe, economic, and readily available. Other sugars—fructose, sorbitol, glycerol, and xylitol—have been suggested as useful energy sources for specific conditions such as diabetes or post-trauma but are not readily available or used. Glucose in PN solutions is in the monohydrous form containing 3.4 kcal/g and is available commercially in concentrations of 2.5 to 70%. The glucose concentration of PN solutions that can be safely administered is limited by osmolality. Concentrations higher than about 12.5% sclerose peripheral veins, increasing the incidence of phlebitis and shortening the time that a vein could be used for infusion. Glucose solutions higher than 12.5% must be administered in a central vein. Glucose is calculated to provide 60 to 75% of nonprotein calories. Estimated energy requirements are provided in Table 75.4A-7.

Initiation of glucose infusion should occur in an incremental fashion to prevent hyperosmolarity and hyperinsulinemia (Table 75.4A-8). The glucose infusion rate (GIR) can be calculated using the following equation:

$$\text{GIR} = \frac{\text{g/kg/d dextrose} \times 1{,}000}{1{,}440 \text{ min/d}}$$

A GIR of 12 to 14 mg/kg/min is tolerated in a healthy child, and infants can be safely given a GIR of 8 to 12 mg/kg/min. In adult patients, there is no apparent correlation between glucose clearance and the rate of oxidation of glucose.[46] Increases in the rate of glucose infusion from 4 to 7 mg/kg/min are associated with an increase in the rate of glucose oxidation. At higher rates, fat is synthesized without a further increase in oxidation. High glucose loads (ie, those containing more than 25% glucose and delivered at > 26 mg/kg/min) may not be beneficial to infants and may contribute to hepatosteatosis.

TABLE 75.4A-7 ESTIMATES OF PARENTERAL ENERGY REQUIREMENTS

AGE (YR)	ENERGY (KCAL/KG/D)	CARBOHYDRATE (MG/KG/MIN)*
Premature infant	80–120	10–18
Term infant	90–120	11–18
1–3	75–90	9–14
4–6	65–75	8–11
7–10	55–75	7–11
11–18	40–55	7–8.5

Reproduced with permission from Lee PC and Werlin SL.[182]
*Estimate based on 60–75% of nonprotein calories as glucose.

The American Academy of Pediatrics recommends that insulin not be routinely added to PN solutions because responses to the addition of this hormone by infants are unpredictable.[47] For persistently hyperglycemic infants, insulin may improve tolerance to glucose.[48] However, a limited number of studies assess the clinical outcomes of infants treated with insulin.[49–51] Concerns about the use of insulin in the neonate include the possibility that suppression of muscle proteolysis may be undesirable, the composition of the resultant weight gain is not clearly understood, serum glucose is driven to tissues other than brain, and the possibility that glucose is converted to fat rather than being oxidized.[47]

Glucose intolerance can develop in critically ill patients, such as those who have experienced trauma, burns, sepsis, or cancer. For these patients, the GIR is limited to 5 to 7 mg/kg/min.[52] Infusion of excess glucose causes hyperglycemia, glucosuria, dehydration, and the conversion of glucose to fat. When hyperglycemia occurs, the GIR can be decreased or insulin can be added to the therapy. Exogenous insulin enhances the movement of substrate from the periphery to the liver and increases fat synthesis and storage. This can lead to hepatosteatosis; hence, insulin is not commonly used in critically ill patients outside the neonatal age group.

Complications associated with glucose infusion are listed in Table 75.4A-9.

INTRAVENOUS FAT ADMINISTRATION

Lipids are administered to virtually all patients who receive PN. Initially, intravenous fats were given to patients who had no oral intake to overcome EFAD; however, because intravenous lipids are the most calorically dense component of PN, intravenous fats are considered an important source of calories, especially for patients who are fluid restricted. Thus, intravenous lipids have at least two roles: avoiding or treating EFAD and as a source of energy.

There are a number of reasons to choose fat-predominant PN solutions over glucose-predominant ones. Glucose results in a higher specific dynamic action and thus higher resting energy expenditures and higher metabolic rates. Glucose-predominant PN can result in a respiratory quotient (RQ) of greater than 1.0, indicating the use of energy for lipogenesis. Finally, glucose metabolism results in an increased carbon dioxide elimination by the lungs when compared with fat metabolism. This fact can be of importance to mechanically ventilated patients and those with marginal lung function. In general, the ratio of calories derived from glucose to the calories derived from fats in the range of 3:1 or 2:1 minimizes carbon dioxide stress.[53,54]

Fats are bioactive precursors of prostaglandins, leukotrienes, and other mediators of inflammation and metabolism. They are also an essential part of neural tissue and cell membranes. These facts are especially important for the child who is growing, laying down tissue and gaining cells. Thus, intravenous lipids act as more than a source of calories, and in the future, intravenous fats will be used in a more specialized and directed way.[55,56]

Intravenous fats consist of three components: an aqueous phase, a lipid phase, and an emulsifier. The lipid phase supplies the majority of the calories and the essential fatty acids. Glycerin is present in the aqueous phrase

TABLE 75.4A-8 GLUCOSE CONCENTRATION IN PARENTERAL SOLUTIONS

	PREMATURE INFANT (< 1,000 G OR 28 WK GESTATION)	INFANT	CHILD (1–10 YR)	ADOLESCENT (11–18 YR)
Begin infusion	5–7.5% or glucose concentration in current IV solution	5–7.5% or glucose concentration in current IV solution	10% or percent higher than concentration in current IV solution	10% or percent higher than concentration in current IV solution
Advance	2.5% each day as tolerated	2.5% each day as tolerated	5% each day as tolerated	5% each day as tolerated
Usual GIR upper limit (mg/kg/min)	8–12	12–14	8–10	5–6
Peripheral maximum concentration (%)	12	12.5	12.5	12.5
Central glucose concentration (%)	20–25	25	25	25
Monitor at initiation and with every increase	Urine glucose	Urine glucose	Urine glucose	Urine glucose

Adapted from Lee PC and Werlin SL.[182]
GIR = glucose infusion rate; IV = intravenous.

TABLE 75.4A-9 COMMONLY REPORTED COMPLICATIONS OF GLUCOSE

COMPLICATION	USUAL CAUSE	PREVENTION OR TREATMENT
Phelibitis	High osmolarity	Limit glucose concentration to 12.5% for peripheral administration
Refeeding syndrome	Rapid refeeding of a malnourished patient	Refeed slowly; monitor serum phosphorus, potassium, calcium, and magnesium
Hepatosteatosis	All nonprotein calories provided as carbohydrate or excessive calories	Provide 30% of calories as lipid
Cholestasis	Infant fed exclusively by PN	Enteral feeding
Carbon dioxide retention	High GIR in a patient with respiratory failure	Decrease GIR
Hypoglycemia	Abrupt discontinuation of PN or decrease in GIR	Taper PN
Hyperglycemia	High GIR, stress, burns, sepsis, incorrect glucose concentration	Decrease GIR, add insulin

Adapted from Lee PC and Werlin SL.[182]
GIR = glucose infusion rate; PN = parenteral nutrition.

and raises the tonicity as well as incidentally supplying calories. The emulsifier is usually egg phospholipid. Soybean oil, safflower oil, and mixtures of the two have been used as sources of lipid. The caloric density of 10% lipid emulsions is 1.1 kcal/mL, 20% emulsion is 2.0 kcal/mL, and 30% emulsion is 3.0 kcal/mL (available for three-in-one solutions only).

Preventing EFAD is one of the two major reasons for administering intravenous fats. The essential fats for humans are linoleic ($C_{18:2\ \omega\text{-}6}$) and linolenic ($C_{18:3\ \omega\text{-}3}$). EFAD has been described in children and adults who have no exogenous source of fats for 3 weeks or more. In the preterm infant, EFAD is seen after only 1 week without essential fatty acids. Symptoms of EFAD include scaly skin rash, sparse hair, susceptibility to infection, failure to thrive, hypotonia, increased red cell fragility, and electroencephalographic and electrocardiographic changes. In EFAD, the ratio of trienoic to tetraenoic acids increases to greater than 0.2.[57] In older children and adults, EFAD can be prevented by supplying 0.5 to 1.0 g/kg/d as intravenous lipid, and for premature infants, 0.6 to 0.8 g/kg/d prevents EFAD.

For the most part, fats supplied intravenously as a lipid emulsion are handled in the circulation in much the same fashion as very-low-density lipoprotein (VLDL) particles. Lipoprotein lipase, present in the capillary endothelium, reduces the triglycerides at the core of the particle, whereas the polar lipids, at the surface of the VLDL, are removed to form nascent high-density lipoprotein (HDL). Lecithin-cholesterol acyltransferase (LCAT), released into the circulation from the liver, converts the nascent HDL into mature HDL.[58] These two enzymes, lipoprotein lipase at the endothelial surface and LCAT in circulation, seem to be key in clearing the infused lipid from the circulation.

Lipids can be cleared by other mechanisms, such as endothelial cell endocytosis via cell-surface heparin sulfate proteoglycans in a receptor-independent manner.[59]

The amount of lipid that can be given safely by the intravenous route is limited by the rate of clearance of lipids from circulation. Two to three grams/kg/d of lipid can be safely administered to premature infants, term babies, and older children. Serum triglycerides must be monitored to ensure that the clearing mechanism has not been overwhelmed. It has been suggested that heparin might enhance clearance. In a randomized trial of two heparin doses, the drug caused an increase in circulating free fatty acids, presumably because of release of the lipoprotein lipase into circulation. There was no increase in lipid use.[60] It was concluded that heparin was of no benefit for fat metabolism. Administering intravenous lipid over 20 hours while allowing 4 hours for lipid clearing has been proposed as another method of facilitating lipid clearing. Evidence does not support this practice.[61]

Lipid emulsions used for parenteral administration contain phytosterols. These are plant-derived isoflavones that possess estrogenic properties. Their importance, either beneficial or detrimental, as part of PN is not known. Phytoestrogens, present in soy-based formulas, have been suggested as a cause of decreased bone mineral content in premature infants. Whether these compounds figure in PN-associated bone disease is unknown. No consistent effect of soy feeding on menarche and telarche has been found.[62,63] The importance of phytosterols in infused lipid emulsions as feminizing compounds has not been studied. To date, there is no proof that phytosterols play a role in PN-associated cholestasis, although this relationship has been suggested.[64]

MINERALS AND TRACE ELEMENTS

In the context of pediatric PN solutions, calcium, phosphorus, and magnesium are usually termed "minerals." Trace elements are those minerals found in the body in small amounts and include iron, zinc, copper, selenium, chromium, manganese, molybdenum iodine, fluoride, strontium, lithium, nickel, boron, vanadium, and arsenic. The discussion of trace elements will include those present in PN trace element solutions (zinc, copper, manganese, and chromium), as well as iodine, selenium, and iron. Deficiency of iodine, selenium, and iron has been described in children receiving long-term PN. These minerals are not included in trace element solutions, but they are frequently added in the solutions of children receiving

long-term PN. Table 75.4A-3 provides estimates of PN mineral requirements, and Table 75.4A-10 lists PN requirements for trace elements.

In 1988, the American Society for Clinical Nutrition (ASCN) published recommendations for parenteral trace elements for the pediatric age range.[65] These recommendations are based on calculations using the factorial method. As this committee recognized, trace element requirements of children receiving PN are inadequately studied. When the ASCN recommendations were critically assessed, some of the recommendations were found to be inaccurate. For instance, Mouser and colleagues found that children receiving the ASCN recommended amount of chromium had 10 times the serum concentration of children consuming a normal diet.[66]

Calcium, Phosphorus, and Magnesium

The minerals calcium, phosphorus, and magnesium are vital to intermediary metabolism serving in energy transfer. Calcium and phosphorus also serve a structural function, and calcium is a regulator for a variety of intracellular processes. Calcium and phosphorus are presumed to be actively transported across the placenta against a concentration gradient.[67] Between the time a fetus weighs 1,000 g and 4,400 g at term, whole-body calcium content increases by 86%, phosphorus by 82%, and magnesium by 78%.[68] Many chronic diseases that require PN support are associated with calcium depletion. Approximately 99% of total-body calcium is found in bone, whereas 85% of phosphorus is in bone, and 50 to 60% of magnesium is in bone. Bone acts as a reservoir for calcium, phosphorus, and magnesium, so serum levels often do not reflect total-body content. This makes it difficult to identify deficiency states. Less than 1% of the total-body calcium, phosphorus, and magnesium is in the circulation. Because serum calcium is tightly controlled at the expense of bone, calcium deficiency is a chronic problem of bone loss. Calcium circulates in three forms: 45% is in the biologically active ionized form, 45% is bound to protein, mainly albumin, and 10% is complexed to phosphate, lactate, and citrate.[69] Measurement of ionized calcium, because it is the active form, better reflects calcium homeostasis than does measuring total serum calcium. Fifty percent of phosphorus exists in circulation as free ions, 10% is protein bound, and 40% is complexed to calcium, magnesium, and sodium as salts. Phosphorus deficiency can be a serious acute problem when malnourished patients are refed and sufficient phosphorus is not provided.[70] Magnesium is mainly an intracellular anion, so serum magnesium levels may not accurately reflect total-body magnesium status.

The parenteral requirements for calcium and phosphorus can be tremendous, especially in small prematurely born infants and in children with a chronic disease. Peak bone mass is attained in late adolescence and is determined by nutritional factors, genetics, mechanical factors, and the environment.[71] PN should supply adequate minerals to attain optimal peak bone mass. Metabolic bone disease is common in premature infants and in children receiving long-term PN.[72,73] The hypocalcemia associated with metabolic bone disease results from not supplying adequate calcium and from urinary calcium losses. Because at varying concentrations, calcium and phosphorus will complex with each other in PN solutions and form a precipitate, there are limitations to the amounts of calcium and phosphorus that can be supplied in PN solutions.[74] These limitations caused nutritionists to seek creative ways of providing the minerals, such as alternating intravenous solutions of high cal-

TABLE 75.4A-10 BASAL DAILY PARENTERAL REQUIREMENTS BY AGE AND DEVELOPMENTAL STAGE

TRACE ELEMENT	DOSE/D
VERY LOW BIRTH WEIGHT INFANTS	
Iron	100 µg/kg
Zinc	350 µg/kg
Copper	60 µg/kg
LOW BIRTH WEIGHT AND PRETERM NEONATES	
Iron	100 µg/kg
Zinc	400 µg/kg
Copper	20 µg/kg
Selenium	2 µg/kg
Chromium	0.2 µg/kg
Manganese	0.1 µg/kg
Molybdenum	0.25 µg/kg
NORMAL INFANTS	
Iron	100 µg/kg
Zinc	250 µg/kg*
	400 µg/kg†
Copper	20 µg/kg
Selenium	2.0 µg/kg
Chromium	0.2 µg/kg
Manganese	1.0 µg/kg
Molybdenum	0.25 µg/kg
Iodine	1.0 µg/kg
CHILDREN	
Iron	1.0 mg
Zinc	5.0 mg
Copper	300 µg
Selenium	30 µg
Chromium	5.0 µg
Manganese	50 µg
Molybdenum	5.0 µg
Iodine	50 µg
ADOLESCENTS	
Iron	1–3 mg‡
Zinc	2.5–4.0 mg
Copper	0.5–1.5 mg
Selenium	30–60 µg
Chromium	10–14 µg
Manganese	150–800 µg
Molybdenum	20–120 µg
Iodine	150 µg
Zinc	350 µg/kg
Copper	60 µg/kg

Adapted from Schanler RJ et al[183] for very low birth weight infants, Zlotkin SH et al[184] for low birth weight infants, Greene HL et al[65] for term infants, with the amount for children being the maximal daily dose, and the American Medical Association (AMA)[185] for adolescents. The nutrients not addressed by the AMA panel consensus levels from diverse literature as discussed by Solomons N[186] have been listed.

The table is adapted from Solomons NW and Ruz M.[187]

*For full-term infants 0–3 months.

†For infants 3–12 months.

‡Depends on gender; extra is needed in females to compensate for menstrual losses.

cium concentration with those of high phosphorus content. These strategies have no proven benefit. Amino acid solutions designed for children under 1 year have added cysteine. Cysteine lowers the pH enough to permit the addition of calcium and phosphorus in amounts that may meet daily requirements.[75] In premature infants, it may not be possible to deliver these minerals in quantities that simulate intrauterine mineral accretion, but severe bone disease can be reduced. Because the amount of calcium that can be delivered is limited, maneuvers that decrease urinary calcium losses have been explored. Factors that promote hypercalciuria are increased calcium intake,[76] decreased phosphate intake,[76] increased amino acid infusion,[77] metabolic acidosis,[78] and cycling PN infusions.[79]

Children on long-term PN do not experience bone pain or fractures because the metabolic disease associated with PN is mostly subclinical.[80] Aluminum present as a contaminant in PN solutions and in additives can lead to decreased bone mineralization.[81] Parenteral amino acid solutions prepared from casein hydrolysates were contaminated with aluminum. Aluminum contamination is associated with osteopenia, growth arrest, fractures, and pain.[82–84] Since the discontinuation of these solutions, metabolic bone disease has become less of an issue. However, osteopenia remains a problem for children.[85,86] A study of children who had bowel resections of varying length, necessitating PN support for 1 to 67 months, showed appropriate bone mineralization for their weight and height after the PN solutions were discontinued.[87] The bone mineralization was less than that of children of the same age. Although this observation is encouraging, the findings must be viewed with caution because more than half of the children had relatively small amounts of bowel removed and required PN for less than 8 months.

Refeeding syndrome frequently accompanies the nutritional rehabilitation of malnourished individuals. The refeeding syndrome is a set of metabolic and functional complications that occurs as a result of intracellular shifts of elements. During rapid nutritional rehabilitation, profound hypophosphatemia, hypomagnesemia, and hypokalemia can precipitate acute respiratory and circulatory collapse. Neurologic manifestations of the refeeding syndrome include weakness, lethargy, paralysis, and confusion. Deaths have been documented.[88] Phosphorus, magnesium, and potassium must be carefully monitored in malnourished patients. Aggressive supplementation and correction of low values prevent the adverse outcomes of refeeding syndrome.

Zinc

Zinc is essential for growth. It is involved in chromosome replication and regulation of the translation of genetic information, provides structure for "zinc finger" proteins, stabilizes ribosomes and membranes, and is a component of a number of enzymes.[89] Zinc deficiency in humans impairs cell-mediated immunity.[90] Signs of zinc deficiency include dermatitis, alopecia, diarrhea, and immune deficiency. Severe PN-associated zinc deficiency mimics acrodermatitis enteropathica.[91] With zinc deficiency, relatively more fat accrues than lean tissue.[92] Zinc status is difficult to monitor. Stress, infections, and trauma all alter circulating zinc levels. Merely administering PN diminishes plasma zinc by about 30%.[92] Despite these shortcomings, serum zinc is commonly used to monitor zinc nutriture. Zinc is lost from the body through urine, sweat, and stool. Zinc status should be assessed and corrected if necessary in the face of extra loss, as in the case of diarrhea or high ostomy output. The composition of the tissue acquired during nutritional rehabilitation may be affected by zinc status. Acute zinc toxicity has resulted in pancreatitis.[93] Chronic toxicity has not been described in pediatric patients receiving PN.

Copper

Copper is important in many enzyme systems, especially those that involve oxygen, hydrogen peroxide, and superoxide. It is necessary for the formation of melanin, catecholamine synthesis, and crosslinking of elastin and collage.[94] Copper circulates bound to ceruloplasmin, which may have antioxidant properties of its own. Copper deficiency has been described in long-term PN patients frequently.[95,96] Premature infants are at special risk of becoming copper deficient because copper accumulates in the fetus during the third trimester. Signs of copper deficiency include hypochromic, microcystic anemia, depigmentation of skin and hair, hypothermia, and hypotonia.

Manganese

Manganese has two known functions: as cofactor for the enzyme pyruvate carboxylase and as part of the mitochondrial enzyme superoxide dismutase. Manganese deficiency in long-term PN has not been described; however, toxicity has been described. High levels of manganese have been implicated as a causative factor in the cholestatic effect of PN and also in basal ganglia damage resulting in Parkinson disease in adults on long-term PN.[97] Fell and colleagues have monitored basal ganglia damage associated with manganese accumulation in children on long-term PN using magnetic resonance imaging. This group also followed the cholestatic effects of manganese in children. This study supports a causative role for manganese in both of these complications of PN.[98]

Chromium

The metabolic role of chromium is poorly understood. Proof that chromium plays a part in a "glucose tolerance factor," thought to activate insulin, is lacking. Two cases of chromium deficiency have been described in adults receiving PN. The symptoms of the described cases include glucose intolerance, hyperosmolarity, dehydration, and glucosuria. Symptoms responded poorly to insulin, but insulin responsiveness was restored with administration of chromium.[99] Chromium deficiency in children receiving PN has not been described; however, chromium excess has been reported.[100] Chromium toxicity results in skin irritation and carcinogenesis in animals.[101] The trivalent chromic ions present in PN solutions are not highly toxic, so excessive chromium from PN is not likely to be harmful.

Iodine

Iodine is essential, and its deficiency has been described in children.[102] However, iodine deficiency had not been described in children receiving PN until recently. Ibrahim and colleagues studied a group of extremely premature infants on PN who were in negative iodine balance.[103] Because the absorption of iodine is good, the parenteral dose should be similar to the enteral recommendation. One microgram/kg/d administered parenterally is recommended for the very low birth weight infant, whereas the enteral recommendation is 30 µg/kg/day.[104,105] Iodine is a minor contaminating component of many PN solutions and is present in antiseptics used to cleanse the skin at the catheter insertion site. PN patients receive measurable iodine from both of these sources.[106] No toxicity has been found in subjects receiving 20 times the Recommended Dietary Allowance for iodine. Any pediatric patient receiving long-term PN requires supplemental iodine.

Selenium

Selenium is bound at the catalytic site of glutathione peroxidase, an enzyme that catalyzes the reduction of H_2O_2 to H_2O. Selenium is also at the active site of type 1 and 3 iodothyronine deiodinase, which is important in the synthesis and degradation of triiodothyronine.[107] However, one study did not show a correlation between selenium supplementation and thyroid function in very low birth weight infants receiving long-term PN.[108] Selenium deficiency has been reported in children receiving PN.[109–111] The ASCN guidelines list the parenteral selenium requirements at 2.0 µg/kg/d for preterm and term infants as well as children.[65] Selenium toxicity is described.[112]

Cholestasis

Because copper and manganese are excreted in the bile, patients with cholestasis who require PN are at risk of developing increased levels of manganese and copper. Therefore, the blood levels of these micronutrients should be carefully monitored and the content of manganese and copper in the PN solutions adjusted as indicated. Similarly, selenium, chromium, and molybdenum should be monitored and the PN content of these minerals adjusted as needed in children with renal disease.

Iron

Iron deficiency is the most common cause of anemia in children.[113] However, anemia is a manifestation of the severe end of the iron deficiency spectrum because iron is used for erythropoiesis at the expense of other tissues, including brain. Iron deficiency adversely affects behavior and neurodevelopment.[114] Iron deficiency is common among children receiving long-term PN. It occurs as a result of the underlying condition necessitating PN rather than as a result of the PN itself. Long-term PN without iron supplementation results in iron deficiency and iron deficiency anemia. Causes of anemia other than iron deficiency may be present and should be searched for before assuming that anemia is due to iron deficiency. These include the anemia of chronic disease, zinc deficiency, vitamin E deficiency, and hemolysis. Chronic blood loss from stool, urine, or vomitus can lead to iron deficiency. These losses should be corrected when possible. A number of tests are useful in assessing iron nutriture: ferritin, free erythrocyte protoporphyrin, zinc protoporphyrin, transferrin saturation, transferrin receptors, and hemoglobin and hematocrit. Hemoglobin and hematocrit are readily accessible but represent severe, end-stage iron deficiency. Ferritin is an early marker of iron deficiency, reflecting liver stores of iron; however, ferritin is also an acute-phase reactant, making interpretation of this test problematic. Changes in protoporphyrin levels reflect early alteration of erythropoiesis. In the context of a child receiving PN, a combination of these tests is recommended.[115]

Prematurely born infants are at particular risk for deficiency because iron is transferred to the fetus via the placenta during the last trimester. Iron precipitates as iron phosphate from lipid emulsions and three-in-one solutions. Iron dextrans are compatible with PN solutions that do not contain lipid.[116]

See Table 75.4A-10 for recommended daily amounts of iron for parenteral administration. Iron can be given either intravenously or intramuscularly as iron dextran.[117] Inorganic salts of iron are used in PN solution in Europe.[118] Some report increased infection rates in children receiving iron supplementation.[119] Other reports have not found this association.[120] Iron homeostasis is regulated at the level of GI absorption. Parenterally administered iron bypasses this control mechanism, making iron overload possible. Iron administration should be reduced or eliminated if overload is present.[121]

VITAMINS

Vitamins are essential nutrients and must be provided by the PN solution, with the possible exception of vitamin D, to avoid deficiency. For the purposes of PN, both water-soluble and fat-soluble vitamins are available in a single solution. Several parenteral vitamin preparations are available for children and adults. Table 75.4A-11 lists some of the preparations, the US Food and Drug Administration (FDA) requirements for adult vitamin solutions (there is no FDA guideline for pediatric multivitamins), and the recommendations of the Committee on Clinical Practice Issues of the ASCN. These recommendations are based on the requirements for stable patients. Adjustments may need to be made for patients who have specific vitamin deficiencies or organ dysfunction, require ventilatory support, or are catabolic. Carnitine is likely a conditionally essential nutrient in the neonate and should be provided after deficiency has been confirmed.[39] To avoid potential toxicity, vitamin preparations designed for preterm infants should not be formulated with propylene glycol or polysorbate.[65]

CENTRAL VERSUS PERIPHERAL ADMINISTRATION OF PN

Central administration of PN solution is required in the infant and the premature infant to deliver meaningful nutrition support.[122] In the older child, peripheral administration can be maintained for a few days, but central

TABLE 75.4A-11 VITAMINS FOR PARENTERAL NUTRITION

	ML	A (IU)	D (IU)	E (IU)	K (MG)	B_1 (MG)	B_2 (MG)	B_3 (MG)	B6 (MG)	B_{12} (µG)	FOLIC ACID (µG)	PANTOTHENIC ACID (MG)	BIOTIN (µG)	C (MG)	CARNITINE (MG/KG)
FDA*		4,000–5,000	400	12–15	2–4/wk	1–1.5	1.1–1.9	12–20	1.6–2.0	3	400	5.0–10	130–300	45	—
Infuvite Adult (Sabex 2002, Boucherville, QC, Canada)†‡	10	3,300	200	10	150	6	3.6	40	6	5	600	15	60	200	—
M.V.I.Adult 12 (aaiPharma, Wilmington, NC, USA)†	10	3,300	200	10	0	3	3.6	40	4	5	400	15	60	100	—
ASPEN§		2,300	400	7	200	1.2	1.4	17	1.0	1.0	140	5	20	80	2–10‖
M.V.I. Pediatric (aaiPharma, Wilmington, NC, USA)†#	5	2,300	400	7	200	1.2	1.4	17	1.0	1.0	140	5	20	80	—
Infuvite Pediatric (Sabex 2002, Boucherville, QC, Canada)†‡	5	2,300	400	7	200	1.2	1.4	17	1.0	1.0	140	5	20	80	—

*US Food and Drug Administration (FDA) conditions for marketing an effective adult (ages 11 years and older) parenteral multivitamin.[189]
†Manufacturer's product information.
‡Baxter.
§As recommended by American Society for Parenteral and Enteral Nutrition.[39]
‖May be conditionally essential in neonates.[190] Confirm deficiency.
#Neosan.

access is necessary for long-term (greater than 1 week) administration of PN. Because PN is not recommended for a duration less than a week,[123] peripheral PN should be thought of as a bridge until a central line can be placed. Although it is possible to provide peripheral PN over a long period of time in children and adolescents, it requires multiple intravenous site changes, risks local complications, and is inconvenient for both the patient and the caregivers. Therefore, central intravenous access for PN should be sought. The technique of peripherally accessed central catheterization (PICC line) for establishing central lines is relatively easily accomplished.[124]

INTRAVENOUS CATHETERS

There are three categories of PN central lines: (1) tunneled lines such as the Broviac or Hickman lines, (2) subcutaneous port lines such as the Medi-port or Hide-a-Port, and (3) PICC lines. No one type of catheter is clearly superior to the others in all situations.[125] PICC lines are easily placed and removed but are associated with the highest incidence of malposition, inadvertent removal, thrombophlebitis, and mechanical obstruction.[126,127] Subcutaneous ports are associated with the least number of infections[128,129] and have a smaller risk of intraluminal thrombosis when compared with the tunneled catheters.[130] Ports are the most difficult to place and require repeated percutaneous access.

In general, reducing the number of lumens reduces the risk.[131] Double-lumen PICC lines are often too large to be of use in young children. Subcutaneous reservoir devices are available with double lumens and divided ports but may be too large to be practical, especially in the small child. It is possible to place a second separate central line if additional access is required.

Catheters should be placed into the superior vena cava or right atrium. The superior vena cava can be reached via the internal jugular vein, the subclavian vein, and peripheral veins. Use of the femoral vein is discouraged. Femoral lines are associated with higher rates of venous thrombosis and catheter-related sepsis.[132] With subclavian access, there is a greater risk of pneumothorax when compared with internal jugular access.[133] Internal jugular insertion is associated with higher rates of hematoma formation, arterial injury, and catheter-related infections.[134] In a large series, radiologic insertion technique was superior to surgical insertion. The radiologic insertion led to fewer infections (1.9 vs 4.0 per 1,000 catheter days), fewer pneumothoraces, and fewer complications overall.[135] Maintaining central venous access long term can be challenging. When all conventional sites have been exhausted, thoracotomy with cannulation of the azygos vein or direct right atrial cannulation[136] has been used. More recently, use of a transhepatic approach for establishing central access for PN has been described.[137]

COMPLICATIONS

Complications associated with PN fall into three general categories: mechanical, metabolic, and infectious. Mechan-

ical complications include misplaced lines and lines that move, fall out, are pulled out, break, kink, or become occluded. Table 75.4A-12 lists some examples of the specific injuries that can occur with mechanical misadventures. Some mechanical complications represent significant life-threatening events, and their occurrence is evident almost immediately or within a short period of time. Others, such as development of a thrombus, occur over a longer time and are heralded by the progressive difficulty in aspirating blood through the catheter. Children experience higher catheter occlusion rates than adults: 10% of children receiving home PN support,[87] 13 to 16% of oncology patients,[138,139] 31% of infants,[140] and 41% of patients with cystic fibrosis.[141] Catheters can be occluded by a thrombus, a kink in the line, too tight sutures, deposition of drugs, mineral precipitation, and lipid deposition within the lumen of the line or abutment of the catheter tip against the vessel wall. Mechanical maneuvers should be attempted as soon as a line is identified as occluded to restore the patency of the line. These maneuvers are not often successful and can be associated with dislodgment of a thrombus and pulmonary embolization, line rupture, or malposition of the catheter.

Thrombosis in central catheters is relatively common. Clot may form as a fibrin sheath at the catheter tip, or a thrombus may form on the outside wall of the catheter and/or on the wall of the vessel adjacent to the catheter. The fibrin sheath is a consistent finding on all indwelling catheters regardless of the type of material, but the amount of sheath varies with the thrombogenicity of catheter material.[142] Mural thrombi occur within 48 hours of catheter insertion. Many strategies have been attempted to prevent the development of catheter-related thrombosis, including the administration of salicylates, dextran, and heparin. Catheters have also been coated with salicylates, heparin, and antibiotics. Some advocate the routine addition of heparin to infused solutions (500–2000 U/L) because this may decrease thrombotic complications with long-term catheter use.[143] But saline flushes are equally efficacious in maintaining catheter patency.[144] Although heparin has been used for many years, some caution is necessary. Heparin can cause a transient fall in platelet count 1 to 3 days after initiation of treatment. In most patients, this is of no clinical significance; the platelet levels return to normal within a few days of the discontinuation of heparin. In some patients, heparin-induced thrombocytopenia, a life- and limb-threatening complication of heparin therapy, can occur. Heparin-induced thrombocytopenia is likely an immune-mediated syndrome that can be precipitated even by minute quantities of heparin given to flush catheters.[145,146] To restore line patency to a fibrin-occluded catheter, streptokinase, urokinase, and tissue plasminogen activator have been used successfully. Currently, recombinant tissue plasminogen activator is the agent of choice to restore patency of thrombin-occluded catheters.[147] Treatment of thrombotic occlusions with tissue plasminogen activator has resulted in removing the obstruction in over 90% of catheters treated[148] and has a low complication rate compared with treatment with either streptokinase or urokinase. Catheter occlusions by some drugs and minerals may be resolved with the instillation of 0.2 to 1.0 mL of 0.1N HCl for 30 minutes to 1 hour.[149–151] More than one instillation may be required. For other drugs, an alkaline solution may be necessary. In clinical situations that do not respond to usual treatment, the possibility of fragmentation or disconnection of part of the indwelling catheter with embolization should be considered.[152] Figure 75.4A-2 is an algorithm for the diagnosis and treatment of catheter occlusion in adult patients with short small bowel syndrome.

Infections related to the catheter can occur systemically or locally at the insertion site. A number of maneuvers have been demonstrated to reduce the risk of infection. These include (1) cleansing the skin prior to insertion with chlorhexidine,[153] (2) avoiding catheter insertion in a febrile child, (3) radiologic insertion by an expert team,[135] and (4) using full-barrier precautions during insertion.[154] Prophylactic antibiotics at the time of insertion do not decrease the risk of infection, and antibiotic ointment at the insertion site should be avoided because it results in the acquisition of resistant organisms and does not decrease the risk of infection.[155] Catheters impregnated with antimicrobial materials such as chlorhexidine, silver sulfadiazine, minocycline, and rifampin are associated with a lower incidence of line infection, but their higher cost may limit their use.[153,156] Certainly, these special catheters do not obviate the need to use all other techniques to reduce infections. Systemic infections occur in 10 to 60% of patients, often require removal of the catheter to clear,[138,157–159] and can be complicated by urinary tract infection, osteomyelitis, and abscess formation. Infections can be caused by bacteria such as those that occur on the skin, populate the GI tract, or have no identifiable source or fungi, the most common of which is *Candida*. The most important route of catheter-related contamination is thought to be migration of organisms from the skin along the subcutaneous tract into the vein. Another important source of infection is catheter hub contamination during tubing manipulations. One study noted that the infectious agent was often found on the skin of nurses who changed the PN fluid bag.[160] Some have hypothesized that a GI tract injured by inflammation, ischemia, surgical manipulation, or malnutrition may be more permeable to infectious organisms.[139,161] If a child with a central line develops a significant fever (38°C), whether or not there is another source of infection, central and peripheral cultures should be obtained, and the child should be treated with intravenous antibiotics covering both gram-positive cocci and gram-negative bacteria. If a source of fever other than the catheter is identified, such as an ear infection or a urinary tract infection, neither the catheter nor the other site can be assumed to be the source of the fever; therefore, antibiotic coverage should include coverage for organisms associated with catheter infections and possible non–catheter-related sources. Catheter-related infections can be eradicated with appropriate antibiotics; however, tunnel-related infections are more difficult to eliminate.[128,162] Catheter removal is usually curative if antibiotic coverage is extended 48 hours after the catheter is

TABLE 75.4A-12 COMPLICATIONS ASSOCIATED WITH PARENTERAL NUTRITION

COMPLICATION	CAUSE	INTERVENTION
Catheter related		
Cardiac arrhythmia	Catheter tip in heart	Remove catheter
Air embolus	Inadvertent injection of air; accidental uncoupling of infusion system	Place patient on left side and lower the head of the bed; this may keep air within apex of the right ventricle until it is absorbed
Thrombosis of central vein	Uncoupling of infusion system; catheter induced	Anticoagulation therapy; remove catheter if unsuccessful (see figure75.4-2)
Catheter occlusion	Hypotension; failure to maintain line patency; formation of fibrin sheath outside the catheter; lipid or mineral precipitates	Anticoagulation therapy (see Figure 75.4A-2)
Perforation and/or infusion leak	More common with polyvinyl polyethylene catheters	Check device connection sites; replace faulty devices, tubing, catheter
Infusion system obstruction	Catheter kinking, clot; bacterial filter airlock; clamping of line	Attempt mechanical manipulation
Phlebitis	Peripheral administration of hypertonic solution (osmolarity ≥ 900 mOsm/kg); line infiltration	Change peripheral line site
Infectious		
Sepsis	Improper aseptic technique; catheter insertion; catheter care; mixing of parenteral solutions	Blood cultures; antimicrobial medication (see Figure 75.4A-3)
Local infection	Improper aseptic technique	Local and/or systemic antimicrobial medications (see Figure 75.4A-3)
Metabolic		
Osmotic diuresis	Hyperglycemia; increased glucose in urine	Reduce infusion rate or decrease dextrose concentration; give insulin
Hyperammonemia	Hepatic dysfunction; excess free ammonia; insufficient arginine; excessive amino acid infusion	Reduce protein concentration; increase nonprotein calorie-to-nitrogen ratio
Azotemia or elevated blood urea nitrogen	Dehydration; calorie-nitrogen imbalance; renal dysfunction	Correct dehydration; increase nonprotein calorie-to-nitrogen ratio or reduce amino acid concentration; give insulin if hyperglycemia is present
Hyperglycemia	Excessive infusion rate or concentration of glucose; concurrent steroid therapy; possible sepsis; insulin insufficiency	Reduce rate or concentration of dextrose; rule out sepsis; give insulin
Hypoglycemia	Abrupt interruption of infusion of a high glucose concentration	Start dextrose infusion; monitor for seizures
Metabolic acidosis	Renal or gastrointestinal losses; infusion of hydrogen ion; inadequate amount of base-producing substance in solutions to neutralize acid products of amino acid degradation; excessive protein and/or calories; acute postinjury phase with increased metabolic rate	Increase acetate in solutions; decrease chloride in solutions with hypochloremic acidosis; provide maintenance calories and protein
Metabolic alkalosis	Base introduction or nasogastric drainage	Provide additional fluid and NaCl, KCl, or NH_4Cl
Fluid overload	Cardiac or renal insufficiency or excessive infusion rate	Decrease fluid; can increase the nutrient concentration of nutrient solutions
Respiratory acidosis	Increased calorie load with high fraction of calories as dextrose	Decrease total caloric intake; decrease fraction of calories from dextrose and increase fraction of calories from lipid
Cholestasis	Idiopathic; lack of enteral feeding	Provide some enteral nutrition, even if only 1–2 mL/h
Hypophosphatemia	Inadequate phosphate; refeeding syndrome; increased losses (diarrhea); high dextrose concentration infusion may increase phosphorus needs	Increase phosphate in solution; monitor solution for compatibility; monitor serum levels
Hyperphosphatemia	Renal insufficiency	Decrease phosphorus in nutrient solution but do not omit; check for other phosphorus source
Hypocalcemia	Refeeding syndrome; inadequate calcium in solution	Increase calcium in solution; monitor compatibility; monitor serum levels
Hypomagnesemia	Inadequate magnesium in solution; refeeding syndrome; gastrointestinal or renal losses	Increase magnesium in solution; monitor solution compatibility; monitor serum levels
Hypokalemia	Gastrointestinal or renal losses; refeeding syndrome; inadequate potassium in solution; high dextrose infusion; hypomagnesemia	Increase potassium in solution; may require 4 mEq K/kg/d; monitor serum levels
Hyponatremia	Gastrointestinal or renal losses; inadequate sodium in solution; excessive fluid intake	Increase sodium in solution; decrease fluid volume; check all sources of intake; monitor intake and output, serum sodium and urine-specific gravity
Essential fatty acid deficiency	Fat-free solutions	Provide at least 2–4% of calories as fat (0.5–1.0 g/kg/d)
Hypertriglyceridemia	Lipid infusion dose too high; acute inflammatory state	Increase duration of infusion; decrease total g/kg/d lipid infused
Rash	Possible allergy	Discontinue lipid administration or change lipid preparation
Trace element deficiency	Inadequate or incomplete trace element supplementation; gastrointestinal losses (zinc)	Routine trace element supplementation, monitor nutritional trace element status
Vitamin disorders	Inadequate intake; excess needs	Monitor serum levels; provide additional vitamins

Adapted from Davis AM.[191]

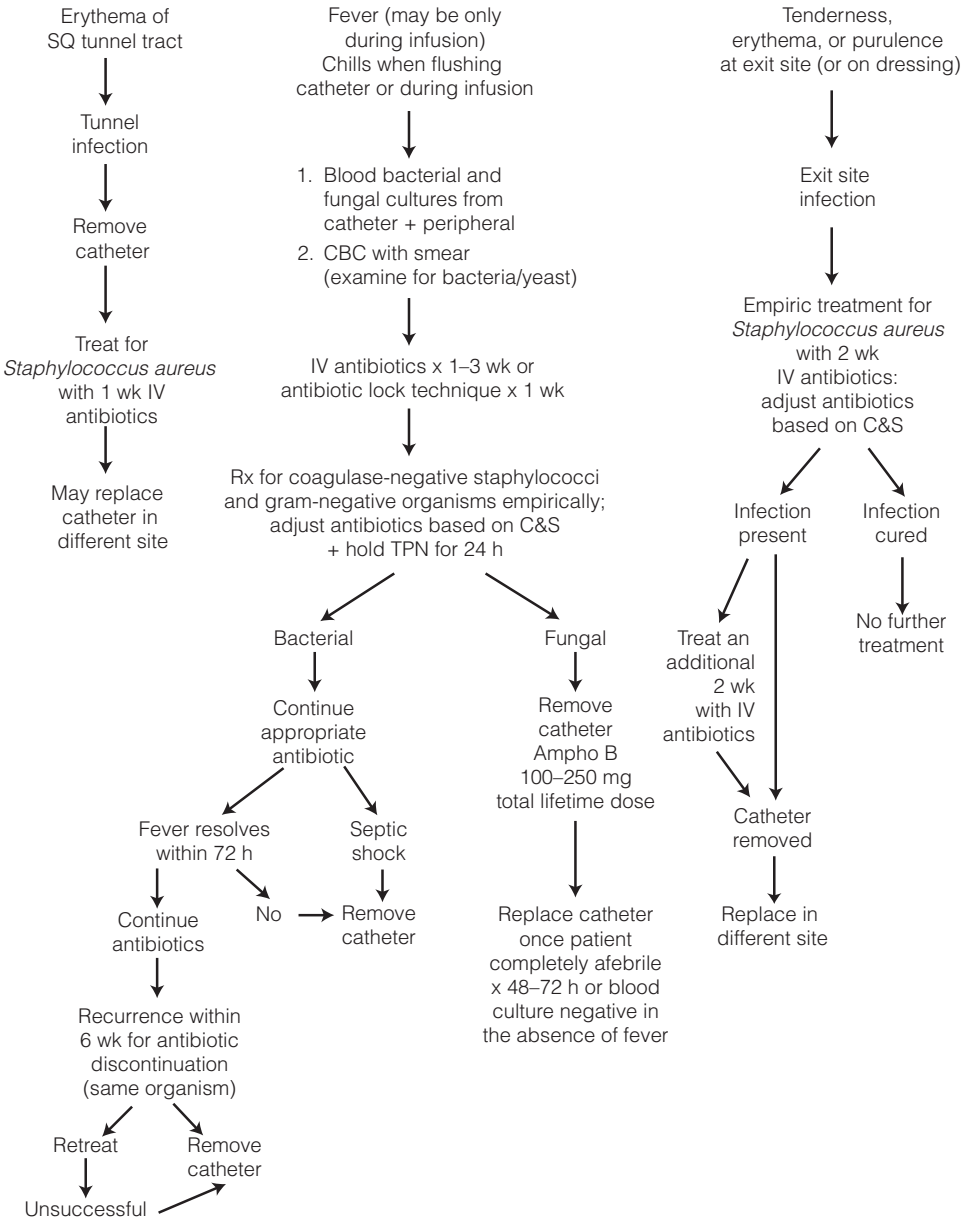

FIGURE 75.4A-2 Algorithm for the diagnosis and treatment of catheter occlusion. Reproduced with permission from the American Gastroenterological Association.[188] Ampho B = amphotericin B; C&S = culture and sensitivity; CBC = complete blood count; IV = intravenous; SQ = subcutaneous; TPN = total parenteral nutrition.

taken out. The catheter should be removed in the face of clinical deterioration, continued bacteremia, and suspected cardiac sepsis or after failing two courses of intravenous antibiotics. Figure 75.4A-3 is an algorithm for the diagnosis and treatment of catheter-related infection in adults with short small bowel syndrome.

The most common metabolic complications associated with PN are over- or underhydration. Too much fluid is sometimes administered when all fluid sources (other intravenous lines, enteral feedings, oral feedings, water flushes) are not considered. Underhydration can develop when inadequate fluids from all routes are provided, during extended periods of hyperglycemia, and when conditions arise that increase fluid requirements (ie, fever, increased renal solute load, ostomy losses, diarrhea). Other metabolic complications include hypoglycemia, electrolyte imbalances, inadequate growth, substrate intolerance, hypersensitivity reactions, and specific nutrient deficiencies. Hypoglycemia can occur if PN is abruptly stopped. Cholestasis associated with PN support is a major problem. The etiology is unknown. Medications such as phenobarbital and ursodiol have been used with limited success. Factors associated with cholestasis include lack of enteral feeding; excessive carbohydrate, fat, and/or calories; inappropriate quality and quantity of amino acids; and deficient trace elements.[163] The only effective treatment is discontinuation of PN.

INITIATION

There is no standard pediatric patient. An individualized plan for initiation, advancement, and maintenance goals should be formulated before starting PN. The plan is based on estimates of requirements and should account for increased or decreased needs of each patient. Before starting PN, baseline laboratory values should be measured and electrolyte and mineral abnormalities corrected. The steps in devising a PN plan are as follows: (1) Determine fluid volume and rate. This determination is based on the fluid requirement and ability of

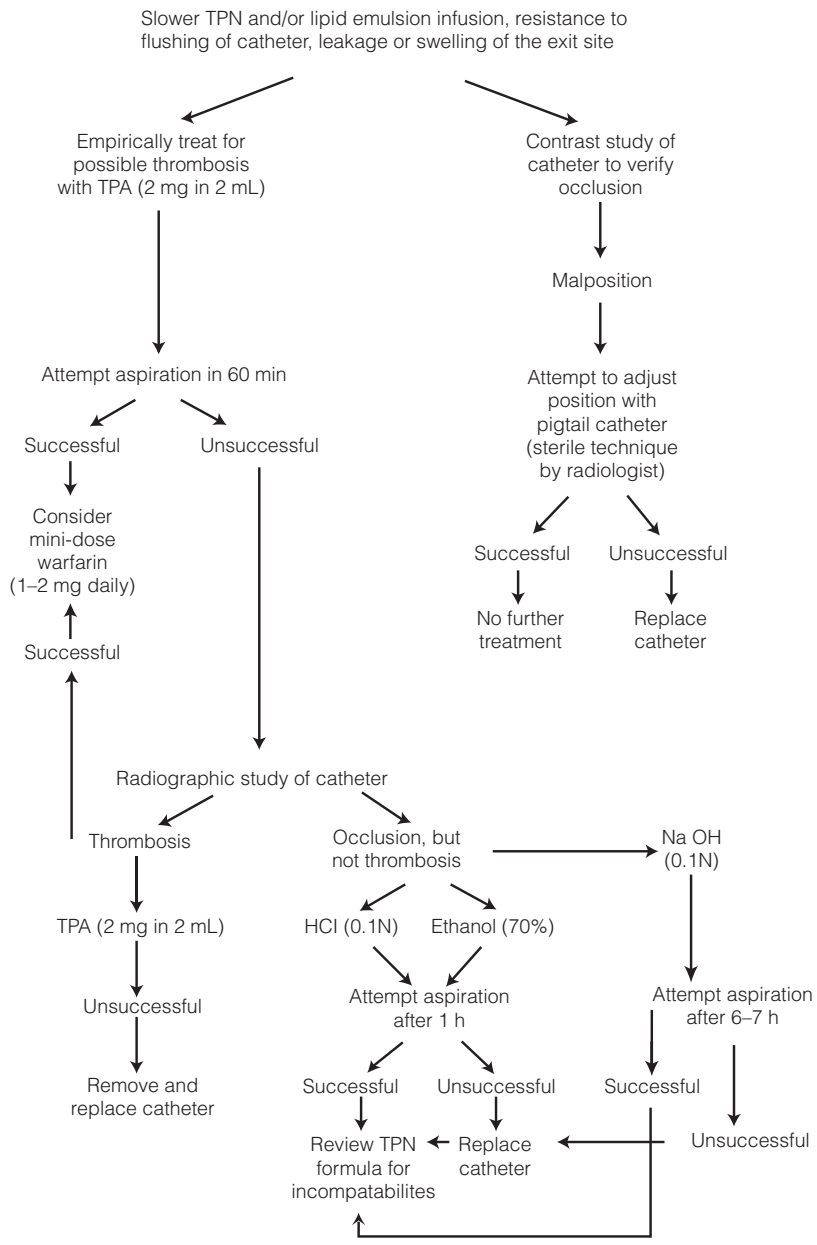

FIGURE 75.4A-3 Algorithm for the diagnosis and treatment of catheter-related infection. Reproduced with permission from the American Gastroenterological Association.[188] HCl = hydrochloric acid; Na OH = sodium hydroxide; TPA = tissue plasminogen activator; TPN = total parenteral nutrition.

the patient to tolerate a fluid load. (2) Calculate the amount of dextrose to be used initially, during advancement, and for maintenance. (3) Determine the amount of protein to be given at initiation, during advancement, and at maintenance. (4) Determine the dose of lipid, again taking into account the three phases, initiation, advancement, and maintenance. (5) Determine the amounts of micronutrients, electrolytes, minerals, trace elements, and vitamins. Vitamins and mineral and trace elements come as prepackaged units. Special consideration of acid-base balance is necessary. Increasing the amount of acetate while decreasing the chloride can shift the balance toward base.[164] Suggested ranges for macronutrient provision in pediatric PN solutions are given in Table 75.4A-13. The distribution of calories derived from macronutrients is quite flexible and depends on the clinical setting: from 7 to 20% of calories in the PN solution can be derived from protein, carbohydrate can account for 20 to 60% of calories, and lipid can range from 20 to 50%.

MONITORING

During initiation, while advancing, and at maintenance rates, patients need to be monitored for complications and to ensure that the goals of the PN are being achieved. A suggested list of laboratory determinations and a schedule for monitoring are provided in Table 75.4A-14. The PN plan as outlined in the previous section should be continually assessed and revised based on the results of monitoring and the changing clinical status of the patient.

DISCONTINUATION AND CYCLING

Sudden discontinuation of high glucose infusion has resulted in hypoglycemia, especially in children under 3 years of age.[165,166] Blood glucose levels should be monitored following cessation of high glucose infusions. PN should be tapered by decreasing to half the rate for 1 hour and to one-fourth the

TABLE 75.4A-13 SUGGESTED PEDIATRIC PARENTERAL SUBSTRATE PROVISION

	NUTRIENT	AMOUNT
INITIATION	Carbohydrate	10% dextrose (6–8 mg/kg/min)
Amino acids	50–100% of goal	
Lipid		0.5–1.0 g/kg/d
ADVANCEMENT	Carbohydrate	5% dextrose per day (2–4 mg/kg/min)
Amino acid	100% of goal	
Lipid		0.5–1.0 g/kg/d
USUAL UPPER LIMIT	Carbohydrate	8–18 mg/kg/min
Peripheral	12.5% dextrose	
Central	25–35% dextrose	
Amino acid		3.0 g/kg/d

original rate for another hour before complete discontinuation. A similar strategy for tapering the PN and monitoring blood sugars should be followed when cycling PN.

There are physiologic and psychological advantages to cycling PN administration, that is, infusing the PN solution in less than 24 hours. For the older child, time without the infusion apparatus allows freedom for greater activity, more social interaction, and school attendance. Continuous PN infusion leads to continuously high serum insulin levels. This hyperinsulinemia causes hepatic lipid deposition and increased hepatic lipogenesis. EFAD may occur because insulin inhibits the release of free fatty acids from adipose tissue. These physiologic effects of continuous PN are reversed by cycling.[167] However, as discussed, cycling increases urinary calcium losses.

CRITICAL CARE

Changes in energy requirements in critically ill or injured children can be quite large, proportionately larger than the changes seen in adults. Especially during early childhood, the energy requirements for growth account for a significant proportion of total requirements. During growth, nutrients need to be supplied in amounts that accommodate tissue accretion, but during the catabolic response to illness or injury, somatic growth is not possible. At times when children are catabolic, overfeeding results from including a share for growth when calculating requirements. The dangers of overfeeding during critical illness are well recognized.[168] It remains a challenge to supply the correct nutrients for each phase of the acute response, allowing for changes in metabolic rate and for growth when it occurs while avoiding overfeeding.

The acute-phase response is proportional to the magnitude and duration of the injury. The response can be divided into three phases: the immediate response lasts minutes to hours and is characterized by autonomic stimulation, resulting in tachycardia, fever, and hypoglycemia. The second, or "ebb phase," is heralded by a sharp decrease in metabolic rate. Insulin levels and body temperature fall, whereas catecholamines, glucose, lactate, and free fatty acids increase. Cardiac output decreases. The second phase lasts from one to several days. The third phase is termed the "flow phase" and is characterized by hypermetabolism. The flow phase can be further subdivided into an early flow phase characterized by hypermetabolism and a negative nitrogen balance. During the late flow phase, hypermetabolism persists, but anabolic repair of tissues and resumption of growth are signaled by a positive nitrogen balance.[169]

Nutritional assessment of a critically ill child includes measuring the constitutive protein pool, the acute-phase protein pool, nitrogen balance, and energy expenditure. All of these measurements can be easily accomplished in the pediatric intensive care unit setting. Prealbumin is a measure of the short-term status of constitutive proteins. C-reactive protein measures acute-phase reactants. These two tests, prealbumin and C-reactive protein, are inversely related and, when measured daily during an acute illness or injury, track the progression of the response. Levels of serum C-reactive protein less than 2 mg/dL are associated with a resumption of anabolic metabolism.[170] Prealbumin begins to rise once an anabolic state is achieved. There are a number of equations for calculating energy expenditure.[171] However, all of these equations are estimates and, especially in infants, can be inaccurate.[172] Indirect calorimetry, using a metabolic cart, measures oxygen consumption and carbon dioxide production. Resting energy expenditure (REE) can be calculated using the Weir equation:

Complete formula:
$$REE = [3.9(VO_2) + (VCO_2)]1.44 - 2.17(UN)$$

Abbreviated formula:
$$REE = [3.9(VO_2) + (VCO_2)]1.44$$

where REE is resting energy expenditure (kcal/d), VO_2 is oxygen consumption (mL/min), VCO_2 is carbon dioxide production (mL/min), and UN is urinary nitrogen (g/d). The RQ can be calculated from the measurements obtained by indirect calorimetry:

$$RQ = VCO_2 / VO_2$$

The RQ is dependent on the substrate being used. Thus, when only fat is being oxidized, the RQ = 0.71. When only carbohydrates are oxidized, the RQ = 1.00. A mixed substrate yields an RQ of about 0.85. Some caution with regard to interpreting RQ; it does not reflect substrate oxidation during hyperventilation, metabolic alkalosis, or for about 6 to 8 hours after general anesthesia.[173]

A number of studies in adults suggest that in nutritional support of a critically ill patient, the enteral route is

TABLE 75.4A-14 SUGGESTED MONITORING PARAMETERS AND SCHEDULE DURING PARENTERAL NUTRITION IN PEDIATRICS

	SUGGESTED FREQUENCY	
PARAMETER	**INITIAL/HOSPITALIZED**	**FOLLOW-UP/HOME**
GROWTH		
Weight	Daily	Daily to monthly
Height/length	Weekly	Weekly to monthly
Head circumference	Weekly	Weekly to monthly
Body composition	Monthly	Monthly to annually
METABOLIC/SERUM*		
Electrolytes	Daily to weekly	Weekly to monthly
BUN/creatinine	Weekly	Monthly
Ca, PO_4, Mg	Twice weekly	Weekly to monthly
Acid-base status	Until stable	Weekly to monthly
Albumin/prealbumin	Weekly	Weekly to monthly
Glucose	Daily to weekly	Weekly to monthly
Triglycerides	Daily while increasing lipids	Weekly to monthly
Liver function tests	Weekly	Weekly to monthly
Complete blood count/differential	Weekly	Weekly to monthly
Platelets, PT/PTT	Weekly	As indicated
Iron indices	As indicated	Biannually to annually
Trace elements	Monthly	Biannually to annually
Fat-soluble vitamins	As indicated	Biannually to annually
Carnitine	As indicated	As indicated
Folate/vitamin B_{12}	As indicated	As indicated
Ammonia	As indicated	As indicated
Cultures	As indicated	As indicated
METABOLIC (URINE)		
Glucose	2 to 6 times/d	Daily to weekly
Ketones	2 to 6 times/d	Daily to weekly
Specific gravity	As indicated	As indicated
Urea nitrogen	As indicated	As indicated
CLINICAL OBSERVATION		
Vital signs†	Daily	Daily
Developmental milestones	As indicated	Annually
Intake and output	Daily	Daily to as indicated
Adminsitration system	6 to 12 times/d	6 to 12 times/d
Catheter site/dressing	6 to 12 times/d	6 to 12 times/d

Adapted from Davis AM.[191]
BUN = blood urea nitrogen; PT = prothrombin time; PTT = partial thromboplastin time.
Frequency depends on clinical conditions.
*For metabolically unstable patients, may need to check more frequently.
†Vital signs include respiratory rate, heart rate, temperature, and blood pressure.

preferred over PN.[174–176] In severe injury, enteral nutrition results in a greater and earlier visceral protein response.[177]

The aims of PN support in the critically ill child are to minimize the effects of catabolism and hypermetabolism that are part of the acute-phase response, to return the patient to positive nitrogen balance quickly, and, finally, to support growth when it resumes. In striving to achieve these aims, caution must be exercised to avoid both under- and overfeeding.

CONCLUSION

PN has saved lives and continues to do so. However, its use is fraught with complications: it is difficult to administer and requires knowledgeable personnel with technical expertise to design complete nutritional fluids, administer them, and monitor for complications. There are limited indications for PN. Its use has declined as medical personnel have become more proficient in the use of enteral feedings, and evidence-based medicine that focuses on outcomes has shown that benefit is limited to defined groups of patients.

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4B. *Enteral* Nutrition

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Pediatric patients unable to tolerate adequate oral feedings may be nutritionally managed with enteral nutrition (EN). Commonly used EN routes include nasogastric (NG) tube, gastrostomy, nasojejunal (NJ) tube, and jejunostomy. The nutritional goal for pediatric patients with chronic illness should be the provision of nutrients appropriate to the patients' metabolic and physiologic limitations and capable of promoting continued growth and development. Although both enteral and parenteral nutrition can provide nutritional support to pediatric patients unable or unwilling to take in adequate oral feedings, EN support is generally considered the preferred modality for critically and chronically ill pediatric patients because they are more physiologic and economic and are easier and safer to administer than parenteral nutrition. Absence of a central venous catheter decreases infection and thrombotic complications. Delivery of nutrients to the intestine minimizes gut atrophy and decreases the risk for bacterial translocation. EN reduces the risk for infectious complications compared with parenteral nutrition.[1] Furthermore, EN allows for better physiologic control of electrolyte levels through endocrine modulation of intestinal absorption and serves as effective prophylaxis against stress-induced gastropathy and gastrointestinal (GI) hemorrhage. EN also offers more complete nutrient provision, including glutamine, nucleotides, trace elements, short-chain fatty acids, and fiber. EN avoids the hepatic complications associated with parenteral nutrition. In addition, EN provides trophic stimulation to the gut by promoting pancreatic and biliary secretions as well as endocrine, paracrine, and neural factors, which promote the physiologic and immunologic integrity of the GI tract.[2–4] Timely initiation of EN is also important, with the greatest metabolic benefits resulting from initiating early EN within less than 72 hours of injury or admission.[4]

Initial attempts at EN should be via the oral route. If the oral route is not emphasized during infancy, the suck reflex may be lost. Because it may take 8 to 9 months for the child to develop sufficiently for use of a cup, oral aversion and language delay may occur during this time interval. Some common indications for initiating EN include oral-motor or esophageal dysfunction owing to prematurity, morphologic abnormalities of the head or neck, or neurologic disease. Excessive respiratory demands from cardiorespiratory disease may compromise coordination of respiration and swallowing.[5] Although nasoenteric feedings are effective in the short-term support of these patients, long-term nutritional support often requires the placement of a feeding gastrostomy. This may be particularly important in the infant who develops oral aversion owing to the prolonged noxious stimulus of a NG tube and to minimize the risks associated with prolonged NG feedings, including sinusitis and otitis media.

This discussion reviews the pathophysiologic mechanisms and nutritional aspects of various pediatric disorders that have been successfully managed with EN. Formula selection and modification as well as enteral feeding techniques and equipment are presented. The administration and monitoring of pediatric EN techniques and the management of common complications are also discussed.

INDICATIONS FOR ENTERAL FEEDINGS: MANAGEMENT OF NUTRITION-RELATED DISORDERS

Table 75.4B-1 lists common conditions under which EN may be warranted in pediatric patients.

Preterm Infants

Feeding methods for preterm infants should be individualized to gestational age, birth weight, and medical status.[6] Preterm infants present a unique nutritional challenge owing to their GI and immunologic immaturity; increased requirements for specific nutrients such as protein, fat, sodium, calcium, and phosphorus; limited renal function; and predisposition to specific metabolic and clinical com-

TABLE 75.4B-1 CONDITIONS UNDER WHICH ENTERAL NUTRITION MAY BE WARRANTED

Preterm infants
Cardiorespiratory disease
Bronchopulmonary dysplasia
Congenital heart disease
Cystic fibrosis
Gastrointestinal disease and dysfunction
Biliary atresia
Inflammatory bowel disease
Gastroesophageal reflux disease
Pancreatitis
Protacted diarrhea of infancy
Short-gut syndrome
Critical illness and postoperative malnutrition
Burn injury
Cancer
HIV/AIDS malnutrition
Trauma/head injury
Renal disease
Neurologic disease and/or impairment

plications such as hypoglycemia, periodic dysmotility, and necrotizing enterocolitis.[6–8] As the coordination of sucking and swallowing appears at approximately 34 weeks gestation, intragastric or jejunal feedings are often used before this time, or beyond, in infants unable to tolerate adequate oral feedings. A number of studies in preterm infants suggest that minimal EN or trophic feedings, defined as the provision of low-volume feedings (10–24 cc/kg/d over 5–14 days) and administered within the first week of life, enhance intestinal adaptation to extrauterine conditions.[8–10] These feedings promote maturation of the mucosal lining, decrease cholestatic jaundice, aid maturation of intestinal motility, and allow earlier progression to full enteral feedings.[9–11] Intermittent bolus feedings may better promote gastrocolic reflexes, gallbladder emptying, and GI hormone release in preterm infants, thus promoting the trophic response,[12,13] and increase accommodation of the stomach to volume loads. Continuous feedings are reserved for neonates with short-gut syndrome, severe gastroesophageal reflux (GER) disease, or persistent feeding intolerance. Transpyloric feedings may promote less GER and overcome gastric dysmotility; however, they are technically more difficult and bypass the gastric phase of fat absorption promoted by lingual lipase and may therefore decrease total fat absorption.[6] This may be a particular concern when bile salt deficiency from prematurity or short-bowel syndrome exists. Early initiation of fat digestion in the stomach may partially offset the effect of bile salt deficiency by earlier formation of mixed micelles.

Cardiorespiratory Illness

Infants and children with cardiorespiratory disorders often require EN support during acute exacerbations of their primary disease, for nutritional rehabilitation of chronic secondary malnutrition, or for promotion of growth prior to surgical procedures. The etiology of growth failure and delayed neurointellectual development in patients with bronchopulmonary dysplasia (BPD) is related to the combined effects of prolonged hypoxia, elevated metabolic rates, inefficient suck and swallow mechanisms, poor appetite, prolonged periods of caloric and nutrient deficits, recurrent emesis, and GI dysmotility.[12–14] Potassium, sodium, and chloride supplementation of the formula is often required in conjunction with diuretic therapy. Increasing formula caloric and nutrient density through either formula concentration or the addition of carbohydrate and fat may be used to achieve caloric densities of 30 to 33 kcal per ounce (1.0 to 1.3 kcal per mL). Excess reliance on carbohydrate in infants who are fluid restricted, however, may result in CO_2 retention and suboptimal protein, vitamin, and mineral delivery if the base formula is excessively diluted.[15] Children with cystic fibrosis (CF) present additional challenges owing to maldigestion.[16] Although behavior modification techniques to improve intake and provision of appetizing high-calorie supplements should be instituted as routine components of medical nutrition therapy for children with CF, EN using semielemental or intact nutrient formulas supplemented with pancreatic enzymes is often necessary for patients who have failed owing to noncompliance.[16,17] Pancreatic enzyme replacement is usually dosed based on lipase units per gram of long-chain triglycerides (mean of 1,800 lipase units). NG feedings have resulted in increased caloric intake and significant weight gain for patients with CF, but long-term effectiveness is hampered by noncompliance.[15] Nocturnal gastrostomy feedings provided over 8 to 10 hours for long-term management may be superior for selected patients.[16]

Infants with congenital heart disease are also at significant nutritional risk generally owing to inadequate calorie intake.[5] Growth failure results from poor nutritional intake, increased respiratory rates, and elevated energy expenditure. It may be exacerbated by tissue hypoxia, suboptimal nutrient absorption, delayed gastric emptying, or more generalized GI dysmotility.[18,19] Owing to their elevated nutritional needs and limited fluid tolerance, these infants often require high–caloric density formulas achieved through formula concentration to a maximum of 24 to 27 kcal per ounce, depending on GI tolerance. Concentration beyond 27 kcal per ounce may not allow enough free water for excretion of the renal osmotic load. Additional calories can be provided through the addition of carbohydrate or fat. Infants with congenital heart disease often benefit from the use of nocturnal continuous feeds while combined with oral intake ad libitum during the day. Alternatively, provision of oral feedings intermittently with NG supplementation of the remainder of the required intake volume may also facilitate provision of the nutritional goals.[5,20,21] Ultimately, effective EN can promote significant catch-up growth and facilitate surgical correction of cardiac defects.

Gastrointestinal Disease

Pediatric patients with acute and chronic GI disease and dysfunction, such as inflammatory bowel disease, short-bowel syndrome, and pancreatitis, often benefit from EN regimens. The etiology of growth failure in children with Crohn disease is multifactorial but often related to inadequate nutrient intake. Semielemental and elemental diets administered orally and nasogastrically have been clinically demonstrated to induce remission in selected patients and produce a significant improvement in nutritional status.[22,23] Clinical remission is more likely in Crohn disease of the small bowel than of the colon.[23] Elemental, semielemental, and polymeric diets have been shown to produce similar clinical results.[24,25] Supplementation of an oral polymeric formula with transforming growth factor-β_2, a polypeptide normally found in human and cow's milk, resulted in clinical remission of 79% in 29 children with intestinal Crohn disease, as well as mucosal healing and a reduction in proinflammatory mediators.[26] For simple weight gain, however, emphasis on high-calorie appetizing food is the gold standard before implementation of less palatable enteral supplements.

The nutritional management of the infant or child with short-bowel syndrome involves the initial use of total parenteral nutrition with a gradually increasing amount of EN. The period of transition to complete EN may take

weeks to years depending on the location and length of intestinal resection, associated dysmotility, presence of the ileocecal valve, and extent of colon preservation. Parenteral nutrition, however, must be continued until it is clinically evident that positive fluid and nutrient balance and weight gain can be maintained on EN alone.[27] Water and sodium may be considered the first limiting nutrients in short-bowel syndrome. Often stool sodium losses or negative water balance or both obscure weight gain. Important considerations for provision of adequate EN include the method of administration, volume, osmolality, and nutrient quality (polymeric versus elemental). Polymeric formulas with intact protein, long-chain fats, and complex carbohydrates may be less tolerated in the initial stages of the enteral feeding progression than glucose and glucose polymers, medium-chain triglycerides (MCTs), and hydrolyzed protein and dipeptides, which require less digestion, are less allergenic, and are more easily tolerated.[27,28] Long-term parenteral nutrition in these infants is associated with cholestatic liver disease, a significant cause of death in children with short-gut syndrome. To prevent liver disease, cyclic parenteral nutrition with provision of a 4- to 6-hour "window" in infants has been shown to reduce the incidence of cholestasis.[29] In older children and adolescents, provision of cyclic parenteral nutrition over 10 to 12 hours with continuous or intermittent enteral feedings, as tolerated, while promoting oral intake during the day can usually be managed in the home setting.[30] Furthermore, treatment of small bowel bacterial overgrowth, provision of supplemental ursodeoxycholic acid, and prevention of sepsis are also adjunctive therapies to decrease the risk of cholestatic liver disease. Prevention of oral aversion involves careful attention to persistent use of oral feedings and avoidance of noxious stimuli to the oral area. Proactive involvement of speech pathology experts during enteral feeding of infants is extremely helpful.

Several other GI diseases impacting nutritional intake and status can be successfully managed with EN. Infants with biliary atresia frequently experience reduced intake associated with liver disease and infection.[31] Following surgical procedures or liver transplant, nutritional support of these infants with continuous NG feedings using a semielemental formula rich in MCTs can promote energy and nitrogen balance.[31,32] Once the postoperative infant or child is clinically stable, transition to an intact nutrient formula or to an oral diet should be made.[33] Careful monitoring of fat-soluble vitamin levels and supplementation with water-soluble varieties using multiple doses during the day may help in the prevention of fat-soluble vitamin deficiency. Infants with GER disease who have failed conventional therapy with acid inhibition, thickened feeds, and upright positioning and have subsequently experienced growth failure have been shown to benefit from continuous NG feedings with improved intake, reduction or cessation of vomiting, and catch-up growth.[34] Children with acute and chronic pancreatitis may be nutritionally managed with NJ feedings of a standard pediatric or, if needed, semielemental formula administered beyond the ligament of Treitz. Clinical studies in adult patients with pancreatitis have demonstrated improved clinical outcomes with fewer infectious complications, decreased incidence of hyperglycemia, and decreased incidence of multiorgan failure and mortality in enterally fed patients compared with those on parenteral nutrition.[35,36]

Critical Illness and Postoperative Malnutrition

EN for the critically ill or postoperative pediatric patient has improved in recent years owing to improvements in EN products, equipment, and techniques.[3] Early EN within 48 hours of injury or surgery reduces sepsis and enhances GI immune function through maintenance of the gut mucosal barrier and enteric lymphoid tissue. Early postoperative EN helps prevent mucosal damage and bacterial translocation, results in decreased sepsis, and blunts the hypermetabolic response in some critically ill patients.[2,4] Clinical studies have demonstrated that GI function can be adequately maintained with improved nitrogen balance and nutritional status in the postsurgical trauma patient.[37,38]

Critically ill patients in hypermetabolic states, such as those with cancer or human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS), may also benefit from EN support. Cancer patients at high nutritional risk who have minimal GI symptoms and adequate platelet counts may be enterally fed via nocturnal or 24-hour feedings depending on the extent of oral intake.[39] Various studies have demonstrated the effectiveness and safety of gastrostomy tube feedings in malnourished children with cancer.[40,41] Pediatric HIV patients with protein-calorie malnutrition who are unable to meet their elevated energy requirements with oral intake alone may also benefit from enteral NG feedings with pediatric or other high–caloric density formulas. Nocturnal enteral feeding is the preferred method of EN in this patient population because it allows the child to eat during the day.[42] Gastrostomy tube feedings for the provision of supplemental nutrition have resulted in improvement in weight gain and reduced morbidity and mortality in pediatric HIV patients.[43]

Renal Disease

Chronic renal failure in infants and children commonly results in growth failure and developmental delay, particularly in those patients with congenital renal disease early in life.[44] The etiology of growth failure in these children is thought to be related to protein-calorie deficiency, renal osteodystrophy, chronic metabolic acidosis, and endocrine dysfunction.[45] Despite aggressive medical management and use of specialized formulas of high caloric density, poor growth and development may persist. Early nutritional intervention and dialysis can result in improved growth and development.[44,45] Nocturnal NG feedings over a period of 8 to 12 hours in patients with renal insufficiency have resulted in catch-up growth.[45]

Neurologic Disease and/or Impairment

The specific nutritional requirements and feeding approach for neurologically impaired children are highly variable and depend on the degree of impairment, oral-

motor function, mobility, and muscular tone. Children with Down syndrome, Prader-Willi syndrome, or myelomeningocele have decreased energy needs, growth rates, and motor activity compared with healthy children.[46] Children with cerebral palsy, however, are generally underweight for height and may have increased energy needs, particularly if they are severely spastic or have choreoathetoid movements. Those patients who are severely affected often require high–caloric density enteral feedings and are often managed via continuous nocturnal gastrostomy feedings and intermittent bolus feedings during the day when oral intake is inadequate.[46] Important considerations for provision of EN to these patients include method of feeding, risk of aspiration, formula caloric density, osmolality and fiber content, fluid intake, and effect of enteral feeding therapy on current and future oral-motor function and intake.[47] Often daytime bolus feedings are preferred by caregivers because they are more easily given and avoid the risk of nighttime aspiration from continuous feedings. The goal of nutritional support for these children should not necessarily be persistent weight gain because increased weight may further compromise muscular ability and complicate the caregiver's ability to move the child. These children are also often at risk for constipation, which can compromise formula tolerance.

NUTRITIONAL NEEDS OF THE ENTERALLY FED CHILD

Preterm Infant

Caloric requirements to support a daily weight gain of 15 g/kg are estimated at 105 to 130 kcal/kg in the appropriate for gestational age preterm infant. Higher energy needs are required depending on the infant's thermal environment, cardiorespiratory status, presence of intrauterine growth retardation, and metabolic stress.[48,49] Infants who are small for gestational age or those with BPD may have caloric requirements of between 130 and 150 kcal/kg.[15] Intake of formulas with whey-to-casein ratios similar to those of breast milk results in metabolic indices and plasma amino acid profiles closer to those of breastfed infants.[50] Because of their GI immaturity, preterm infants demonstrate improved nutrient absorption when fed a mixture of MCT and long-chain unsaturated fatty acids and a mixture of lactose and glucose polymers as their fat and carbohydrate sources, respectively. Owing to the high accretion rates for calcium, phosphorus, and trace elements during the final trimester of gestation, preterm infants have elevated requirements for these nutrients.[8,49]

Infants and Children

The nutritional requirements of infants and children are outlined elsewhere in the literarure.[48,49,51] It must be emphasized that these recommended allowances are intended for healthy active children and represent the average intake of nutrients that would maintain good health for an extended period.[51] As previously discussed, tube-fed children often have illnesses that result in malnutrition and inactivity and thus require adjusted allowances for energy and other nutrients. The specialized nutritional requirements of nutrition-related illnesses have been reviewed extensively in the literature.[5,12–16,28,32,39,42,44,46,49]

EN support of critically ill pediatric patients requires careful attention to prevent overfeeding of calories with exacerbation of the underlying clinical status. Energy and protein requirements in the critically ill child are significantly different from those of healthy children. Although basal metabolic needs may be elevated owing to the stress response and its associated elevation of counterregulatory hormones and cytokines, inhibition of growth and reduced physical activity may result in an overall decrease in energy requirements.[52] Studies in critically ill pediatric patients have underscored the importance of not using the published Dietary Reference Intakes for estimating energy requirements but rather using the child's basic energy expenditure multiplied by a factor correlated to the underlying disease or injury process.[53] Overfeeding cannot reverse the catabolic process until the acute metabolic stress response resolves; furthermore, overfeeding of calories may result in iatrogenic hepatic and respiratory disease and decreased survival.[54]

In the child with failure to thrive, particular attention should be given to the estimation of the energy and protein for achievement of catch-up growth, which occurs when the cause of growth impairment is removed and requires the provision of calories and protein in excess of normal needs.[55] It is best to allow the child's appetite to be the determinant of intake whenever possible because overfeeding during the initial stages of rehabilitation may be associated with edema and refeeding syndrome in the severely malnourished child.[56] Estimated catch-up growth requirements can be calculated from the following equation[55]:

$$\frac{\text{kcal}}{\text{kg}} = \frac{\text{RDA kcal/kg for weight age} \times \text{ideal body weight (kg)}}{\text{actual weight (kg)}}$$

Weight age is the age at which the present weight is at the 50th percentile, ideal weight is at the 50th percentile for age or ideal weight for height, and RDA means Recommended Dietary Allowance.

Fluid requirements can be calculated by estimating normal water requirements adjusted for specific disease-related factors; special consideration must be given to monitoring the fluid balance of children receiving high-calorie, high-protein formulas; those who have short-bowel syndrome or severe neurologic impairment; or those with emesis, diarrhea, fever, or polyuria.[2,57] The provision of extra water to prevent slow dehydration or "tube feeding syndrome" is especially important for neurologically devastated or immature children who cannot communicate their thirst to the care provider.

INFANT AND PEDIATRIC FORMULA SELECTION FOR ENTERAL FEEDINGS

Selection of an optimal infant enteral formula depends on a number of factors, including diagnosis, associated nutritional problems and requirements, and GI function.

Important formula factors include osmolality, renal solute load, caloric density, viscosity, and composition. Figure 75.4B-1 presents an algorithm that identifies appropriate infant and pediatric formulas based on indication for use. Table 75.4B-2 lists and describes the nutrient sources of a variety of infant and pediatric formulas.

Preterm Infant Formulas

Specialized formulas have been developed that are uniquely suited to the physiologic needs of the preterm infant. Physiologic factors in the preterm infant that call for alterations in their nutritional management include limited oral-motor function, lactase deficiency, limited bile salt pool, decreased energy and nutrient stores, limited gastric volume, decreased intestinal motility, and limited renal function.[7,8]

There are several differences in nutrient content between preterm and term infant formulas. Preterm formulas provide a combination of both lactose and glucose polymers versus lactose alone in standard formulas, thus decreasing osmolality and improving digestibility and calcium absorption.[50,58] Preterm formulas use a fat blend containing both long-chain triglycerides, very-long-chain triglycerides such as docosahexaenoic acid (DHA) and arachidonic acid (ARA), and MCTs, which promote improved weight gain and fat, nitrogen, and calcium absorption.[59,60] Documented improvement in weight gain, linear growth, and central nervous system development in preterm infants fed DHA- and ARA-supplemented formula[61,62] has been observed and subsequently resulted in the addition of very-long-chain triglycerides to preterm and term infant formulas. An elevated protein content and 60:40 whey-to-casein formulation promote plasma amino acid profiles closer to those of the breastfed infant.[50,63] Increased amounts of sodium, calcium, and phosphorus compensate for the increased urinary sodium losses seen in the preterm infant and promote bone mineralization closer to intrauterine rates.[50] The concentration of various vitamins, including vitamin E, in preterm formula is greater than that found in term infant formula owing to preterm infants' limited stores and wide variability in absorption. Vitamin D content is also high for promotion of bone mineralization. Owing to the lower birth weight and initial hemoglobin concentration of preterm infants, iron has been added to preterm infant formulas to provide approximately 2 mg/kg of iron per day when fed at a level of 120 kcal/kg.[50]

Discharge preterm formulas for infants weighing less than 2,000 g provide additional calories and nutrients to promote optimal growth during the first year of life. Several studies have demonstrated benefits with their use, including improved weight gain and linear growth[64]; improved calorie, calcium, and phosphorus intakes[65]; and improved bone mineral content.[66] The use of discharge preterm formulas such as EnfaCare (Mead Johnson, Evansville, IN) and Similac NeoSure (Ross Products Division, Abbott Laboratories, Columbus, OH) has become standard practice in most neonatal intensive care units. These formulas are iron fortified and commercially available in the ready to feed 22 kcal/oz form for hospital use and in the powdered form for home use. Infants whose volume intakes continue to be limited owing to cardiorespiratory illness may be fed formulas concentrated to 24 to 30 kcal/oz using alternative mixing methods provided by the manufacturers. Both discharge preterm formulas contain a blend of lactose and glucose polymers and provide 20 to 25% of the fat as MCT oil. Vitamin and mineral levels are greater than those found in standard formulas. Preterm infants consuming a minimum of 165 cc/kg do not require additional multivitamin supplementation.[8]

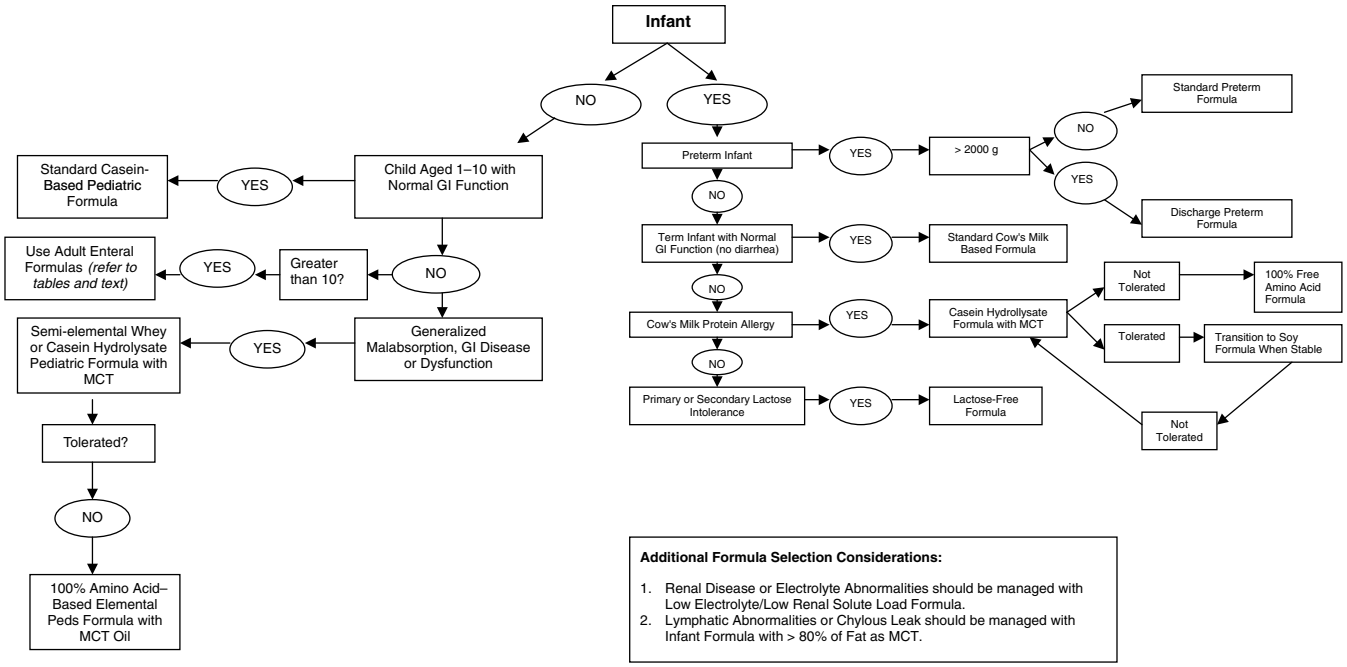

FIGURE 75.4B-1 Appropriate infant and pediatric formulas based on indication for use. GI = gastrointestinal; MCT = medium-chain triglycerides.

TABLE 75.4B-2 INFANT AND PEDIATRIC FORMULAS

PRODUCT NAME	KCAL/ OZ	CARBOHYDRATE (G/100 ML)	FAT (G/100 ML)	PROTEIN (G/100 ML)	MOSM/ KG	NUTRIENT SOURCES (CARBOHYDRATE; FAT; PROTEIN)
Preterm infant formulas						
Enfamil Premature Lipil	24	9.0	4.1	2.4	310	Corn syrup, lactose; MCT, soy, and sunflower oil; whey, casein
Similac Special Care 24	24	8.6	4.4	2.2	280	Corn syrup, lactose; MCT, soy and coconut oil; whey, casein
EnfaCare 22 Lipil	22	7.9	3.9	2.1	230	Maltodextrin, lactose; MCT, sunflower oil, soy, and coconut oil; whey, casein
Similac NeoSure 22	22	7.7	4.1	1.9	250	Maltodextrin, lactose; soy and coconut oil, MCT; whey, casein
Term infant formulas*						
Enfamil Lipil 20	20	7.3	3.5	1.4	300	Lactose; palm olein, soy, coconut, and sunflower oil; whey and casein
Similac Advance 20	20	7.2	3.6	1.4	300	Lactose; high-oleic safflower, coconut, and soy oil; whey, casein
Enfamil Lactofree	20	7.3	3.5	1.4	250	Corn syrup solids; palm olein, soy, coconut, and sunflower oil; whey and casein
Carnation Good Start	20	7.5	3.4	1.5	265	Lactose, maltodextrin; high-oleic safflower, palm olein, soy, and coconut oil; whey hydrolysates
Soy infant formulas†						
Prosobee 20	20	7.1	3.4	1.7	200	Corn syrup solids; palm olein, soy, coconut, and sunflower oils; soy protein + L-methionine
Isomil 20	20	6.9	3.4	1.6	200	Corn syrup, sucrose; high-oleic safflower, coconut, and soy oil; soy protein + L-methionine
Carnation Alsoy	20	7.4	3.3	1.9	200	Corn, maltodextrin, sucrose; palm olein, soy, coconut, and sunflower oil; soy protein + L-methionine
Specialized infant formulas†						
Nutramigen	20	7.4	3.4	1.9	320	Corn syrup, tapioca starch; palm olein, soy, coconut, and sunflower oil; casein hydrolysate
Pregestimil	20	6.9	3.8	1.9	320	Corn syrup, tapioca starch; MCT (55%), corn, soy, and sunflower oil; casein hydrolysates and amino acids
Alimentum	20	6.8	3.8	1.9	370	Tapioca starch, sucrose; MCT (50%), safflower and soy oil; casein hydrolysates and amino acids
Portagen	20	7.8	3.2	2.4	230	Corn syrup, lactose, sucrose; MCT (88%), corn oil (12%); sodium caseinate
Neocate	20	7.8	3.0	2.1	375	Corn syrup solids; safflower and soy oil, MCT (5%); free amino acids
Pediatric enteral formulas						
Kindercal with Fiber	31	13.5	4.4	3	325	Maltodextrin, sucrose; MCT, canola and high-oleic sunflower oil; calcium and potassium caseinates
PediaSure with Fiber	30	10.9	5.0	3	345	Corn syrup, sucrose, lactose; high oleic safflower and soy oil, MCT; sodium caseinate and whey
Peptamen Junior	30	13.7	3.9	3	260–360	Maltodextrin, corn starch; MCT, soy and canola, lecithin; hydrolyzed whey
Neocate Junior	30	10.4	5.0	3	607	Corn syrup solids; MCT, canola and safflower oil; free amino acids
EleCare	30	10.7	4.76	3	596	Corn syrup solids; high oleic safflower and soy oil, MCT; free amino acids

MCT = medium-chain triglyceride.

Enfamil products are Mead Johnson, Evansville, IN; Similac products are Ross Products Division, Abbott Laboratories, Columbus, OH; Carnation Infant Formula Products are Nestle Carnation, Wilkes Barre, PA.

*Term infant formulas are available in 24 kcal/oz ready to feed for hospital use.

†Can be prepared to 24 kcal/oz by adding less water to concentrate or powder base.

Breast milk continues to be the preferred EN source for preterm and sick neonates because it provides numerous nutritional, immunologic, and psychosocial advantages over formulas. Breast milk contains numerous trophic peptides, such as epidermal growth factor, which enhance GI growth and development.[67] Preterm breast milk is higher in protein, sodium, chloride, magnesium, and iron than mature human milk and is thus more suitable for the enteral feeding of the preterm infant.[50] Despite these advantages, preterm milk is still relatively deficient in calcium and phosphorus for the needs of the growing preterm infant.[8,50] Breast milk fortification is generally recommended for preterm infants (1) born at fewer than 34 weeks gestation, (2) born at less than 1,500 g, and (3) receiving parenteral nutrition for more than 2 weeks. Commercially available fortifiers include Enfamil Human Milk Fortifier (Mead Johnson), Similac Human Milk Fortifier (Ross Products Division, Abbott Laboratories), and Similac Natural Care (Ross Products Division, Abbott Laboratories), a liquid fortifier mixed 1:1 with pumped breast milk. The addition of one packet of Enfamil Human Milk Fortifier to 25 mL of human milk increases the caloric density by 4 kcal/oz and also increases the levels of protein, calcium, phosphorus, and other minerals. Most notably, the Enfamil fortifier contains added iron equal to that of iron-fortified term formulas and additional fat, 20% of which comes from MCTs. Beneficial outcomes in terms of infant growth, development, and nutritional status have resulted from fortification of breast milk.[68,69]

Term Infant Formulas: Standard, Soy, and Specialized

Term infant formulas meet the nutritional requirements of term infants during the first year of life. The protein, carbohydrate, and lipid macronutrient sources vary depending on whether the formula is standard, soy based, or partially hydrolyzed. The protein source may be casein and whey, soy protein isolate, or hydrolysates of casein or whey.[70,71] Infant formulas contain a blend of vegetable oils as the fat source and either lactose, glucose polymers, sucrose, or a combination of these as the carbohydrate source.[72,73] Vitamins and minerals are added to all infant formulas in accordance with the recommendations set by the American Academy of Pediatrics (AAP) and the Infant Formula Act of 1980.[74,75] Specialized whey hydrolysate formulas have resulted in reduced incidence of intolerance in infants with a family history of allergy.[76–78] Follow-up formulas for infants 4 months or older follow the European Society of Pediatric Gastroenterology and Nutrition guidelines for follow-up formulas[79] and the US Food and Drug Administration (FDA) nutritional requirements guidelines. Specialized milk-based formulas such as lactose-free formulas and formulas with added rice starch such as Enfamil AR (Mead Johnson) have been introduced to manage GI intolerance and reflux. Infant formulas generally have a caloric density of 20 kcal/oz but can be concentrated to 24 kcal/oz or greater using both concentration and the addition of caloric additives to meet the needs of infants with cardiorespiratory illness or failure to thrive. The use of sterile liquid ready to feed or concentrate is recommended by the FDA for the preparation of infant formulas within the institutional setting.[80]

Specialized formulas such as soy formulas, partially hydrolyzed formulas, and elemental formulas are indicated for a variety of uses. Soy formulas are used in the management of primary and secondary lactase deficiency and galactosemia.[81–85] Specialized formulas such as Nutramigen (Mead Johnson), Pregestimil (Mead Johnson), and Alimentum (Ross Products Division, Abbot Laboratories) are indicated for infants with allergies to intact protein of cow's milk or soy and generalized malabsorption, respectively. Portagen (Mead Johnson), which contains 88% of the fat as MCT oil, is used in the management of severe steatorrhea or chylous leak.[82,86] Finally, elemental formulas such as Neocate (SHS North America, Gaithersburg, MD) are used in the management of infants with severe protein allergies who are intolerant of hydrolysate formulas.[87,88]

Formulas for Children 1 to 10 Years of Age

Over the past 15 years, a number of pediatric enteral formulas, including polymeric, semielemental, and elemental formulas, have been developed for the support of children with a variety of disease states.[89] Figure 75.4B-1 presents an algorithm for selection of an appropriate pediatric formula based on GI function. Polymeric pediatric formulas meet the specialized needs of the 1- to 10-year-old child and are available in the tube feeding and flavored varieties and with or without fiber. The caloric and nutrient density and the osmolality and macronutrient sources can be found in Table 75.4B-2. Pediatric patients with GI disease or severe protein allergies may require EN support with a semielemental or an elemental formula. These formulas contain either peptides, free amino acids, or a combination of these, and part of the fat is provided as MCT. These formulas are also lactose and fructose free to optimize tolerance.

ADULT ENTERAL TUBE FEEDING FORMULAS

Children over the age of 10 years may be effectively managed with an adult enteral formula selected to meet the child's individual nutritional needs. Table 75.4B-3 lists the nutrient sources in a variety of selected adult formulas. These adult formulas are generally categorized into standard polymeric, semielemental, elemental, and specialized. Standard polymeric formulas contain mixtures of protein isolates, oligosaccharides, vegetable oil, MCTs, and added vitamins and minerals.[2,90,91] Soluble and insoluble fiber is added in a variety of forms. It serves as a fuel source for colonic mucosal cells and may also decrease the severity of diarrhea.[92] Older children and adolescents with markedly elevated calorie and protein requirements who require fluid restriction may be optimally managed with high-calorie, high-nitrogen formulations.[93] Semielemental and elemental formulas have been used effectively for the continuous enteral feeding support of patients with Crohn disease, CF, and short-gut syndrome[94–96] because nitrogen is more rapidly and effectively absorbed in the compromised bowel in the form of di- and tripeptides.[97,98]

TABLE 75.4B-3 SELECTED ADULT ENTERAL FORMULAS

PRODUCT NAME	KCAL/ CC	CARBOHYDRATE (G/1,000 ML)	FAT (G/1,000 ML)	PROTEIN (G/1,000 ML)	MOSM/ KG	NUTRIENT SOURCES (CARBOHYDRATE; FAT; PROTEIN)
Standard enteral formulas						
Isocal	1.06	135	44	34	270	Maltodextrin; soy, MCT; sodium and calcium caseinates and soy protein isolate
Osmolite	1.06	151	35	37	300	Maltodextrin; high-oleic safflower, canola, MCT; lecithin; sodium and calcium caseinates, soy protein
Nutren 1.5	1.5	169	68	60	510	Maltodextrin; MCT, canola, corn, soy lecithin; calcium and potassium caseinates
Nutren 2.0	2	196	106	80	745	Maltodextrin; MCT, canola, corn, soy lecithin; calcium and potassium caseinates
Osmolite HN	1.06	144	35	44	300	Maltodextrin; high-oleic safflower, canola, MCT; lecithin; sodium and calcium caseinates, soy protein
Osmolite HN Plus	1.2	139	35	49	360	Maltodextrin; high-oleic safflower, canola, MCT, lecithin; sodium and calcium caseinates, soy protein
Fiber-containing formulas						
Ultracal (cellulose and soy fiber)	1.06	142	39	44	360	Maltodextrin; canola, MCT, high-oleic sunflower, corn oil; milk protein concentrate and casein
Jevity (soy fiber)	1.06	155	35	44	300	Maltodextrin, corn syrup; high-oleic safflower, canola, MCT, lecithin; sodium and calcium caseinates
Nutren 1.0 with Fiber (soy polysaccharide)	1	127	38	40	320–380	Maltodextrin, corn syrup; canola, MCT, corn, soy, lecithin; calcium-potassium caseinates
Probalance (soy polysaccharide + gum arabic)	1.2	156	41	54	350–450	Maltodextrin, corn syrup solids; canola, MCT, corn, soy lecithin; calcium-potassium caseinates
IsoSource 1.5 (soy fiber + guar gum)	1.5	170	65	68	650	Hydrolyzed corn starch, sucrose; canola, MCT, soybean oil; sodium and calcium caseinates
Semielemental and elemental formulas						
Peptamen	1	127	39	40	270-380	Maltodextrin, corn starch; MCT, soybean, soy lecithin; enzymatically hydrolyzed whey
Peptamen 1.5	1.5	188	56	68	550	Maltodextrin, corn starch; MCT, soybean, soy lecithin; enzymatically hydrolyzed whey
Subdue	1	127	34	50	440–525	Maltodextrin, modified corn starch; MCT, canola, high-oleic sunflower, corn; hydrolyzed whey protein concentrate, casein hydrolysate
Vital HN	1	185	10.8	42	500	Maltodextrin, sucrose; safflower oil, MCT; partially hydrolyzed whey, meat, soy, free amino acids
Specialized adult enteral formulas						
Impact (immune enhancing)	1	130	28	56	375	Hydrolyzed corn starch; palm kernel, sunflower, menhaden oil; sodium and calcium caseinates, arginine
Crucial (immune enhancing)	1.5	135	68	94	490	Maltodextrin, corn starch; MCT, fish oil, soybean, soy lecithin; hydrolyzed casein, L-arginine
Nutri-Hep (hepatic)	1.5	290	21	40	690	Maltodextrin, modified corn starch; MCT, canola, soy lecithin, corn oil; amino acids, whey (50% branched-chain amino acids)
Suplena (renal—predialysis)	2	256	96	30	600	Maltodextrin, sucrose; high-oleic safflower, soy, lecithin; sodium and calcium caseinates
Nepro (renal—dialysis)	2	222	96	70	665	Corn syrup, sucrose, FOS; high-oleic safflower, canola, lecithin; calcium, magnesium and sodium caseinates
Respalor (pulmonary)	1.5	146	68	75	400	Corn syrup, sucrose; canola, MCT, high-oleic safflower, corn oil; calcium and sodium caseinates
Pulmocare (pulmonary)	1.5	106	93	63	475	Sucrose, maltodextrin; canola, MCT, corn, high-oleic safflower, lecithin (55% of kcal/fat); caseinates
Choice DM (glucose intolerance)	1.06	119	51	45	300	Maltodextrin; canola, high-oleic sunflower, corn oil; milk protein concentrate; fiber: soy fiber and cellulose
Glucerna (glucose intolerance)	1	96	54	42	355	Maltodextrin, fructose; high-oleic safflower, canola oil, lecithin; sodium and calcium caseinates

FOS = fructose oligosaccharide; MCT = medium-chain triglyceride.

Osmolite, Jevity, Vital HN, Glucerna, Pulmocare, Suplena, and Nepro products are Ross Products Division, Abbott Laboratories, Columbus, OH; Isocal, Ultracal, Subdue, Respalor, and Choice DM are Mead Johnson, Evansville, IN; the Nutren products, Probalance, Crucial, Nuri-Hep, and the Peptamen products are Nestle Clinical Nutrition, Deerfield, IL; Isosource products and Impact are Novartis Nutrition, Minneapolis, MN.

Specialized enteral formulas have been designed for the nutritional support of patients with a variety of specific diseases. Glutamine, arginine, ω-3 fatty acids, nucleotides, and other pharmacologic additives are provided in some formulas.[2] These formulas are expensive, and their clinical efficacy over standard polymeric formulas for the management of clinical conditions, including trauma, sepsis, diabetes, and renal failure, remains controversial.[99–108]

Oral Supplements and Modular Components

Milk-based and polymeric formulas may be used as oral supplements and are usually of moderately high lactose and residue owing to their high lactose content. Oral supplements such as Carnation Instant Breakfast (Nestle Clinical Nutrition) mixed with milk are often better accepted by children than are the lactose-free commercial supplements. Flavored polymeric formulas such as Boost (Mead Johnson) and Ensure Plus (Ross Products Division, Abbott Laboratories) are well accepted and palatable. Table 75.4B-4 presents the nutrient content of a variety of oral supplements.

Owing to the unique and often elevated nutritional requirements of the enterally fed pediatric patient, modification of enteral formulas with modular components is often necessary.[109] In these clinical situations, standard and specialized infant and pediatric formulas may be supplemented with caloric modulars, including carbohydrate, fat, and protein modules. Table 75.4B-5 reviews the caloric density and nutrient composition of selected modular products.

Gastrostomy and NG and NJ Feedings

When the requirement for EN support has been established, the optimal route of delivering nutrients must be determined. Many practitioners recommend the placement of NG or NJ tubes when the estimated course of therapy will not exceed 1 to 3 months. If the risk of aspiration is not significant, gastric feedings are preferable owing to the bactericidal effects of acid, the action of lingual lipase, ease of management, and ability to use bolus feedings. If GER is present, aspiration is a significant risk, and the duration of tube feeding may be relatively short. In this situation, NJ feeding is preferable to NG feeding. Tubes made of polyurethane and silicone rubber are soft and pliable and may be left in place for indefinite periods of time. Polyvinyl chloride tubes become stiff and nonpliable when left in place for more than a few days; however, they are useful for intestinal decompression or short-term feeding. They should be changed every 2 to 3 days to avoid skin necrosis or intestinal perforation. Some feeding tubes made of polyurethane or silicone rubber have a weight at the tip that makes them useful for duodenal or jejunal feedings. Tube sizes of 5F to 8F are appropriate for most pediatric patients. The weight on tubes that are 7F or 8F may be too great for easy passage in a young infant.

Children who require long-term tube feeding are candidates for placement of a gastrostomy tube. GER, which may occur in neurologically disabled children or even in normal infants following gastrostomy tube placement, may necessitate an operative antireflux procedure (eg, Nissen fundoplication). Although the procedure is effective in reducing GER, postoperative complications can be troublesome. Intractable retching episodes, dumping syndrome, continued problems with swallowing, impaired esophageal emptying, slow feeding, and gas bloating have all been reported. Controversy currently exists over the necessity of an antireflux procedure in neurologically impaired children who require a feeding gastrostomy. Our current policy when there is a question of GER with continuous or bolus enteral feedings is to administer the feeding on a trial basis through a NG tube before a decision is made on the need for a Nissen fundoplication. We do not feel that preoperative endoscopy or pH probe studies allow one to predict who will have pulmonary complications with GER. Indeed, many times GER improves, especially with continuous feedings.

Percutaneous endoscopic gastrostomy (PEG) tubes can be placed without a laparotomy and, in some older adolescents, without general anesthesia. PEG tubes can also be placed fluoroscopically without endoscopy. The most frequent complication of percutaneous gastrostomies appears to be localized cellulitis. This may be controlled by careful attention to adjusting the tension on the external bolster, which will vary during the postoperative period depending on edema at the insertion sight. There is controversy as to whether preoperative antibiotics prevent postoperative

TABLE 75.4B-4 SELECTED ORAL SUPPLEMENTS

			GRAMS PER SERVING		
PRODUCT NAME	SERVING SIZE, ML (OZ)	KCAL PER SERVING	CARBOHYDRATE	FAT	PROTEIN
Boost Drink	237 (8)	240	40	4	10
Ensure	237 (8)	240	40	6	9
Resource Standard	237 (8)	240	40	6	9
Boost High Protein	237 (8)	240	33	6	15
Boost Plus	237 (8)	360	45	14	14
Ensure Plus	237 (8)	360	50	11.5	13
Carnation Instant Breakfast (mixed with low-fat milk)	270 (9)	250	39	5	12
Carnation Instant Breakfast (no sugar added)	270 (9)	190	24	5	12
Mighty Shake	120 (4)	200	32	5	6
Mighty Shake (sugar free)	120 (4)	200	18	10	8

Boost products are Mead Johnson, Evansville, IN; Ensure products are Ross Products Division, Abbott Laboratories, Columbus, OH; Carnation products are Nestle Clinical Nutrition, Deerfield, IL; Mighty Shakes are Hormel Healthlabs, Austin, MN.

TABLE 75.4B-5 SELECTED CALORIC AND NUTRIENT MODULAR PRODUCTS

PRODUCT NAME	CALORIC DENSITY	NUTRIENT COMPOSITION
Polycose Powder	8 kcal/tsp	Glucose polymers
Polycose Liquid	2 kcal/cc	Glucose polymers
Light Karo Syrup	4 kcal/cc	Corn syrup, sugar, vanilla
Microlipid	4.5 kcal/cc	Safflower oil emulsion
Vegetable oil	8.6 kcal/cc	Oleic, linoleic, linolenic acids
Resource BeneProtein	25 kcal/scoop	Whey protein isolate, 6 g/scoop
Duocal	14 kcal/tsp	Corn starch, vegetable oil, MCT

Polycose is Ross Products Division, Abbott Laboratories, Columbus, OH; Microlipid is Mead Johnson, Evansville, IN; Resource Beneprotein is Novartis Nutrition, Minneapolis, MN; Duocal is SHS NorthAmerica, Gaithersburg, MD.

local cellulites. PEGs are sometimes contraindicated after previous abdominal surgery, in the presence of an abdominal tumor or significant organomegaly, or when obesity complicates placement.

Another complication of PEG placement is inadvertent perforation of a loop of bowel, generally the transverse colon. The colon may be enlarged if there is preoperative constipation and also may enlarge owing to excessive gas production, generally from air swallowing. Occasionally, this occurs if a child cries and swallows air for an extended period just prior to the procedure. Skillful, rapid placement of intravenous lines for sedation and other placating tactics may minimize this. Careful observation of the gas pattern during transillumination of the stomach prior to tube placement can also hint at inadvertent colon enlargement.

The gastrostomy button is a feeding device that can be used to form an effective one-way valve at the gastrostomy site. These products fit flush with the skin and attach to commercial feeding tubes that lock onto the button in a variety of ways. The button is less prone to accidental removal. However, the valve system makes button devices difficult to use for decompression of the stomach unless a tube is inserted through the valve directly into the stomach. It is possible to modify the external PEG tube into a button using a commercial conversion kit. This temporary button may last up to a year after initial PEG placement. It must be removed endoscopically by pulling it out through the mouth. It can then be replaced with a standard button.

Two types of buttons are currently available on the market. One has an inner deformable bolster that must be stretched with a trocar and forcibly inserted into the gastrostomy orifice. There is a 2 to 5% risk of tearing or perforating the site during insertion. It has the advantage of being very durable and not prone to breakage. The other variety of button has an inflatable bolster that is easier to insert. After insertion, the balloon is inflated. The inner seal provided by the inflatable bolster products is somewhat adjustable by varying the amount of water used to inflate the bolster. A disadvantage is that the balloon or the button itself is more prone to breakage and generally must be changed every 3 months.

If short-term enteral support is necessary and GER, delayed gastric emptying, or chronic pancreatitis is present, transpyloric jejunal feedings are an alternative. Placement of transpyloric nasoduodenal or NJ tubes is more complicated than NG tube placement and requires confirmation of position by radiography or pH analysis of aspirates. Placement of transpyloric tubes can be facilitated by the use of fluoroscopy and intravenous metoclopramide. Special catheters that measure pH using an electrode system are also available to confirm placement. Transpyloric tubes can also be placed endoscopically through existing gastrostomies. Extreme care must be exercised to be certain that retching or emesis has not moved the tip of the tube into the esophagus. Retrograde continuous delivery of formula into the esophagus virtually ensures aspiration. When tubes are placed beyond the pylorus, gastric decompression may be required to prevent distention that could impair small bowel motility or lead to aspiration. Feeding jejunostomies generally do not tolerate large bolus feeding over short intervals without producing dumping syndrome.

Two different methods are employed for delivery of enteral feedings. Intermittent bolus feedings deliver the formula over a period of time similar to that for an oral feeding (ie, 10–20 minutes). This technique is simple, requires minimal supplies, and may facilitate the transition to home care. Intolerance of this method is indicated by gastric residuals, malabsorption, dumping syndrome, aspiration, or persistent regurgitation. Bolus feeding is not well tolerated when feedings are delivered distal to the pylorus. When there is intolerance of intermittent bolus feeding, continuous infusion using an infusion pump is an alternative. When compared with hourly bolus feeding in adult burn patients, continuous feeding resulted in fewer stools and reduced the time to reach nutritional goals. Continuous feeding appears to be particularly beneficial when used for patients with impaired absorption, such as chronic diarrhea or short-bowel syndrome.[110,111] For chronic diarrhea, the reason for success of continuous feeding may be related to decreased gastric distention, which, in turn, affects the gastrocolic reflex. In some patients, this lack of gastrocolic reflex may potentiate constipation, which is a frequent complication of enteral feedings. Also, lack of gastric distention may decrease the usual postcibal GER, which is directly correlated to increases in feeding volume.[112]

We have found that chronic constipation is often an occult complication of enteral feedings that is manifest by early satiety, distention, and poor gastric emptying or even emesis. Often it is unsuspected because overflow diarrhea is present. An abdominal radiograph and/or digital examination will often reveal large amounts of fecal material. Disimpaction with enemas and establishment of regular

bowel movements using routine laxative administration is often extremely beneficial.

Similar symptoms of emesis and diarrhea during EN may also be observed with bacterial overgrowth. Bacterial overgrowth may occur as a result of altered anatomy, disorganized motility, and/or chronic acid inhibition. It may be diagnosed by enteral intubation and culture or inferred from high basal or early postcibal breath hydrogen analyses. However, neither of these measurements totally excludes bacterial overgrowth. Therefore, we prefer an empiric trial of treatment, generally with metronidazole for anaerobic bacteria or, less commonly, trimethoprim-sulfamethoxazole for gram-negative overgrowth. When the problem is recurrent, therapy with regular 3- or 4-day courses of treatment every 3 to 4 weeks allows for smoother progression of feedings. Alternatively, use of a probiotic such as *Lactobacillus* GG may be of benefit. We have also observed in two patients that use of a high-fat formula seemed to decrease the troublesome recurrent symptoms of bacterial overgrowth. This might be due to slower motility or less carbohydrate available as bacterial substrate.

WATER

It must be emphasized that the most important nutrient deficiency to be avoided is water deficiency (dehydration or tube feeding syndrome) Often this occurs insidiously over several days or even months. Those patients who are unable to voluntarily control their water intake, owing to limitations on absorption, developmental immaturity, or neuromuscular diseases, are at particular risk for the development of tube feeding syndrome. This deficiency will become particularly critical during times of increased water losses owing to fever, vomiting, or diarrhea. Even ambient weather conditions can contribute to deficiency. The patient discharged to a home without air-conditioning during the summer months may have an enormously increased requirement for water. If the patient wears diapers, it is useful to ask the caregiver how many urine voids there are during the day. If the diaper contents are always mixed urine and stool, it is important to ask how many "urine-only" diapers are changed during the day. During routine visits to the physician, urine-specific gravity, serum sodium, and blood urea to creatinine concentrations should be studied in a flowchart representation to help spot trends.

The practice of adding supplemental calories and electrolytes to feedings can exacerbate tube feeding syndrome water deficiency by producing increased GER[112] or diarrhea. Simplistically, clinicians often feed to the point of emesis or diarrhea in an attempt to maximize weight gain. This puts the patient at particular risk for tube feeding syndrome. The goal should be steady, modest weight gain with positive fluid balance.

Mechanical complications are also common with enteral feedings. The small-bore tubes can easily become clogged or kinked. Clogged feeding tubes can be a major problem, requiring repeated reinsertions. Newer enteral feeding tubes have wider openings to diminish clogging. Additional ports at the connection site allow for medication administration and flushing without interruption of the feeding.

To prevent clogging, liquid medications should be used whenever available. If medication in tablet form is necessary, it should be crushed to a fine powder. Adequate suspension in solution can sometimes be achieved by allowing the tablet to dissolve in water rather than attempting to dissolve the crushed tablet. Medications that congeal, such as Metamucil or cholestyramine, easily clog small-bore tubes and should be avoided when possible. If they are necessary, these medications should be administered and cleared quickly. Cholestyramine products that have aspartame instead of sugar are often finer powders and less likely to clog the tube. Feeding tubes should be flushed with water before and after intermittent bolus feedings and periodically (every 4 to 6 hours) during continuous feeding. An investigation of nine nontoxic substances (including digestive enzymes, proteolytic enzymes, and cranberry juice) theoretically useful in clearing clogged feeding tubes demonstrated that successful declogging occurred with chymotrypsin, papain, and distilled water. Preventing feeding tubes from clogging is easier than attempting to clear them.[113] Other mechanical complications include irritation from transnasal tubes, which can produce sinusitis, otitis media, GER, and nasopharyngeal and gastric irritation.

SUMMARY AND CONCLUSIONS

We have discussed the indications for specialized enteral formulas and routes of administration. The enteral route is the preferred route of nutrient administration. Even in the face of relative compromise of the GI tract, specialized products and techniques promote positive nutrient balance. Partial use of the enteral route during parenteral nutrition prevents atrophy of the intestine and reduces the tendency toward the cholestasis associated with intravenous feeding. If possible, the oral route is preferable, and appetizing, nutritious foods should be emphasized. Enteral feeding is cheaper, simpler, more effective, and safer than parenteral feeding.

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5. Special Dietary Therapy

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SPECIAL DIETARY NEEDS

Malnutrition in children with chronic gastrointestinal disorders includes underfeeding (protein-energy malnutrition [PEM]) and overfeeding (overweight or obesity). In childhood, malnutrition is characterized by growth failure (weight, height/length), behavioral and cognitive deficits, and increased susceptibility to infections secondary to immune system dysfunction. Repeated infections may further alter nutrient intake and requirements, making this a vicious cycle. PEM often occurs as a result of inadequate nutrient and energy supply or in response to an injury or illness with increased requirements in a marginally nourished child. Malnutrition is common in hospitalized pediatric patients, with an estimated prevalence of 25 to 60% in North America.[1,2] Therefore, it is essential to meet the special nutrition needs of children with significant medical conditions. An overview of the nutritional considerations for selected disease states is presented in Table 75.5-1.

In many disease states, PEM occurs as food intake fails to increase in response to the increased energy and nutritional demands of inflammation and/or malabsorption. Most illnesses exert some effect on nutrient status and requirements. In certain disorders, the ability of the body to use specific nutrients may be altered (eg, vitamin E deficiency seen in abetalipoproteinemia). Nutritional deficiencies can have long-lasting effects, especially in growing children. Therefore, it is important to thoroughly understand the pathophysiology of the patient's disorder and its impact on nutritional status as part of nutrition support planning. Because drug therapy is part of the management of many diseases, drug–nutrient interactions must be considered. Medication side effects, as well as therapeutic effects, may contribute to changes seen in nutritional status owing to anorexia, nausea, vomiting, diarrhea, malabsorption, and maldigestion.[3,4]

Previously, the 1989 Recommended Dietary Allowances (RDAs) guided macronutrient and micronutrient intake for healthy children and those with acute and chronic illness. The RDAs have been replaced by the new Dietary Reference Intakes (DRIs). The DRIs are evidence based and represent recommendations for healthy US and Canadian populations for nutrient intake for good health, as well as for the prevention of chronic disease.[5] Additional information on nutrient requirements of children is available in Chapter 75.3, "Nutritional Assessment and Requirements."

The role of nutrition in disease and disease prevention is recognized in the medical community. Studies have shown that poor nutritional status negatively affects health outcomes in chronic disorders of childhood such as pulmonary status in patients with cystic fibrosis[6] and survival rates following a liver transplant.[7] Additionally, foods such as those containing prebiotics and probiotics may be used to modulate disease activity and the immune system. Many children with chronic diseases require additional calories, as well as other specific dietary interventions, to achieve optimal growth and development. These therapeutic diets are modifications of a normal diet pattern and are intended to counteract specific nutrient inadequacies that are associated with the disease states. An example is the provision of a high-protein diet in patients with protein-losing enteropathy. Some disease states such as acute diarrhea require dietary modifications for a limited time. A lactose-free diet in patients with acute diarrhea may provide symptomatic relief. However, for the majority of children with chronic diseases, the dietary modifications will be long term and will require follow-up as well as ongoing age- and culturally appropriate education. Re-evaluation of the diet therapy and monitoring of nutrient and nutritional status are essential to ensure adherence and comprehension. The goal is optimization of micronutrient and macronutrient intake based on age, nutritional status, and medical condition. Nutritional assessment plays a key role in achieving this goal.

Specific Aspects of Nutrition Assessment

Detailed information on nutritional assessment in infants, children, and adolescents is available in Chapter 75.3. Nutrition assessment is a component of the medical physical examination for all children, especially for those with chronic disease. Monitoring nutrition status is critical to ensure that the patient's nutritional goals are met. A registered dietitian often contributes to this assessment, which includes nutrition-focused medical and nutrition history, including infant feeding history, typical current intake, food aversions, allergies or intolerances, recent weight loss or gain, chewing and swallowing difficulties, and dietary, vitamin, mineral, or herbal supplements. Physical examination and growth assessment include anthropometric measurements: weight, height or length, head circumference, body mass index (for children > 2 years), midarm muscle circumference, triceps skinfold thickness, an assessment of fat and muscle stores, and signs of

TABLE 75.5-1 SELECTED DISEASE CONDITIONS AND RELATED NUTRITIONAL CONSIDERATIONS

CYSTIC FIBROSIS
High-calorie, high-fat diet
Salt replacement, especially in hot weather
Evaluation of essential fatty acids and bone health
Fat-soluble vitamin supplementation
Enzyme therapy for pancreatic insufficiency
Oral supplements for calories
INFLAMMATORY BOWEL DISEASE
High-calorie diet
Lactose-free diet
Vitamin B_{12} supplementation with ileal disease
Vitamin D, calcium, iron, zinc, folic acid supplementation
Multivitamin supplementation
Elemental diet for inducing remission and providing caloric support
Bone disease evaluation
CONGENITAL HEART DISEASE (in infants)
High-calorie formula in infants
Concentrated formulas
Fortified breast milk in infants
Tube feeds
Sodium restriction
Fluid restriction
CANCER
High-calorie diet
Oral supplements
Tube feeds
Parenteral nutrition for extensive nausea and vomiting
Appetite stimulants
Small, frequent feedings
RENAL DISEASE
Fluid restriction
Fortified breast milk or concentrated formula
Sodium restriction
Provide RDA for protein
Phosphorus restriction
Phosphorus binder
Supplement with vitamin D, calcium
Bone disease evaluation
LIVER DISEASE
Fluid restriction for end-stage disease
Fat-soluble vitamin/multivitamin supplementation
Fat malabsorption may occur with biliary atresia or Alagille syndrome
Evaluation of essential fatty acid status
Evaluation of bone health
Protein restriction with encephalopathy
Adequate calories and protein for growth
METABOLIC DISORDERS
Dietary restriction of the offending nutrients
Adjusted intake of offending amino acids and fats to promote optimal growth and development
Monitor compliance to diet
Replacement of deficient coenzymes
CEREBRAL PALSY
Supplemental tube feeds
Fiber-containing formula
Speech evaluation if chewing/swallowing difficulties
May require modified diet (puree, thickened liquids, etc)
Supplemental multivitamin and minerals
Ketogenic diet (patients with intractable seizures)
Bone disease evaluation
DIABETES MELLITUS
Blood glucose control
Dietary balance with regularly scheduled meals and snacks
Meal planning, carbohydrate counting, insulin education when indicated
Monitor cholesterol and triglycerides and recommend low-fat diet when indicated
Adequate calories for proper growth and development
CELIAC DISEASE
Gluten-free diet
Lactose-free diet may be indicated until complete mucosal healing
Possible infant sensitivity to cow's milk protein
Vitamin and mineral therapy
Adequate calories for catch-up growth
Bone disease evaluation
Monitor compliance to diet

RDA = Recommended Dietary Allowance.

micronutrient excesses and deficiencies. Alternative anthropometric measures for linear growth (lower leg length and upper arm length) should be performed on patients who are unable to stand. A midarm circumference may be a useful measure to follow in patients with significant dependent edema that results in an unreliable weight. This measurement can be done every 2 weeks in infants and every 4 weeks in children and adolescents as a gross estimate of growth and response to therapy. To ensure reliability and accuracy, an experienced clinician should obtain these measurements using quality calipers that have been maintained regularly. Signs of malnutrition may be identified with physical assessment of body fat and muscle stores, the oral cavity, skin, hair, and eyes.

Evaluating pubertal development is also an important part of nutritional assessment. For those patients at nutritional risk, resting energy expenditure measurements by indirect calorimetry are recommended to determine individual caloric needs. Obtaining a dual-energy x-ray absorptiometry (DXA) scan provides body composition information such as fat and muscle stores and bone health. Biochemical analysis, including prealbumin, albumin, electrolytes, minerals (calcium, phosphorus, and magnesium), trace minerals, iron studies, and liver function tests, is important in selected clinical settings. Specific nutrient analysis will depend on the underlying disease state (eg, triene-to-tetraene ratio to evaluate for essential fatty acid deficiency in cystic fibrosis or serum alkaline phosphatase, parathormone, vitamin D [25-hydroxy and 1,25-dihydroxy], calcium, and phosphorus to evaluate for rickets).

Specific Dietary Therapy by Nutrient Groups

Dietary therapy during chronic illness is directed at providing calories, macronutrients, and micronutrients to support normal growth and, in many cases, nutritional rehabilitation. In an effort to meet the increased nutritional needs associated with chronic illness, infants and children may require dietary modifications. Typically, infant formulas are calorically concentrated from the standard 20 calories per ounce to 27 calories per ounce by mixing with less water. When higher caloric content is required, additional macronutrients can be added, usually in the form of modular components. The addition of carbohydrate, protein, and fat modular components to increase the caloric density of a formula is a com-

mon practice in pediatric nutrition support and is indicated for patients who are unable to meet nutrient needs with standard amounts of formula. Attention must be paid to the renal solute load when concentrating formulas, especially for patients with renal dysfunction. Generally, caloric density should be advanced by 2 to 4 calories per ounce in a 24-hour period as tolerated. The selection of the modular component (carbohydrate, protein, or fat) is dependent on the clinical situation. See Table 75.5-2 for a list of some commonly used modular components and their indications.

CARBOHYDRATES

The DRIs state that 45 to 65% of total calories should come from carbohydrates (Tables 75.5-3 and 75.5-4).[5] No more than 25% of total calories is recommended as added sugars because more may result in reduced intakes of certain micronutrients.[5] A number of popular weight loss diets recommend very low intakes of carbohydrate. A growing body of scientific evidence has shown an increased risk for adverse health effects with chronic consumption of any diet that has a pattern of increased or decreased micronutrients. Therefore, low-carbohydrate weight loss diets are not recommended for growing children. An exception is the ketogenic diet, which provides the majority of calories from fat with minimal protein and carbohydrate and is used to treat children with intractable seizure disorders. The DRIs recommend that children > 1 year consume at least 130 g of carbohydrates each day. Adequate intake of carbohydrate is specifically important for brain health and to spare metabolism of protein for energy.

Human milk and most infant formulas contain 40 to 50% carbohydrate, with lactose being the primary source. Lactose plays a key role in infant feeding because it maintains lactobacilli in the gut, thereby preventing the growth of less desirable bacteria. Lactose also lowers the pH of the intestinal contents, thereby providing the optimal calcium absorption environment. Lactose intolerance commonly occurs in older children and adolescents. In infancy, lactose intolerance is often secondary to damage to the intestinal epithelium following acute gastrointestinal infections and celiac disease. Symptoms include bloating, flatulence, and diarrhea with ingestion of lactose-containing foods. Treatment may include supplemental lactase enzymes, restrictions of dietary lactose (lactose-free formula, reduced-lactose diet), and provision of adequate calcium and vitamin D intake. These conditions are discussed in more detail elsewhere in this text. See Table 75.5-5 for a list of selected common infant and childhood formulas and indications for use.

A well-appreciated role of dietary carbohydrates is in blood glucose control in diabetes. Nutritional management for children with diabetes includes carbohydrate counting and distribution of carbohydrates evenly throughout each meal or snack of the day. The number of carbohydrate

TABLE 75.5-2 SELECTED THERAPEUTIC MODULAR DIET COMPONENTS

MODULAR COMPONENT	INDICATION	CARBOHYDRATE	PROTEIN	FAT	CALORIC DENSITY
Polycose liquid (glucose corn polymer)	Added to supplements and formulas or foods to increase calories	X			2 kcal/mL
Polycose powder (glucose corn polymer)	Added to supplements and formulas or foods to increase calories	X			23 kcal/tbsp
Duocal powder (SHS Ltd, Rockville, MD, USA) (corn syrup solids; vegetable, corn, coconut oil)	Added to supplements and formulas or foods to increase calories	X		X	42 kcal/tbsp
Promod powder (whey protein)	Added to oral supplements, enteral tube feedings, or food to increase protein		X		28 kcal/scoop; 5 g protein/scoop
Additions (must be mixed with hot food) (corn syrup solids, whey protein isolate, canola oil, soy lecithin)	Added to foods to increase calories and protein	X	X	X	43 kcal/tbsp; 2.5 g protein/tbsp
MCT oil (fractionated coconut oil)	Added to formulas or tube feedings to increase calories; often used for fat malabsorption disorders; does not contain essential fatty acids			X	7.7 kcal/mL
Microlipids (safflower oil)	Added to formulas or tube feedings to increase calories; also used in treatment or prevention of fatty acid deficiencies; contains essential fatty acids			X	4.5 kcal/mL
Vegetable oil (canola, corn, soybean, safflower, or sunflower)	Added to formulas and foods to increase calories			X	8.3 kcal/mL
Margarine, butter (long-chain triglycerides)	Added to foods to increase calories			X	102 kcal/tbsp

MCT = medium-chain triglyceride.

TABLE 75.5-3 DIETARY REFERENCE INTAKES: RECOMMENDED INTAKES FOR INDIVIDUALS FOR MACRONUTRIENTS

AGE GROUP	CARBOHYDRATE (g/d), AI OR RDA	FAT (g/d), AI	PROTEIN (g/d), AI OR RDA
Infants			
0–6 mo	60 (AI)	31	9.1 (AI)
7–12 mo	95 (AI)	30	13.5 (RDA)
Children			
1–3 yr	130 (RDA)	ND	13 (RDA)
4–8 yr	130 (RDA)	ND	19 (RDA)
Males			
9–13 yr	130 (RDA)	ND	34 (RDA)
14–18 yr	130 (RDA)	ND	52 (RDA)
19–30 yr	130 (RDA)	ND	56 (RDA)
Females			
9–13 yr	130 (RDA)	ND	34 (RDA)
14–18 yr	130 (RDA)	ND	46 (RDA)
19–30 yr	130 (RDA)	ND	46 (RDA)

Adapted from Institute of Medicine.[5]
AI = Adequate Intake; ND = not determinable; RDA = Recommended Dietary Allowance.

servings is adjusted in relation to a child's growth and energy needs.

There are other clinical disorders in which digestion or absorption of carbohydrate is altered, resulting in carbohydrate intolerance. These can be seen in children with transient lactase deficiency owing to mucosal damage from an acute viral illness and newly diagnosed patients with celiac disease with villous atrophy and congenital/genetic disorders (eg, sucrase-isomaltase deficiency, alactasia, and glucose-galactose malabsorption). In addition, some metabolic disorders of carbohydrate metabolism such as aldolase-B deficiency, fructose 1-6-diphosphatase deficiency, and galactosemia require the elimination of certain carbohydrates. Table 75.5-5 lists selected enteral products that have been developed to accommodate these dietary restrictions.

Humans cannot digest and absorb many plant carbohydrates. The DRIs define fiber as the nondigestible carbohydrate and lignin components from the plant cell wall that are composed of soluble and insoluble fiber. This includes fiber contained in oat and wheat bran. Soluble fiber is fermented by colonic bacteria and converted into short-chain fatty acids that are subsequently absorbed by the colon. In this process, fructo-oligosaccharides (FOSs) are formed and promote the growth of probiotic bacteria. FOSs have been shown to be beneficial in inflammatory bowel disease (IBD). Functional fiber is the dietary fiber that has potential beneficial physiologic effects and includes FOS, pectins, and gums.[5]

The potential health benefits of certain dietary fibers include a role in the management of diabetes by delaying glucose uptake and reducing the insulin response. In addition, certain dietary and functional fibers may have a protective effect against coronary heart disease by decreasing serum cholesterol levels. Fiber also delays gastric emptying, which may play a key role in weight control by increasing satiety.[8–10] In children, the consumption of fiber is important to prevent constipation, which accounts for 25% of pediatric gastroenterology office visits.[11] The 2002 DRIs provide the first recommendation for dietary fiber intake and refer to total fiber (sum of dietary fiber and functional fiber) (Table 75.5-6). These recommendations were generated in response to evidence that shows an increased risk for heart disease when low-fiber diets are consumed.

PROTEIN

Protein intake should constitute 5 to 35% of total daily calories (see Tables 75.5-3 and 75.5-4). Protein requirements are altered in many pediatric illnesses. Inborn errors of amino acid metabolism require that certain dietary proteins be restricted to minimize exposure to indicated amino acids. Care must be taken in these conditions to ensure an adequate energy and total protein energy intake to support normal rates of growth. Examples include phenylketonuria, maple syrup urine disease, propionic acidemia, and methylmalonic acidemia. Table 75.5-5 lists selected enteral products that have been developed to accommodate these dietary restrictions.

In chronic renal failure not requiring dialysis, the DRI for protein should be provided to ensure adequate growth. No data exist to show that protein restriction below the DRI delays the progression of renal disease. Restricting dietary phosphorus also leads to protein restriction. For those children undergoing dialysis, it is important to supply adequate amounts of protein to compensate for protein lost in the dialysate.

Protein requirements are increased in the pediatric patient with burn injury owing to an accelerated breakdown of tissue and from protein losses from the wound before skin grafting. Failure to meet protein requirements can decrease wound healing and increase the risk for infection.

Selected amino acids may become conditionally essential during periods of rapid growth and serious illness (trauma, severe infection, and cancer). This occurs because synthetic rates do not adequately increase during periods of increased requirements. Arginine is a precursor of nitric oxide, and it modulates hepatic protein synthesis, mediates the vasodilatory effects of the endotoxins, and reduces tumor and bacterial growth.[12] Enteral arginine supplementation has been shown to be most beneficial in adult postoperative, critically ill, and trauma patients.

TABLE 75.5-4 DISTRIBUTION RANGES FOR MACRONUTRIENT INTAKE

MACRONUTRIENT	RANGE (PERCENT ENERGY)	
	CHILDREN, AGE 1–3 YR	CHILDREN, AGE 4–18 YR
Fat	30–40	25–35
ω-6 Polyunsaturated fatty acids (linoleic acid)	5–10	5–10
ω-3 Polyunsaturated fatty acids (linolenic acid)	0.6–1.2	0.6–1.2
Carbohydrate	45–65	45–65
Protein	5–20	10–30

Adapted from Institute of Medicine.[5]

TABLE 75.5-5 SELECTED ENTERAL PRODUCTS AND COMMON INDICATIONS

FORMULA TYPE/INDICATION	PRODUCT	AGE		
		< 1 YR	1–10 YR	> 10 YR
HYPOALLERGENIC				
Cow's milk protein allergy, soy protein allergy, impaired GI function	Nutramigen* (Mead Johnson, Evansville, IN, USA)	X		
	Neocate (SHS Ltd, Rockville, MD, USA)	X		
	Neocate One Plus		X	
	Elecare (Ross, Columbus, OH, USA)		X	
	Pediatric E028*(SHS Ltd, Rockville, MD, USA)			X
SEMIELEMENTAL				
Malabsorption, impaired GI function, cow's milk protein allergy	Nutramigen	X		
	Pregestimil (Mead Johnson, Evansville, IN, USA)	X		
	Alimentum (Ross, Columbus, OH, USA)	X		
	Peptamen Jr. (Nestle, Deerfield, IL, USA)		X	
	Peptamen			X
	Peptamen 1.5			X
ELEMENTAL				
Pancreatitis (if enteral nutrition initiated)	Vivonex T.E.N.(Novartis, Minneapolis, MN, USA)			X
	Tolerex (Novartis, Minneapolis, MN, USA)			X
Malabsorption, impaired GI function, protein allergy	Elecare		X	
	Neocate	X		
	Neocate One Plus		X	
	Pediatric E028			X
	Pediatric Vivonex		X	
	Tolerex			X
	Vivonex T.E.N.			X
LOW LONG-CHAIN TRIGLYCERIDES				
Chylothorax	Portagen (Mead Johnson, Evansville, IN, USA)	X	X	
	Lipisorb (Mead Johnson, Evansville, IN, USA)			X
	Vivonex T.E.N.			X
	Tolerex			X
LIVER				
Biliary atresia, alagille syndrome, maldigestion, malabsorption	Alimentum	X		
	Pregestimil	X		
	Peptamen Jr.		X	
	Peptamen			X
	Peptamen 1.5			X
RENAL				
Alterations in electrolytes and protein	Good Start Supreme (Nestle, Deerfield, IL, USA)	X		
	Suplena (Ross, Columbus, OH, USA) (predialysis)			X
	Nepro (Ross, Columbus, OH, USA) (dialysis)			X
FIBER-CONTAINING FORMULAS				
To normalize bowel function	Pediasure with Fiber (Ross, Columbus, OH, USA)		X	
	Kindercal with Fiber (Mead Johnson, Evansville, IN, USA)		X	
	Nutren Junior with Fiber (Nestle, Deerfield, IL, USA)		X	
	Nutren 1.0 with Fiber			X
	Jevity 1 Cal (Ross, Columbus, OH, USA)			X
	Ensure with Fiber (Ross, Columbus, OH, USA)			X
STANDARD FORMULAS				
	Pediasure		X	
	Kindercal		X	
	Nutren Jr.		X	
	Nutren 1.0			X
	Ensure			X
	Isosource (Novartis, Minneapolis, MN, USA)			X
SPECIALTY FORMULAS				
Acute respiratory distress syndrome	Oxepa (Ross, Columbus, OH, USA)			X
Carbohydrate intolerance, malabsorption fat/protein,	Mead Johnson 3232A (Mead Johnson, Evansville, IL, USA)	X	X	X
Carbohydrate intolerance, ketogenic diet	Ross Carbohydrate-Free (RCF) (Ross, Columbus, OH, USA)	X	X	X
	Ketocal (SHS Ltd, Rockville, MD, USA)		X	X
Phenylketonuria	Phenex-1 (Ross, Columbus, OH, USA)	0–3 yr		
	Phenex 2		> 4 yr	

(continues)

TABLE 75.5-5 Continued

FORMULA TYPE/INDICATION	PRODUCT	AGE		
		< 1 YR	1–10 YR	> 10 YR
Maple syrup urine disease	Ketonex-1 (Ross, Columbus, OH, USA)	0–3 yr		
	Ketonex-2		> 4 yr	
Propionic acidemia	Propimex-1(Ross, Columbus, OH, USA)	0–3 yr		
	Propimex-2		> 4 yr	
Methylmalonic acidemia	Propimex-1	0–3 yr		
	Propimex-2		> 4 yr	
Tyrosinemia	Tyrex-1 (Ross, Columbus, OH, USA)	0–3 yr		
	Tyrex-2		> 4 yr	
Galactosemia	Isomil (Ross, Columbus, OH, USA)	X		
	Prosobee (Soy) (Mead Johnson, Evansville, IL, USA)	X		
	Fortified rice milk		X	
Glycogen storage disease	Prosobee	X		
Pyruvate dehydrogenase deficiency	Ross Carbohydrate-Free (RCF) + Microlipid (Mead Johnson, Evansville, IL, USA) + Polycose (Ross, Columbus, OH, USA)or Ketocal	X	X	X
VLCAD deficiency	Portagen (Mead Johnson, Evansville, IL, USA)	X	X	X
LCAD deficiency	Provimin (Ross, Columbus, OH, USA) + MCT oil + Polycose	X	X	X
CONCENTRATED FORMULAS				
Fluid restriction, increased energy needs or volume intolerant	Nutren 1.5			X
	Peptamen 1.5			X
	Crucial 1.5 (Nestle, Deerfield, IL, USA)			X
	Nutren 2.0			X
	Nu Basics 2.0 (Nestle, Deerfield, IL, USA)			X
	Deliver 2.0 (Mead Johnson, Evansville, IL, USA)			X
	Jevity 1.2 Cal (Ross, Columbus, OH, USA)			X
	Jevity 1.5 Cal			X
	Ensure Plus			X

GI = gastrointestinal; LCAD = long-chain hydroxyacyl CoA dehydrogenase deficiency; MCT = medium-chain triglyceride; VLCAD = very-long-chain acyl-CoA dehydrogenase deficiency.

*These products are not completely hypoallergenic.

This chart does not represent a comprehensive list of enteral products but rather a listing of commonly used enteral products. Note that in some special circumstances, an adult formula (used in children > 10 years old) may be used in children < 10 years old under the close supervision of a registered dietitian. Computerized diet analysis is required in these instances to determine nutrient imbalance.

Glutamine is the most abundant nonessential amino acid and is a precursor for glutathione, and it, too, may be conditionally essential during selected clinical conditions. It has a major role as an energy source for enterocytes, colonocytes, lymphocytes, and macrophages.[13,14] It may reduce bacterial translocation in the gut, thereby reducing the risk of bacteremia and consequent mortality in adult patients with cancer undergoing chemotherapy. Glutamine supplementation has been found to be safe in premature infants,[12,15,16] to be beneficial in patients with short-bowel syndrome, to improve nitrogen balance in critically ill and postsurgical patients,[17] to preserve gastrointestinal mucosal structure,[18] and to decrease sepsis and shorten the hospital stay in some adult bone marrow transplant patients.[19] Glutamine is currently added to some specialized enteral formulas and parenteral nutrition solutions for patients with short-bowel syndrome and those undergoing bone marrow transplants. It is unstable in solution, but newer formulations of glutamine dipeptides are stable, allowing its addition to enteral products.

Taurine is essential for the conjugation of bile acids early in infancy, especially in small premature infants. It is added to infant formulas at concentrations similar to those in breast milk and at higher levels in premature infant formulas. Carnitine is important for intracellular fatty acid oxidation and energy production. It is a nonessential amino acid but may become conditionally essential when requirements increase. Carnitine has been used in the treatment of various genetic defects in organic and fatty acid metabolism in pharmacologic doses. Nucleotides are important during periods of metabolic stress and rapid growth. Beneficial effects on the immune system have been observed,[20] and infant formulas are now supplemented with nucleotides at concentrations seen in human milk.

FAT

It is recommended that 20 to 40% of total daily calories come from dietary fat (see Tables 75.5-3 and 75.5-4). Certain inborn errors in fatty acid oxidation require restriction of dietary fats. Deficiency of the essential fatty acids linoleic and linolenic acids results when adequate long-chain fat is not provided in the diet. Table 75.5-5 lists selected enteral products that have been developed to accommodate these dietary restrictions. Recently, there has been an increased interest in two fatty acids that occur naturally in breast milk. Docosahexaenoic acid (fish and organ meat) and arachidonic acid (meat, eggs, and milk) are ω-3 and ω-6 polyunsaturated fatty acids that are important constituents of the brain and the

TABLE 75.5-6 DIETARY REFERENCE INTAKES: RECOMMENDED INTAKES FOR TOTAL FIBER

AGE GROUP	AVERAGE INTAKE (AI) TOTAL FIBER (g/d)
Children	
0–6 mo	ND
7–12 mo	ND
1–3 yr	19
4–8 yr	25
Males	
9–13 yr	31
14–18 yr	38
19–30 yr	38
Females	
9–13 yr	26
14–18 yr	26
19–30 yrs	25

Adapted from Institute of Medicine.[5]
AI = Adequate Intake; ND = not determinable. AI based on 14 g total fiber/1,000 kcal and average kcal intake for age range; decrease fiber goal if kcal intake is less than amount recommended for age.

retina. Arachidonic acid is essential for growth and functions as a precursor for eicosanoids. Several infant formulas are now supplemented with docosahexaenoic acid and arachidonic acid.

Essential fatty acid deficiency (EFAD) occurs in patients receiving long-term parenteral nutrition without adequate intravenous fat, with prolonged fasting, or with extended use of a formula predominantly containing medium-chain fats. In addition, EFAD may occur in patients who have fat malabsorption. Signs of EFAD include poor growth, thrombocytopenia, and rough, scaly skin. EFAD is commonly diagnosed by a serum triene-to-tetraene ratio of greater than 0.4. Fatty acids are precursors of prostaglandins and leukotrienes. The ω-3 fatty acids have been shown to affect the clinical course of patients with IBD, rheumatoid arthritis, cardiovascular disease, and respiratory distress syndrome.[21–24]

Medium-chain triglycerides (MCTs) are found in coconut oil and contain fatty acids with a chain length of 6 to 12 carbons. MCTs are absorbed directly into the portal system and do not require bile salts for absorption. Typical diets do not contain large amounts of MCTs; however, they are often used as an energy source and as a component of specialized diets for patients with fat malabsorption or pancreatic insufficiency.

MICRONUTRIENTS

Supplemental vitamin and mineral use is increasing,[25,26] and there is much interest in the role of the antioxidants (vitamins A, E, and C; selenium; and β-carotene) for disease prevention. Additionally, there is evidence to support increased use of specific vitamins and minerals in specific disease states. Examples include conditions in which deficiencies exist owing to malabsorption, as seen in patients with cystic fibrosis and cholestatic liver disease who need fat-soluble vitamin supplementation. Certain micronutrients are required as cofactors for enzyme reactions in a variety of metabolic disorders, such as the role of thiamine in Kearns-Sayre syndrome.[24] Supplemental micronutrients may also be required to counterbalance the depletion caused by medications, for example, folic acid and sulfasalazine in patients with IBD. Enhanced outcomes in certain disease states can also be related to supplemental micronutrients such as vitamin A supplementation of extremely low birth weight infants with bronchopulmonary dysplasia[27] and to treat ichthyosis and psoriasis.[28] The general public is using supplements in variable dose ranges without medical supervision, and the US Food and Drug Administration does not regulate these products. Serious adverse side effects have been reported with the use of these dietary supplements.[29]

Evidence is accumulating to support the use of specific micronutrients in the prevention and treatment of adult chronic disease. Many of these diseases, especially atherosclerotic cardiovascular disease and osteoporosis, begin in childhood. This leads to consideration of whether prevention measures should be initiated in children. The Dietary Guidelines for Americans recommend consuming a balanced diet and five servings of fruits and vegetables a day to provide sufficient levels of micronutrients.[30] Further research is required to evaluate whether dietary modifications in children may help prevent the development of adult chronic diseases.

The following examples help illustrate how certain micronutrients have been used to treat and prevent diseases. Vitamin E has been shown to decrease atherosclerotic heart disease[31] and lung cancer.[32] Riboflavin has been used to decrease migraine headaches.[33] Patients with infantile lactic acidosis, skeletal myopathy, and Leigh disease can improve following riboflavin administration.[34] Nicotinic acid is used to treat atherosclerotic heart disease.[35] The benefits of folic acid are seen not only in megaloblastic anemia but also in pregnancy, in which a decrease in spontaneous abortion is noted. Periconceptional folate decreases birth defects, especially neural tube defects. Public health policy worldwide recommends that women in the childbearing age group should be supplemented with folate.[36] Folate supplementation also reduces homocysteine levels, and trials are under way to see if this effect will reduce cardiovascular disease risk.[37] Folate has been shown to be effective in the prevention of certain types of cancer.[38] In the United States, grains intended for processed foods are fortified with folate.

Glucose tolerance has been shown to improve with the use of chromium. This effect is due to improved efficiency of insulin and improved blood lipid profiles. Adult subjects with some degree of impaired glucose tolerance were more responsive to the effects of chromium, and no effect was seen in those with normal glucose tolerance. Improved glucose control was also seen in patients with diabetes mellitus.[39] Zinc supplementation has been used in diarrhea and pneumonia prevention.[40] Beneficial effects of zinc supplementation are also seen in growth, neuropsychological performance, fetal growth, and birth outcomes.[13] The benefits of iron supplementation in infants and children with iron deficiency anemia are well known, with improvement

in work capacity, behavior and cognitive function, body temperature regulation, immunity, and resistance to infections.[41] The use of calcium and vitamins in osteoporosis prevention is discussed later in the chapter.

Evidence suggests that antioxidant levels are decreased in chronic disease.[42,43] Oxidative stress that occurs in chronic inflammation leads to increased requirements of antioxidant vitamins and consequent depletion, resulting in a relative deficiency state. Vitamin A, β-carotene, and vitamin C are added to specialty formulas (for trauma, critical care, and IBD) to enhance healing and reduce inflammation.

Transforming growth factor-β_2 is a cytokine that is present in human milk and gut epithelial cells. It has anti-inflammatory effects, reduces epithelial permeability, and regulates cellular growth. It has been added to formulas used to treat children with IBD, with preliminary reports suggesting improved mucosal healing.[44–46]

COMPLEMENTARY AND ALTERNATIVE MEDICINE

Complementary and alternative medicine (CAM) covers a broad range of healing philosophies and therapies that are not commonly part of mainstream Western (conventional) medicine. This includes diet-based therapies that often employ herbal and botanical supplements. Concerns about the use of CAM in pediatric health care relate to the potential of CAM products to adversely influence growth and development. Limited efficacy and safety information is available. Dosing information for body size and herb–drug interaction is also unavailable.

CAM use in pediatric patients with IBD has been reviewed.[47] It was noted that 50% of patients used CAM therapies, which included nutritional supplements (43%), special diets (22%), alternative health systems (8%), and herb medicines (5%). No association was seen with a history of previous surgery and hospitalizations. Higher use was noted among patients receiving immunomodulatory therapy, suggesting that patients who are more ill may be more likely to seek adjunctive therapies. In an international CAM study, in patients with IBD, 41% used CAM (megavitamin therapy in 19%, dietary supplements in 17%, and herbal medicine in 14%). The reasons for CAM use included a history of previous side effects from conventional medications, hope for a cure, and prescribed medications not working as well as anticipated. Additionally, 59% of respondents not taking CAM were interested in learning about it, suggesting that patients with chronic illness are interested in CAM.[48]

The use of herbal compounds is increasing in pediatric and adolescent patients,[49] with limited scientific data to guide this use. Epogam (Scotia Pharmaceuticals Ltd, Auckland, Australia) evening primrose oil treatment was evaluated in pediatric patients with atopic dermatitis and asthma. Increased plasma essential fatty acid levels and significant improvement were seen with eczema symptoms in the subjects who received primrose oil, but no differences were seen between the control and treatment groups with reactive airways disease.[50] Certain oils (evening primrose, borage, and black currant) have anti-inflammatory effects and have been used in rheumatoid arthritis. The active ingredient is γ-linolenic acid (18:3, ω-6), which is anti-inflammatory and immune regulating.[51,52] Trials of probiotics and fish oil in adult patients with IBD have suggested potential efficacy, but similar studies have yet to be done in children.

Probiotics are recognized as being beneficial in normal health (maintaining a healthy gastrointestinal ecosystem) and for immune modulation and disease prevention.[53,54] Probiotics help control gastrointestinal inflammation, normalize mucosal function, and down-regulate hypersensitivity reactions. They have been used for the treatment of infectious diarrhea, IBD, radiation-induced diarrhea, traveler's diarrhea, antibiotic-associated diarrhea, *Helicobacter pylori* infection, and food allergy, with varying success.[55,56] There are several proposed mechanisms of action. These include stimulation of the immune response to pathogens, competition for nutrients required for the growth of pathogens, synthesis of compounds that inhibit or destroy pathogens, competitive inhibition of bacterial adhesion, normalizing intestinal permeability, altering gut flora, decreasing intestinal inflammatory responses, and controlling the balance between pro- and anti-inflammatory cytokines. *Lactobacillus acidophilus*, *Lactobacillus* GG, *Lactobacillus planatarum* 299V, *Bifidobacterium bifidum*, *Bifidobacterium longum*, *Streptococcus thermophilus*, *Enterococcus faecium* SF68, and *Saccharomyces boulardii* are among the probiotics that have been studied.[13,57]

Prebiotic compounds (FOS) support the growth of probiotic organisms and are found in foods (eg, onions, artichokes, and bananas). They are not digested in the small bowel and pass into the colon, where they are metabolized into short-chain fatty acids, promote sodium and water absorption, and serve as an energy source for colonocytes. Their use has been studied in constipation, irritable bowel syndrome, and lipid metabolism and necrotizing enterocolitis.[58,59] They have a low cariogenic potential, improve lipid metabolism, protect against colorectal cancer and infectious colitis, increase the bioavailability of calcium and magnesium, and may enhance host defenses.[60–62] Fructans (inulin and oligofructose) and soybean oligosaccharides are among the prebiotics that have been added to foods.[63,64] In both human and animal studies, beneficial effects have also been seen in calcium, magnesium, zinc, and iron nutriture.[65,66]

BONE HEALTH

Bone tissue is continuously being laid down during infancy, childhood, and adolescence, so disease processes that affect the normal pattern of bone accretion will affect ultimate bone health. Peak bone mass (PBM) is the maximum amount of bone mineral that is achieved during the life cycle in all bones in the body. It determines bone health later on in life, and it has been noted that 90% of PBM is achieved by 18 years of age.[67,68] Childhood and adolescence are crucial periods for bone health, not only for healthy children but also for those with chronic disease. Factors

adversely affecting bone development include poor weight and height gain, delayed puberty, immobilization, reduced weight-bearing activity, malnutrition, insufficient calcium intake, vitamin D deficiency, inflammatory conditions, high levels of circulating cytokines, and corticosteroid use.[69]

Bone disease is often suspected when a radiograph is obtained for an unrelated reason and is reported to be "osteopenic." Plain films should not be used to screen for bone disease because these radiographic changes represent a late effect. Indeed, bone disease should be suspected long before a radiograph shows "washed out" bones. In instances in which bone disease is suspected, a DXA assessment should be conducted. This is a safe test, with approximately only one-twentieth of the radiation of a chest radiograph and less radiation than a transcontinental airplane flight. The results are expressed in terms of bone mineral content in grams and bone mineral density (BMD) in g/cm^2. New technology allows for quick scanning of the patient. In children and adolescents, lumbar spine and whole-body scans are obtained. Sufficient normative data using the current technology do not exist for infants and children less than 4 years of age. Reference data are available for lumbar spine scans, and the results are expressed in terms of standard deviation or z-scores. Normative data do not exist for whole-body scans. DXA also evaluates body composition as fat and lean body mass. The use of DXA for body composition analysis has been validated, and DXA is an accepted modality to determine body composition.[70–73]

Although a valuable diagnostic tool, DXA does have some drawbacks. Interpretation of scans is sometimes difficult, especially if there is delayed growth and a significant difference between chronologic age and height age. This is especially true in the patient with delayed pubertal development. Additionally, the reference data for the lumbar spine scans are based on relatively small numbers of subjects. Studies are in progress to combine data sets, collect additional data, and thus improve the reference standards. DXA has clinical value in many disease states. There may be difficulty interpreting the DXA scan in the clinical setting of delayed puberty and growth. In these cases following BMD over time, taking into account that increases in BMD may just be due to maturation and growth and not necessarily due to therapy is important.

Therapy for bone disease is limited in pediatric patients. Important therapeutic options include (1) improving nutritional status and normalizing weight and height; (2) controlling the underlying chronic disease and decreasing inflammation and malabsorption; (3) ensuring adequate or therapeutic intake (diet and/or supplements) of calcium, vitamin D, magnesium, and vitamin K and monitoring levels as indicated; (4) monitoring pubertal status and optimizing pubertal development; and (5) increasing weight-bearing physical activity as appropriate. Those pediatric patients with significantly abnormal BMD and those with increased fracture risks should be prescribed noncontact, weight-bearing physical activity. There are limited data regarding intervention studies using the antiresorptive medications commonly used in adults, and routine use in pediatric patients is not advised at this time.

Osteopenia[74,75] and vertebral compression fractures[76] have been shown in children with IBD. Possible reasons for the osteopenia include malnutrition, poor dietary intake, malabsorption, inflammation, and elevated cytokine levels.[77] Medications can also affect BMD, for example, glucocorticoids and immunosuppressives, which are frequently used in children and adolescents with more severe forms of the disease.[74,75,78] Although lower BMD z-scores have been noted, once these results have been adjusted for bone age, the difference between control subjects and patients is less.[79] Similarly, bone disease has been noted in patients with chronic liver disease.[80] Possible factors include malnutrition, micronutrient deficiency, and malabsorption of vitamins D and K.[81,82] Despite normalizing vitamin D levels, some patients still persist in having bone disease.[83] Bone disease has also been noted in patients following liver transplant, but in children, improvement in BMD frequently occurs.[84–86] Bone disease is also seen in patients with celiac disease.[87] A gluten-free diet may result in some improvement in BMD.[88–90]

CONCLUSION

Nutritional therapy is important in the treatment and prevention of many disorders. This chapter has attempted to provide guidelines for nutritional therapy in selected pediatric disorders and to illustrate how the manipulation of macronutrients and micronutrients can be used in disease treatment and rehabilitation. There is an increase in the use of CAM in the United States, and many pediatric patients, including those with gastrointestinal diseases, are using CAM. Familiarity with these modalities and an open climate for discussion with patients are key to optimal patient care. The foundation for healthy bones is laid down in childhood and adolescence. Attention must be paid to the prevention and treatment of bone disease in those patients with chronic gastrointestinal disease.

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6. *Protective* Nutrients

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The gastrointestinal (GI) tract is a major interface between the host and the environment. It has a dual function, acting as the portal of entry for nutrients used for cell growth and function while serving as a barrier for noxious agents. The epithelial cells and other cells of the GI mucosa rely on nutrients supplied from both the bloodstream and intestinal lumen. These nutrients serve to preserve the integrity and function of the GI mucosa. Protective nutrients or "functional foods" are terms used to describe nutrients or foods that have an effect on the physiologic function of the GI mucosa that is separate from their established nutritional functions.[1] The cells of the GI tract are capable of specific adaptive responses to ingested nutrients. Undernutrition, starvation, inflammation, and noxious agents impair intestinal adaptation and mucosal integrity. The physiologic function of the GI mucosa also varies with age, specifically the premature intestine, infantile intestine, and adult GI tract.[2] Thus, human adult clinical trials may not be relevant to infants or children. Animal studies have suggested that the presence of luminal nutrients is important to intestinal mucosal metabolism, regardless of overall nutritional status.[3,4] Human studies have often not been conclusive and suffer from uncontrolled variables. This chapter reviews the basic physiologic development of the gut and the purported effect of protective nutrients on cell function and immunity. Candidate protective nutrients reviewed include glutamine, arginine, polyunsaturated fatty acids (PUFAs), nucleotides, and zinc. The roles of prebiotics, non-nutritive ingredients in foods that foster growth of beneficial bacteria, and transforming growth factor-β (TGF-β) are briefly explored.

EFFECT OF NUTRITION OR INFLAMMATION ON GUT DEVELOPMENT AND FUNCTION

Because the GI tract undergoes postnatal maturation, the importance of protective nutrients on mucosal integrity may vary with postgestational age. The basic structure of the GI tract is the crypt-villus unit. Mucosal cells are formed at the base of the crypt and, once formed, migrate to the villus tip. During this migration, the cell functionally matures and develops a thick microvillus membrane with numerous glycoprotein enzymes.[2,5,6] These enzymes function in digestion, absorption, and antigen recognition. When the cell reaches the villus tip, programmed cell death occurs, and the cell is extruded. Cell division requires 24 hours, whereas cell migration requires 3 to 6 days.[6] In states of malnutrition, inflammation, or toxins, there is an imbalance of cell renewal and cell death.

Malnutrition resulting from starvation results in a thinning of the mucosa and muscularis. There is a marked increase in the cell-cycle time, which decreases crypt cell proliferation or cell renewal, resulting in a shortening of both the villi and crypts.[7] As a consequence of reduced protein synthesis, there is a decrease in disaccharidase activity.[8] Additionally, decreased cellular immunity and secretory immunoglobulin A produce an environment conducive to bacterial overgrowth.[9] Bacterial overgrowth may produce mucosal injury and bile salt deconjugation, resulting in steatorrhea, diarrhea, and increased nutrient loss.[10] Many chronic disorders, such as Crohn disease, liver or renal disease, malignancy, cystic fibrosis, and celiac disease, are exacerbated by undernutrition and produce a lesion morphologically similar to marasmus.

Diseases that result in protein deficiency often cause a flat villus lesion. The mucosal thickness appears normal owing to marked elongation of the crypt.[11] This flattening results from alterations in protein metabolism, such as hypoalbuminemic protein-calorie malnutrition (kwashiorkor), or an inflammatory response to a specific protein, as in celiac disease, allergic enteropathy, or severe cow's milk or soy protein intolerance. Additionally, profound inflammation or infection may produce a similar lesion. Morphologically, there is an increase in the crypt-to-villus ratio. Physiologically, there is a reduction of the absorptive surface, resulting in a loss of disaccharidase activity and of mucosal integrity. Clinically, this is manifested as malabsorption of macro- and micronutrients.

The intestinal mucosa is highly dynamic in terms of cell renewal and response to nutrients, circulating or local hormones, and neurohumoral influences.[12] The presence of specific glycoprotein enzymes varies with gestational age, and gene expression can also be affected by diet. Expression of the disaccharidase sucrase-isomaltase is modulated by dietary source. Diets high in sucrose and fructose induce gene transcription, whereas diets low in these nutrients have a decreased enzymatic specific activity.[13] Mucosal development and integrity of the gut are dependent on numerous endogenous factors, such as thyroxine, cortisol, gastrin, enteroglucagon, pancreatic

glucagon, growth hormone, and epidermal growth factor.[14] Neurohumoral influences include enteroglucagon, gastrointestinal inhibitory peptide, motilin, and neurotensin. These agents also influence gastric, pancreatic, and biliary secretion.

Human clinical trials cannot control for the various factors affecting gut development and integrity; therefore, the results of clinical studies are often difficult to interpret. Nutrients that may be advantageous in a preterm infant may have no observable advantage for children or adults. Nutrient supplements may be beneficial, harmful, or unhelpful depending on the underlying status of the GI mucosa and the specific disease. Intestinal mucosal requirements may be different in disorders resulting from intestinal inflammation or septic shock compared with requirements for recovery from massive surgical resection, ischemia, toxic injury, or physiologic stress from severe burns. Thus, published human studies evaluating protective nutrients and factors must be interpreted cautiously, and the results cannot be extrapolated to patients of different ages or disease states. Additionally, animal models used to study these factors may not be applicable for human requirements or disease states.

PROTECTIVE NUTRIENTS

Functional food ingredients and nutrients may influence intestinal growth, maturation, and adaptation. Essential nutrients are those that are required for physiologic function and cannot be endogenously synthesized. In states of immaturity or severe catabolism, intracellular stores of specific essential nutrients may become depleted and lead to deficiency, and/or existing biosynthetic pathways of normally nonessential nutrients cannot meet increased metabolic demands. These nonessential nutrients then may become "conditionally essential" and may be potentially useful ingredients in functional foods.

GLUTAMINE

Glutamine is the most abundant amino acid in the human body. It is defined as a nonessential amino acid because it can be synthesized by a wide variety of tissues rich in glutamine synthetase. Glutamine, a structural component of proteins, functions in nitrogen transfer between tissues, is a precursor in the synthesis of nucleotides, and serves as an important nutrient for renal excretion of ammonia.[15] Importantly, glutamine also serves as the substrate for production of glutathione, a crucial antioxidant found in high concentrations in the GI mucosa. Inhibition of its synthesis results in mucosa degeneration, diarrhea, and growth failure.[16,17] Animal studies suggest that GI epithelial cells are highly dependent on glutathione, and oral or intravenous administration of glutathione may be protective against toxicity associated with inflammatory disease, ischemia, oxidative damage, chemotherapy, and radiation.[18]

Glutamine is of interest as a protective nutrient because it is the major fuel source for rapidly dividing cells such as GI epithelial cells, lymphocytes, fibroblasts, and reticulocytes.[16] Although glutamine is an abundant nonessential amino acid, it has been proposed to become a conditionally essential amino acid in catabolic conditions. In severe catabolic stress, humans show an increased efflux of glutamine from skeletal muscle and hepatocytes.[18] Severely ill patients nutritionally dependent on total parenteral nutrition (TPN) are at risk for glutamine deficiency owing to the combination of increased turnover, reduced dietary intake, and absence of this amino acid in parenteral solutions. Glutamine is relatively unstable and is therefore not included in standard intravenous protein solutions.[19] Premature or very low birth weight infants are also at risk for nutritional insufficiency and catabolism. Sparse energy reserves are rapidly depleted, and enteral sufficiency is delayed owing to the immaturity of the GI tract. Additionally, full enteral feeding is often delayed owing to the risk of necrotizing enterocolitis (NEC).

Some adult human studies have shown that glutamine-supplemented TPN has significantly reduced hospital-acquired infection, decreased gut permeability to lactulose, and improved nitrogen balance and protein synthesis after bone marrow transplant.[20,21] Other studies have shown no advantage from enteral or parenteral glutamine supplementation on clinical infections after bone marrow transplant in adults[22] or children (Krebs N, et al, unpublished data, 2000). Enteral supplementation of very low birth weight infants resulted in improved tolerance to enteral feedings and a decrease in the incidence of hospital-acquired infection.[23]

Numerous animal and human studies support a benefit of supplemental glutamine on gut function, including reversal of gut atrophy associated with TPN, radio- or chemotherapy, improved immune function, and reduced episodes of bacterial translocation and sepsis.[16] Although several studies suggest that glutamine supplementation is advantageous, problems with sample size, randomization, and comparable control groups limit interpretation. For example, studies using glutamine-supplemented TPN have been designed to provide isonitrogenous amino acid solutions to patients, but different amino acid admixtures were also used to ensure adequate intake of essential amino acids without excessive nitrogen intake in the test groups. A recent review of clinical trials of glutamine supplementation highlights the challenges of conducting adequately controlled human studies.[24] Short-term studies using intravenous glutamine have generally reported no safety concerns. An exception may be with preexisting liver disease, in which case, further elevation of transaminases has been observed.[24,25] With the putative beneficial effects from controlled trials, glutamine-supplemented enteral formulas are now available (Table 75.6-1). Although glutamine supplementation in parenteral nutrition remains investigational, newer approaches using glutamine dipetides, which have better solubility and heat stability, are expected to enable the development of glutamine-enriched enteral formulas. Well-designed and -controlled trials will be necessary, however, to determine whether such formulations will benefit clinical outcomes.

TABLE 75.6-1 COMMERCIALLY AVAILABLE FORMULAS CONTAINING PROTECTIVE NUTRIENTS

FORMULA	MANUFACTURER	FORM	AMINO ACID SUPPLEMENT	OMEGA-3 PUFA
Vivonex–Pediatric Vivonex–Plus Vivonex–TEN	Novartis Nutrition	Powder	L-Glutamine + L-arginine	–
Impact–1.0/1.5 calories/cc Impact–Fiber Impact–glutamine	Novartis Nutrition	Liquid	L-Arginine	+
Impact–Recover	Novartis Nutrition	Powder	L-Glutamine + L-arginine	–
Immune-Aid	McGraw	Powder	L-Arginine + L-glutamine	–
Crucial	Nestle Nutrition	Liquid	L-Arginine	+
Internical	Mead Johnson	Liquid	L-Arginine	+
Optimental	Ross Laboratories	Liquid	L-Arginine	+
Periactive	Ross Laboratories	Liquid	L-Arginine	–
Resource–Arginade Extra	Novartis Nutrition	Liquid	L-Arginine	NA
Resource–Gluta Solve	Novartis Nutrition	Powder	L-Glutamine	NA
Ristope-X	Cambridge Neutraceuticals	Powder	L-Glutamine + L-arginine + *N*-acetylcysteine	NA

NA = not added; PUFA = polyunsaturated fatty acid.

ARGININE

Arginine is a nonessential amino acid important in the transport, storage, and excretion of nitrogen. Similar to glutamine, arginine has been postulated to be a conditionally essential amino acid under conditions of catabolic stress.[26] The importance of arginine as a protective nutrient is related to its role as a precursor of nitric oxide (NO). Arginine is converted to NO via a family of enzymes, the nitric oxide synthases (NOSs). There are two identified forms of NOS: the constitutive form (cNOS) and the inducible form (iNOS). Calcium and calmodulin activate cNOS, whereas iNOS is calcium independent. cNOS produces small amounts of NO, which acts as a biologic mediator previously referred to as endothelium-derived relaxing factor. NO derived from vascular endothelium plays an important role in maintaining baseline vasodilator tone. NO derived from cNOS is secreted from peripheral nonadrenergic-noncholinergic neurons meditating neurogenic vasodilatation and regulating various GI, respiratory, and genitourinary tract functions.[27] Compared with cNOS, iNOS is found in a wide variety of cells, including macrophages, neutrophils, mast cells, fibroblasts, hepatocytes, vascular endothelial cells, smooth muscle cells, and cardiac myocytes. Inflammatory cytokines and bacterial endotoxins induce iNOS, whereas glucocorticoids inhibit the induction of iNOS but have no effect on the activity of either the constitutive or the inducible enzyme.

The role of arginine and NO and its relation to immunity and inflammation have been studied in various animal models. Animals supplemented with arginine have higher thymic weight and thymic lymphocyte counts and improved wound healing.[28,29] Tumor-bearing rat models have decreased tumor protein synthesis with improved whole-body protein synthesis. Intestinal integrity was improved with arginine supplementation in rodents subjected to intestinal ischemia, radiation, resection, or transplant.[16] Additionally, bacterial translocation was decreased in rats recovering from radiation enteritis receiving supplemental arginine.[26]

Inflammatory bowel disease (IBD) and neonatal NEC are examples of two human disease states marked by inflammation and increased intestinal permeability. The role of iNOS in adult chronic IBD has been investigated using surgically resected intestine obtained from patients suffering from active ulcerative or Crohn colitis. When compared with intestine resected from adults for other reasons, there was a marked increase in iNOS expression in patients with colitis compared with control specimens.[30] Low levels of serum arginine[31] and glutamine have been demonstrated in premature infants prior to and during an episode of NEC. Using a hypoxia-reoxygenation–induced NEC model in young mice, supplemental arginine or carnitine greatly attenuated hypoxia-induced tissue damage.[32] In a recent prospective double-blind randomized placebo-controlled arginine supplementation trial, 152 premature infants were followed for 28 days. There was a significantly lower incidence of NEC in the arginine-supplemented group. Serum arginine levels decreased in all infants who developed NEC; however, they were significantly higher in the supplemented group than in the control group.[33] Although NEC resembles IBD microscopically, the histopathologic response may result from different mechanisms. Additionally, the apparent protective role of arginine in NEC may not be related to NO production but to other functions of arginine, such as the production of glutamine, nucleotides, and polyamines. Thus, the role of NO and its production from cNOS or iNOS is not completely defined because it appears that NO has both anti- and proinflammatory effects.

Human clinical trials have evaluated arginine supplementation in healthy adults and healthy adult surgical patients. Dietary supplementation in these patients increased lymphocytic immune response and increased exhaled NO.[34] Many clinical trials using arginine supplementation in adult patients at risk of intestinal stress because of trauma, cancer, or critical illnesses have been reported.[16] It is difficult to compare results owing to the variety of other nutrients supplied in these so-called "immune-enhancing" formulations. Two meta-analyses comparing commercially available formulas

have been published. An analysis of 12 trials with a total of 1,557 patients[35] and 22 trials among 2,419 patients[36] showed a decrease in the length of hospitalization, duration of ventilator support, and lower overall infection rates in patients receiving an arginine-supplemented diet. The effect on mortality was not clear. In planned subgroup analysis, patients who were undergoing elective surgery benefited more than did critically ill patients. These studies suggest that the use of arginine-supplemented formulas may be beneficial for discreet patient conditions such as surgical or cancer patients. Further studies are needed to ensure that there is no detrimental effect from such formulas in septic patients or solid organ transplant recipients. Although several supplemented formulas designed to enhance immune function are commercially available (see Table 75.6-1), indiscriminate use of these formulas is not justified.

LONG-CHAIN PUFAS

Long-chain PUFAs are essential for membrane structure, fluidity, and function, specifically in neural and retinal tissues. PUFAs are precursors for the production of prostaglandins, prostacyclins, thromboxanes, and leukotrienes. PUFAs are divided into two broad classes: ω-3 and ω-6 fatty acids. Omega-3 fatty acids are represented by α-linolenic acid (ALA), whereas ω-6 fatty acids are represented by linoleic acid. This nomenclature refers to the location of the first double bond from the methyl terminus. Mammals are unable to insert double bonds into fatty acid molecules closer than the 9 position and thus are dependent on dietary consumption of these essential fatty acids (EFAs). Linoleic acid is found abundantly in the seeds of most plants and is especially high in corn, safflower, and soy oils but is present in only limited amounts in coconut, cocoa, and palm oils. ALA is found in soy and canola oils, as well as marine plants, algae, and phytoplankton. ALA is transformed in the marine food chain, and its derivatives are present in large quantities in some fish oils. Omega-6 fatty acids are the most abundant PUFAs in the Western diet. It is estimated that over the last 100 years, the ratio of consumption between ω-6 and ω-3 PUFAs has changed from 1 to 20:1. This change is associated with the increased use of vegetable oil and changes in agriculture practices. Domestic beef are fed grain rich in ω-6 fatty acids, and modern aquaculture produces fish that contain less ω-3 fatty acids than fish grown in the wild.[37]

Animal cells modify these EFAs into long-chain PUFAs by elongation, desaturation, and β-oxidation. Linoleic acid is modified to arachidonic acid (AA). ALA is modified to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Premature infants may be limited in their ability to make EPA and DHA from ALA,[38] although quantitative data are very limited. Importantly, ω-6 long-chain PUFA derivatives induce a stronger inflammatory response than ω-3 fatty acid derivatives.

The nutritional importance of specific fatty acids was first reported in weanling rats fed a fat-free diet. These animals developed scaly skin, tail necrosis, impaired fertility and growth retardation, which was reversed or prevented with the addition of either linoleic acid or ALA to their diet.[39] EFAs were initially considered of marginal importance for humans. Infants fed either a fat-free milk–based formula or lipid-free parenteral nutrition developed growth failure and dry thickened skin, which was primarily associated with linoleic acid. ALA deficiency resulted in more subtle clinical symptoms affecting neurodevelopment, visual function, and a peripheral neuropathy.[40]

EFA concentrations in breast milk vary with diet. In breast milk from women in most Western countries, the ratio of AA to DHA is 2:1, whereas in women in Asian or fish-eating communities, the ratio is 1:1 or lower.[37] Determination of the optimal ratio of EFAs awaits further studies. Although the addition of PUFAs to commercially available formulas (Table 75.6-2) may better mimic human breast milk and increase measured fatty acids in plasma and erythrocyte membrane phospholipids, the long-term functional significance of these findings is presently unclear.

Laboratory studies on animal models and clinical trials in human adults suffering from IBD have investigated the immunomodulatory effects of ω-3 PUFAs. The conflicting results in reducing inflammation, prolonging remission, or decreasing steroid requirements are likely due to the multifactorial nature of the disease and the complexity of the immune system.[38] Clinical studies vary in the disease type (Crohn disease or ulcerative colitis), disease location and activity, and the composition and dose of fatty acids supplied. The role of ω-3 fatty acids for the treatment of IBD has yet to be defined.

The role of PUFAs as a protective nutrient in NEC has been investigated in a hypoxia-induced mouse model of NEC. Mice fed a diet deficient in ω-3 fatty acids developed more severe ischemia and had increased levels of platelet activating factor and leukotriene B_4 than did control animals.[41] In a human clinical trial, preterm infants were fed a diet supplemented with egg phospholipids, which contain increased levels of esterified choline, AA, and DHA. When compared with infants fed a nonsupplemented formula, there was significantly less stage II and III NEC.[42] The advantages of routine supplementation for gastrointestinal protection are not clearly defined for preterm and term infants. Special conditions such as NEC and IBD warrant further carefully designed studies.

TABLE 75.6-2 PEDIATRIC FORMULAS CONTAINING POLYUNSATURATED FATTY ACIDS VERSUS HUMAN MILK*

POLYUNSATURATED FATTY ACID	HUMAN MILK	ENFAMIL LIPIL	SIMILAC ADVANCE	PREMATURE ENFAMIL LIPIL
Docosahexaenoic acid	0.15–0.3	0.32	0.15	0.33
Arachidonic acid	0.5–0.6	0.64	0.40	0.67

*Numbers represent percentage of fatty acid content.

NUCLEOTIDES

Nucleotides are nonprotein nitrogen compounds composed of a nitrogenous base, a pentose sugar, and one or more phosphate groups. Nucleotides serve as nucleic acid precursors, physiologic mediators, components of coenzymes, and sources of cellular energy and participate in immunity. Nucleotides can be synthesized de novo, but the process is metabolically costly. An alternative mechanism is the salvage pathway, where preformed nitrogen bases or nucleosides are converted to nucleotides. Nucleotides have been postulated to be conditionally essential during severe malnutrition, rapid cellular growth, or severe disease states. Therefore, nucleotides may also be conditionally essential in conditions such as prematurity, small for gestational age infants, severe diarrhea, short-gut syndrome, NEC, intestinal transplant, or IBD.

Much of the interest in nucleotides arose from the observation that the nucleotide content of human breast milk is significantly higher than that of cow's milk protein formulas. The nonprotein nitrogen content of human milk is approximately 25 to 30% of the total nitrogen content. Nucleotides comprise 2 to 5% of the nonprotein nitrogen. In contrast, nonprotein nitrogen accounts for only 2% of the nitrogen content of cow's milk.[43] Additionally, the nucleotide profile differs in cow's milk compared with human milk, with a lower proportion of cytidine and adenosine derivatives in cow's milk.

Beneficial effects of dietary nucleotides have been demonstrated in several animal models. Dietary nucleotides have been associated with increased mucosal deoxyribonucleic acid (DNA), protein synthesis, villus height, enterocyte proliferation and maturation, and reduced bacterial translocation during recovery from malnutrition.[44–46] Dietary nucleotides promote healing of small bowel ulcers in experimental ulcerative ileitis, decrease the inflammatory response to ischemia and reperfusion, and improve morphologic development of small intestinal mucosa after transplant.[47–49]

The beneficial effects of supplemental dietary nucleotides in humans are less certain. Initial reports suggested that infants fed a nucleotide-supplemented formula (NSF) had an increase in stool bifidobacteria and enterobacteria that mimicked human milk–fed infant stool microflora.[50] In contrast, a more recent study reported that infants fed NSF had reduced amounts of bifidobacteria and enterococci and increased amounts of *Bacteroides* and *Escherichia coli* when compared with human milk–fed infants.[51] Increased postprandial mesenteric artery blood flow has been demonstrated in both term and preterm infants following NSF formula compared with either human milk–fed or nonsupplemented formula-fed infants.[52] Infants fed NSF had an increased antibody response to *Haemophilus influenzae* type b and diphtheria but not to tetanus or oral polio vaccine.[53]

NSF first became available in Japan in 1965 and in the United States in 1989.[53] Currently, most infant formulas contain supplemental nucleotides. To date, no deleterious effects have been reported. The European Commission's Scientific Committee for Food has provided several published guidelines for nucleotide supplementation suggesting that the total nucleotide concentration should be less than 1.2 mg/100 J, which is the same order of magnitude as the free nucleotides in human milk. The latest recommendations from the United States suggest a maximum of 5 mg/100 J for term infant formula and 7 mg/100 J for preterm infant formula.[54] Human milk is considered to be the gold standard for infant nutrition. Thus, the rationale for nucleotide supplementation of infant formula is to mimic the composition of human milk. Because the role of human milk nucleotides for breastfed infants is not known, the indications for nucleotide supplementation of infant formula and the optimal amounts remain somewhat controversial.[55]

ZINC

As an essential trace element, zinc is second only to iron in its abundance in the human body and has a very broad range of functions. For example, approximately 80% of total-body iron in the human is localized to the erythrocyte mass alone, whereas similar total amounts of zinc are spread among thousands of proteins.[56] Zinc functions as a cofactor to more than 100 metalloenzymes involved in DNA synthesis and repair, cellular integrity, bone and liver metabolism, and multiple dehydrogenase and carboxypeptidase reactions.[57] As a component of the zinc finger transcription factors, zinc is critical to both transcription and regulation of gene expression and to cell differentiation and proliferation in rapidly turning over tissues such as the bone marrow, thymus, and GI tract. Acrodermatitis enteropathica is a congenital defect of zinc absorption and transport and represents the prototype of severe zinc deficiency. Affected infants have a characteristic erythematous, vesiculobullous, pustular rash, which is most prominent around the orifices, along with mucous membrane damage, alopecia, nail loss, growth retardation, diarrhea, and recurrent infections. Acquired zinc deficiency is often of mild to moderate severity and typically results from inadequate intake of bioavailable zinc or from excessive losses. Mild zinc deficiency is associated with growth impairment, anorexia, and immune dysfunction, with impairment of both T- and B-cell function in animal models and pediatric populations. Zinc deficiency has been associated with a wide range of malabsorptive conditions, including protein-energy malnutrition, short-gut syndrome, celiac disease, cystic fibrosis, or IBD. Zinc deficiency is associated with decreased resistance to parasitic, fungal, and viral infections.[58] Complicating the assessment of zinc status in disease conditions are the lack of a sensitive biomarker of zinc status and the hypozincemia resulting from redistribution of circulating zinc in conjunction with the acute-phase response. These realities result in dependence on response to carefully controlled zinc supplementation trials to demonstrate preexisting zinc deficiency.[59]

Consideration of the role of zinc as a protective nutrient for the GI tract is particularly complex owing to the circular relationship between zinc deficiency, which causes diarrhea, and diarrhea, which may cause zinc deficiency. The critical role of the GI tract in normal zinc homeostasis is well established, both in terms of absorption of dietary

zinc and modulation of secretion and reabsorption of endogenous intestinal zinc. Thus, pathologic conditions of the GI tract predictably perturb zinc homeostasis and predispose the individual to zinc deficiency, possibly by morphologic changes that alter transit, permeability, and/or absorptive surface, thereby interfering with absorption of exogenous zinc and reabsorption of endogenous zinc. On the other hand, zinc deficiency is associated with induction of proteins that may increase fluid and possibly endogenous zinc secretion into the GI tract. Candidate proteins that have been proposed include uroguanylin and iNOS, both of which reportedly have increased expression during zinc deficiency. The effects of zinc deficiency on the immune system or on the integrity of the mucosal surface may also have adverse effects on the GI tract and predispose the individual to diarrhea.[60]

Regardless of the questions remaining about mechanisms, there is now little doubt that zinc supplementation in vulnerable populations reduces the severity of diarrhea. In a pooled analysis of randomized blinded controlled zinc supplementation trials conducted in developing countries, infants and children who received zinc supplements had a significant reduction in the length of an episode of acute diarrhea, a decreased probability of developing chronic diarrhea, and a lower probability of associated morbidity compared with children who did not receive supplemental zinc.[61] Although the dietary intake and the nutritional status of the subjects in these studies were generally not characterized in detail, they were presumed to have a high prevalence of zinc deficiency, and the positive response was interpreted to be due to correction of the deficiency rather than a pharmacologic effect of the supplement. Since this pooled analysis, similar findings have been reported in other settings of poverty and high infectious burden.[62,63] Ongoing studies are also investigating the role of zinc-supplemented oral rehydration formulas for the treatment of acute diarrhea in developing countries.[16]

Among the most urgent remaining issues are identification of the populations at most risk for zinc deficiency and therefore to be targeted for interventions; the optimal doses, modes, and schedules of supplemental and therapeutic zinc; and, ultimately, identification of sustainable and culturally appropriate strategies to reduce the prevalence of zinc deficiency through changes in dietary practices. In addition to the exigency for eradication of zinc deficiency on a global basis, there is also substantial need for investigations to clarify the potential contribution of perturbations in zinc homeostasis to some of the clinical manifestations of other GI diseases, including especially those with chronic inflammatory components. Investigations to better characterize mechanisms of altered GI function in relation to zinc metabolism and homeostasis will be especially important to optimally define the role of zinc in therapy.

PREBIOTICS

Prebiotics are nondigestible food ingredients that promote the growth and/or activity of natural or supplemented beneficial bacteria residing in the colon, thus enhancing the health of the host (see Chapter 77.2, "The Pediatric Ostomy").[64] Criteria for a classification as a prebiotic are as follows: the substance should not be hydrolyzed or absorbed in the upper GI tract; it is selectively beneficial for growth of commensual bacteria in the colon; and it alters the flora to a healthy balance by inducing favorable luminal or systemic effects in the host.[65] Prebiotics have many of the same attributes of dietary fiber, with an added benefit for selective colonic bacteria, and include short-chain carbohydrates such as inulin and fructo-oligosaccharides (FOSs), soybean oligosaccharides, and galacto-oligosaccharides (GOSs).[65] The putative nutritional and physiologic effects of prebiotics include alteration of the composition of colonic flora, effects on bowel function, enhanced bioavailability of minerals (especially calcium), and possibly beneficial effects on lipid metabolism and on the risk of colon cancer.[66]

The fructans, which include inulin and FOS, have been extensively studied and are naturally present in more than 36,000 plant species as storage carbohydrates.[65] The most popular natural sources of these indigestible ingredients include wheat, onion, bananas, garlic, tomatoes, and chicory. In addition to their natural availability in foods, prebiotics have been used in food manufacturing for calorie reduction. Used as a replacement for fat and sugar in foods such as ice cream, dairy products, confections, and baked goods, they not only add fiber but also maintain a creamy, fat-like feel in the mouth of the consumer.[16]

GOSs are among numerous disaccharides primarily found in human milk,[67] and it seems likely that they survive digestion in the GI tract of the human infant.[68–70] Colonic bifidobacteria possess an enzyme that facilitates preferential use of the oligosaccharide and thereby allow a prebiotic effect of the GOSs. The GOSs thus comprise one of the components of breast milk that facilitates the preferential growth of *Bifidobacterium* and *Lactobacillus* in the colon[67] and may also provide anti-inflammatory effects in the intestine.[71] These protective colonic bacteria ferment the prebiotic substance to release lactic and acetic acid and thus create a desirable acidic environment. There is intense interest in the potential for addition of oligosaccharides to infant formula to provide some of the protective function of human milk. One randomized, double-blinded trial comparing an infant formula containing a mixture of GOSs and FOSs to a similar standard formula without these additives found a higher proportion of bifidobacteria in the infants' stools after 6 weeks of consuming the supplemented formula.[72]

There are few studies of prebiotic therapeutic efficacy in children. A double-blind, randomized, controlled study compared the use of oligofructose-supplemented formula and unsupplemented formula among 123 nonbreastfed infants. The supplemented formula group had a decrease in both the severity and duration of acute diarrhea episodes.[73] A large-scale community-based randomized controlled trial of oligofructose-supplemented infant cereal conducted in infants from a shantytown near Lima, Peru, was not associated with any benefit on the prevalence of

diarrhea, use of health care resources, or response to immunization. The Peruvian infants were between 6 and 12 months of age and were receiving both human milk and complementary foods. The authors concluded that the effects of breastfeeding may mitigate the potential benefit of prebiotic supplementation.[74] Thus, despite the purported benefits of prebiotic consumption, large-scale clinical studies demonstrating the beneficial effects of prebiotics in children are currently lacking.[67] This is likely to remain an area of strong clinical interest, however, because of the potential positive effects on GI health and function.

TRANSFORMING GROWTH FACTOR-β

TGF-β has been shown to antagonize certain proinflammatory cytokines, including interferon-γ, tumor necrosis factor-α, and interleukin-2.[75] Many different authors have proposed that the intestinal mucosa is under a state of "controlled inflammation" owing to exposure to a large variety of antigens.[76] Interferon-γ is known to stimulate the expression of major histocompatibility (MHC) class II proteins on the surfaces of cells. This expression attracts inflammatory mediators to the location of the MHC class II proteins within the small intestine, resulting in inflammation. Enteral formulas that contain certain bioactive molecules may be therapeutic by stimulating the maturation and differentiation of cells and by modulating the immune and inflammatory responses.[75]

Enteral nutrition may pose fewer potential side effects than the current medication treatments used for IBD. Formulas that include bioactive molecules such as TGF-β have been developed for this purpose. In an uncontrolled study, 29 children with active Crohn disease received a TGF-β–supplemented formula. Clinical remission was achieved in 79% of children after 8 weeks of therapy. There was a reduction of interferon-γ and interleukin-8 messenger ribonucleic acid, suggesting an immunologic downregulation.[76] Randomized controlled clinical trials are needed to confirm these findings and to support the routine use of TGF-β–supplemented formulas as a primary treatment for IBD.

SUMMARY

This chapter summarized some of the published animal and human studies evaluating various protective nutrients and substances in relation to maintaining health and function in the GI tract. Some nutrients seem promising for specific disease states, whereas others appear safe but have minimal clinical impact. Despite great strides in medical molecular biology over the last decade, particularly in genomics and proteomics, the ability to detect clinically important changes in GI function remains limited. Clinical trials are often poorly designed and suffer from inadequate numbers and a lack of appropriate control population. Despite the relative lack of controlled studies and questionable clinical efficacy, commercial products containing "protective nutrients" are actively marketed. Indiscriminate use of these products is not warranted. Large carefully designed randomized multicenter studies are needed to further investigate the health claims of these products and to better quantify their potential for primary or adjunctive clinical treatments.

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CHAPTER 76

DRUG THERAPY

1. *Immunosuppressive Therapies*

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Significant progress continues to occur in the medical treatment of autoimmune diseases and graft rejection. Drugs previously studied in clinical trials (eg, mycophenolate, infliximab, sirolimus) have now received regulatory approval from the US Food and Drug Administration (FDA) and are being increasingly used in the treatment of a variety of autoimmune diseases and transplant rejection. Genotyping and blood level metabolite monitoring of 6-mercaptopurine (6-MP) and azathioprine now enable the clinician to improve efficacy and decrease the risk of side effects such as pancytopenia. In addition, basic scientists have continued to elucidate the cellular and molecular mechanisms of T-lymphocyte activation, graft rejection, and tolerance to foreign antigens.

There are three principal uses of immunosuppressive therapies in medicine: prevention of organ transplant rejection, treatment of diseases with a presumed autoimmune etiology, and treatment of graft-versus-host disease. In the case of organ rejection, the primary goal of immunosuppression is to abolish the normal host response against foreign tissues. In contrast, the treatment of autoimmune diseases involves the control of an aberrant immune response that has developed against the host's own tissues.

This chapter first reviews components of the immune response that are potential targets for immunosuppressive therapies. The second portion of the chapter reviews currently available and commonly used immunosuppressive agents. The emphasis is on the mechanisms of action and the pharmacology of these agents. Clinical uses in pediatric gastrointestinal disease are discussed in a limited manner. For further information regarding the therapy of specific diseases, the reader is referred to other chapters within this textbook. Lastly, novel immunotherapies not currently in widespread use are considered.

COMPONENTS OF THE IMMUNE RESPONSE

Immunosuppressive medications block both "classic" immune responses (ie, the response of the immune system to a foreign pathogen or self-antigen) and "nonclassic" immune responses (ie, those seen in rejection, the response of the host's immune system to an allogeneic graft). Classic immune responses are characterized by major histocompatibility complex (MHC)-associated antigen presentation (Figure 76.1-1): CD4 T lymphocytes (which include most T helper [Th] cells) recognize peptides presented by antigen-presenting cells (APCs) expressing MHC class II molecules, whereas CD8 T lymphocytes (which include most suppressor and cytotoxic T cells) recognize antigenic peptides in association with MHC class I expressing cells.[1] In contrast, transplant rejection involves alternative immune responses, including the attack of graft tissue and endothelium by preformed immunoglobulin (Ig)G antibodies (hyperacute rejection), attack of donor tissues by CD4 and CD8 T cells (acute rejection), and the stimulation of fibrosis by leukocytes producing fibrogenic cytokines (chronic rejection). The modes of T-lymphocyte activation also vary in rejection and may include antigen presentation by APCs of the donor graft, and direct recognition by T lymphocytes of MHC molecules without antigen.[2] In general, the magnitude of the immunologic response observed in rejection (allogeneic response) is much greater than that seen in an immune response to microbial infections.

COMPONENTS OF THE CLASSIC IMMUNE RESPONSE

Antigen Uptake and Delivery. An immune response is a humoral and cellular response to either a self- or a foreign antigen, with most antigens being protein fragments (peptides), proteins, or polysaccharides. The classic immune response is summarized in Table 76.1-1 and described in the following subsections. Foreign antigens, including bacteria and viruses, inhaled antigens (pollen), or ingested antigens (foods or toxins), can be prevented from eliciting an immune response by the barrier function of mucosal epithelia. In the digestive tract, gastric acid and pancreatic enzymes degrade bacterial and food antigens,

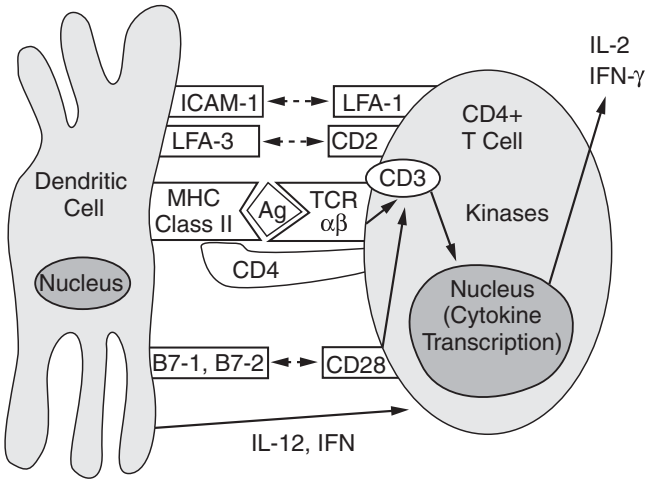

FIGURE 76.1-1 Molecular interactions involved acquired immunity: antigen presentation and helper (CD4) T-cell activation. An antigen-presenting cell (APC; eg, a dendritic cell) endocytoses a dietary or bacterial antigen, digests the protein via endosomes into smaller peptides, and binds the antigen (Ag) to major histocompatibility complex (MHC) class II molecules. The MHC-Ag complex travels to the surface of the APC. Here the MHC-Ag complex binds to the CD4 T cell via the T-cell receptor (TCR). The CD4 molecule strengthens the APC–T-cell interaction by binding to MHC class II. Additional binding occurs by the association of accessory molecules on the APC and T cell, respectively, including intercellular adhesion molecule 1 (ICAM-1)–lymphocyte function–associated antigen (LFA-1), LFA-3–CD2, and B7–CD28. Additional cytokines released by the dendritic cell may determine which cytokines the T cell produces (eg, secretion of interleukin [IL]-12 and interferon [IFN] may promote a T helper 1 cytokine [IL-2, IFN-γ] pattern). The MHC-TCR reaction is the first signal initiating T-cell activation, and B7-CD28 costimulation provides the second signal. If these two events occur, cell signaling via the T cell–CD3 complex and ζ chain occurs (see Figure 76.1-2), resulting in phosphorylation of kinases, cytokine gene transcription through nuclear factor κB, and cytokine synthesis and release.

and intestinal mucin and epithelial cells physically block the passage of antigen. In addition to a simple barrier function, intestinal epithelial cells digest pathogens via intracellular lysosomes and secrete chemokines such as interleukin (IL)-8, which, in turn, recruit polymorphonuclear leukocytes and other cells of the immune system.[3,4] The barrier function of human mucosal epithelia is augmented by secretory IgA, by the cytotoxic properties of intraepithelial lymphocytes, and by the secretion of antimicrobial cryptdins by Paneth cells.[5,6] Both transplanted organs and tissues attacked in autoimmune disease are already located within the host. Therefore, prevention of antigen passage across epithelia will not ameliorate the immune response seen in these disorders.

Antigen Processing and Presentation. The two principal classes of mature T lymphocytes are CD4 cells (which help recruit and activate calls that kill extracellular pathogens) and CD8 cells (which kill cells infected with intracellular pathogens). Helper T cells of the CD4 phenotype are essential in stimulating the inflammatory response to antigen, regulating antibody production, stimulating cytotoxic (CD8) T-cell responses, and recruiting effector cells (such as cytotoxic T cells, neutrophils, and eosinophils). However, CD4 T lymphocytes do not directly bind or respond to antigen unless the antigen is bound to the surface of an APC. APCs are characterized by their ability to phagocytose proteins or peptides, degrade them intracellularly, complex these peptides with proteins of the MHC class II (human leukocyte antigen [HLA] molecules -DP, -DQ, and -DR), and transport these proteins in association with MHC class II molecules to the cell surface of the APC.

The "professional" APC of the body is the dendritic cell. Dendritic cells endocytose antigen and present to T cells via MHC class II molecules (adaptive immunity; see below) and also recognize bacterial constituents (eg, lipopolysaccharide) via either Toll-like receptors present on the plasma membrane or caspase-recruitment domain/nucleotide-binding oligomerization domain receptors in the cell cytoplasm (innate immunity).[7,8] In either case, a dendritic cell stimulated with antigen will express increased levels of costimulatory molecules on the cell surface and also produce cytokines that down-regulate or up-regulate inflammation. The dendritic cell is perceived as a gatekeeper cell, which determines whether an antigen generates an immune response or tolerance (ie, the absence of an immune response).[7] In addition to dendritic cells, other APCs include B lymphocytes, monocytes, and macrophages.[1]

Once ingested, antigen is degraded by proteases in intracellular vesicles termed endosomes. Simultaneously, class II molecules manufactured in the endoplasmic reticulum are transported into the endosomes in association with a transport protein called the invariant chain. Once inside the endosome, invariant chain is cleaved, the MHC class II molecule binds to the antigenic peptide, and the MHCII-peptide complex is transported to the surface of the dendritic or other APC.[9]

Once the antigen-MHC complex has been transported to the surface of the APC, the complex then interacts with the T-cell receptor (TCR) on the surface of the CD4 Th cell (see Figure 76.1-1). The principal component of the TCR is a heterodimeric complex consisting of two covalently linked chains (either the α/β chain or the γ/δ chain). The other components of the TCR complex are the CD3 molecule and the TCR ζ protein; these two proteins mediate intracellular signaling by activation of tyrosine kinases. Binding between the T cell and the APC is strengthened by accessory molecules on the surface of the T cell (eg, CD4, CD28, leukocyte

TABLE 76.1-1 COMPONENTS OF THE IMMUNE RESPONSE

Antigen uptake across mucosal surfaces
Antigen processing
Antigen presentation to T cells
T-lymphocyte activation
B-cell activation and immunoglobulin production
Leukocyte homing and adhesion to tissues
Effector cell recruitment
Cytokine and chemokine production
Release of inflammatory mediators (eg, prostaglandin, leukotriene, complement)

function-associated antigen [LFA]-1, and CD2), which bind to corresponding ligands on the surface of the APC (class II MHC, B7, intercellular adhesin molecule 1 [ICAM-1], and LFA-3, respectively; see Figure 76.1-1).[10,11]

To initiate intracellular events that result in CD4 T-lymphocyte activation, at least two binding events (signals) must occur between the molecules on the surface of the APC and the T cell. The first signal is initiated by binding between the peptide-MHC complex on the APC and the TCR-CD4-CD3 complex on the T cell (Figure 76.1-2). Antigen binding to the TCR initiates a cellular signal transmitted through the CD3 molecular complex to intracellular tyrosine kinases. However, stimulation of a T cell by this signal alone does not induce an immune response and may even result in anergy (ie, the absence of an immune response). The second signal in T-cell activation is a co-

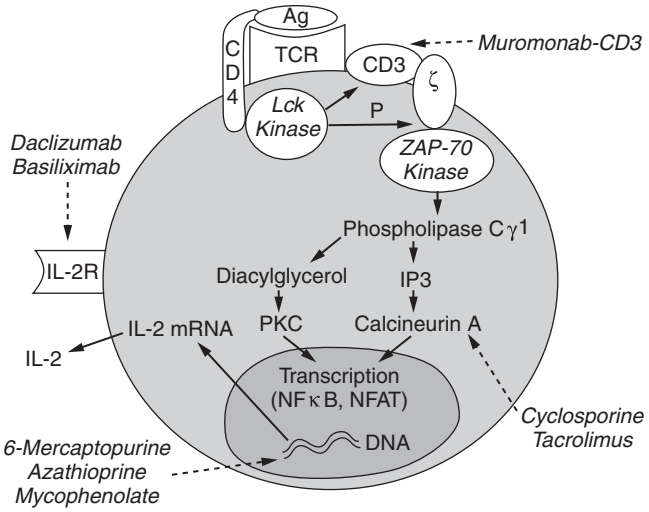

FIGURE 76.1-2 Signal transduction pathways involved in T-lymphocyte activation and sites of action of immunosuppressive therapies. Binding of antigen (Ag) (in association with major histocompatibility complex proteins) to the T-cell receptor (TCR)-CD4-CD3 complex activates intracellular tyrosine kinases. The CD4 molecule is closely associated with the lymphocyte-specific protein tyrosine kinase (Lck), which, in turn, phosphorylates intracellular portions of the CD3 molecule and the ζ chain. The ζ chain, in turn, activates the ZAP-70 kinase, which results in the activation of phospholipase Cγ1. The activated phospholipase converts inositol biphosphate into inositol 1,4,5-triphosphate (IP_3), which, in turn, activates calcineurin A in the cytosol. Calcineurin A is essential in dephosphorylating transcription factors such as nuclear factor κB (NFκB) and nuclear factor of activated T cell (NFAT), which allows the factors to travel to the nucleus. A second pathway activated by phospholipase C and important in promoting transcription involves diacylglycerol and protein kinase C (PKC). The end result is increased intranuclear transcription factor activity, deoxyribonucleic acid (DNA) synthesis, and cytokine (eg, interleukin [IL]-2) production. Cyclosporine and tacrolimus inactivate calcineurin A and inhibit cytokine transcription. Azathioprine, 6-mercaptopurine, and mycophenolate inhibit DNA synthesis. Muromonab-CD3 (OKT3) is a monoclonal antibody that binds to the CD3 molecule, resulting in removal of T lymphocytes from the circulation in vivo. In contrast, the antibodies daclizumab and basiliximab bind to IL-2 receptors (IL-2R) that are present only on activated T lymphocytes. mRNA = messenger ribonucleic acid.

stimulatory signal provided by other APC–T-cell membrane interactions. Although a number of costimulatory signals have been identified, the most important is the interaction between B7 (CD80 or CD86) on the APC with CD28 on the T cell. This interaction promotes T-cell differentiation and cytokine secretion. Additional events include the binding of CD40 on APCs with CD40 ligand on T cells, which may serve to increase the expression of B7 molecules on the APC surface. Cell-cell adhesion is strengthened by binding between ICAM-1 (CD54) on the APC with LFA-1 (CD11a/CD18) on the T cell.[12,13]

T-Lymphocyte Activation. If antigenic stimulation and costimulation occur, a signal is transduced through the CD3 complex, characterized by phosphorylation of tyrosine molecules in the CD3 and ζ chains (see Figure 76.1-2).[14] Subsequently, tyrosine kinases, including lymphocyte protein tyrosine kinase (Lck) and ζ-associated 70 (ZAP-70), are activated and induce phosphorylation of phospholipase Cγ1, which, in turn, converts inositol 4,5-biphosphate to inositol 1,4,5-triphosphate (IP_3).[13,15] IP_3 formation results in increased cytosolic free calcium from intracellular stores and activation of the molecule calcineurin.

A second intracellular signal transduction pathway initiated by phospholipase Cγ1 involves the molecules diacylglycerol and protein kinase C (see Figure 76.1-2).[16,17] These pathways are separate but synergistic, and inhibition of one or the other may abrogate T-cell activation. Calcineurin and protein kinase C enzymes, in turn, promote increased transcription of cytokine gene products mediated by nuclear binding factors, including nuclear factor of activated T cell (NFAT) and nuclear factor κB (NFκB). A third T-cell activation pathway triggered by antigen recognition involves a group of kinases termed mitogen-activated protein (MAP) kinases, which, in turn, activate the transcription factor activator protein 1(AP-1).[18]

Activation of T cells is characterized by increased T-cell deoxyribonucleic acid (DNA) synthesis and proliferation, increased protein synthesis, increased production of cytokines, and increased production of cytokine receptors. Cell membrane proteins (activation markers) present on the surface of activated T cells include the IL-2 receptor CD154 (ie, CD40 ligand, a molecule that promotes B-cell differentiation), CD45RO (a marker for memory T cells), trace amine receptor 1, and signalling lymphocyte activation molecule (a transmembrane receptor that, when activated, induces interferon [IFN]-γ production).[17,19] In addition, activated T cells express a ligand for the protein Fas. Binding of Fas to Fas ligand results in caspase activation and T-cell apoptosis, thereby helping to clear kill activated T cells that have been repeatedly stimulated by antigen.[20] Of importance in immunosuppression, the molecules cyclosporine and tacrolimus (FK506) both inhibit T-lymphocyte activation by decreasing the activity of calcineurin and intranuclear levels of NFAT (see Figure 76.1-2).[21]

Based on studies performed with murine T-lymphocyte clones, Th (CD4) lymphocytes have been categorized into two broad types. Th1 cells promote cellular immune responses and delayed-type hypersensitivity by secreting

IL-2, IFN-γ, and tumor necrosis factor (TNF)-β, whereas Th2 cells promote humoral responses by secreting IL-4, -5, -10, and -13.[22] IL-4, -5, and -13, in turn, promote B-lymphocyte differentiation into plasma cells and antibody synthesis. Both Th1 and Th2 T-cell subsets develop from naive CD4 cells, depending on the type of antigens processed by dendritic cells or macrophages. A Th1 cytokine response promotes macrophage activation with the aim of eliminating intracellular microbes, whereas a Th2 response results in mast cell activation, clearing of parasites, and allergic reactions.[23] Two other groups of regulatory T-cell subsets, Th3 and CD4+CD25+ cells, decrease inflammation and promote tolerance by secreting anti-inflammatory cytokines, such as transforming growth factor-β (TGF-β) and IL-10.[23] Th1 cells are implicated in the pathogenesis of Crohn disease, whereas Th2 cells have been implicated in the pathogenesis of ulcerative colitis and allergic disorders.

Development of Humoral Immunity. In contrast to T lymphocytes, which require processed antigen presented through peptide and MHC, B lymphocytes recognize protein antigen via Ig molecules directly on the cell surface (Figure 76.1-3). Mature B lymphocytes with surface Ig are present in the peripheral blood, spleen, lymph nodes, and lymphoid follicles. Surface Ig on the B-cell membrane binds antigen and provides an initial stimulus for B-cell lymphocyte activation. The antigen–surface Ig complex results in tyrosine phosphorylation, activation of downstream kinases and phospholipase C, and activation of transcription factors. If an activated B cell is to further differentiate into an Ig-producing plasma cell, it requires both physical contact with a T-cell membrane and stimulation by exogenous cytokines produced by T cells (see Figure 76.1-3). IL-2, -4, and -5 promote B-cell proliferation and differentiation, whereas IL-6 perpetuates proliferation of antibody-secreting B cells.[24]

All B cells are initially programmed to synthesize IgM. For a B cell to switch its class of antibody produced to IgG or IgA (isotype switching), several other molecular stimuli need to occur. The CD40 ligand (glycoprotein 39, CD154) is a molecule on the surface of the T cell that binds to CD40 on B cells. This interaction promotes B-cell activation and differentiation and isotype switching from IgM to IgG, IgA, or IgE (see Figure 76.1-3). Conversely, the CD40-CD154 interaction also promotes activation of CD4 T cells. Deficiency of this molecule results in an unusual form of immunodeficiency, termed the hyper-IgM syndrome.[25] Cytokines such as IL-4 are responsible in switching B cells from IgM to IgE production, and TGF-β has been shown to play a role in B-cell switching to IgA production.[26]

Homing and Adhesion. Immune cells that react to a tissue protein must leave the systemic circulation and bind to the tissue or region where they exert their effects. A large number of molecules mediate adhesion between the circulating lymphocytes and the vascular endothelium, extracellular matrix proteins, and tissues. These molecules have been divided into three superfamilies based on their protein structure: the integrin superfamily (including the molecules very late activation [VLA]-1 through VLA-6 and LFA-1), the Ig superfamily (including the molecule ICAM-1), and the selectin superfamily (including the molecules L-selectin and E-selectin).[27,28] Among the more important interactions mediated by adhesion molecules, the β_1 integrin VLA-4 binds the protein vascular cell adhesion molecule 1 (VCAM-1) on the surface of activated endothelial cells. This reaction is blocked by the monoclonal antibody natalizumab (see section entitled "Natalizumab [Antegren]"). The selectin family of molecules, including L-selectin and E-selectin, also promotes binding of lymphocytes to vascular endothelial cells.[27] Soluble cytokines and chemokines promote stronger cell adhesion by increasing the expression of integrins on the cell surface.[29]

Cytokine and Chemokine Production. Activated cells of the immune system, including macrophages, monocytes, and B and T lymphocytes, produce a large number of multifunctional cytokines and chemokines. These can be functionally categorized into Th1-type cytokines, which stimulate cell-mediated immunity and cytotoxicity, and Th2-type cytokines, which stimulate humoral immunity and allergic responses.[30,31] These molecules promote activation of cells of the immune system, recruitment of effector

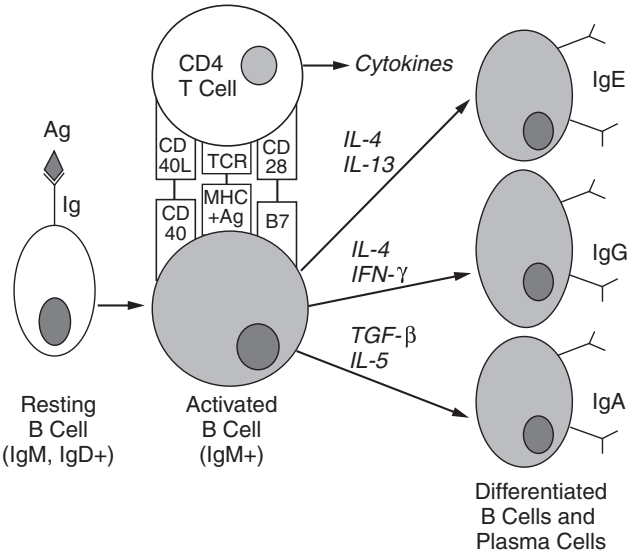

FIGURE 76.1-3 B-cell differentiation and the role of T helper cells. For a resting B cell to differentiate into an antibody-producing plasma cell, three steps are necessary. The first step involves binding of antigen (Ag) onto immunoglobulin (Ig) molecules on the surface of the B cell, which provides an initial signal for B-cell activation. The second step involves physical contact with a T helper lymphocyte, which further activates both the B and the T cell. The three major molecular interactions mediating the B- and T-cell contact involve the CD40-CD40 ligand, major histocompatibility complex (MHC) + antigen with the T-cell receptor (TCR), and B7-CD28. This physical contact promotes B-cell proliferation and differentiation. The third step in B-cell differentiation involves cytokine stimulation. The activated T cell may produce different cytokines, which promote Ig class switching (isotype switching). Differentiation into IgE-producing B cells and plasma cells are promoted by interleukin (IL)-4 and IL-13, IgG-producing B cells are promoted by IL-4 and interferon-γ (IFN-γ), and IgA-producing B cells are promoted by transforming growth factor-β (TGF-β) and IL-5.

cells such as neutrophils and eosinophils, and the production of acute-phase reactants by the liver. Many cytokines have both proinflammatory and anti-inflammatory effects. For example, IL-2 promotes differentiation of Th1 cells, which, in turn, mediate macrophage activation. However, IL-2 can also have anti-inflammatory effects, such as promoting lymphocyte apoptosis and increasing the population of CD4+CD25+ (suppressor type) T cells.[32] Cytokines such as IL-1 and TNF-α mediate the clinical effects (including fever, diarrhea, and hypotension) seen in rejection, shock, and sepsis.[33] IL-5 recruits eosinophils, whereas IFN-γ activates macrophages to phagocytose and kill microbes. Regulatory T lymphocytes release molecules that inhibit inflammation, including IL-10 and TGF-β.[34] Oral tolerance to an antigen develops when dendritic cells exposed to dietary antigens produce IL-10 and TGF-β.[35]

Other Inflammatory Events. The recruitment of effector cells, including eosinophils and neutrophils, together with complement fixation by IgG, follows B- and T-lymphocyte activation. A group of soluble chemoattractant molecules termed chemokines (eg, eotaxin, IL-8) are released by dendritic cells and T cells to recruit eosinophils and neutrophils to the site of inflammation.[36,37] Chemokines also promote the growth of new blood vessels (angiogenesis) and secretion of matrix metalloproteinases.[38] Neutrophils, mast cells, and eosinophils are considered effector cells. Their products (including prostaglandins and leukotrienes by neutrophils, prostaglandins and histamine by mast cells, and eosinophilic chemotactic factor and major basic protein by eosinophils) cause many of the end-stage characteristics of inflammation, including fever, pain, swelling, erythema, cramping, and diarrhea.[39] However, because these events occur late in the inflammatory cascade, therapies targeted against these late manifestations of inflammation (eg, 5-aminosalicylate derivatives, sodium cromolyn, or lipoxygenase inhibitors) are generally less effective at controlling inflammation than immunosuppressive agents that work earlier in the proinflammatory cascade.

Allogeneic Immune Response

The allogeneic immune response to a foreign allograft is a far stronger immune reaction than the classic response to an antigen, involving up to 2% of the total T-lymphocyte population.[40] Multiple mechanisms occur by which a transplanted organ is rejected. First, CD8 cytotoxic lymphocytes from the recipient recognize MHC class I molecules on the surface of the donor tissue. These MHC class I (HLA-A, -B, and -C) molecules differentiate self from nonself, and, in the case of transplant rejection, the HLA molecules of the donor graft are inevitably different from those of the host. The cytotoxic CD8 lymphocytes then bind and destroy foreign cells through the release of cytotoxic molecules (including perforins and granzymes).

Whereas CD8 T cells mediate much of the tissue damage seen in rejection, CD4 T cells are probably even more important. In CD4 knockout mice, allografts are retained indefinitely.[41] In contrast, CD8 knockout mice can still reject allografts. This suggests that CD4 T cells mediate allograft rejection via either stimulation of antibody production or the generation of other cytotoxic leukocytes. The rejection effects of CD4 T cells include enhancing antigen presentation by APCs and promoting the survival and proliferation of cytotoxic T cells and the secretion of proinflammatory cytokines. In addition, CD4 T cells induce dendritic cells to produce IL-12, which induces the proliferation of CD4 cells with a Th1 phenotype.[40,41]

In allograft rejection, CD4 Th cells produce lymphokines such as IL-2 and IFN-γ, which recruit and activate more cytotoxic T cells, thereby propagating both cellular and humoral immune responses. CD4 cells can be activated in one of two ways. The indirect pathway (classic antigen-presenting pathway) involves antigen presentation of foreign (donor) antigens by recipient APCs to recipient CD4 cells. In the indirect pathway, the donor antigens are bound to recipient MHC and are presented to recipient T cells in a way similar to that of a microbial peptide. The second (direct) pathway is unique to transplant recipients and involves recognition of the intact unprocessed MHC of donor APCs by recipient CD4 T cells. The direct T-cell recognition of allogeneic MHC molecules is evaluated by using a mixed lymphocyte reaction.[42]

Despite the complexities and differences between the classic immune response to tissues seen in autoimmune disease and the allogeneic immune response seen in rejection, the final common pathway of both responses involves mononuclear cell activation, generation of proinflammatory lymphokines, and generation and recruitment of cytotoxic effector cells (including macrophages, killer T cells, neutrophils, mast cells, and eosinophils). The most potent immunosuppressive agents currently in use (including cyclosporine, tacrolimus, and monoclonal anti-CD3) either remove host T-lymphocyte populations or inhibit T-lymphocyte activation.

Some individuals, however, develop the ability to tolerate a foreign graft with minimal or no immunosuppression, a condition termed chimerism or transplant tolerance. Tolerance is an ideal outcome because it allows retention of a graft without the long-term toxicity of immunosuppression. Potential mechanisms for inducing tolerance in the host include stimulation of the TCR without the delivery of a second costimulatory signal, infusion of immunosuppressive cytokines at the time of graft delivery, and the promotion of T-cell apoptosis via the Fas–Fas ligand interaction. Another approach to promoting graft tolerance involves the infusion of donor bone marrow cells at the time of solid organ transplant.[43,44] Although the exact mechanism of chimerism or tolerance is not known, it most likely involves the development of a population of CD4 T cells that down-regulate the immune response.[41] At this time, no reliable method exists to consistently promote tolerance in human liver and small bowel transplant recipients.

COMMONLY USED IMMUNOSUPPRESSIVE THERAPIES

Corticosteroids

Corticosteroids are natural or pharmacologically modified molecules that are derivatives of cortisol, which is synthe-

sized from its precursors cholesterol and pregnenolone in the adrenal cortex. Corticosteroids have 21-carbon atoms, with a 2-carbon chain attached at position C_{17} of the sterol molecule; they differ from androgenic steroids, which are 19-carbon steroids.[45] Corticosteroids have a wide variety of endocrinologic, anti-inflammatory, and immunosuppressive effects. Commonly used immunosuppressive corticosteroids, their half-lives, and relative potencies are shown in Table 76.1-2.

Mechanism of Action. Corticosteroids are transported through the circulation to tissues by steroid binding proteins. They then dissociate from the binding protein, cross the cell membrane, and bind to nuclear receptors. The steroid-receptor complex stimulates the production of the NFκB inhibitor IκB, which down-regulates NFκB (the transcription factor that is critical in promoting synthesis of cytokines; see Figure 76.1-3). The end result is decreased synthesis of a wide variety of proinflammatory cytokines, including IL-1, IL-2, TNF-α, and IFN-γ.[46]

Corticosteroids reduce the numbers of lymphocytes in the circulation. In addition, corticosteroids decrease prostaglandin and leukotriene production by blocking arachidonic acid synthesis through effects on the enzyme phospholipase A_2.[47] Corticosteroids do not inhibit phospholipase A_2 directly but rather potentiate the release of lipocortin, a powerful inhibitor of phospholipase A_2.[48] Corticosteroids also down-regulate leukocyte recruitment into inflamed areas by modulating chemokine production.[49] Therefore, corticosteroids have multiple immunosuppressive and anti-inflammatory effects and act on multiple levels of the proinflammatory cascade.

Pharmacology. Corticosteroids are well absorbed from the gastrointestinal tract, principally from the proximal jejunum[50]; up to 30% of corticosteroids are absorbed from retention enemas.[51] In the systemic circulation, 90% of cortisol is bound to serum albumin and corticosteroid binding globulin. Cortisol and other corticosteroids are metabolized by both reduction and glucuronidation in the liver. Inducers of hepatic conjugation, including phenobarbital and rifampin, increase hepatic metabolism and excretion of steroids.[45] Although the plasma half-life of cortisol and other steroids is less than 5 hours, the biologic half-life (as measured by adrenal suppression and tissue effects) is far longer (see Table 76.1-2).[45] The most prominent pharmacologic effect of corticosteroids, suppression of the hypothalamic-pituitary adrenal axis, can occur with as little as 5 days of high-dose (50 mg/d) oral prednisone and is almost universally seen with 14 days of therapy.[50,51]

Adrenal suppression with corticosteroid therapy is assessed by screening of morning cortisol levels or more formal assessment after adrenal stimulation with metyrapone or corticotropin.[52] The degree of adrenal suppression is a function of the dose of the steroid, the mode of delivery, the half-life of the steroid, and the frequency of the doses given. Therefore, twice-daily dosing of prednisone, even with a lower dose, increases the degree of adrenal suppression, and alternate-day prednisone therapy reduces the adrenal suppression. In contrast, alternate-day treatment with dexamethasone has significant side effects because of its longer half-life.[45,51] Inhaled corticosteroids, such as beclomethasone, which are given twice per day to asthmatics, can suppress pituitary function and inhibit linear growth.[52,53] As little as 3 mg/m^2 of prednisone given daily for any longer than 6 months may decrease growth.[54] In contrast, there is no evidence that low-dose alternate-day prednisone therapy results in suppression of growth velocity.

Adverse Events. The large number of side effects (Table 76.1-3) seen with chronic high-dose steroids limits both the dose and duration of therapy.[50] Some side effects (eg, hypertension, fluid retention, diabetes mellitus) may be seen within 2 weeks of the onset of treatment, but most side effects are seen with prolonged (> 1 month) treatment. Loss of trabecular bone and a decrease in bone density occurs in adults treated with more than 7.5 mg/d of prednisone, with the greatest degree of bone loss occurring during the first

TABLE 76.1-2 COMMONLY USED GLUCOCORTICOID PREPARATIONS

STEROID PREPARATIONS	EQUIVALENT DOSE (MG)	PLASMA HALF-LIFE (MIN)	TISSUE HALF-LIFE (H)
SHORT ACTING			
Cortisone*	25	30	8–12
Hydrocortisone*	20	90	8–12
INTERMEDIATE ACTING			
Prednisone	5	60	12–36
Prednisolone	5	200	12–36
Methylprednisolone	4	180	12–36
Budesonide†	1.25†	180	??
LONG ACTING			
Dexamethasone	0.5	100–300	36–54
Betamethasone	0.6		

Adapted from Yang Y and Lichtenstein G,[46] Truhan A and Ahmed A,[50] and Spencer C and McTavish D.[82]

*Strongest mineralocorticoid (sodium retaining) effects.

†Locally acting preparation, with high first-pass metabolism and 15% or less systemic absorption. In clinical trials, 9 mg of enteric-coated budesonide has been compared with 40 mg prednisone.

few weeks of therapy, when dosages are highest.[55,56] Aseptic necrosis of the hip is also reported to complicate long-term corticosteroid use, but this may be related, in fact, to the underlying disease process.[57] The child on chronic steroid therapy is at increased risk for opportunistic infection, such as disseminated varicella. Ideally, children should be immunized against varicella prior to the institution of corticosteroid therapy. An unexposed, unimmunized child should receive zoster Ig after exposure and acyclovir for active infection. Gastrointestinal side effects (peptic ulcers and pancreatitis) occur only rarely.[58]

TABLE 76.1-3 SIDE EFFECTS OF CORTICOSTEROID THERAPY

CARDIOVASCULAR
Hypertension
? Atherosclerosis
DERMATOLOGIC
Cushingoid appearance
Moon facies
Striae
Alopecia
Hirsutism
Acne
Thinning/friability of skin
Telangiectasia
Impaired wound healing
ENDOCRINOLOGIC
Adrenal suppression
Impaired stress response
Growth failure
Diabetes mellitus/glucose intolerance
Hyperlipidemia
GASTROINTESTINAL
Nausea/vomiting
Fatty liver
Gastritis
Peptic ulcer
Pneumatosis intestinalis
Pancreatitis
HEMATOLOGIC
Leukocytosis
Lymphocytopenia
INFECTIOUS
Viral: especially varicella, herpes zoster
Bacterial: staphylococcal/pseudomonal
Fungal: especially *Candida*, *Aspergillus*
Parasitic: *Pneumocystis*
Mycobacterial: reactivation of tuberculosis
NEUROLOGIC
Headache
Pseudotumor cerebri
MUSCULAR
Proximal myopathy
OPHTHALMOLOGIC
Posterior subcapsular cataracts
Increased intraocular pressure
Papilledema
Exophthalmos
Eyelid swelling
ORTHOPEDIC
Osteoporosis
Fractures
Aseptic necrosis
Spontaneous tendon rupture
PSYCHIATRIC
Depression
Mania
RENAL
Sodium retention
Nephrocalcinosis
Hypercalciuria

Adapted from Swartz S and Dluhy R,[45] Truhan A and Ahmed A,[50] and Plevy S.[55]

Another adverse event of corticosteroid therapy is the development of corticosteroid dependence or corticosteroid resistance. In both autoimmune hepatitis (AIH) and inflammatory bowel disease, the proportion of patients relapsing after weaning or cessation of corticosteroids is greater than 70%.[59,60] In adults with asthma, corticosteroid resistance is associated with decreased levels of the glucocorticoid receptor and increased expression of the cytokine transcription factor AP-1 and other phosphorylated signaling proteins.[61,62] Thus, patients who are corticosteroid resistant may acquire a population of leukocytes resistant to the pharmacologic effects.[63]

Clinical Indications. Corticosteroids are used to treat a wide variety of gastrointestinal diseases, including allergic and eosinophilic gastroenteritis, graft-versus-host disease, liver transplant rejection, inflammatory bowel disease, and AIH. Corticosteroids have no proven efficacy in the therapy of many gastrointestinal diseases, including Reye syndrome, fulminant liver failure, primary sclerosing cholangitis (PSC), Ménétrier disease, and Whipple disease.

Inflammatory Bowel Disease. Corticosteroids are used as induction therapy in moderate to severe active ulcerative colitis. For patients with severe colitis, intravenous methylprednisolone twice daily in a total daily dose of 40 to 60 mg (or 1–2 mg/kg/d) is used, whereas for patients with moderate colitis, 1 mg/kg/d up to 40 mg/d of prednisone is used. Assuming that remission is achieved, one author recommends that the prednisone dosage be reduced by 5 to 10 mg/wk to 20 mg/d and then decreased by 2.5 to 5 mg each week until complete withdrawal of the prednisone.[55] Some clinicians prefer to taper to a dose of between 10 and 20 mg and then wean to an alternate-day regimen. For patients with ulcerative proctitis or left-sided colitis, the use of topical hydrocortisone enemas or foam preparations (Cortenema, Cortifoam) may induce and maintain remission with fewer systemic side effects.[64]

Two large studies in adults have demonstrated the superiority of corticosteroids over placebo in the treatment of active Crohn ileitis and ileocolitis.[65,66] In both the National Cooperative Crohn's Disease Study and the European Cooperative Crohn's Disease Study, 60 to 80% of patients treated with prednisone for approximately 4 months entered remission compared with 30 to 40% of patients given placebo. However, up to 30% of patients persist with active disease despite steroid treatment (ie, corticosteroid resistance), and another 30% of patients will relapse within 1 to 3 months of steroid discontinuation (corticosteroid dependence).[60,67] Risk factors for the devel-

opment of corticosteroid dependence include smoking, young age, and colonic Crohn disease.[68]

Whether alternate-day steroid administration helps prevent the relapse of Crohn disease is controversial. Early studies suggested that alternate-day prednisone was useful in preventing relapse in Crohn disease or ulcerative colitis, but this approach has not been validated in a long-term prospective placebo-controlled trial.[69,70] Given the availability of other effective maintenance therapies, such as 6-MP, this strategy is unlikely to become the subject of a randomized controlled trial.

Autoimmune Hepatitis. AIH is characterized by a mixed cellular inflammatory infiltrate of the hepatic portal areas and parenchyma and by the presence of hypergammaglobulinemia. Older children and teenagers often have anti–smooth muscle antibodies in the serum, whereas younger children frequently present with anti–liver-kidney microsomal antibodies.[71] Prednisone at a dose of 40 mg/d in adults or 1 mg/kg/d in children will improve both symptoms and biochemical parameters of AIH and prolong survival. The response rate to corticosteroid induction in AIH exceeds 80%.[72,73] As with inflammatory bowel disease, AIH has a high rate of relapse once steroids are either tapered or discontinued.[59,74] Therefore, steroids should be slowly tapered over a 2- to 3-month period to a low daily dose while monitoring liver biochemistries and serum Ig levels. To maintain remission once corticosteroid weaning is undertaken, physicians frequently add azathioprine (1.5–2 mg/kg/d) to the therapeutic regimen because azathioprine has been shown to be effective in preventing relapse.[75] Cyclosporine and tacrolimus have been used with success to treat corticosteroid-resistant cases of AIH.[76,77]

Eosinophilic Esophagitis. This increasingly recognized condition presents in children with symptoms of solid food dysphagia, heartburn, and dyspeptic symptoms. Affected individuals frequently have an atopic history. Endoscopy demonstrates a thick, irregular esophageal wall, and biopsies demonstrate large numbers of eosinophils.[78] Both oral prednisone and swallowed fluticasone successfully treat this condition.[79] Teitelbaum and colleagues demonstrated that induction therapy with fluticasone results in improved symptoms and decreased numbers of antigen-presenting T cells.[80] Although fluticasone therapy relieves symptoms, it may be complicated by the development of *Candida* esophagitis. Moreover, relapse following the cessation of therapy is frequent.

Budesonide (Entocort)

Budesonide is a synthetic corticosteroid structurally related to 16α-hydroxyprednisolone. An enteric-coated form designed for controlled ileal release is available in the United States, whereas budesonide enemas also are available in Canada and many European countries. The mechanism of action is similar to other corticosteroids, but because of the rapid first-pass metabolism, budesonide may have fewer corticosteroid-associated side effects.

Pharmacology. Budesonide is available in two principal forms: an oral form (3 mg controlled ileal release capsule), which provides controlled release into the ileum and cecum, and an enema (2 mg/100 mL) designed for drug delivery into the left colon. In both children and adults, budesonide has a low systemic bioavailability (approximately 10 to 15% of the ingested dose). However, following ingestion of a single 9 mg dose of budesonide, plasma cortisol is decreased by approximately 50% in adults and 60% in children.[81] After enema infusion, maximum plasma concentrations of 3 nmol/L are achieved 90 minutes after rectal administration. In the body, budesonide is rapidly metabolized to 6β-hydroxybudesonide and 16α-hydroxyprednisolone, which have 1 to 10% the glucocorticoid activity of budesonide. The elimination half-life of budesonide is approximately 3 hours.[82]

Adverse Effects. Because of the low systemic bioavailability and rapid metabolism of budesonide, it has been proposed to have fewer side effects than conventional corticosteroids. Volovitz and colleagues found no growth suppression in 15 children with asthma who inhaled 100 μg of budesonide 4 times daily for 3 years or longer.[83] In contrast, two asthma studies documented decreased adrenal cortisol output following a 1.2 mg oral dose of budesonide.[84,85] A number of studies in adults with ulcerative colitis suggest that the enema form of budesonide does not decrease basal plasma cortisol levels but may decrease adrenal gland reserve, as measured by adrenocorticotropic hormone testing.[82]

A number of clinical trials in adults using oral budesonide as induction and maintenance therapy in Crohn disease suggest that steroid side effects (moon face, acne, hirsutism) are reduced, but not eliminated, in patients receiving budesonide.[86] In one multicenter study, 67% of patients receiving prednisone (40 mg/d) compared with 44% of patients receiving budesonide (9 mg/day) reported cushingoid side effects.[87] The long-term effects of budesonide on bone density are unclear. In one study, methylprednisolone was noted to impair osteoblast activity (as measured by serum osteocalcin), whereas budesonide did not.[88]

Clinical Use. The principal gastrointestinal indication for use of budesonide has been in the therapy of Crohn disease and ulcerative colitis. Budesonide enemas (2 mg/100 mL) are effective in treating distal ulcerative colitis.[89] For Crohn disease in adults limited to the ileocecal region, 9 mg of budesonide given orally has comparable efficacy to 40 mg/d of prednisone.[90] However, oral budesonide may be less effective than prednisone for disease involving the distal colon and rectum.[87] Lower-dose budesonide (either 3 mg or 6 mg/d) may have some efficacy in the maintenance of remission, but this is controversial. In three identically designed studies, patients were randomized to placebo, budesonide 3 mg/d, or budesonide 6 mg/d. Two studies noted that budesonide prolonged the time to relapse, with the 6 mg/d dose being more effective.[91,92] However, a third study showed no difference in the median time to relapse between groups.[86]

There are extensive data on inhaled budesonide in children with asthma but less information on children with inflammatory bowel disease. In an initial randomized comparison of budesonide and prednisone for the treatment of children with active Crohn disease, budesonide 9 mg/d and prednisone 40 mg/d had comparable response rates after 12 weeks of therapy.[93] However, a subsequent retrospective study by the same investigators suggested that prednisone was more effective induction therapy for active Crohn disease, with a response rate of 77% for prednisone versus 48% for budesonide. In addition, prednisone was effective as rescue therapy in 73% of children who did not achieve a remission with budesonide.[94]

Although budesonide may be less effective as an induction agent, it has the benefit of producing fewer cosmetic side effects, such as moon face, acne, and hirsutism.[93] Whether budesonide suppresses growth with long-term use remains unclear. A large cohort of asthma patients treated with inhaled budesonide and followed for a mean of 9 years demonstrated reduced growth velocity initially but achieved normal final adult height.[95] Kundhal and colleagues described a cohort of children with Crohn disease treated with budesonide who demonstrated a reduced height velocity (mean 2.3 cm/yr) while on therapy.[96] It is unclear from this study, however, whether the growth suppression was secondary to the drug itself or subclinical disease activity. There are limited data on bone turnover and osteoporosis in budesonide-treated children, but results from asthmatics suggest that budesonide likely causes less bone resorption than prednisone.[97]

Cyclosporine (Sandimmune, Neoral)

Cyclosporine is a cyclic 11–amino acid peptide produced by the fungus *Tolypocladium inflatum Gams*. Its potent immunosuppressive properties revolutionized the field of transplant in the early 1980s and made it possible for solid organ transplant (including liver and small bowel transplant) to become a recognized mode of therapy for specific illnesses. Dosages, adverse effects, and pharmacokinetics of cyclosporine and other immunomodulatory agents are summarized in Table 76.1-4.

Mechanism of Action. Cyclosporine enters the cytosol of T cells and binds to the cytosolic protein cyclophilin A (Cyp-A). In the cytoplasm, the cyclosporin A–cyclophilia complex binds to the calcium-dependent phosphatase calcineurin (see Figure 76.1-2). The calcineurin molecule is essential in T-lymphocyte activation and in the transcription of cytokines by Th cells because it dephosphorylates the transcription factor NFAT, thereby facilitating migration of NFATs across the nuclear membrane, leading to decreased transcription and release of IL-2 and other proinflammatory cytokines.[21,98] Of note, only calcium-dependent pathways of T-lymphocyte activation are affected by cyclosporin A. Activation of T lymphocytes via the cell surface molecule CD28 (a calcium-independent process) is not inhibited by cyclosporin A.[99]

The most prominent immunologic effect of cyclosporine is the suppression of IL-2 release by Th cells, resulting in marked inhibition of cell-mediated immunity. The synthesis and release of other cytokines, including IL-6 and -8, are also impaired.[98] Cyclosporin A exhibits a wide variety of other effects on nonhelper T cells (Table 76.1-5). Cyclosporin A impairs the cytotoxic action of CD8 (killer) T cells.[100] Paradoxically, in some animal models, cyclosporin A prevents the development of tolerance and can exacerbate autoimmune disease by blocking the suppressive effects of CD8 suppressor T cells.[101] Cyclosporine also modulates antibody production by B lymphocytes, as well as the release of proinflammatory and chemotactic cytokines by mast cells. Although the in vitro effects of cyclosporine are varied, the net effect of cyclosporine administration to humans is to suppress the cellular immune response without lowering antibody levels.[98,102]

Pharmacology. Cyclosporine is a highly lipophilic molecule. The initial formulation of cyclosporine (Sandimmune, Sandoz Pharmaceuticals Corporation, East Hanover, NJ) consisted of cyclosporine in an oil base. Thus, the oral absorption was dependent on intact fat absorption and bile flow, and the bioavailability of this formulation was quite low (approximately 20–30%). The bioavailability was further impaired in conditions that reduced fat absorption, such as cholestasis and enteropathies.[103] Coadministration of oral D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) (the water-soluble vitamin E derivative) increases the oral bioavailability of oil-based cyclosporine.[104]

In the late 1990s, an oral-microemulsion formulation of cyclosporine (Neoral, Sandoz Pharmaceuticals Corporation) was made available and is now almost exclusively used by liver and renal transplant centers.[105] The new formulation has improved bioavailability, thus necessitating a reduction in drug dosage of approximately 10 to 15% when patients are converted from the oil-based product.[98] In liver transplant recipients, the frequency of graft survival and patient survival is similar regardless of the formulation.[98] Adverse effects are also comparable in both Sandimmune- and Neoral-treated patients.[106]

Cyclosporine has a large volume of distribution (ranging from 3 to 5 L/kg in adults) and is 90% bound to plasma proteins and erythrocytes. Cyclosporine is hydroxylated and demethylated in the liver and excreted in the bile, with less than 10% of each dose excreted in the urine. Clearance of the drug is biphasic, with an initial elimination half-life ($t_{1/2\alpha}$) of 1.2 hours and a terminal elimination half-life ($t_{1/2\beta}$) of 8 to 27 hours. As a result of dependence on hepatic metabolism, cyclosporine can be used even in patients with renal failure. However, drugs that induce P-450 microsomal enzymes (including phenobarbital, phenytoin, and carbamazepine) decrease cyclosporin A concentrations, whereas compounds that inhibit P-450 enzymes (including ketoconazole, erythromycin, and grapefruit juice) increase cyclosporin A concentrations (Table 76.1-6).[98,103]

The exact protocol for monitoring of cyclosporine levels varies between transplant centers. Traditionally, trough values of cyclosporin A levels are obtained, with a target

TABLE 76.1-4 COMMONLY USED IMMUNOSUPPRESSIVES (PHARMACOLOGY)

DRUG	DOSAGES	HALF-LIFE	METABOLISM AND EXCRETION	ABSORPTION/ BIOAVAILABILITY	DRUG INTERACTIONS	COMMENTS
Cyclosporin A (CyA)	5–10 mg/kg/d orally twice a day (adjust by blood levels); intravenous dose approximately 30% of oral dose	10 h (range 4–48 h)	Hydroxylation and methylation; 95% biliary excretion < 5% urinary excretion	Poor (~ 30%); dependent on bile flow, intestinal absorption; microemulsion (Neoral) has improved absorption.	See Table 76.1-6; multiple medications will either increase or decrease half-life	Liver failure increases half-life
Tacrolimus (FK-506)	0.2–0.4 mg/kg/d orally twice a day (adjust by blood levels)	9 h (range 5–16 h)	Bound to erythrocytes; whole blood levels are 10 times serum levels; 99% hepatic metabolism (hydroxylation/ demethylation) absorption	Poor (~ 30%); less dependent on bile flow or mucosal integrity than CyA	See Table 76.1-6; multiple medications will either increase or decrease half-life	Intravenous form is more toxic than oral; useful in liver bowel transplant because of more reliable oral absorption
Methotrexate	15 mg/m^2/wk intramuscular or subcutaneous	Biphasic: initial phase 1.5–3.5 h; final phase 8–15 h	Hepatic 7-hydroxylation; intracellular polyglutamate formation; 60–90% renal excretion; < 10% biliary excretion	Poor (30–50% absorbed by gut)	Sulfonamides, salicylates, tetracycline, phenytoin may displace methotrexate from plasma proteins	Bioavailability decreases with increasing oral dosage; may enter pleural or ascitic fluid
6-Mercaptopurine (6-MP)	1.5–2.0 mg/kg orally once a day	Triphasic 45 min, 2.5 h, and 10 h	Degradation by xanthine-oxidase *S*-methylation in liver; 40% renal excretion	Variable (10–50% absorbed)	Allopurinol, probenecid, increase level	Wide genetic variations in metabolism, determined by thiopurine methyltransferase activity
Azathioprine	2.–2.5 mg/kg/d orally once a day	5 h	Metabolized to 6-MP, 6-thioinosinic acid	Well absorbed from the gastrointestinal tract	See 6-MP	See 6-MP
Mycophenolate mofetil	20 mg/kg/d in 2 divided doses	18 h (adult)	Hepatic glucuronidation 90% renal excretion	99% bound to plasma albumin		Significant gastrointestinal side effects in 20–30% of patients
Sirolimus (rapamycin)	2–5 mg/d, adults 3 mg/m^2, children	60 h	Hepatic by CYP3A4; excretion by P-glycoprotein	15% oral bioavailability; food interferes with absorption	Multiple, including cyclosporine and other drugs metabolized by cytochrome 3A4	Hyperlipidemia and myelosuppression major side effects

CyA = cyclosporin A; 6-MP = 6-mercaptopurine.

TABLE 76.1-5 IMMUNOLOGIC EFFECTS OF CYCLOSPORIN A

CELL	EFFECTS
CD4 T cell	Decreases IL-2, IFN-γ release Decreases IL-2 receptor expression
Cytotoxic T cell	Decreases proliferation Decreases IL-2 responsiveness Possible decrease in cytotoxicity
B cells	Decreases activation gene expression Decreases anti-immunoglobulin responsiveness
Mast cells	Decreases TNF-α release

IFN = interferon; IL = interleukin; TNF = tumor necrosis factor.

therapeutic range of 150 to 400 µg/L. However, because the half-life is variable, trough values poorly predict the actual concentration of the drug over time (area under the curve [AUC]) and correlate less well than true AUC in predicting rejection or nephrotoxicity.[98] For this reason, many centers calculate AUC by performing a trough level and levels 2 hours and 4 hours after a dose.

Adverse Effects. Cyclosporine, even at therapeutic levels, has a wide variety of adverse effects and toxicities (Table 76.1-7). Nephrotoxicity, manifested by a decrease in creatinine clearance and a rise in serum creatinine, occurs in 25 to 75% of patients. Nephrotoxicity usually reverses on discontinuation of the drug or lowering of the dose. Other adverse effects include hypertension, neurotoxicity, insulin-dependent diabetes mellitus, elevated transaminases, hirsutism, and gingival hyperplasia.[102] In liver transplant recipients receiving cyclosporine, up to two-thirds of patients will have bacterial infection and up to 20% will have infections with opportunistic pathogens such as cytomegalovirus, Epstein-Barr virus, *Candida* species, and *Aspergillus*.[107] Lymphoproliferative disorders also occur in patients receiving cyclosporine, usually correlating with serologic or molecular evidence of Epstein-Barr virus infection.[108] Although lymphoproliferative disease can be reversible if immunosuppression is halted, in a minority of patients, the disease progresses to lymphoma.

Clinical Uses. Cyclosporine has been used primarily in the prevention of rejection in liver transplant recipients. More recently, it has been applied to autoimmune liver and bowel diseases, including AIH, ulcerative colitis, Crohn disease, and autoimmune enteropathy. In pediatric liver transplant recipients, cyclosporine is typically begun on the first postoperative day. Whereas the old preparation (Sandimmune) was given intravenously, the microemulsion preparation (Neoral) can be given orally or via nasogastric tube. In the early post-transplant period, clinicians adjust the cyclosporine dosage to maintain a trough level between 150 and 250 ng/mL, as determined by high-performance liquid chromatography. The oral maintenance dosage of cyclosporine is approximately 5 to 10 mg/kg/d in two divided doses, but higher doses may be necessary with the older preparation.[109] In one Canadian study, 32 children undergoing liver transplant were randomly assigned to receive either the lipid-soluble form or the microemulsion in the post-transplant period. Pharmacokinetic studies performed 3 weeks after transplant demonstrated that patients receiving the microemulsion had a mean 8-hour AUC of 950 µg/L/h compared with 300 µg/L/h with the standard formulation.[110] Thus, in children, the Neoral microemulsion gives higher levels of cyclosporine for a longer period of time. However, it remains unclear if this pharmacokinetic benefit translates into improved graft and patient survival.

Two studies emphasize the efficacy of cyclosporine in children with AIH. Debray and colleagues used cyclosporine (mean dose 4.7 mg/kg/d) alone as primary induction therapy in children with type 2 AIH and obtained remission in six children. In another portion of the same study, the authors successfully used cyclosporine (5.6 mg/kg/d) to induce remission in five patients with type 2 AIH resistant to steroids and azathioprine.[111] In another study, 30 patients with AIH were given cyclosporine as monotherapy for 6 months. Twenty-five of the 30 children had normal alanine transaminase (ALT) levels within 6 months.[112] Therefore, early use of cyclosporine can induce remission in AIH without the use of high-dose corticosteroids.

Dosages used in the therapy of Crohn disease and ulcerative colitis are lower. Brynskov and colleagues demonstrated that an oral dose of 5 to 7.5 mg/kg/d promotes remission of Crohn disease in 22 of 37 adults with steroid-refractory Crohn disease.[113] In contrast, a large multicenter placebo-controlled study using low-dose (2.5 mg/kg) cyclosporine as an adjunct to standard treatment for adults with Crohn disease did not demonstrate any benefit of low-dose cyclosporin A.[114]

For ulcerative colitis, Lichtiger and colleagues demonstrated that intravenous cyclosporin A was effec-

TABLE 76.1-6 IMPORTANT DRUG INTERACTIONS WITH CYCLOSPORINE OR TACROLIMUS

CLASS OF MEDICATION	RAISES CYCLOSPORINE AND TACROLIMUS LEVELS	LOWERS CYCLOSPORINE AND TACROLIMUS LEVELS
Antimicrobials	Erythromycin, clarithromycin, clotrimazole, ketoconazole, itraconazole, fluconazole, doxycycline	Isoniazid, nafcillin, rifampin
Antihypertensives	Diltiazam, verapamil, nicardipine	
Anticonvulsants		Phenobarbital, primidone, carbamazepine, phenytoin
Other	Amiodarone, methylprednisolone, danazol, grapefruit juice, sirolimus	Octreotide, St. John's wort

Adapted from Dunn C et al,[98] Kahan B,[102] Kosmach B et al,[109] and Peters D et al.[124]
Potential synergistic nephrotoxicity: acyclovir, aminoglycosides, nonsteroidal anti-inflammatory drugs, amphotericin, vancomycin, certain antineoplastic agents.

TABLE 76.1-7 SIDE EFFECTS OF CYCLOSPORIN A OR TACROLIMUS (FK506) THERAPY

ORGAN SYSTEM	ADVERSE EFFECT
Renal and electrolyte	Hypertension; nephrotoxicity: increased creatinine, oliguria, kidney failure; hypomagnesemia, hyper- and hypokalemia
Nervous system*	Tremor, headache, paresthesia, myalgias, muscle spasm
Gastrointestinal	Nausea, vomiting, diarrhea, anorexia, constipation
Endocrine and metabolic	Hyperglycemia and diabetes,* hypercholesterolemia†
Cardiovascular	Arrhythmia,† cardiomyopathy*
Infectious	Opportunistic infections: eg, herpes, CMV, EBV, parvovirus, *Pneumocystis*
Malignancy	EBV-associated lymphoproliferative disorder, lymphoma
Other	Gingivitis and gingival hyperplasia,† hirsutism,† anemia, pruritus

Adapted from Dunn C et al,[98] Kahan B,[102] Plosker G and Foster R,[123] Peters D et al,[124] and Atkison P et al.[131]
CMV = cytomegalovirus; EBV = Epstein-Barr virus.
*More common with tacrolimus.
†More common with cyclosporine therapy.

tive treatment in steroid-refractory fulminant colitis. In this trial, 9 of 11 adults treated with cyclosporin A responded within 7 days compared with 0 of 9 receiving placebo.[115] A subsequent study suggested that intravenous cyclosporine (4 mg/kg/d) is effective as monotherapy compared with corticosteroids in the treatment of ulcerative colitis, with a response rate of 60%.[116] In children, given either orally or intravenously at a dose of 4 mg/kg, cyclosporine will bring about remission of ulcerative colitis in up to 80% of children.[117] However, withdrawal of the medication both in children and in adults is associated with a high rate of relapse, even if azathioprine is added as maintenance therapy. Long-term remission rates (> 1 year) are less than 50%.[118] Because the remission induced by cyclosporine is short, it remains a matter of debate whether the benefits of short-term remission outweigh the complications of potent immunosuppressive therapy.

Cyclosporine also has been used to treat autoimmune enteropathy, an idiopathic intestinal inflammatory disease occurring in young children in association with antienterocyte antibodies and evidence of systemic autoimmunity (including renal disease, diabetes, and arthritis). Two groups have treated patients with autoimmune enteropathy with cyclosporin A and noted improved growth and increased nutrient absorption.[119]

Cyclosporine has been used in conjunction with corticosteroids for the treatment of graft-versus-host disease, although the benefit over corticosteroids alone is unclear.[120,121] However, in one study comparing tacrolimus with cyclosporine for prophylaxis of graft-versus-host disease, the incidence of graft-versus-host disease was 18% in the tacrolimus group compared with 48% in the cyclosporine group.[122]

Tacrolimus (FK506, Prograf)

Tacrolimus is a macrolide lactone produced by the fungus *Streptomyces tsukubaensis*. The principal uses of tacrolimus include primary therapy in liver, bowel, and liver-bowel transplants; treatment of cyclosporine-refractory organ rejection; and treatment of graft-versus-host disease. Although the molecule is structurally different from cyclosporine, tacrolimus also works by inhibiting cytokine transcription via calcineurin (see Figure 76.1-3).

Mechanism of Action. Tacrolimus is a lipid-soluble molecule that is internalized into the intracellular compartment of the lymphocyte. Once in the cytosol, tacrolimus complexes with a group of proteins termed FK binding proteins (FKBP, especially FKBP-12). In a manner similar to that of cyclosporine, the tacrolimus-FKBP complex binds calcineurin to inhibit the calcium-dependent pathway of lymphocyte activation. Tacrolimus inhibition of calcineurin results in decreased levels of the NFAT and decreased transcription of T-cell activation genes.[123] Transcription of cytokines produced by activated T cells, such as IL-2, is also drastically decreased. Therefore, T-cell lymphocyte proliferation and cytokine production are blocked by tacrolimus.[124] As with cyclosporine, calcium-independent mechanisms of T-lymphocyte activation are not affected by tacrolimus. In addition to effects on T lymphocytes, tacrolimus inhibits B-cell proliferation and the production of IgM and IgG. At higher doses, tacrolimus also inhibits cytokine production by monocytes and prostaglandin production by mast cells.[125,126] Although tacrolimus and cyclosporine have similar immunologic effects, work in animal models and in humans suggests that tacrolimus also inhibits anti-inflammatory cytokines, such as IL-10, whereas cyclosporine has stronger effects on humoral immunity.[127,128]

Pharmacology. Tacrolimus is 10 to 100 times more potent than cyclosporine. The oral bioavailability of tacrolimus is similar to that of cyclosporine (approximately 30% absorption), but tacrolimus is less dependent on bile flow than cyclosporine for absorption. In fact, tacrolimus absorption is independent of the presence of bile acids, and absorption is less affected by enteropathies. However, coadministration with foods will decrease bioavailability. Once absorbed, tacrolimus is distributed in tissues and in erythrocytes, with a volume of distribution of approximately 1 L/kg.[123]

As with cyclosporine, metabolism of tacrolimus occurs almost exclusively in hepatocytes, with demethylation and hydroxylation occurring via the cytochrome P-450 system. The half-life of tacrolimus is variable, with a mean of 12 hours in both adult and pediatric transplant recipients. Although the half-life is unchanged in renal disease, even mild hepatic impairment will significantly prolong the

half-life. Drugs that inhibit P-450 metabolism, such as ketoconazole and erythromycin (see Table 76.1-7), will raise tacrolimus levels, whereas drugs that accelerate P-450 metabolism decrease tacrolimus levels.[124] Children require higher mg/kg doses than adults because of more rapid drug clearance and higher volumes of distribution.[129] McDiarmid and colleagues compared the dose requirements and clearances of the pediatric and adult liver transplant patients. The overall mean pediatric oral dose for the first year was 0.46 mg/kg/d, a dose approximately three times the mean adult dose.[130]

Toxicities of tacrolimus are similar to those seen with cyclosporine (see Table 76.1-7). Side effects include nephrotoxicity, electrolyte abnormalities (hypokalemia, hypomagnesemia), diabetes mellitus, tremor, hypertension, diarrhea, post-transplant seizures, and lymphoproliferative disease. In addition, patients receiving tacrolimus are predisposed to opportunistic bacterial, viral, and fungal infections. Some side effects seen with cyclosporin A, including hirsutism, coarsening of facial features, and gingival hyperplasia, are not seen with tacrolimus.[124] Cardiomyopathy, a rare but serious complication of tacrolimus therapy, typically resolves after lowering the dose of tacrolimus or changing to cyclosporine.[131]

Clinical Uses. The principal indication for the use of tacrolimus is in the prevention of solid organ transplant rejection.[132] As primary therapy immediately after liver transplant in children, tacrolimus is usually given in a dose of 0.3 mg/kg/d, and the dosage is adjusted to give a whole blood level between 10 and 15 µg/L. Over the first few months after transplant, the drug dosage is weaned to achieve levels of 5 to 10 µg/L. Concomitant therapies include corticosteroids, azathioprine, or mycophenolate mofetil; corticosteroids are weaned over time. A European multicenter trial reported that patients treated with tacrolimus (vs cyclosporine) are more likely to wean off corticosteroids.[133] In one pediatric series, 233 children were given tacrolimus as primary therapy, and, when compared with historical controls treated with cyclosporine, the patient survival was 86% in the tacrolimus group versus 69% in the cyclosporine group. However, this difference may simply reflect improved care of liver transplant patients over time.[134] Histologic rejection rarely occurs in children receiving tacrolimus as primary therapy.[135] Tacrolimus has also been used as rescue therapy for children who have developed either chronic rejection despite cyclosporine therapy or adverse effects of cyclosporine.[136,137]

Tacrolimus has been the mainstay of antirejection therapy for patients having received bowel, combined liver and bowel transplants, or multivisceral transplants.[138] In these settings, tacrolimus is usually given with other concurrent immunosuppression, such as corticosteroids and monoclonal antibodies (eg, daclizumab). Initially, levels may need to be kept higher (eg, 15–25 µg/L) to prevent bowel rejection.[139] Both rejection and lymphoproliferative disease occur more commonly in this patient population.

Tacrolimus has also been employed in a wide variety of nontransplant conditions, including as an alternative to cyclosporine in both type 1 and type 2 AIH.[72,140] Children with autoimmune enteropathy that is resistant to cyclosporine can develop restoration of bowel function and histologic improvement after tacrolimus therapy.[141,142] Open-label studies suggest that tacrolimus is effective in treating severe mucosal and fistulizing Crohn disease, but the rate of adverse events is high.[143,144] In a multicenter open-label experience of tacrolimus in the treatment of severe ulcerative colitis and Crohn colitis in children, 70% of patients responded, but more than 50% underwent colectomy within a year.[145] In graft-versus-host disease, tacrolimus can stabilize or improve cases refractory to cyclosporine; however, in one open-label study of 39 patients, the long-term response was approximately 30%.[146]

Azathioprine (Imuran) and 6-MP (Purinethol)

Both azathioprine and 6-MP are purine derivatives that are incorporated into DNA and inhibit DNA synthesis. Azathioprine is metabolized in vivo to 6-MP, and its biologic effects are essentially identical to those of 6-MP; therefore, both the mechanisms of action and the toxicities of these drugs are identical. In contrast to 6-MP, azathioprine is metabolized more slowly and has a longer half-life. Therefore, azathioprine has been more widely used as an immunosuppressive agent in transplant recipients. Both drugs are useful in the treatment of liver transplant rejection, as well as in the therapy of inflammatory bowel disease and chronic active hepatitis.

Mechanism of Action. 6-MP is converted to 6-thioguanine (6-TG) ribonucleotides by the enzyme hypoxanthine-guanine phosphoribosyltransferase (HGPRT). The ribonucleotides produced by HGPRT are then incorporated into the DNA of rapidly dividing cells and are cytotoxic. In vitro, 6-MP can inhibit Th cell–dependent Ig production. In addition, 6-MP inhibits Ig production by IL-6–stimulated B-cell lines.[147,148]

Pharmacology. The absorption of 6-MP from the gastrointestinal tract is variable; as little as 10% or much as 50% may be absorbed. In contrast, azathioprine is well absorbed from the gastrointestinal tract. 6-MP is primarily metabolized in the liver, but up to 40% can be excreted in the urine.

There are three major pathways of 6-MP metabolism (Figure 76.1-4). The first route involves transformation by HGPRT and other enzymes into 6-TG nucleotides. Because inhibition of lymphocyte proliferation depends on 6-TG, concentrations of 6-TG in the body may predict the therapeutic efficacy of 6-TG. A second metabolic pathway involves breakdown of 6-MP via xanthine oxidase to 6-thiouric acid; because xanthine oxidase can be inhibited by allopurinol, this drug is relatively contraindicated by individuals taking 6-MP. The third pathway is the principal route of catabolism of 6-MP and involves *S*-methylation by the enzyme thiopurine methyltransferase (TPMT) to generate 6-methylmercaptopurine (6MMP).[147]

The half-life of 6-MP is triphasic, with the half-life of the final phase being 10 hours. Allopurinol increases levels of 6-MP by inhibiting xanthine oxidase metabolism. In

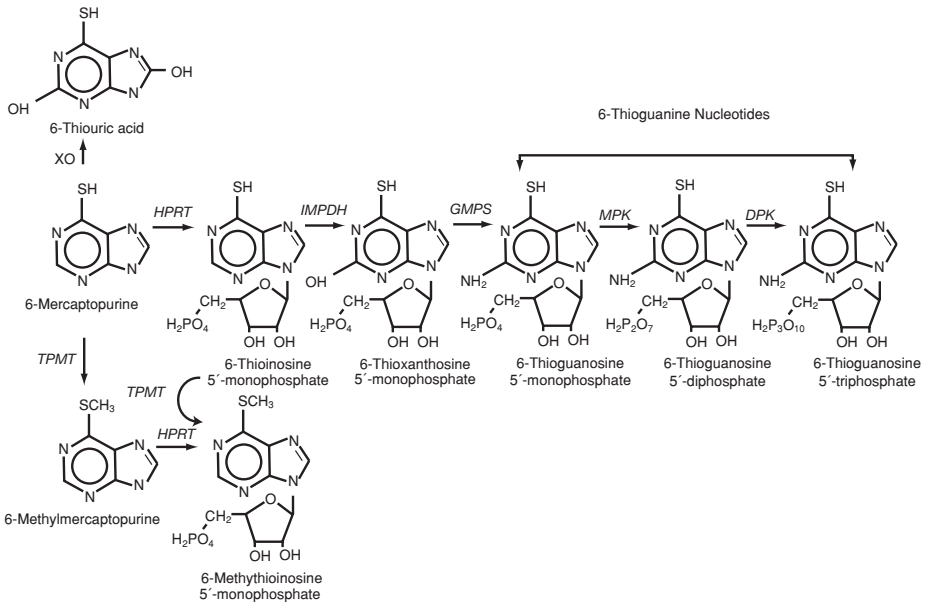

FIGURE 76.1-4 Metabolism of 6-mercaptopurine (6-MP). The initial metabolism of 6-MP may occur via one of three competing enzymatic pathways: thiopurine methyltransferase (TPMT), xanthine oxidase (XO), and hypoxanthine phosphoribosyltransferase (HPRT). Further metabolism of the thinucleotide is catalyzed by inosine monophosphate dehydrogenase (IMPDH) and guanosine monophosphate synthetase (GMPS) to produce 6-thioguanine nucleotides, which are principally responsible for the immunosuppressive effects of 6-MP. Reproduced from Cuffari C et al,[147] with permission of Dr. E. Seidman and BMJ Publishing Group.

addition, probenecid increases the level of 6-MP by inhibiting urinary excretion.[148] There is extensive variability in TPMT activity in human hepatocytes, presumably on the basis of genetic polymorphisms. Approximately 90% of individuals have normal or high TPMT activity and break down 6-MP at a normal rate. Those patients with high TPMT activity have lower concentrations of 6-TG. Lennard and colleagues reported that patients with higher TPMT activity have a higher relapse rate of acute lymphoblastic leukemia, suggesting that the more rapid metabolism of the drug leads to decreased efficacy.[149] Ten percent of individuals have low TPMT activity and have higher concentrations of 6-TG in their tissues and greater potency of the drug; however, this subset of patients may also be at increased risk of myelosuppression. Approximately 0.3% of individuals have negligible TPMT activity, this group is at the highest risk of developing myelosuppression associated with very high 6-TG levels.[147,150] Laboratory tests to determine TPMT genotype, blood TPMT activity, and 6-TG levels are now commercially available.

Adverse Effects and Toxicity. The principal toxic effects of 6-MP are myelosuppression, pancreatitis, elevated transaminases, increased risk of viral infection, and a theoretical increased risk of lymphoma. In one large series of inflammatory bowel disease patients taking 6-MP, 4% developed pancreatitis, up to 10% developed systemic infections, and 1 patient in 400 developed a cancer potentially attributable to the therapy.[151] Whether 6-MP increases the risk of lymphoma remains controversial. Connell and colleagues tracked 755 adults who received 2 mg/kg of azathioprine for up to 15 years (median 12.5 months) and found no increased risk of cancer.[152] However, an increased incidence of Epstein-Barr virus–associated lymphoma was reported in a cohort of 1,200 adults with inflammatory bowel disease treated with either 6-MP or azathioprine.[153] Kirschner described a case series of 95 children who received 6-MP or azathioprine for a mean of 2.2 years. Overall, 82% of children tolerated 6-MP, with the most common complications being elevated aminotransferases (13% of patients), leukopenia (10%), infections (8%), nausea (5%), and pancreatitis (4%).[154]

Clinical Utility. *Inflammatory Bowel Disease.* 6-MP came into widespread use for the treatment of Crohn disease after 1980, when a randomized placebo-controlled trial demonstrated a 70% response rate in patients with steroid-refractory Crohn disease.[155] It is now recognized that 6-MP has multiple beneficial effects in the therapy of inflammatory bowel disease, including a decrease in disease activity, steroid-sparing effects, and the healing and closure of fistulae. In children and adolescents, treatment of Crohn disease with 6-MP (at a dose of 1.5 mg/kg/d) results in similar improvement and response rates.[156] However, there is a 3- to 4-month lag time between the initiation of 6-MP treatment and a clinical response. A 6-TG level of > 235 pmol/10^8 erythrocytes appears to correlate with an improved clinical response.[147,157,158] A subset of patients who may be resistant to treatment with 6-MP includes those with low levels of 6-TG nucleotides and a high ratio of 6MMP to 6-TG, suggesting rapid breakdown of 6-MP and a decrease in levels of active metabolites.[159] 6-TG has been associated with an increased risk of liver toxicity, so this drug cannot be recommended for use.[160]

Children with Crohn disease may benefit from the early use of 6-MP as a maintenance agent. In an important study by Markowitz and colleagues, 55 children with newly diagnosed Crohn disease were induced with prednisone and then randomized to either 6-MP or placebo as the steroid therapy was withdrawn. After 18 months, the relapse rate was 9% in the 6-MP group compared with 47% in the placebo group. In addition, the 6-MP–treated group received a lower cumulative dose of corticosteroids over the 18-month period.[161]

Another potential use of 6-MP in Crohn disease is the prevention of postoperative disease recurrence. One retrospective study in children suggested that early use of 6-MP decreases the recurrence risk in patients undergoing ileal

or colonic resection.[162] Another study suggested that azathioprine heals recurrent ileitis developing in the neoterminal ileum.[163]

Azathioprine also has been widely used in the therapy of inflammatory bowel disease. The results suggest clinical efficacy comparable with that of 6-MP. In one study of the pharmacokinetics of azathioprine, oral azathioprine achieved 6-TG levels comparable to those of 6-MP, although name brand azathioprine (Imuran) gave higher 6-TG levels than the generic drug.[164] In adults with Crohn disease, high-dose intravenous azathioprine is reported to shorten the time to remission.[165] A case series reporting azathioprine use in children with steroid-refractory inflammatory bowel disease identified a response rate of 75%.[166]

Few controlled trials have been performed to evaluate the efficacy of 6-MP or azathioprine in ulcerative colitis. Roughly 60% of patients with ulcerative colitis refractory to steroid tapering demonstrate an apparent clinical response to 6-MP (as defined by both improvement in clinical symptoms and a decrease in steroid dosage).[167] Another study reported a comparable response rate to 6-MP in children with ulcerative colitis.[168] 6-MP and azathioprine maintenance therapy may be particularly useful following treatment of severe colitis with cyclosporine.[118] A small case series of three children suggested that intravenous azathioprine can be effective as primary therapy for fulminant colitis.[169] It is unclear whether therapy with immunosuppressive agents such as 6-MP and azathioprine increases the risk of colon cancer in children with ulcerative colitis. However, for some patients, such therapy is beneficial and can allow the children and the family to psychologically prepare themselves for colectomy.

Liver Disease. Azathioprine is used in liver transplant patients in conjunction with cyclosporine and prednisone (triple-drug immunosuppression); the usual dose used is 1 to 2 mg/kg/d. Azathioprine is not effective in treating acute rejection. The use of azathioprine and the duration of therapy varies with individual transplant centers; some centers discontinue use of the drug within a year of transplant.[170] A retrospective study by van Hoek and colleagues found that patients receiving azathioprine were less likely to develop ductopenic rejection and vanishing bile duct syndrome, 14 of 66 patients (21%) without azathioprine, compared with 1 of 98 patients (1%) receiving 2 mg/kg/d of azathioprine.[171]

Complicating the use of azathioprine in liver transplant recipients is the occasional development of azathioprine-induced hepatotoxicity, including cholestasis, peliosis hepatis, nodular regenerative hyperplasia, and veno-occlusive disease. Withdrawal of azathioprine in patients with azathioprine-associated induced hepatitis generally results in an improvement of liver enzyme tests within a week.[172]

Azathioprine also has been widely used in the treatment of steroid-refractory autoimmune chronic active hepatitis.[173] Traditionally, the therapy of this entity involved high-dose steroid therapy with a slow steroid taper. However, many patients relapse once the steroid dose is tapered. Chronic active hepatitis can be kept in prolonged remission if patients continue to take azathioprine.[75] Thus, a commonly used alternative to prednisone as monotherapy for AIH involves the addition of 50 mg, or more, of azathioprine as a maintenance agent. In adults who relapse on low-dose azathioprine, the dose may be increased up to 150 mg/d.[72] Since up to 80 to 90% of children with autoimmune chronic active hepatitis relapse once corticosteroids are tapered, azathioprine is useful in the treatment of pediatric type I and type II AIH. The addition of azathioprine will also decrease the long-term toxicities and growth-impairing effects of chronic corticosteroid therapy in childhood.

Methotrexate

In 1948, methotrexate was used by Sidney Farber to treat pediatric leukemia. Since then, it has been discovered that lower doses of this drug could also be used to treat autoimmune diseases such as psoriasis, PSC, and primary biliary cirrhosis (PBC). In 1989, the first open trial using methotrexate in patients with refractory inflammatory bowel disease was conducted.

Mechanism of Action. Methotrexate is an inhibitor of the enzyme dihydrofolate reductase and decreases the synthesis of the reducing agent tetrahydrofolate. Tetrahydrofolate is essential in the synthesis of thymidylate, a pyrimidine nucleoside required for DNA synthesis. At high doses, methotrexate inhibits DNA synthesis and is cytotoxic to rapidly proliferating cells. At low dosages, however, methotrexate has no cytotoxic or antiproliferative effects. Instead, methotrexate polyglutamates increase intra- and extracellular levels of adenosine by indirectly inhibiting adenosine deaminase. In neutrophils, this effect leads to a diminished oxidative burst, decreased adhesion to endothelial cells, and decreased production of leukotriene B_4 and TNF-α. In monocytes and macrophages, adenosine inhibits expression of TNF-α, IL-6, and IL-8 and promotes transcription of the anti-inflammatory agents IL-10 and IL-1 receptor antagonist.[174,175]

Pharmacology. Methotrexate can be administered orally, subcutaneously, intramuscularly, or intravenously. The enteral bioavailability varies widely between 45 and 100%.[176] Although oral methotrexate generally has good bioavailability at doses < 15 mg, higher doses can result in poorer absorption. Therefore, high-dose methotrexate is traditionally administered parenterally. The steady-state volume of distribution is approximately 1 L/kg. Although methotrexate undergoes 7-hydroxylation in the liver, the bulk of its excretion (80–90%) occurs in the urine. Less than 10% of methotrexate is excreted into the bile, and there is essentially no extrahepatic circulation. Methotrexate has a biphasic half-life, with the initial phase being 1.5 to 3.5 hours and the terminal half-life 8 to 15 hours.[177] The half-life is increased in patients with renal failure. There is evidence that certain other chemotherapeutic agents, such as cytarabine, lower red blood cell methotrexate levels, but it is unclear whether lower blood levels decrease drug efficacy because methotrexate levels are not routinely monitored.[178]

Adverse Effects and Toxicity. The most common side effects of methotrexate include anorexia, nausea, stomatitis, diarrhea, hepatitis, renal toxicity, bone marrow suppression, opportunistic infections, and, possibly, neoplasm. Severe toxicity usually occurs when high-dose methotrexate is given to treat hematologic malignancies. If leucovorin rescue is not given to patients undergoing high-dose methotrexate therapy, the consequences can be fatal. For autoimmune diseases, however, far lower doses are used, and the primary toxicity of concern has been hepatic fibrosis.[179]

Several studies in patients with rheumatoid arthritis or psoriasis receiving low-dose methotrexate suggest that over a period of years, fatty change in the liver, hepatic lobular necrosis, fibrosis, and cirrhosis rarely occur. As the use of methotrexate has increased, it has become evident that clinically serious liver disease is far less common in patients with rheumatoid arthritis compared with those with psoriasis. Therefore, the recommendations for monitoring by the American College of Rheumatology differ from those of the American Association of Dermatology, with pretreatment liver biopsy recommended only if there is prior excessive alcohol consumption, chronic hepatitis B or C infection, or persistently abnormal baseline aspartate aminotransferase (AST). During therapy, serum AST, ALT, and albumin are monitored at 4- to 8-week intervals. Subsequent liver biopsy is recommended only if the AST is abnormal in 5 of 9 determinations within a 12-month period (6 of 12 determinations if tests are obtained monthly) or if the serum albumin is below normal.[180] Because there is a lack of correlation between liver function abnormalities and liver histology in patients with psoriasis, some physicians still suggest that a liver biopsy be performed after each cumulative dose of 1 to 1.5 g of methotrexate.[181] The effects of methotrexate on liver histology may be formally classified using published criteria.[181]

Until recently, few data regarding hepatotoxicity in patients receiving methotrexate for inflammatory bowel disease have been available.[182] In a study of 32 patients with inflammatory bowel disease receiving a cumulative methotrexate dose of ≥ 1,500 mg, liver chemistry tests were obtained before and during therapy. Twenty patients underwent liver biopsies and had little hepatoxicity despite cumulative doses up to 5.4 g. Although the majority of biopsied patients had mild to moderate steatosis or portal tract inflammation, only one had evidence of fibrosis.[183] In a recent review, it was recommended that liver function tests be checked every 3 months in patients with inflammatory bowel disease receiving methotrexate. If there is an elevation of transaminases, they should be checked monthly. If they are elevated on three consecutive tests or are > 120 IU/L on any one occasion, methotrexate should be stopped or a liver biopsy performed. Some still suggest that surveillance biopsies be considered after 1.5 g of methotrexate or 2 years after starting treatment, whichever is sooner.[184]

Guidelines for biopsy in children are not well established. In one study, 33 children with juvenile rheumatoid arthritis receiving methotrexate (duration of therapy 17–140 months) underwent liver biopsy; 18% had histologic changes, including hepatocellular inflammation or mild fibrosis. In this study, elevated AST or ALT was the best predictor of histologic abnormalities.[185]

In addition to hepatotoxicity, hypersensitivity pneumonitis is a rare but potentially life-threatening event that occurs in < 1% of patients on methotrexate. The onset of fever, cough, shortness of breath, tachypnea, or hypoxia should prompt further evaluation with a chest radiograph and formal pulmonary function testing, and the methotrexate should be discontinued.[186] There have also been case reports of patients on low-dose methotrexate having an increased susceptibility to opportunistic infections, including herpes zoster and *Pneumocystis carinii*. Therefore, although methotrexate is considered to be a less potent immunosuppressive agent than cyclosporine or tacrolimus, the risk of opportunistic infection is still present.

Clinical Uses. In 1989, Kozarek and colleagues used parenteral methotrexate to treat 21 patients with steroid-refractory ulcerative colitis and Crohn disease. Sixteen of 21 subjects had clinical and endoscopic improvement with decreased diarrhea, lowering of the corticosteroid dose, and improvement in disease activity index parameters.[187] Subsequently, the same investigators demonstrated, in an open-label trial, that patients who failed 6-MP or azathioprine therapy could respond to parenteral methotrexate. Further follow-up studies suggest that Crohn disease patients can be maintained in remission with methotrexate, whereas ulcerative colitis patients tend to relapse and require colectomy.[187]

In 1995, Feagan and colleagues conducted a large multicenter trial of intramuscular methotrexate (25 mg weekly) for active Crohn disease. Methotrexate was more effective than placebo in achieving remission (39% vs 19%, respectively) and reducing requirements for prednisone.[188,189] A subsequent multicenter study was conducted to determine the use of methotrexate in the maintenance of remission in Crohn disease. Patients with chronically active Crohn disease who had entered remission after 16 to 24 weeks of treatment with 25 mg intramuscular methotrexate once weekly were included in this study. At week 40, 65% of the methotrexate-treated patients were in remission compared with 39% who received placebo ($p = .01$). Less than 50% of the patients in the methotrexate group had relapsed by the end of the 40 weeks. In addition, fewer patients in the methotrexate group required prednisone for relapse compared with the placebo group (28% vs 58%; $p = .01$). Although nausea and vomiting occurred more frequently among patients receiving methotrexate, none had a severe adverse event. Therefore, patients with Crohn disease can achieve remission with methotrexate therapy, and a low dose is effective in maintaining remission.[190]

In children, Mack and colleagues conducted an open-label trial of methotrexate (15 mg/m^2/wk subcutaneously) in 14 patients with inflammatory bowel disease who had failed 6-MP therapy. After 3 months of therapy, seven patients had inactive Pediatric Crohn's Disease Activity Index (P-CDAI) scores, six still had active P-CDAI scores, and one patient died (most likely unrelated to the methotrexate).[191]

Methotrexate also has been used to treat PBC and PSC. However, studies of methotrexate efficacy in the treatment of PBC have given conflicting results.[192–194]

In a randomized double-blind trial involving 24 patients with PSC, those on methotrexate had lowering of alkaline phosphatase levels but no improvement of liver histology. However, these patients had advanced liver disease, with 50% having cirrhosis at the start of therapy.[195] In another study of 19 patients with PSC, the use of methotrexate, in combination with ursodeoxycholic acid (UDCA), was associated with toxicity without further improvement in liver biochemistries compared with UDCA alone.[196] Therefore, there is little evidence that methotrexate is of benefit in the treatment of PBC or PSC.

Mycophenolate Mofetil (CellCept)

Mycophenolate mofetil (MMF) is an antiproliferative agent that inhibits lymphocyte DNA synthesis and that was given FDA approval in 1995 for the treatment of renal transplant rejection. The drug is now undergoing widespread use in renal, heart, and liver transplant recipients and has been preliminarily studied in inflammatory bowel disease.

Mechanism of Action. MMF is an ester prodrug of the immunosuppressant mycophenolic acid. MMF inhibits lymphocyte proliferation by blocking the de novo synthesis of guanosine nucleotides from inosine via reversible inhibition of the enzyme inosine monophosphate dehydrogenase (IMPDH). Mycophenolic acid is a more potent inhibitor of the type II isoform of IMPDH, which is found in activated lymphocytes, as opposed to the type I isoform, which is expressed in most cell types.[197] Unlike other cell types that can use a salvage pathway for synthesis of guanosine, B and T lymphocytes are dependent on the de novo pathway for generation of guanosine. The effects of this drug are wide ranging and include inhibition of cytotoxicity by T lymphocytes and the inhibition of antibody production by B cells. In vitro, MMF inhibits DNA synthesis and the proliferation of peripheral blood lymphocytes in response to both T- and B-cell mitogens. In addition, MMF alters cell adhesion and decreases the recruitment of inflammatory cells into target tissues by inhibiting glycosylation of adhesion molecules.[198]

Pharmacokinetics. Oral MMF is generally well absorbed, even though intake with food will decrease absorption. The volume of distribution is approximately 3.6 L/kg in healthy adults. In the body, MMF is rapidly converted to mycophenolic acid, the active metabolite. Enterohepatic cycling contributes about 40% to the AUC of mycophenolic acid.[199] Elimination of mycophenolic acid involves glucuronidation by the liver, with renal elimination of more than 90% of the glucuronidated compound and fecal excretion of the remainder. The half-life of the mycophenolic acid is approximately 18 hours in adults. Patients with renal failure (Cr_{Cl} < 25 mL/min) have an increased AUC and a prolonged half-life. In children, AUC increases with increasing age, suggesting that young children metabolize the drug more rapidly.[198,200] Most studies in adults agree that therapeutic drug monitoring can help to avoid adverse effects and optimize immunosuppression. There is increasing evidence that children may benefit from the same monitoring, particularly in the early postoperative period. A trough of 2 to 5 mg/L and an average AUC_{12} of 59 mg/L/h, corresponding to 50% inhibition of IMPDH, is proposed to be sufficient for immunosuppression.[199]

Adverse Effects. The principal side effects of MMF are gastrointestinal and hematologic. Gastrointestinal side effects, including diarrhea, constipation, nausea, dyspepsia, vomiting, esophagitis, and ulcers, occur in up to 30% of patients. Severe neutropenia may occur in up to 2% of patients receiving MMF. Anemia, thrombocytopenia, and leukopenia also occur in 10 to 25% of patients, a rate comparable to that seen with azathioprine.[198] Opportunistic infections (including cytomegalovirus, herpes zoster, and herpes simplex infections) and malignancies (including lymphoproliferative disease and lymphoma) also occur in transplant patients treated with MMF.

Clinical Use. MMF was first studied as a supplemental antirejection medication in renal transplant patients. In a multicenter trial, MMF at doses of 2 or 3 g/d combined with cyclosporine and steroids reduced the incidence of acute rejection to 31% compared with 47% in patients receiving azathioprine.[201] Multiple studies in liver transplant patients also suggest that MMF can provide effective rescue therapy in patients who develop chronic rejection.[202] In one study, 12 of 19 patients who had failed treatment with azathioprine, prednisone, and cyclosporine had complete histologic resolution of rejection when converted from azathioprine to MMF (from 0.25 to 2 g twice a day).[203]

In a study using MMF as part of induction therapy, 350 liver transplant patients were randomized to receive either double-drug therapy (tacrolimus and steroids) or triple-drug therapy (tacrolimus, steroids, and MMF 1 g twice a day). Patients in the double-drug arm who developed acute rejection received MMF as rescue therapy. The rate of rejection was significantly lower at 3 months in the group that received triple-drug therapy ($p < .03$). However, there was no difference in the overall rejection rate or graft survival at the end of follow-up (mean 33.8 ± 9.1 months). This study suggests that MMF does not need to be started immediately post-transplant but can be added to the drug regimen if rejection develops.[202,204] In a prospective study comparing the efficacy of MMF versus azathioprine in 63 liver transplant patients, who also received thymoglobulin and methylprednisolone, there was a trend toward a lower incidence of acute rejection in the MMF group ($p = .06$). There was, however, a decreased rate of thrombocytopenia in patients who received MMF compared with those who received azathioprine (19% vs 47%, respectively).[205] In a pediatric series of 26 patients, MMF (10–12.5 mg/kg/dose twice a day), the combination of microemulsion cyclosporine and prednisone, was found to be effective primary antirejection therapy, with only 20% of patients needing conversion to tacrolimus for persistent rejection.[206]

MMF also has been used as adjunctive therapy in adults with Crohn disease with variable success.[207,208] In one open-label trial involving six patients with refractory

perianal or ileal Crohn disease unresponsive to azathioprine, patients were given 1 g twice a day of MMF. All six patients improved, and four entered full remission.[207] A larger randomized trial involving 70 patients used MMF or azathioprine as a steroid-sparing agent. Response rates, tolerability, and side effects were comparable in both arms of the trial.[208] In an open-label trial, 11 patients with Crohn disease and 13 with ulcerative colitis were treated with MMF 2 g/d and prednisone (starting dose of 60 mg/d followed by a taper of 5 mg/wk to reach a final dose of 5 mg/d). Four patients with Crohn disease and six with ulcerative colitis achieved remission at 3 months. However, remission was maintained in only one Crohn disease patient at the end of the 6-month study.[209]

A pilot study comparing MMF versus azathioprine in 24 patients with active ulcerative colitis found that 42% (5 of 12) of patients who received MMF and prednisolone achieved remission in the first 4 weeks and remained in remission at 1 year. When compared with the azathioprine and prednisone group, however, the rates of remission were lower, the number of relapses higher, and the number of patients weaned off steroids lower. Current data suggest that in inflammatory bowel disease, MMF should only be used as a rescue therapy if more conventional immunomodulatory agents (eg, 6-MP, azathioprine, methotrexate, infliximab) are ineffective or not tolerated.[210]

Although the frequency of intestinal transplant is increasing, there still are limited data regarding the use of MMF in this setting. In one center's experience with 77 intestinal transplant patients, there did not appear to be improved graft survival with MMF compared with three other treatment arms. In addition, it was noted that patients who received MMF tended to have a higher incidence of graft-versus-host disease and post-transplant lymphoproliferative disease compared with the other treatment regimens.[211]

Sirolimus (Rapamycin, Rapamune)

Sirolimus is a hydrophobic macrocyclic lactone produced by *Streptomyces hygroscopicus*. Although structurally similar to tacrolimus, the mechanism of action and adverse effect profiles of the two drugs are quite different. Sirolimus is a potent immunosuppressive drug that has been used in renal transplant and is being increasingly studied in liver transplant as well.

Mechanism of Action. Sirolimus, like tacrolimus, readily penetrates the plasma membrane and binds to FKBP12. Whereas tacrolimus blocks cytokine gene transcription by binding calcineurin, the sirolimus-FKBP12 complex binds to a specific cell-cycle regulatory protein, mammalian target of rapamycin (mTOR). This binding to mTOR then leads to suppression of cytokine-driven (IL-2, -4, -7, and -15) T-cell proliferation, interrupted progression from the G_1 to the S phase of the cell cycle, and inhibition of IL synthesis.[212]

Pharmacokinetics. Sirolimus is manufactured as an oral solution and as a tablet, which are bioequivalent in nature. The oral bioavailability is 14%, and there is rapid intestinal absorption. Intake with food and drugs such as cyclosporine, diltiazem, and ketoconazole will increase exposure to sirolimus. There are interactions with drugs metabolized by cytochrome P-450 3A.[213] More than 90% of drug is excreted in feces, with urine representing only a minor route of elimination (2.2%). The mean half-life is 60 hours, but there is significant interpatient variation, particularly in patients with hepatic impairment and in children. For this reason, therapeutic drug monitoring is recommended.[214]

Adverse Effects. The principal adverse effects associated with sirolimus include hyperlipidemia and bone marrow suppression. Sirolimus raises high-density lipoproteins in serum but also produces much greater elevations of low-density lipoproteins, cholesterol, and triglycerides.[215] Hyperlipidemia occurs in about 40% of renal transplant recipients receiving sirolimus.[214] Bone marrow suppression is reversible and concentration dependent and occurs in 61% of sirolimus-treated patients. Inhibition of critical cytokine signals that promote maturation and proliferation of bone marrow precursors are likely responsible for sirolimus-related myelosuppression. Patients with trough levels ≥ 15 ng/mL tend to have an increased risk for lipotoxicity and myelotoxicity. Other less common adverse effects include diarrhea, epistaxis, noninfectious pneumonitis, arthralgia, and potentiation of adverse reactions to cyclosporine, namely hypertension, acne, and hirsutism.[214,215] The incidence of post-transplant lymphoproliferative disorders in renal transplant patients receiving sirolimus was not significantly different compared with placebo ($p = .162$).[216]

Clinical Uses. Sirolimus has recently been studied in phase III trials in renal transplant and has been used in liver and small bowel transplant. In a multicenter, double-blind, placebo-controlled trial of 576 renal transplant patients, subjects treated with cyclosporine and corticosteroids were then randomly assigned to receive either placebo or sirolimus at either 2 mg/d or 5 mg/d. The primary composite end point (ie, first occurrence for biopsy-confirmed acute rejection, graft loss, or death) at 6 months post-transplant was lower in the sirolimus-treated groups compared with the placebo group. Also, the incidence of biopsy-proven rejection at 12 months was lower in both of the sirolimus groups (2 mg/d and 5 mg/d) compared with the placebo group (24.7% and 19.2% vs 43.3%; $p \leq .002$).[216]

Flechner and colleagues conducted a prospective randomized trial of sirolimus versus cyclosporine in 61 adult primary renal transplant patients treated with basiliximab and MMF.[217] There was no statistically significant difference in the 1-year patient survival, graft survival, and biopsy-confirmed acute rejection rates between the two study arms. At 6 and 12 months, the sirolimus-treated patients had significantly better serum creatinine levels and creatinine clearances compared with cyclosporine-treated patients. This would suggest that calcineurin inhibitor drug avoidance with sirolimus provides comparable immunosuppression while diminishing the nephrotoxic side effects.

A pilot study of 15 liver transplant patients suggested that sirolimus reduces the incidence of rejection when added to prednisone and cyclosporine.[217] In a retrospective review of 14 liver transplant patients with renal insuffi-

ciency or acute mental status impairment, immunosuppression with MMF, corticosteroids, and sirolimus (dose between 1 and 4 mg/d) was used. Six patients developed acute rejection; however, only one required antilymphocytic therapy. The findings suggest that sirolimus may be an alternative immunosuppressive agent when calcineurin inhibitors are undesirable or contraindicated.[218] In another study, 39 liver transplant patients were treated with either tacrolimus or cyclosporin A and sirolimus (dose 2 mg/d), with a 3-day tapered dose of steroids. Patient and graft survival rates were identical to those of historical controls. The incidence of rejection and muromonab-CD3 use was lower than that of historical controls. These findings suggest that liver transplant may be successfully performed with sirolimus and minimal corticosteroid use.[219]

Florman and colleagues have reported on intestinal transplant patients treated with tacrolimus-based immunosuppression and steroids. In addition, subjects received either sirolimus and basiliximab or daclizumab. There was a decreased incidence of rejection among the sirolimus-treated group both at 30 days ($p < .002$) and at 90 days ($p < .02$). There was no significant difference in actuarial 1-year patient survival or graft survival.[220]

Infliximab (Anti-TNF, cA2, Remicade)

Infliximab is a murine-human chimeric monoclonal antibody directed against TNF-α. Over the past decade, infliximab has been used extensively in the treatment of inflammatory conditions such as rheumatoid arthritis and Crohn disease.

Mechanism of Action. TNF-α is a potent proinflammatory cytokine whose effects are mediated by ligation to the receptors, tumor necrosis factor receptor (TNFR)-I and TNFR-II. In addition, both monocytes and lymphocytes express a membrane-bound form of TNF-α that is thought to activate other cells by direct cell-cell contact. When bound to soluble TNF-α, infliximab is able to neutralize its effects, preventing release of other cytokines, such as IL-6.[221] When bound to the membrane-bound form of TNF-α, infliximab is able to fix complement and mediate destruction of TNF-α–producing cells such as T lymphocytes and is thereby able to induce apoptosis of macrophages.[222,223]

Pharmacokinetics. Infliximab is administered as an intravenous infusion, usually given over 2 hours. Recommended adult dosing is as follows:

1. Fistulizing Crohn disease: 5 mg/kg given at weeks 0, 2, and 6
2. Moderate to severe Crohn disease, induction: 5 mg/kg given at weeks 0, 2, and 6
3. Moderate to severe Crohn disease, maintenance: 5 mg/kg every 8 weeks

Infliximab is distributed primarily within the vascular compartment. Serum concentrations of infliximab may be detected up to 8 to 12 weeks after infusion.[224]

Adverse Effects. Infusion reactions are a well-described complication of infliximab therapy, occurring in 4 to 13% of adult patients (Table 76.1-8). Reactions may be immediate or delayed and range in degree from a mild reaction to anaphylaxis. Typical immediate reactions include flushed sensation, shortness of breath, dizziness, hypotension, hives, nausea, vomiting, rash, and numbness. Delayed reactions include joint pain or swelling, rash, and fever. For immediate reactions, patients generally respond to a decreased rate of infusion. Occasionally, however, diphenhydramine or even epinephrine may be required.[225]

Other adverse effects include a risk for reactivation of tuberculosis (TB), development of autoantibodies, risk for infection, and risk of malignancy. Between 1998 and 2002, 101 cases of TB were reported in patients receiving infliximab.[226] Current recommendations include screening for TB prior to the initiation of infliximab therapy.

In the ACCENT I trial of 573 patients with Crohn disease receiving infliximab as a maintenance therapy, 32% had infections (most commonly, upper respiratory tract and urinary tract infections) requiring treatment. More serious infections occurred in 4% of patients.[227]

The development of antinuclear antibodies (ANAs) and rare cases of lupus-like syndrome have been reported. A recent report of 125 patients with Crohn disease receiving infliximab found that 56.8% of patients have developed a positive ANA (titer > 1:40) during treatment. To date, two patients developed symptoms, which resolved after the cessation of therapy.[228] The clinical implication of an elevated ANA in the absence of symptoms has yet to be determined. Eight cases of lymphoma, occurring a median of 6 weeks after initiation of the infliximab treatment, were reported to the FDA between May 1999 and December 2000. There are insufficient data to determine if infliximab directly contributed to the development of the malignancy.[229]

Clinical Use. Infliximab was first used for Crohn disease in 1993.[230] Since then, it has been employed in the treatment of adults with moderate to severe Crohn disease and/or fis-

TABLE 76.1-8 ADVERSE EFFECTS REPORTED IN INFLAMMATORY BOWEL DISEASE AND RHEUMATOID ARTHRITIS PATIENTS TREATED WITH INFLIXIMAB

Infusion reactions	Chest pain, dyspnea, vomiting, urticaria, anaphylaxis
Immune-mediated reactions	Aseptic meningitis
	Antinuclear antibodies and lupus-like syndrome
	Adult respiratory distress syndrome
Opportunistic infections	Tuberculosis, histoplasmosis, *Salmonella*, *Listeria*, *Cryptococcus*, cytomegalovirus, aspergillosis
Hematologic	Neutropenia, thrombocytopenia
Other	Headache, nausea, upper respiratory infection, pharyngitis, sinusitis

Adapted from Cunnane G et al,[275] Cheifetz A et al,[276] Lee JH et al,[277] and Lipsky PE et al.[278]

tulizing disease with inadequate response to more conventional therapies. In a retrospective review of 19 pediatric children with Crohn disease who received one to three infusions of infliximab at a dose of 5 mg/kg over a 12-week period, there was a reduction in the P-CDAI in all patients.[231] In a prospective open-label trial of a single 5 mg/kg infusion of infliximab in 15 children with medically refractory Crohn disease, 14 of 15 (94%) had a decrease of both P-CDAI and daily steroid use. Ten patients (67%) achieved complete remission by 10 weeks.[232] In a multicenter, open-label, dose-blinded trial, 21 pediatric patients received a single infusion of infliximab at a dose of 1, 5, or 10 mg/kg. Improvement in the P-CDAI was observed with all infliximab dosages (median improvement 50%). All achieved clinical response, and 10 of 21 (48%) achieved clinical remission. Adverse events included upper respiratory tract infection, pancreatitis, sinusitis, and appendicitis.[224]

Although infliximab has been effective in inducing clinical response and remission after a single infusion, the benefit of infliximab as maintenance therapy is not as well established. In a multicenter randomized controlled trial of 573 adults with active Crohn disease, subjects received an initial 5 mg/kg infusion and then were randomized to receive repeat infusions of placebo or 5 or 10 mg/kg of infliximab at 8-week intervals. Patients receiving infliximab as maintenance infusions were more likely to maintain remission and to discontinue steroids compared with those who received placebo. However, a cautionary note is highlighted by two patients who died from sepsis.[227]

Randomized trials have not yet established efficacy of infliximab in ulcerative colitis. Nonetheless, initial studies suggest that infliximab may be effective in the treatment of this disease. A double-blind placebo-controlled trial of infliximab conducted in 11 patients with severe, active, steroid-refractory ulcerative colitis was terminated early because of slow enrolment. Nevertheless, 4 of 8 patients who received infliximab were considered treatment successes compared with none of the 3 receiving placebo.[233] In another study, eight patients with refractory ulcerative colitis scheduled for surgical colectomy were treated with a single 5 mg/kg infusion of infliximab. All patients experienced marked improvement in clinical symptoms, colonoscopic findings, and histopathology.[234] Nine children with moderate to severe ulcerative colitis unresponsive to conventional therapies were treated with infliximab infusions. Seven (77%) patients had improvement in activity of their disease at a median follow-up of 20 weeks.[235]

The formation of antibodies to infliximab (ATI) is associated with an increased risk of infusion reactions and diminished efficacy of repeated infusions. The use of concurrent immunosuppressants and the administration of a second infusion of infliximab within 8 weeks of the first both reduce ATI formation.[236] In a cohort of 125 patients with Crohn disease treated with infliximab, ATI were detected in 61% of patients. The presence of antibody was associated with an increased risk of infusion reactions and a reduced duration of response to treatment.[237] Another study found that 19 of 53 patients (36%) developed ATI. Loss of initial response and incidence of infusion reactions were strongly related to ATI formation. Following this finding, 80 patients were randomized to receive intravenous hydrocortisone 200 mg or placebo prior to infusions of infliximab. At week 16, whereas ATI levels were lower among hydrocortisone-treated patients (1.6 vs 3.4 µg/mL, $p = .02$), there was no reduction in infusion reactions.

Antilymphocyte Antibody Preparation: Muromonab-CD3 (Orthoclone, OKT3, Monoclonal Anti-CD3)

Polyclonal antisera to human T cells (antilymphocyte globulins) were developed in the 1970s for the treatment of organ rejection and hematologic malignancies. However, such preparations were variable in their potency (because they were prepared by generating immune responses in vivo in animals) and contained extraneous antibodies. Muromonab-CD3 is a purified monoclonal IgG2a antibody obtained from murine ascites directed against the CD3 antigen on human T lymphocytes. Treatment with muromonab CD3 results in the short-term depletion of systemic T lymphocytes and a dramatic inhibition of lymphocyte cytotoxic reactions. This drug is now accepted therapy for the treatment of acute cellular graft rejection.

Mechanism of Action. Muromonab-CD3 binds to virtually all differentiated human T lymphocytes through the CD3 molecule. The CD3 complex is essential for signal transduction between the TCR and the intracellular kinases, resulting in T-lymphocyte activation. In vitro effects of anti-CD3 are complex; anti-CD3 can cause polyclonal T-lymphocyte activation, but only when a second signal (such as phorbol myristate acetate or a monocyte–T-cell interaction) is delivered in addition to the anti-CD3. In vivo, however, muromonab anti-CD3 opsonizes T cells; the coated T cells are subsequently removed by cells of the reticuloendothelial system or lysed by complement. Within 1 hour of muromonab-CD3 administration, CD3+ T lymphocytes are cleared from the systemic circulation.[238]

Pharmacologic Aspects. Muromonab-CD3 must be given intravenously to prevent degradation. Goldstein and colleagues demonstrated in adult renal transplant recipients treated for rejection that a once-daily dose of 5 mg maintains a steady-state level of 800 to 1,000 µg/L, a level adequate to block cytotoxic T-cell function and facilitate T-cell clearance.[239] Cessation of treatment results in a rapid return of peripheral blood CD3 T cells, which reach normal levels within a few days. Therefore, muromonab-CD3 must be administered for 10 to 14 days to have a sustained effect. Alloway and colleagues demonstrated that a lower dose of muromonab-CD3 (2 mg daily) as prophylactic therapy for rejection of renal allografts is as efficacious as the higher (5 mg) dose.[240] Dosage for smaller pediatric recipients is 2.5 mg/d (weight < 30 kg) and 5 mg/d for those with body weight > 30 kg.[241]

Adverse Effects. The adverse effects of muromonab-CD3 require that the first few doses be given in hospital and currently limit this therapy to transplant recipients

(Table 76.1-9). The administration of muromonab-CD3 is associated with a variety of adverse effects known as the cytokine release syndrome. This occurs as a result of the systemic release of cytokines (including TNF-α and IFN-γ) by activated or lysed T lymphocytes.[242] Symptoms generally include fever, chills, shortness of breath, nausea, vomiting, diarrhea, headaches, and myalgias. Less commonly, patients experience wheezing, thrombocytopenia, hypertension, and rash.[238] More serious, and rarer, complications of muromonab-CD3 include pulmonary edema (which generally develops in patients with fluid overload) and aseptic meningitis.[238]

Long-term side effects include overwhelming infection with conventional and opportunistic pathogens (particularly herpes simplex virus and cytomegalovirus).[243,244] In addition, treatment with anti-CD3 places transplant patients at increased risk for the development of lymphoproliferative disease or other hematologic malignancies.[245]

TABLE 76.1-9 ADVERSE EFFECTS OF MUROMONAB-CD3 (OKT3)

"First-dose syndrome": > 50% of patients
Fever
Chills
Tremor
Dyspnea
Wheezing
Nausea/vomiting
Diarrhea
Rash
Joint pain
Tachycardia, hypertension (rare)
Pulmonary edema (rare)
Aseptic meningitis
Interstitial nephritis
Infections (especially cytomegalovirus, adenovirus, herpes simplex)
Lymphoproliferative disease

Adapted from Todd P and Brogden R,[238] McDiarmid S et al,[243] and Fung J and Starzl T.[249]

Clinical Use. Pediatric gastroenterologists use muromonab-CD3 almost exclusively in the treatment of liver transplant recipients. Currently, muromonab-CD3 is used to treat episodes of acute rejection usually after courses of high-dose steroid therapy have failed. The drug also is useful in patients who have renal dysfunction, which limits the use of cyclosporine.[243]

Treatment of acute rejection with muromonab-CD3 reverses rejection up to 70 to 80% of cases in adult series and improves allograft survival.[246] A randomized trial found muromonab-CD3 better than high-dose steroids (73% vs 23% response rate) in treating the first episode of rejection following liver transplant.[247] However, a large pediatric study using muromonab-CD3 to treat liver transplant rejection reported a success rate in treating rejection of only 59%. In addition, the majority of patients still had greater than 5% CD3+ cells in their peripheral blood during therapy. The investigators concluded that pediatric patients may be less responsive to muromonab-CD3 than are adults.[243]

Administration of a second course of muromonab-CD3 is less effective than the initial course of therapy owing to the development of anti-idiotype antibodies, which inactivate the molecule. Antibody production can be measured, however, and patients who develop only low-titer antibodies after the first course of muromonab-CD3 may be successfully treated with a second course.[238,248] Muromonab-CD3 also has been used as prophylaxis in the immediate postoperative period following liver transplant.

Although clearly effective in prevention of rejection, muronomab does not appear to offer any demonstrable benefit over conventional immunosuppression (calcineurin inhibitor, azathioprine, and prednisone). The cost and future risks of opportunistic infections and malignancy limit its use.[249]

IL-2 Receptor Antibodies

Another target for monoclonal antibodies is the IL-2 receptor (IL-2R). T cells involved in acute rejection are characterized by increased expression of activation markers such as IL-2R. The IL-2R is composed of three chains (a 55 kD α chain, a 75 kD β chain, and a 64 kD γ chain) and is present on activated T cells. Binding of IL-2 to IL-2R results in T-cell proliferation and clonal expansion of activated T lymphocytes. Anti–IL-2R antibodies (antibodies to the α chain of the IL-2R) are designed to provide greater selectivity against only those lymphocytes directly involved in the immune response because resting T lymphocytes express low levels of the IL-2R.

IL-2R antibodies were initially developed for use in induction protocols for renal transplant patients. There have been good results in this setting, with a decreased incidence of acute rejection and minimal side effects. More recently, use has been applied to the prophylaxis of acute rejection in liver transplant patients. Two antibodies are now commercially available, both of which contain the hypervariable regions of the murine anti–IL-2R antibody (anti-Tac) involved in binding the α subunit. The main differences between the two are the half-life, the proportion of murine to human sequences, and the affinity to its target.[250]

Daclizumab (Zenapax). In a study by Koch and colleagues, the pharmacokinetics and pharmacodynamics of daclizumab in liver transplant patients were investigated. Patients were given daclizumab at a dose of 1 mg/kg within 6 hours of graft reperfusion on day 0 and then a dose of 0.5 mg/kg on day 4 following transplant. This dosing regimen provided effective blockade of the IL-2Rα for at least 14 days after transplant. CD25+ cells are decreased for 28 days following administration of daclizumab. Whereas the half-life of daclizumab in renal transplant patients is 273 hours, in liver transplant patients, it is significantly shorter (99 hours). Drug clearance through drained ascites, postoperative blood loss, or loss of antibody into intercellular and extravascular spaces may account for this difference. To date, no human antibody responses against daclizumab have been reported.[250]

An initial study by Hirose and colleagues investigated the use of daclizumab (1 mg/kg on day 0 then at 2-week intervals for a total of five doses) as part of an induction therapy with mycophenolate and corticosteroids but with-

out calcineurin inhibitors. Treatment with daclizumab in the absence of calcineurin inhibitors was ineffective, with a 100% acute rejection rate. One possible explanation was inadequate dosing of daclizumab.[251] In a subsequent study of 39 adult liver transplant patients, daclizumab was used as adjunctive therapy to an induction regimen of corticosteroids and cyclosporine or tacrolimus. The daclizumab group experienced less acute rejection (18% vs 40%; $p = .02$) in the first 6 months compared with the control group.[252] Heffron and colleagues conducted an open-label prospective study of daclizumab in pediatric liver transplant recipients. Twenty patients received a single dose of daclizumab (1 mg/kg) within 24 hours after liver transplant, and tacrolimus was withheld for 7 days following surgery. A historical control group of patients who received tacrolimus immediately following tranplant was used for comparison. Both groups of children also received mycophenolate and corticosteroids. There were fewer episodes of acute rejection in the daclizumab-treated group compared with historical controls ($p = .002$).[253]

Basiliximab (Simulect). In comparison with daclizumab, basiliximab has a shorter half-life (approximately 6.5 days in renal transplant patients) and is less humanized but has a higher affinity for IL-2Rα. The role of basiliximab in liver transplant is still being developed. In a multicenter randomized controlled trial, 381 adult liver transplant patients received basiliximab 20 mg on days 0 and 4 in addition to cyclosporine and corticosteroids. There was a decreased acute rejection rate in patients receiving basiliximab (33.1% vs 47.6%; $p = .034$).[254] In a smaller study, seven pediatric liver transplant recipients with steroid-resistant rejection received one or two doses of basiliximab (10 mg for patients < 30 kg, 20 mg for patients > 30 kg; doses were given 3 to 7 days apart). Primary antirejection therapy consisted of cyclosporine, azathioprine, and prednisolone in four patients and tacrolimus and prednisolone in three others. All had received high-dose steroids for acute rejection and were converted to tacrolimus, followed by the addition of mycophenolate. Five of the patients had improvement, as measured by biochemical responses and by liver biopsy. There were no immediate side effects associated with basiliximab. However, two children went on to develop chronic rejection and subsequently died.[255]

OTHER AGENTS

Leflunomide (Arava)

Leflunomide is a relatively new immunosuppressive agent that inhibits dihydroorotate dehydrogenase.[256] It is an isoxazole derivative that is converted by first-pass metabolism in the liver and gut to an active metabolite, A77 1726, which inhibits de novo synthesis of pyrimidines.[257] In addition, leflunomide also inhibits lymphocyte activation by blocking intracellular tyrosine kinases.[258] In rat xenograft models, leflunomide is effective antirejection therapy.[259] More recently, the active metabolite of leflunomide was shown to halt the replication of herpes viruses, particularly cytomegalovirus and herpes simplex virus.[260]

In 1998, leflunomide was approved in the United States for the treatment of rheumatoid arthritis. A 2-year double-blind randomized controlled trial compared the efficacy of leflunomide versus methotrexate in 235 patients with rheumatoid arthritis. The results demonstrated that leflunomide is safe and equally effective treatment for active rheumatoid arthritis compared with methotrexate. Adverse effects reported include upper respiratory tract infections, diarrhea, nausea and vomiting, rash, reversible alopecia, and transient elevations in liver enzymes.[261]

Efficacy trials have not yet been reported in human transplant recipients. One study by Williams and colleagues, however, reports experience with leflunomide in both renal and liver transplant patients with regard to pharmacologic properties. There was marked interpatient variability (> 300%) in the dose required to reach a targeted serum level. The terminal half-life also varied between 5.2 and 15.1 days. In renal transplant patients, the principal side effect observed was anemia, and in those with liver transplants, there were elevations of serum transaminases.[262]

Thalidomide (Thalomid)

Thalidomide (α-*N*-phthalimidoglutarimide) is a glutamic acid derivative with multiple biologic properties. It is a sedative, a teratogen, an inhibitor of angiogenesis, and an inhibitor of TNF-α production.[263] A number of open-label trials purport that thalidomide is efficacious in a variety of autoimmune and intestinal conditions, including erythema nodosum, aphthous stomatitis, Behçet syndrome, human immunodeficiency virus (HIV)-associated mucosal ulceration, and graft-versus-host disease.[263] Open-label trials also suggest that up to 50% of patients with Crohn disease refractory to other therapies will respond to thalidomide. The drug is typically started at a dose of 50 mg in adults and advanced as high as 200 mg, with clinical improvement usually occurring about 4 to 6 weeks after instituting therapy.[263,264] The primary side effect of thalidomide is neurotoxicity. In addition, the drug is contraindicated in women of childbearing age because of severe limb deformities (phocomelia) in the fetus. Because of the toxicities, this agent should be used with caution in patients with intestinal inflammation.[265,266]

Etanercept (Enbrel)

Etanercept is a genetically engineered fusion protein composed of two identical chains of the recombinant human TNFR p75 monomer that is fused to the Fc domain of human IgG1. This protein binds to and inactivates TNF and lymphotoxin, a cytokine that activates lymphocytes. One of the potential benefits of etanercept is that it does not contain nonhuman material, thereby rendering it less immunogenic. There is extensive clinical experience in the treatment of rheumatoid arthritis with etanercept. More recently, its use in the treatment of refractory Crohn disease also has been studied. In an open-label trial, 10 patients with active Crohn disease were treated with subcutaneous etanercept (25 mg) twice weekly for 12 weeks. Although there was an improvement in CDAI in 6 of 10 patients ($p < .03$), there was no improvement in follow-up terminal ileal or colonic

biopsies. In addition, all patients who initially responded to therapy reported relapse of symptoms within 4 weeks after discontinuation.[267] In a randomized, double-blinded, placebo-controlled trial of 43 patients with moderate to severe Crohn disease, subcutaneous etanercept (25 mg) was administered twice weekly. There was no difference in clinical response at week 4 in patients treated with etanercept compared with placebo (39% vs 45%; $p = .763$). In addition, there was no clear benefit for fistulizing Crohn disease. Common adverse events include injection-site reactions, headache, asthenia, abdominal pain, and skin rash.[268] A report of two patients with Crohn spondyloarthropathy found that although these patients experienced complete resolution of spinal pathology with etanercept, the activity of the Crohn disease did not improve. These findings indicate that binding of TNF alone is not sufficient to ameliorate the inflammatory response in the intestinal mucosa.[269]

Natalizumab (Antegren)

Natalizumab is humanized monoclonal antibody targeted against α_4 integrin. The integrins are a group of heterodimeric proteins composed of noncovalently bound α and β chains that promote cell-cell or cell-matrix adhesion. The α_4 integrins heterodimerize with either α_1 or α_7 subunits. Integrins containing the β_1 subunit are also known as the VLA antigens and are the predominant integrin found on the surface of T lymphocytes. $\alpha_4\beta_1$ (VLA-4) is expressed only on leukocytes and mediates their attachment to endothelial cells via binding to VCAM-1. VCAM-1 is expressed on cytokine-activated endothelial cells and is one of the principal surface proteins that regulates trafficking of lymphocytes to the endothelium. In the gut, the $\alpha_4\beta_7$ integrin binds to mucosal addressin cell adhesion molecule (MAdCAM-1), which mediates homing of T cells to sites of inflammation in the intestinal tract.[270] MadCAM-1 expression is enhanced in inflammatory bowel diseases.[271]

Natalizumab is effective in controlling the exacerbations of inflammatory bowel disease. In a double-blind placebo-controlled dose-ranging multicenter trial of natalizumab of 248 adults with active Crohn disease, clinical responses were higher in all three natalizumab groups at weeks 4, 6, and 8 than in the placebo group. The highest rate of remission (71%) occurred at 6 weeks in the group of patients given two infusions of 3 mg/kg. Drug-associated adverse events were mild; two patients had infusion reactions during the second infusion, and one of these patients developed antinatalizumab antibodies.[272] In a pilot study, 10 patients with ulcerative colitis had improved clinical scores 2 and 4 weeks after a single (3 mg/kg) infusion of natalizumab.[273]

Rituximab (Rituxan, Monoclonal Anti-CD20)

Rituximab is a chimeric (mouse-human) monoclonal antibody that binds to the CD20 antigen on the surface of B lymphocytes. Binding results in antibody-dependent and complement-dependent cytotoxicity. Initially, rituximab was used to treat low-grade B-cell lymphomas but more recently has been used in the treatment of post-transplant lymphoproliferative disease. In one case series reporting six pediatric liver transplant recipients with Epstein-Barr virus–associated post-transplant lymphoproliferative disease, rituximab was given intravenously (375 mg/m^2 once a week for a total of 4 weeks in four patients and a total of 3 weeks in the other three). Infusion with rituximab was associated with complete remission in five patients. Disappearance of tumor masses occurred within 1 to 2.5 months after initiating treatment. Adverse effects included headaches, hypogammaglobulinemia, transient neutropenia, and one case of anaphylactic reaction.[274]

CONCLUSIONS

The optimal treatment of organ rejection and autoimmune diseases continues to evolve dramatically. Newer immunosuppressive agents are effective and potent and seem to have fewer adverse effects, at least in the short term. Instead of agents with broad physiologic and immunologic effects (eg, corticosteroids, azathioprine, calcineurin inhibitors, cyclosporine), the clinician can now use monoclonal antibodies that target one point in the T-cell activation pathway (eg, sirolimus), one cytokine (eg, infliximab, anti-TNFα), or one cell type (eg, rituximab, anti-CD20 B cells). The principal drawback of these new agents is that patients receiving the recombinant proteins develop antibodies, which puts them at risk for infusion reactions, decreased antibody efficacy, and, possibly, other autoimmune disorders. It remains to be seen whether the favorable safety profile of these new biologics will persist with long-term follow-up. Currently, however, these new medications have dramatically improved the quality of life for children and adolescents following organ transplant and those with refractory inflammatory bowel disease.

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2A. *Antimicrobials*

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This section deals with antibiotic modulation of the pattern of colonization at a site or sites within the gastrointestinal tract. Eradication of single pathogens such as *Helicobacter pylori*, *Giardia lamblia*, and parasitic worms from the gastrointestinal tract by antimicrobials is covered in other sections.

The gastrointestinal tract provides the major reservoir of colonizing microorganisms. Determinants of bowel colonization are poorly understood but include mode of delivery (vaginal/cesarean), diet, health status, age, travel, and gastrointestinal physiology and pathology. The impact of the bowel flora on the human host is complex, with a wide range of metabolic, nutritional, and immunologic effects.[1] In health, the bowel flora provides many benefits for the host, including competitive exclusion of pathogens, the recovery of usable energy sources from cellulose, and provision of butyrate to the colonic mucosa. The gastrointestinal microflora and interactions with the mucosa are reviewed in Chapter 2, "Intestinal Flora and Microbial Epithelial Interactions."

Determinants of the resistance of the microflora to perturbation are poorly understood. There is broad agreement that increasing diversity of an ecosystem promotes stability and increases the resistance to perturbation,[2] yet administration of most antimicrobials, by a systemic route to most individuals, will lead to modulation of the bowel flora. Microbial colonization of the gut with commensal flora benefits the host by preventing colonization or overgrowth with pathogens,[3] so it follows that use of antimicrobials for the prevention or treatment of disease can lead to a detrimental change in colonization.[4] The concept of colonization resistance is often taken to imply that the intestinal flora, once established, is relatively static. However, molecular analysis of the bifidobacterial and lactobacillus composition of the microflora in humans suggests that this may not be so and that the pattern of bacterial flora is dynamic in healthy people, regardless of selection pressure caused by the use of antimicrobials.[5] Gastrointestinal side effects are a common consequence of use of antimicrobials. The most frequent side effects are changes in bowel habit and overgrowth with fungi, and a large proportion of these disturbances probably result from alterations of the bowel flora.

The clinical consequences of use of antimicrobials reflect not only modulation of the distribution of microorganisms in the gastrointestinal tract but also changes in microbial physiology and the production of microbial virulence determinants, such as lipopolysaccharide, toxins, and colonization factors.[6–10] Antimicrobials, by modifying microbial metabolic activities, can induce changes in intraluminal conditions, for example, pH, which may also have profound effects on microbial growth and physiology and modify the interaction with the host.[11] Acquisition of antibiotic resistance may be associated with changes in the production of microbial virulence or colonization factors.[12] Even a subinhibitory concentration of antibiotics can have major effects on the transcription of bacterial genes.[13]

There is increasing evidence that the early pattern of colonization is an important determinant of development of the intestine.[14,15] Gram-positive bacteria, such as *Lactobacillus* spp and *Bifidobacterium* spp, may have a role in the prevention of the development of atopy.[16] Neonatal antibiotic treatment of mice leads to a long-term T helper 2 skewed immunologic response, which can be prevented by the introduction of intestinal bacteria.[17]

The effect of administration of antimicrobials in the peripartum period on the early pattern of colonization in the newborn has not been extensively studied, but there is some support for the idea that use of antibiotics in the peripartum period alters the pattern of subsequent microbial colonization and the risk of systemic infection of the newborn.[18] The effects of antibiotic treatment may be more marked in infants than in adults. In a recent study of the fecal flora of 1- to 3-month-old infants, bifidobacteria and lactobacilli were suppressed to undetectable levels in most infants during treatment with amoxicillin, pivampicillin, cefaclor, cefadroxil, erythromycin, or cotrimoxazole. [19]

Antimicrobials may facilitate the acquisition of novel colonizers, a strategy that can be used to facilitate bowel colonization by probiotic strains, but can also reduce the infective inoculum of microbial pathogens.[20] Antimicrobials also provide a selection pressure for colonization or overgrowth of the gut with antimicrobial-resistant microorganisms. The density and diversity of the microflora facilitate the spread of transmissible genetic elements,[21] so the gastrointestinal tract can become a major reservoir for antimicrobial-resistant bacteria and resistance genes, with important consequences for both the colonized individual and others. This is particularly important in hospital environments, such as intensive care units. In preterm infants cared for in a neonatal intensive care unit, use of antibiotics for suspected or proven episodes of infection encourages colonization with a limited range of antibiotic-resistant bacteria, while at the same time the opportunities are reduced

for acquisition of bacteria that normally colonize healthy infants. The intestine, as a major reservoir for antibiotic-resistant bacteria, can predispose the individual infant to systemic infection but also puts at risk other infants in the hospital setting.[22,23] The abnormal pattern of colonization also has been implicated in the pathogenesis of neonatal necrotizing enterocolitis (NEC).[24]

Older infants and children nursed in intensive care units generally arrive in the unit with a complex gastrointestinal microflora but while in hospital acquire novel colonizers, which are frequently antibiotic resistant. Changes in gastrointestinal motility, use of drugs that reduce gastric acid production, and use of biomedical devices such as nasogastric tubes also contribute to modification of the gut flora in children nursed in intensive care units.

CLASSES OF ANTIBIOTIC

A wide range of antimicrobials are available, including β-lactams (cephalosporins and penicillins), related compounds such as carbapenems and monobactams, and macrolides, quinolones, aminoglycosides, tetracyclines, and peptides. Few novel classes of antimicrobial agent have been marketed over the last 10 years, and most that are currently marketed are representative of classes of antimicrobial agents that have been available for over 20 years.

PENETRATION OF THE GASTROINTESTINAL TRACT BY ANTIMICROBIAL AGENTS

The main routes by which antimicrobial agents reach the lumen of the gastrointestinal tract are by oral ingestion, in bile, by transudation across the gut wall, and by rectal administration. Erythromycin, ampicillin-related drugs (including ureidopenicillins), and rifamycins are excreted in high concentrations in bile. In contrast, sulfonamides, chloramphenicol, and aminoglycosides are relatively poorly excreted in bile.

DETERMINANTS OF ANTIMICROBIAL ACTIVITY

Antimicrobial agents differ in the extent to which they impact on the gastrointestinal flora. Activity is dependent on a wide range of factors, including drug characteristics such as spectrum of activity, modes of administration, and routes of excretion (bile, urine) and the extent to which there is an enterobiliary circulation. Important factors specific to individuals include diet, transit time, intraluminal conditions (eg, pH, redox potential, ionic conditions, nonspecific binding to macromolecules such as proteins and mucins, presence of inhibitors, degrading enzymes, growth substrate availability, and bacterial growth phase), and bowel flora components and complexity. For example, the concentration of divalent cations, such as calcium, is an important determinant of the activity of gentamicin, iron may interfere with the activity of tetracyclines, and sulfonamides are inhibited by para-aminobenzoic acid. Some antimicrobials, such as colistin, are rapidly inactivated.[25]

EFFECTS OF ANTIMICROBIAL AGENTS ON THE MICROBIAL FLORA OF THE GASTROINTESTINAL TRACT

The impact of antimicrobials on the culturable components of the oral and fecal flora has been extensively studied,[26–28] frequently in healthy adult volunteers. On the other hand, relatively little is known about the impact of antimicrobial agents in patients with specific disease states or at less accessible sites, such as the small intestine and proximal colon. Attempts to describe the impact of antimicrobials on the flora at inaccessible sites using both in vitro continuous culture and animal models demonstrate that the fecal flora does not accurately represent changes in other parts of the gastrointestinal tract.[29]

Recent molecular studies from subjects undergoing colonoscopy confirm that whereas the fecal flora is different from that of the colon, mucosa-associated bacteria are relatively uniformly distributed along the colon.[30] There is some evidence that host-related factors are important in determining the intestinal microflora, and, as a result, attempts to modulate the gut flora in different individuals may give quite variable results.[30]

In vitro models are an attractive approach for studies of bowel flora modulation by antimicrobials but also may not accurately describe changes in vivo. For example, studies of microbial responses in models of the gastrointestinal flora in vitro have historically used planktonic (ie, free floating) populations of bacteria.[31,32] In contrast, in vivo bacteria usually grow in surface-associated communities referred to as biofilms.[33] Biofilm growth may prove an important element in the pathogenesis of intestinal infections.[34] Bacteria growing in biofilms show important differences from planktonic cells in metabolic activity, gene transfer, and susceptibility to both antimicrobial agents and host defense factors.[35,36]

Another area of uncertainty is the impact of antimicrobials on the unculturable components of the gastrointestinal flora. Recent molecular studies suggest that 50 to 90% of the human fecal flora is unculturable using conventional techniques.[37,38] Current technology precludes studies of the unculturable components of the bowel flora of more than a few individuals. Accordingly, there is little information on the impact of antimicrobials on the uncultured components of the bowel flora. It may be that the use of nucleotide sequence arrays will facilitate further research in this area.

Most studies have concentrated on the impact of antimicrobials on facultative gram-positive cocci (such as staphylococci, enterococci, and streptococci), Enterobacteriaceae, anaerobic species (such as clostridia) and yeasts, and the emergence of antimicrobial resistance. In general, bacteria that are susceptible to the antimicrobial agent administered tend to decrease in numbers, and those that are resistant increase in numbers. For example, administration of penicillin reduces the numbers of strep-

tococci in the mouth, whereas the numbers of Enterobacteriaceae increase. There is considerable interindividual variation in the effect of antimicrobial exposure on the microflora. In addition, antimicrobials within the same class may vary in their impact on the bowel flora.[39] The results of individual studies also will vary depending on the population studied and on the constituents of the microflora, such as the proportion of resistant components. Some of the effects of antimicrobial administration cannot be explained by antimicrobial susceptibility. For example, oral vancomycin reduces the numbers of *Bacteroides* spp despite poor antimicrobial activity in vitro.[40] Some antibiotics, such as macrolides, have effects on gastrointestinal motility through binding and activation of the motilin receptor,[41] and these changes can modify the bowel flora indirectly.

It is still widely assumed that there is a direct and exclusive relationship between the use of a particular antimicrobial agent and the development of resistance. This assumption underlies antibiotic use policies of clinics and hospitals and even national policies. However, it has been known for more than two decades that, at least in the case of Enterobacteriaceae, development of multidrug-resistant strains is generally not the result of accumulation of single point mutations. Instead, there is simultaneous acquisition of genetically linked resistance genes carried on transmissible genetic elements such as transposons, integrons, and plasmids.[42,43] Extensive horizontal gene transfer allows passage of resistance in the intestine between both closely related and distantly related bacteria.[21] Earlier assumptions that bacteria pay a fitness penalty in maintaining antimicrobial resistance in the absence of any selective pressure have proven to be incorrect.[42,44]

USE OF ANTIBIOTICS FOR PREVENTION OR TREATMENT OF DISEASE

Antimicrobials may be used to eradicate a specific pathogen from the gastrointestinal tract (see specific sections) or to modulate the pattern of colonization at a site or sites in the gastrointestinal tract. Antimicrobials may be prescribed for the prevention or treatment of disease and may be used as a sole treatment strategy but are also commonly used in combination with other strategies. Some classes of antimicrobial have highly selective activities, so, for example, aztreonam is active only against gram-negative bacteria, and there are a number of agents with specific activity against gram-positive bacteria. These agents may have a particular role in elucidating the contribution of specific elements of the bowel flora to the pathogenesis of disease.

Table 76.2A-1 summarizes the bowel flora activities of selective antimicrobials and potential indications for their use in the prevention or treatment of disease. Some recently introduced antimicrobials have not been evaluated for bowel flora modulation, and some classes of agent have bowel flora activity only when administered orally or rectally, such as glycopeptides. Carbapenems do not reach high intraluminal concentrations so may have less impact on the bowel flora than antimicrobials such as cephalosporins, which have a similar spectrum of activity.

TREATMENT OF CONDITIONS ASSOCIATED WITH ABNORMAL DISTRIBUTION OF BACTERIA IN THE GASTROINTESTINAL TRACT

There is evidence that some groups of patients with small bowel bacterial overgrowth may benefit from use of antimicrobials over weeks or months.[45–48] However, there are few long-term follow-up studies of patients treated for small bowel stasis. Relapse of symptoms is common unless underlying defects can be corrected. Recent studies do not support the use of small bowel decontamination following liver transplant.[49,50]

Antimicrobials chosen for control of small bowel bacterial overgrowth include tetracyclines, quinolones such as ciprofloxacin, and metronidazole. Each of these classes of agent has a different spectrum of activity and produces a different range of effects on the fecal flora of healthy volunteers. There are no randomized trial data available regarding the comparative efficacy of agents used to treat and control small bowel bacterial overgrowth.

TABLE 76.2A-1 BOWEL FLORA ACTIVITIES OF SELECTIVE ANTIMICROBIALS AND POTENTIAL INDICATIONS FOR THEIR USE IN THE PREVENTION OR TREATMENT OF DISEASE

ANTIMICROBIAL AGENT	BOWEL FLORA ACTIVITY	POTENTIAL INDICATIONS
Cephalosporin	Broad (depends on cephalosporin); generally includes gram-negative bacilli; staphylococci	Prophylaxis of infection—surgery, intensive care (selective decontamination)
Monobactam (aztreonam)	Gram-negative bacilli	? Prophylaxis of necrotizing enterocolitis
Aminoglycosides	Oral administration—gram-negative bacilli; staphylococci	Prophylaxis of infection— surgery, ? neonatal necrotizing enterocolitis, intensive care (selective decontamination)
Penicillins	Broad (depends on penicillin); penicillinase inhibitors further broaden spectrum (tazobactam, clavulanate)	Same as for cephalosporins
Quinolones	Gram-positive bacilli; staphylococci	Small bowel overgrowth; prophylaxis of infection—surgery, immunocompromised
Tetracyclines	Broad	Small bowel overgrowth
Metronidazole	Anaerobes	Small bowel overgrowth
Azole antifungals		Prevention of fungal infection

PROPHYLAXIS OF INFECTION IN THE IMMUNOCOMPROMISED HOST

Antifungal agents have been used to prevent fungal overgrowth of the gastrointestinal tract and thereby reduce the risk of both local and systemic fungal infections in a wide variety of groups of immunocompromised patients, such as in patients being treated for malignancies and those with human immunodeficiency virus (HIV) infection. A recent systematic review concluded that systemic antifungal prophylaxis reduces the severity of oral mucositis and the frequency of oral candidiasis in patients treated with chemotherapy for cancer.[51] Systemic antifungal prophylaxis probably also prevents invasive fungal infection in patients with neutropenia.[52] Antimicrobials also have been used to prevent gram-negative sepsis in patients undergoing treatment for cancer, particularly hematologic malignancies.[53] However, there are concerns that this approach increases the likelihood of serious infection involving antimicrobial-resistant strains and modification in the pattern of infecting agents causing disease, with marginal benefit for patient outcomes.[54,55]

PROPHYLAXIS OF INFECTION IN THE INTENSIVE CARE UNIT

Selective decontamination of the digestive tract has been advocated as a strategy to reduce the risk of ventilator-associated pneumonia in patients undergoing intensive care. The aim of selective decontamination of the digestive tract is to reduce colonization of the upper gastrointestinal tract with aerobic gram-negative rods and *Candida* species. Selective decontamination of the digestive tract involves the administration of a topical antimicrobial preparation to the oropharynx, usually combined with a systemic antimicrobial agent. The use of selective decontamination of the digestive tract has been subject to a number of meta-analyses.[56] The implementation of a selective decontamination of the digestive tract strategy carries considerable costs. Between 13 and 39 patients would require selective decontamination of the digestive tract to prevent one death from ventilator-associated pneumonia.[57] The methodologic quality of these studies also has been criticized.[58] Moreover, there are concerns about the long-term consequences of selective decontamination of the digestive tract on levels of antimicrobial resistance.[59,60] Given that critically ill patients are so heterogeneous and that the antibiotic treatments may be quite different (in duration, type of antibiotic, and route of administration), it is hardly surprising that no overall consensus has emerged. In the largest single trial of selective decontamination of the digestive tract (546 predominantly surgical adult patients), mortality was reduced significantly (number needed to treat = 12). In this study, there was no observed increase in colonization by resistant gram-negative organisms, but there was increased colonization of all patients by ciprofloxacin-resistant coagulase-negative staphylococci and enterococci.[61] Concerns about antibiotic resistance could be reduced if oropharyngeal decontamination alone were enough to improve survival. In a recent study of oropharyngeal decontamination, the incidence of ventilator-associated pneumonia was reduced, but overall patient survival was not improved.[62]

PROPHYLAXIS AND/OR TREATMENT OF INFLAMMATORY BOWEL DISEASE

In animal models of inflammatory bowel disease, the colonizing microflora has an important etiologic role.[63,64] However, evidence supporting the role of antimicrobial agents in the treatment or prevention of inflammatory bowel diseases in humans is limited.[65–68] Metronidazole, in high doses, has been shown to modify the course of Crohn disease in some patients, such as those with perianal fistulae.[69] There is little evidence of a role for antimicrobial agents in the control or treatment of ulcerative colitis.[70] The use of live microbial feed supplements (probiotics) has been shown in one randomized controlled trial to benefit patients with pouchitis.[71] Antimicrobial agents also can be used to facilitate probiotic colonization, and it may be that in the future, attempts to modify the luminal flora to benefit patients with inflammatory bowel disease will use an approach in which antimicrobial agents are first used to facilitate subsequent colonization with probiotics.

PROPHYLAXIS OF INFECTION ASSOCIATED WITH ABDOMINAL SURGERY

There is an extensive literature and broad agreement on the benefit of antimicrobial agents for the prophylaxis of infection associated with gastrointestinal tract surgery.[72–74] Current recommendations are that there should be a suprainhibitory concentration of appropriate antimicrobial agents present at the site of operation and at the time of bacterial contamination of the operative site. There is little evidence that extending prophylaxis beyond the period of operation is of any benefit, and it may be of harm.[75] Antimicrobial agents chosen for prophylaxis should be active against both facultative bacteria and strict anaerobes. Metronidazole alone is less effective than when it is combined with an agent active against facultative bacteria, such as a cephalosporin. However, the optimum regimen for prophylaxis in the perioperative setting has not been defined.

NEONATAL NECROTIZING ENTEROCOLITIS

Attempts to reduce the incidence of NEC by the oral administration of antibiotics have not provided conclusive results. This subject has been systematically reviewed,[76] with the conclusion that the incidence of NEC may be reduced by the oral administration of antibiotics, but also concluding that there are concerns about the selection of antibiotic-resistant bacteria and identifying a need for further studies of sufficient size and duration to allow the risks and benefits to be assessed. The antibiotics used to treat infants for episodes of suspected sepsis may also modify the bowel flora and influence the incidence of NEC.[77] There are no randomized comparative studies of antimicrobial efficacy in the treatment of neonatal NEC.

SHIFTING THE FLORA TO A MORE HEALTHY COMPOSITION

Recent research suggests that the pattern of bowel colonization, particularly in early life, may have an important influence on subsequent health and disease, such as on the risk of development of atopy.[78] The use of antibiotics to modulate the bowel flora of healthy individuals with a view to long-term health benefits, however, has not been systematically investigated.

It is now well established that there are major differences between the intestinal flora of formula-fed and breast milk–fed infants. These differences are particularly marked in very low birth weight infants.[79] The mode of delivery also is important, and babies born by cesarean section have a different flora, which persists at least until 6 months after birth, presumably owing to the use of prophylactic antibiotics (a single dose of ampicillin) and reduced exposure to the mother's bacterial flora at the time of delivery.[80]

There is no agreement regarding the age at which the intestinal microflora becomes comparable to that of an adult. In a recent study using viable bacterial counts, 16S ribosomal ribonucleic acid, and generic probes, it was found that there were higher numbers of bifidobacteria and clostridial species in the pediatric population compared with healthy adults. Most strikingly, the carriage of Enterobacteriacae was 100-fold higher in children (16 months to 7 years) than in healthy adults (21–34 years).[81]

PROMOTING COLONIZATION OF PROBIOTICS

Antimicrobial agents may have a role in changing patterns of colonization to a more healthy composition and perhaps facilitate colonization of the intestinal tract by probiotic bacteria. There is a need for further research in this area.

CONCLUSIONS

Antimicrobial agents can be used to modify the gastrointestinal flora in ways that may benefit the host, such as for prophylaxis of infection associated with gastrointestinal tract surgery. Antibiotics may also have other indirect effects on the flora that are not readily apparent. Current strategies emphasize the eradication of pathogens or suppression of flora rather than modulation to a more healthy composition. Future strategies may well see the use of antimicrobial agents to facilitate colonization of the bowel with microbes that contribute to the maintenance of health (ie, probiotics) rather than simply for the eradication or suppression of those microbes that cause disease.

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2B. *Probiotics*

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Probiotics are live microbial food supplements or components of bacteria that have been demonstrated to have beneficial effects on human health. Oral introduction of probiotics reinforces various lines of gut defense, including immune exclusion, immune elimination, and immune regulation. Probiotics also stimulate nonspecific host resistance to microbial pathogens and thereby aid in their eradication. Correction of the properties of unbalanced indigenous microbiota forms the rationale of probiotic therapy. The application of probiotics currently lies in reducing the risk of diseases associated with gut barrier dysfunction; the most fully documented probiotic intervention is the treatment and prevention of acute infectious diarrhea. Recent clinical and nutritional studies and characterization of the immunomodulatory potential of specific strains of the gut microbiota, beyond the effect on the composition of the microbiota, may lead to future applications not only for different infectious diarrheas but also for allergic and inflammatory diseases.

The probiotic potential of strains differs; different bacterial species, and even strains of the same species, are each unique and have defined adherence sites, specific immunologic effects, and varied effects in the healthy versus the inflamed mucosal milieu. Current probiotic research aims at characterization of the healthy individual gut microbiota and understanding the microbe-microbe and host-microbe interactions. The goal is to use the defined microbiota both as a tool for nutritional management of specific gut-related diseases and as a source of novel microbes for future probiotic bacteriotherapy applications.

HEALTHY GUT MICROBIOTA

The human gastrointestinal tract harbors a complex collection of microorganisms, which form a typical individual microbiota for each person.[1] This specific microbiota is dependent on genetic factors and the environment. The total number of microbes in the intestinal tract is estimated to reach 10^{12} bacteria per gram of intestinal contents. Several hundred bacterial species can be identified using traditional culture methods.[1] The development of novel means of characterizing gut microbiota, in particular molecular methods, has uncovered new microbial species in intestinal mucosa and contents.[1,2]

The microbiota is metabolically active, and its composition is related to multiple disease states within the intestine and also beyond the gastrointestinal tract. Components of the human intestinal microbiota or organisms entering the intestine may, however, have both harmful or beneficial effects on human health.

The basis of healthy gut microbiota lies in early infancy and the initial process of intestinal colonization. The generation of immunophysiologic regulation in the gut depends on the establishment of indigenous microbiota.[3–5] The microbiota of a newborn develops rapidly after birth and is initially strongly dependent on the mother's microbiota, mode of delivery, and birth environment.[6] Subsequently, feeding practices and the home environment of the child influence the composition. Major changes in the composition occur during breastfeeding, weaning, and introduction of solid foods.[6,7]

The establishment of the gut microbiota has traditionally been considered a stepwise process with facultative anaerobics such as the enterobacteria, coliforms, and lactobacilli first colonizing the intestine with rapid succession by bifidobacteria and lactic acid bacteria.[7–9] New molecular methods indicate, however, that lactic acid–producing bacteria account for less than 1% of the total microbiota in infants, whereas bifidobacteria can range from 60 to 90% of the total fecal microbiota in breastfed infants.[2,10] Moreover, the new techniques indicate that the greatest difference in the microbiota of breastfed and formula-fed infants lies in the bifidobacterial composition of intestinal microbiota, whereas the lactic acid bacteria composition appears to be rather similar. *Bifidobacterium breve*, *Bifidobacterium infantis*, and *Bifidobacterium longum* are frequently found in fecal samples of breastfed infants, whereas the most common lactobacilli in breastfed and formula-fed infant feces constitute *Lactobacillus acidophilus* group microorganisms such as *L. acidophilus*, *L. gasseri*, and *L. johnsonii*.[10,11]

Healthy microbiota is defined as the normal microbiota of an individual that both preserves and promotes well-being and absence of disease, especially in the gastrointestinal tract.[12] The collective composition of the colonizing strains in infancy also provides the basis for healthy gut microbiota later in life. In addition, the development of the disease-free state of the gut lies in the host-microbe interaction in infancy.[13,14]

DEFINITIONS OF PROBIOTICS

The history of probiotics dates back to ancient times, but the scientific work on health benefits was initiated by Metchnikoff early last century.[15] Health-promoting fermented foods are used for the treatment and prevention of infant diarrhea in countries around the world without knowledge of the specific microbial composition of such products.[16] Beneficial bacteria in fermented foods that promote health have only more recently been called probiotics. These have been variously defined, according to their initial application, in animal feeds. For the purpose of human nutrition, a probiotic is currently defined as a live microbial food ingredient beneficial to health.[12] However, inactivated probiotic bacteria also may have beneficial health effects. The history of the definition and the current status are presented in Table 76.2B-1.[15,17–22]

Probiotics were initially selected to provide strains with good food-processing conditions, but, more recently, the physiologic properties of probiotics in the human gastrointestinal tract have formed the basis for selection. These criteria have been redefined to include the healthy human intestinal or mucosal microbiota as the main source of new strains. At present, emphasis is placed on survival in the gut, acid and bile stability, temporary colonization of the mucosal surfaces in the intestinal tract, and fecal recovery of the administered probiotic to define the dosage needed for individual target uses.[23] The most frequently used genera fulfilling these criteria are lactobacilli and bifidobacteria. Currently, most probiotics have been selected from members of normal healthy adult microbiota.

RATIONALE FOR PROBIOTIC INTERVENTION IN PEDIATRIC PRACTICE

The therapeutic and prophylactic interventions by probiotics derive from the concept of a well-functioning gut barrier and a normal balanced microbiota. In addition to its principal physiologic function, digestion and absorption of nutrients, the intestinal mucosa provides a protective interface between the internal environment and the constant challenge from antigens of the external environment, also carrying defense mechanisms against infectious and inflammatory diseases.

Gut microbiota as a component of intestinal barrier has been considered as a physiologic blockade to foreign substances such as antigens. One current view focuses on communication between the host and the resident commensal microbes.[22] This interaction manifests best during early infancy, when the colonization process governs the development of intestinal integrity and host immune defense mechanisms.[3–5] Conversely, the genetic background of the host and development of the immune system influence the collective composition of the intestinal microbiota.

In several gut-related inflammatory conditions, the healthy host-microbe interaction is disturbed, and inflammation is accompanied by an imbalance in the intestinal microbiota in such a way that an immune response may be generated against resident bacteria. For example, an altered gut microbiota is reported in patients with rotavirus diarrhea, inflammatory bowel diseases, rheumatoid arthritis, and allergic diseases,[23] implying that the normal gut microbiota constitutes an ecosystem responding to inflammation both in the gut and elsewhere in the human body. Normalization of the properties of an unbalanced indigenous microbiota by specific strains of the healthy gut microbiota constitutes the rationale for probiotic therapy. Such an approach with oral introduction of specific probiotics may halt the vicious circle in inflammation.

The probiotic effects in conditions involving impaired mucosal barrier function, particularly infectious and inflammatory diseases, lie in normalization of increased intestinal permeability and altered gut microecology, improvement of immunologic barrier functions of the intestine, and alleviation of intestinal inflammatory responses.[22,23]

Although it is well documented that balanced normal microbiota may become aberrant and immunogenic secondary to gut-related disease, it is not known whether changes in the composition of the microbiota can be a primary cause of disease. Such associations recently have been suggested in allergic disease[22] and autism.[24] Differences in the neonatal gut microbiota, in particular the bal-

TABLE 76.2B-1 CURRENT UNDERSTANDING AND HISTORY OF PROBIOTIC DEFINITIONS

DEFINITION	SOURCE
Specific bacteria in yoghurt fermentation balance intestinal microbiota	Metchnikoff (1907)[15]
Substances excreted by one protozoan to stimulate the growth of another	Lilly and Stillwell (1965)[17]
Substances that have a beneficial effect on animals by contributing to the balance of the intestinal biota	Parker (1974)[18]
Live microbial feed supplements that beneficially affect the host animal by improving the intestinal microbial balance	Fuller (1989)[19]
Mono- or mixed cultures of live microorganisms that, when applied to humans, affect beneficially the host by improving the properties of the indigenous microbiota	Huis in't Veld and Havenaar (1991)[20]
Live microbial food ingredients that are beneficial to health (efficacy and safety scientifically documented)	Salminen et al (1998)[12]
Live microbial cell preparations or components of cells that have a beneficial effect on human health	Salminen et al (1999)[21]
Specific live or inactivated microbial cultures that have documented targets in reducing the risk of human disease or in their adjunct treatment	Isolauri et al (2002)[22]

ance between *Bifidobacterium* and *Clostridium* microbiota, may precede the manifestation of the atopic responder type with heightened production of antigen-specific immunoglobulin E antibodies, suggesting a crucial role of the balance of the indigenous intestinal bacteria for the maturation of human immunity to a nonatopic mode.[25] These observations underline the importance of the need for precise characterization of healthy versus aberrant microbiota development and composition. This requires thorough investigation of the infant microbiota by up-to-date techniques; in particular those based on molecular techniques including ribosomal ribonucleic acid sequencing. Indirect methods, such as fecal microbial enzyme activities,[26] may also reflect differences in microbiota development.

CLINICAL EVIDENCE OF PROBIOTIC EFFECTS IN CHILDREN

The potential health effects of normal gut microbiota must be demonstrated by well-controlled clinical and nutritional studies in human subjects.[27] So far, several clinical studies have investigated the use of probiotics, principally lactobacilli and bifidobacteria, as dietary supplements for the prevention and treatment of various gastrointestinal infectious and inflammatory conditions (Table 76.2B-2).

Acute Enteritis

The currently accepted guidelines for treatment of acute diarrhea are based on correcting the dehydration by oral rehydration solutions. In addition, immediately after the completion of oral rehydration, full feedings of a previously tolerated diet can be reintroduced. Well-controlled clinical studies have shown that probiotics such as *Lactobacillus rhamnosus* GG, *Lactobacillus reuteri*, *Lactobacillus casei* Shirota, and *Bifidobacterium lactis* Bb12 can shorten the duration of acute rotavirus diarrhea,[23] above the beneficial effect of rapid refeeding, and thus constitute safe adjunct nutritional management of the condition.

In patients hospitalized for acute rotavirus diarrhea, *L. rhamnosus* strain GG (ATCC 53103) as a fermented milk or as a freeze-dried powder reduced the duration of diarrhea compared with the placebo group given a fermented and then pasteurized milk product.[28] This result has been confirmed in subsequent studies.[29,30] Moreover, probiotics reduce the duration of rotavirus excretion in stools.[31] A multicenter study by the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition working group tested the clinical efficacy and safety of a probiotic administered in an oral rehydration solution.[32] In rotavirus diarrhea, but not in nonspecific or bacterial diarrhea, a decrease in the number of diarrhea episodes was observed. The study also confirmed the safety of administration of a probiotic in an oral rehydration solution and prevention of the evolution of rotavirus-induced diarrhea toward a protracted course. These studies have invariably evaluated patients with mild or moderate dehydration. A recent randomized placebo-controlled study in severely dehydrated male children under 2 years of age showed no clinical benefit of supplementing oral rehydration with *Lactobacillus* GG.[33]

Probiotics, specifically *Bifidobacterium bifidum* (later renamed *B. lactis*) and *Streptococcus thermophilus*, are also effective in the prevention of acute infantile diarrhea.[31] *Lactobacillus* GG supplementation resulted in a decrease in the incidence of diarrhea in undernourished, nonbreastfed Peruvian children followed for 15 months.[34] *Lactobacillus* GG also reduces the incidence of nosocomial diarrhea but has no effect on the prevalence of rotavirus infection.[35] Recently, Mastretta and colleagues confirmed the result when assessing the effects of *Lactobacillus* GG and breastfeeding on nosocomial rotavirus infections in 220 hospitalized infants during one rotavirus epidemic season.[36] The frequency of nosocomial rotavirus infection was 28%. This probiotic preparation was ineffective, whereas breastfeeding was effective in reducing the risk of rotavirus infection.

The effect of probiotic therapy (see Table 76.2B-2) in diarrhea has been explained by a reduction in the duration of rotavirus shedding, normalization of gut permeability

TABLE 76.2B-2 TARGETS OF PROBIOTIC THERAPY

EFFECT	METHOD OF ASSESSMENT	OUTCOME
Nutritional management of disease	Randomized double-blind clinical studies	Reduction in the duration of symptoms
Diarrhea		Eradication of the infectious agent
Allergic/inflammatory diseases		Symptom score
Control of disease activity/reactions/ relapses/inflammation	Clinical follow-up studies	Reduction of disease activity indices specific for the condition
	Crossover challenge studies (double blind, placebo controlled)	Reduction of proinflammatory cytokines specific for the condition and site
Enhanced host defense	Intestinal permeability	Promotion of immunologic and nonimmunologic barrier function
	Immunomodulation in vitro/in vivo	
	Gut microbiota aberrancy	Generation of anti-inflammatory cytokines
Reduction in risk of disease	Randomized double-blind, placebo-controlled study	Reduction in the frequency of the condition after appropriate follow-up
Diarrhea		
Allergic disease		
Gut microbiota stabilization	Modern techniques of evaluation of the gut microecology	Balanced microbiota appropriate for age
Regulation of bowel movement		
Comparative exclusion		

Adapted from Isolauri E et al.[22,23]

caused by rotavirus infection, and an increase in immunoglobulin (Ig)A-secreting cells against rotavirus.[14,23] Moreover, the ability of specific probiotics to increase the expression of mucins may contribute to the barrier effect but also to inhibition of rotavirus replication.[37]

Antimicrobial treatment disturbs colonization resistance of the gut microbiota,[1] which may induce clinical symptoms, most frequently diarrhea. The incidence of diarrhea after single antimicrobial treatment and the effect of probiotics was evaluated in children with no history of antimicrobial use during the previous 3 months.[38] The frequency of diarrhea was 5% in the group given *Lactobacillus* GG and 16% in the placebo group ($p = .05$), supporting the efficacy of probiotics. *Lactobacillus* GG, compared with placebo, reduces stool frequency and increases stool consistency during antibiotic therapy in children aged 6 months to 10 years given oral antibiotics in an outpatient setting.[39] In addition, there are preliminary reports on resolution of *Clostridium difficile* diarrhea and colitis in adults.[40]

The value of probiotic preparations for the prophylaxis of traveler's diarrhea has been studied using *Lactobacillus acidophilus*, *B. bifidum*, *Lactobacillus bulgaricus*, and *S. thermophilus*, but the results have been conflicting, likely owing to differences in probiotic species and vehicles used, in dosage schedules, as well as varying travel destinations with different causes of diarrhea.[12]

Inflammatory Bowel Diseases

An increasing number of clinical and experimental studies demonstrate the importance of constituents within the intestinal lumen, in particular the resident microbiota, in driving the inflammatory responses in these diseases. Intestinal microbiota appears to be responsible for deep colonic lesions and severe inflammatory response.[41] Probiotic bacteria may counteract the inflammatory process by stabilizing the gut microbial environment and the intestine's permeability barrier and by enhancing the degradation of enteral antigens and altering their immunogenicity. Another explanation for the gut-stabilizing effect could be improvement of the intestine's immunologic barrier, particularly intestinal IgA responses. Probiotic effects may also be mediated via control of the balance between pro- and anti-inflammatory cytokines.[14,23]

Preliminary reports indicate benefit in reversing some of the immunologic disturbances characteristic of Crohn disease.[23] In addition, reductions in disease activity and increased intestinal permeability have been achieved in pediatric patients with Crohn disease by probiotic intervention.[42] In adults operated on for the condition, however, *Lactobacillus* GG failed to prevent endoscopic recurrence during 1 year of follow-up.[43] A recent study provides evidence for treatment with a nonpathogenic *Escherichia coli* in maintaining remission in ulcerative colitis.[44] In a clinical trial in adults, a preparation containing four strains of lactobacilli (*L. casei*, *L. plantarum*, *L. acidophilus,* and *L. delbrückii* subsp. *bulgaricus*) and three bifidobacteria strains (*B. longum*, *B. breve*, and *B. infantis*), together with *Streptococcus salivarius* subsp. *thermophilus*, is encouraging for prevention of relapses of chronic pouchitis.[45]

Additional, controlled prospective data in human inflammatory bowel diseases are required to define the effects of specific probiotic strains on distinct forms of inflammatory bowel disease and attendant complications.

Allergic Diseases

The prevalence of atopic diseases, atopic eczema, allergic rhinoconjunctivitis, and asthma appears to have increased over this century throughout the industrialized world.[46] These conditions are associated with the generation of T helper (Th) cell 2–type cytokines, including interleukin (IL)-4, IL-5, and IL-13, which promote IgE production and eosinophilia.[46] Initial signals to counter IL-4, and thereby IgE and atopy, and IL-5–generated eosinophilic inflammation may stem from components of the innate immune system, which generates the necessary initial steps for the targeted and specific function of the adaptive immune system.[22,46]

Two structural components of bacteria, the lipopolysaccharide portion of gram-negative bacteria (endotoxin) and a specified CpG motif in bacterial deoxyribonucleic acid (DNA),[47,48] activate immunomodulatory genes via Toll-like receptors (TLR4 and TLR9, respectively) present on macrophages and dendritic and intestinal epithelial cells[49,50] and elicit an immunosuppressive effect on intestinal epithelial cells by inhibition of the transcription factor nuclear factor-κB signaling pathway.[51] Specific strains of the gut microbiota contribute to a T regulatory cell population amenable to oral tolerance induction[13] and counter allergy by the generation of IL-10 and transforming growth factor-β.[52,53] These activities are associated with suppression of proliferation of Th cells and reduced secretion of proinflammatory cytokines,[53–57] with control of IgE responses[58] and reduced allergic inflammation in the gut.[59] However, different *Lactobacillus* and *Bifidobacterium* strains appear to induce distinct and even opposing responses.[60,61] Thus, specific strains of the gut micobiota and probiotics may play a crucial role in determining the Th1/Th2-driving capacity of intestinal dendritic cells. In parallel, recent observations indicate that the cytokine production patterns induced by intestinal bifidobacteria are strain specific.[22] The results of clinical studies evaluating the effects of probiotics in allergic disease appear to substantiate this suggestion.

In one prospective study, the intestinal microbiota from 76 infants at high risk of atopic diseases was analyzed at 3 weeks of age by conventional bacterial cultivation and two culture-independent methods.[25] A positive skinprick reaction at 12 months was observed in 29% of the subjects. At 3 weeks of age, the bacterial cellular fatty acid profile in fecal samples differed between those infants later developing atopic sensitization and those not developing atopy. Fluorescence in situ hydridization was used to show that atopic subjects have more *Clostridium* species and fewer *Bifidobacterium* species in stools compared with nonatopic subjects.[25] Differences in the neonatal gut microbiota thus appear to precede the manifestations of atopy, suggesting a crucial role of the balance of indigenous intestinal bacteria for the maturation of human immunity to a nonatopic mode.

Improvement in the clinical course of atopic eczema and cow's milk allergy is observed in infants when given probiotic-supplemented extensively hydrolyzed formula compared with placebo-supplemented formula.[59,62] In parallel, markers of systemic[62] and intestinal[59] allergic inflammation were reduced (see Table 76.2B-2). Similar results have been obtained in milk-hypersensitive adults.[63] In these subjects, a milk challenge in conjunction with a probiotic strain prevented the immunoinflammatory response characteristic of the response without probiotics.

The preventive potential of probiotics in atopic diseases has been demonstrated in a double-blind, placebo-controlled study.[64] Probiotics administered pre- and postnatally for 6 months to children at high risk of atopic diseases reduced the prevalence of atopic eczema to half compared with infants receiving placebo.[64] Moreover, the effect extends beyond infancy.[65]

WHAT IS REQUIRED FOR FUTURE PROBIOTICS?

Research interest in the science of nutrition is currently directed toward improvement of defined physiologic functions beyond the nutritional impact of food, including the potential to reduce the risk of diseases. This is also the focus for probiotic research. Future probiotics must have more thoroughly defined mechanisms either to control specific physiologic processes in the evolution of disease for at-risk populations or in the dietary management of specific diseases (Figure 76.2B-1).

Prerequisites for probiotic action include survival in and adhesion to specific areas of the gastrointestinal tract and competitive exclusion of pathogens or harmful antigens.[66] These processes may depend first on specific strain characteristics and second on the age and the immunologic state of the host (Table 76.2B-3). Some probiotic strains adhere better to the small intestine, whereas others bind specifically to different parts of the large intestine.[67] It is likely that strains also adhere differently to healthy versus damaged mucosa.[68] It has also recently been demonstrated that strains with lower total in vitro binding capacity may still effect high competitive exclusion of pathogens or harmful bacteria,[66] indicating a need for further characterization of in vivo adhesion properties to develop preclinical selection methodologies for candidate probiotic strains.

Genetically modified bacteria evincing improved or added functional properties may also achieve probiotic effects. These include probiotics encoding mammalian genes to produce and secrete functional anti-inflammatory cytokines, such as *L. lactis* engineered to produce IL-10 locally.[69] Other methods of probiotic modification are exposure of the microorganism to sublethal stress such as acidic conditions or heat to improve survival in the gastrointestinal tract and tolerance to stress and thereby to furnish the organism with improved competitiveness against pathogens in the intestinal milieu.[70–72] Inactivation may also have potential in the modification of probiotics. The use of inactivated instead of viable microorganisms would have merit in terms of safety, longer shelf-life, and less interaction with other components in food products.

Owing to limited availability of controlled data in humans, more research is required on the effects of specific probiotic strains, in particular when there are modifications of components of these bacteria. Probiotic effects appear to be strain specific.[60,61] Indeed, the effects of even closely related strains can be counteractive. No single probiotic strain alone can influence all of the multifactorial processes controlling the intestinal milieu. Therefore, targets of probiotic intervention should be clearly identified and effective strains and specific strain combinations must be developed with desired properties for both nutritional management and the control of human diseases (see Figure 76.2B-1).

SAFETY ASPECTS OF PROBIOTIC THERAPY

Probiotic therapy forms a relatively new treatment modality for gastrointestinal disorders. The ingestion of large numbers of viable bacteria requires strict assurance of both acute and long-term safety. Probiotics currently used have been assessed as safe for use in fermented foods, but, generally, the safety assessment of microbial food supplements is not well developed.[73] The ability of probiotic strains to survive in gastric conditions and to strongly adhere to the intestinal epithelium may entail a risk of bacterial translocation,[74] bacteremia,[75] and sepsis.[76]

Reports from countries with high probiotic consumption suggest that current preparations are safe for their intended uses.[75,76] However, patients with severe underly-

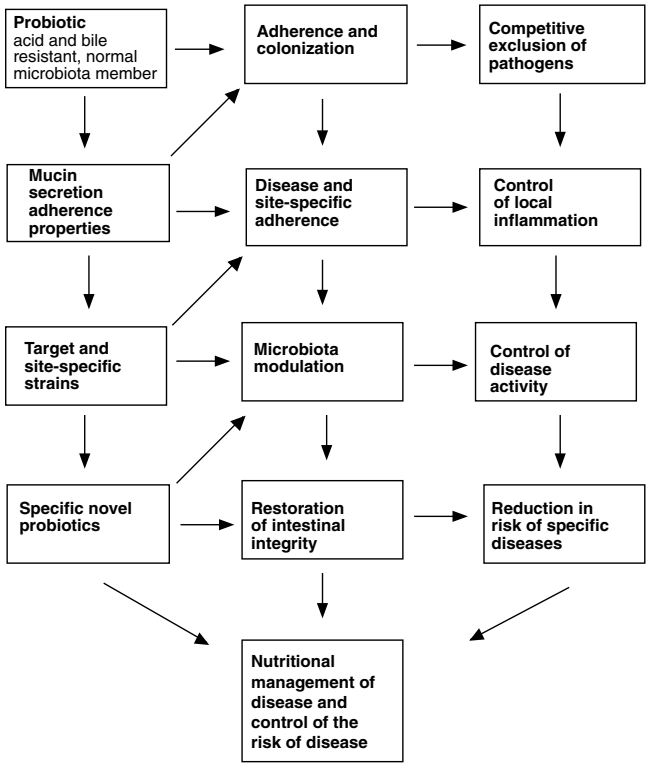

FIGURE 76.2B-1 The selection and use of specific probiotic strains and some targets in disease risk reduction symptom management.

TABLE 76.2B-3 PROPERTIES OF PROBIOTICS TO BE ASSESSED DURING THE DEVELOPMENT OF NEW STRAINS AND NEW APPLICATIONS

PROPERTY	TARGET AND METHOD
Species specificity	Source or origin; healthy human gut microbiota as the source
Resistance to pH	Model systems for gastric and bile effects
Adhesion to intestinal mucosa	Several model systems to be used (eg, cell cultures, mucus, intestinal segments) Fecal recovery in human subjects
Competitive exclusion	In vitro and in vivo model systems for pathogen adherence exclusion
Immune regulation	In vitro and human studies
Generation and balance of cytokines	Cytokine profile Contact with immune cells Adhesion related to immune effects Improvement of gut barrier and permeability disorders
Safety	Exclusion of antibiotic resistance and virulence factors and postmarket surveillance
Technological properties	Stability and activity throughout the processes
Efficacy assessment	Human clinical intervention studies with final product formulations; at least two independent studies to prove efficacy in target populations and safety in all consumer groups

Adapted from Salminen S et al[12] and Isolauri E et al.[22]

ing diseases, particularly immunocompromised subjects, appear to carry an increased risk of bacteremia associated with the use of some current probiotics.[75,76] Translocation of intraluminal bacteria may be one risk factor, but recent data also suggest that some probiotic strains directly interfere with host innate immune functions.[77]

Genetically modified microorganisms could be developed for use in foods. Developments in this area also may provide medical applications. However, a safety concern is the potential for the transfer of antibiotic resistance from modified organisms to gut pathogens.[78] Selection procedures have been developed to monitor the absence of antibiotic resistance, thus far specifically for *Lactococcus*.[79] The use of inactivated bacteria as probiotics has been advocated because their consumption may be safer than the use of viable bacteria.[23] However, information on the effects of inactivation methods on cell wall structure and composition is scarce. Such concerns are not limited to heat treatment; detrimental effects also may be expected in response to lyophilization and irradiation.[80]

To conclude, specific probiotics offer a tool for modification of the gut barrier and microbiota. The microbes used must be obtained from acceptable sources with scientifically proven safety and efficacy to guarantee their application in infectious, allergic, and inflammatory diseases.

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3. *Motility*

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As discussed in Chapter 4, "Motility," the mixing and propulsive actions of the gut are the result of coordinated activity in the enteric nervous system (ENS), modulated by hormones and central nervous system (CNS) input. The ENS contains complex motility "programs" for activities such as the interdigestive migrating motor complex (MMC) and the gastrointestinal components of emesis. Over 30 definite and putative neurotransmitters have been identified in the gut (see Table 4-2, Chapter 4). In many cases, a single neurotransmitter may stimulate several different receptors on different target tissues (nerves, smooth muscle) to produce different effects. As the rapidly advancing discipline of neurogastroenterology expands our understanding of motility and signaling within the gut, many new targets are being identified for specific therapeutic intervention.

This chapter provides an overview of the many drugs currently available and in development for modulating gastrointestinal motility. For each agent, the mechanism of action is briefly discussed, followed by a review of the currently available clinical experience. Wherever possible, data from pediatric trials are reported. However, in many cases, especially with newer medications, in which only scanty or no data exist for pediatric patients, data from trials in adults are discussed. The identification of effective pharmacologic therapies for pediatric gastrointestinal motility disorders using prospective randomized controlled trials has been identified as a research priority in the recently released research agenda of the North American Society of Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN).[1]

Disorders of motility result in abnormalities of contraction amplitude, frequency, or coordination. Although many older motility drugs had nonspecific effects, affecting only contraction amplitude and/or frequency, newer agents also enhance the coordination of gastrointestinal contractions, greatly enhancing their therapeutic utility. The development of drugs targeting specific receptor subtypes in the ENS and enteric smooth muscle has reduced the incidence of systemic side effects that limited the use of previous agents. Drugs that alter motility fall into two broad categories: prokinetics and antimotility agents or antispasmodics. Also, it is increasingly apparent that many patients with motility disturbances also suffer from visceral hypersensitivity. Several of the newer motility drugs have additional antinociceptive qualities. The major drugs in each category are listed in Tables 76.3-1 (prokinetics) and 76.3-2 (antispasmodic and antimotility agents).

PROKINETICS

Prokinetic drugs may stimulate motility by direct actions on enteric smooth muscle or by interacting with the neurons of the ENS. Drugs currently in use include dopamine D_2 receptor antagonists, motilin agonists, 5-hydroxytryptamine subtype 4 (5-HT_4) agonists, and anticholinesterases. The final common pathway of all of these drugs is the M_3 muscarinic acetylcholine receptor on enteric smooth muscle cells.[2]

Dopamine Antagonists

Under normal physiologic conditions, dopamine inhibits the release of acetylcholine in the myenteric plexus by acting at presynaptic D_2 dopaminergic receptors. D_2 receptor antagonists such as metoclopramide and domperidone have prokinetic effects, enhancing the release of acetylcholine.[3] Domperidone is a specific D_2 antagonist, whereas metoclopramide also stimulates 5-HT_4 receptors and is an antagonist at 5-HT_3 receptors.[3]

Metoclopramide. The prokinetic effects of metoclopramide are primarily in the upper gut.[4] In addition to its peripheral effects, metoclopramide crosses the blood-brain barrier and exerts central antiemetic effects. This CNS activity may lead to side effects such as sedation, restlessness, and insomnia. Most concerning are dystonic reactions, such as oculogyric crisis and opisthotonos, which occur more frequently in children than in adults.[5] Tardive dyskinesia has been reported and may be permanent.[6] Rare side effects include neuroleptic malignant syndrome and methemoglobinemia. Despite its drawbacks, metoclopramide is one of the few prokinetic agents available at present. Although metoclopramide is widely used for its antiemetic action, the following focuses on its use as a prokinetic agent.

Randomized controlled trials of high-dose (1 mg/kg) intravenous metoclopramide have shown efficacy in improving delayed gastric emptying in children.[7] Recent experience in adult patients suggests that subcutaneous injection of metoclopramide may be effective in treating gastroparesis[8] and nausea.[9,10] Subcutaneous administration circumvents the problems of unpredictable oral drug bioavailability encountered in patients with severe motility disorders. Although this route of administration is less attractive in pediatric patients, it may well be acceptable to diabetic patients with symptomatic gastroparesis. However, further data from randomized double-blind placebo-controlled trials are required.

TABLE 76.3-1 PROKINETIC DRUGS

DRUG	RECEPTOR	EFFECT ON MOTILITY	REDUCES VISCERAL HYPERSENSITIVITY	POTENTIAL INDICATIONS
Metoclopramide	D_2 receptor antagonist, 5-HT_4 agonist, 5-HT_3 antagonist	↑Motility: proximal > distal[4]	Possible[178–180]	Foregut dysmotility syndromes: GERD, gastroparesis, nausea
Domperidone	D_2 receptor antagonist	↑Motility: proximal > distal gut[4]	Possible[181]	Foregut dysmotility syndromes: GERD, gastroparesis, nausea
Erythromycin	Motilin receptor agonist	↑ Motility: proximal > distal[182]	No; possible worsening[183]	Foregut dysmotility syndromes: gastroparesis, postprandial hypomotility
Octreotide	Somatostatin receptor agonist	Stimulates phase III of MMC, beginning in duodenum[50]; antimotility effects (see Table 76.3-2)	Yes[55]	Small intestinal hypomotility, prevention of bacterial overgrowth, pseudo-obstruction, dumping syndrome, visceral hypersensitivity
Cisapride	5-HT_4 receptor agonist, 5-HT_3 receptor antagonist	↑Motility: proximal > distal gut[4]	No[184]	Foregut dysmotility syndromes: gastroparesis, postprandial hypomotility, pseudo-obstruction, possible role in constipation
Tegaserod	5-HT_4 receptor, potent partial agonist	↑Enhances gastric motility, stimulates peristaltic reflex and intestinal secretion, shortens colonic transit time[91,92]	Yes[92]	Constipation-predominant IBS in adult women
Bethanecol	Muscarinic receptor agonist	↑Contraction amplitude but not transit[97–99,185]	No; increased sensitivity[185]	GERD
Neostigmine	Anticholinesterase	↑Motility and transit: colonic > proximal[185–187]	No; increased sensitivity[185]	Acute colonic pseudo-obstruction, episodic pseudo-obstruction
Baclofen	$GABA_B$ receptor agonist	↓Transient lower esophageal sphincter relaxations[107,108]	Possible (effective in neuropathic pain)[188,189]	GERD
Phenylephrine (topical)	α_1-Adrenergic agonist	↑Internal anal sphincter pressure[119,120]	No data	Fecal incontinence

GABA = γ-aminobutyric acid; GERD = gastroesophageal reflux disease; 5-HT = 5-hydroxytryptamine; MMC = migrating motor complex.

TABLE 76.3-2 ANTIMOTILITY AND ANTISPASMODIC DRUGS

DRUG	MECHANISM OF ACTION	EFFECT ON MOTILITY	REDUCES VISCERAL HYPERSENSITIVIY	POTENTIAL INDICATIONS
Loperamide	Mu opioid agonist	↓ Bowel motility and ↑ anal sphincter tone[4]	Yes[190]	Diarrhea, including IBS-D Hirschsprung disease post–pull-through surgery
Dicylomine, hyoscyamine	Nonspecific muscarinic antagonists	↓ Motility[4]	No data	Decreasing amplitude of painful/noxious visceral contractions, eg, visceral hypersensitivity, IBS, neuropathic pseudo-obstruction
Mebeverine	Complex actions at multiple receptors	Small intestine: mixed effects,[191] colonic motility[192]	No data	Decreasing amplitude of painful/noxious visceral contractions, eg, IBS
Nifedipine, verapamil, diltiazem	L-type calcium channel antagonists	↓ Amplitude contractions[149,150]	No data	Decreasing amplitude of painful/noxious visceral contractions, eg, nutcracker esophagus, diffuse esophageal spasm, esophageal achalasia
Pinaverium	Selective gastrointestinal calcium channel antagonist	↓ Colonic motility[193–195]	No data	Decreasing amplitude of painful/noxious visceral contractions
Peppermint oil	Calcium channel antagonist[157,196]	↓ Motility: upper and lower GIT Esophagus: ↓ simultaneous contractions, ↑ peristaltic contractions	Possible (anesthetic actions)[197]	Decreasing amplitude of painful/noxious visceral contractions, eg IBS, spastic esophageal disorders
Nitrates	Nitric oxide donors	Smooth muscle relaxation	No[198]	Decreasing amplitude of painful/noxious visceral contractions, eg, nutcracker esophagus, diffuse esophageal spasm, anal fissures
Ondansetron	5-HT_3 antagonist	↓ Motility[4,62]	Yes[4,62]	Decreasing visceral hypersensitivity and nausea
Granisetron	5-HT_3 antagonist	↓ Motility[4,62]	Yes[4,62]	Decreasing visceral hypersensitivity and nausea
Alosetron	5-HT_3 antagonist	↓ Motility and tone: slows colonic transit, ↑ fluid absorption[4,92]	Yes[4,92]	Intractable IBS-D in adult women, unresponsive to standard medical therapies
Sumatriptan	5-HT($1_{B/D}$) receptor agonist	↑ Relaxation gastric fundus, colon ↓ gastric emptying[4,62]	No direct effect[199,200]	Increasing gastric receptive accommodation
Octreotide	Somatostatin receptor agonist	↓ Gastric emptying ↓ diarrhea, ↓ intestinal transit, ↓ secretions[54,201]	Yes[55]	Retarding gastric emptying, eg, in dumping syndrome; also reduces visceral hypersensitivity
Glucagon	Glucagon receptor agonist	↓ Motility smooth muscle relaxation	No data	Inhibiting gastrointestinal contractions, eg, during endoscopic procedures
Botulinum toxin	Prevents acetylcholine release from motoneurons	Denervation paralysis smooth muscle	No data	Reducing sphincter tone, eg, esophageal achalasia, anal achalasia, gastroparesis
Clonidine	α_2-Adrenergic agonist	↓ Motility and tone[198, 202]	Yes[55,189,198,202]	Inhibiting noxious/painful gastrointestinal contractions and visceral hyperalgesia, eg, IBS-D
Salbutamol	β-Adrenergic agonist	Smooth muscle relaxation	No data	Inhibiting noxious/painful gastrointestinal contractions, eg, proctalgia fugax

GIT = gastrointestinal tract; 5-HT= 5-hydroxytryptamine; IBS = irritable bowel syndrome; IBS-D = diarrhea-predominant irritable bowel syndrome.

Metoclopramide has proven useful in accelerating the passage of small bowel biopsy capsules in children.[11] Similarly, metoclopramide has been used to assist the placement of transpyloric feeding tubes, although the results of clinical trials are contradictory.[12] Low-dose erythromycin (see below) is more effective for this indication.[12,13]

Although metoclopramide increases lower esophageal sphincter tone in children,[14] the majority of studies have shown little or no effect on gastroesophageal reflux disease (GERD) as measured by prolonged pH monitoring.[14–18] Also, metoclopramide was less well tolerated than placebo, with side effects including irritability and dystonic reactions.[14,15] The current position paper of the NASPGHAN states that although cisapride appears to be a marginally effective prokinetic agent for the treatment of GERD, the effectiveness in children of other prokinetic agents, including metoclopramide, is unproven.[19]

Domperidone. Another dopamine antagonist, domperidone, is available in most countries outside the United States. Unlike metoclopramide, domperidone does not pass the blood-brain barrier, and CNS side effects are rare.[20] Despite this, domperidone does have antiemetic effects, owing to the fact that the chemoreceptor trigger zone is outside the blood-brain barrier, and domperidone exerts its CNS effects here.[20] Domperidone raises lower esophageal sphincter pressure, increases the amplitude of gastric and duodenal contractions, and accelerates small intestinal transit.[21,22]

Domperidone seems effective in gastroparesis. A recent randomized controlled trial demonstrated that domperidone was more effective than cisapride in improving symptoms, diabetic control, gastric emptying, and electrogastrography variables in diabetic children with gastroparesis.[23] As with metoclopramide, there is minimal evidence that domperidone therapy is beneficial in GERD.[19]

Motilin Agonists

The peptide hormone motilin is released by enteroendocrine cells in the small intestine during fasting and has a role in initiating the interdigestive MMC. Motilin excites presynaptic neurons in the ENS to stimulate postsynaptic cholinergic motoneurons and induce phasic contractions in smooth muscle.[24] Motilin also has direct excitatory effects at motilin receptors on gastrointestinal smooth muscle cells, inducing an increase in intracellular calcium.[25] Motilin agonists (motilides) are a promising class of prokinetics that is currently the subject of intensive research.

Erythromycin. Erythromycin is a motilin receptor agonist that in low doses (1–3 mg/kg) stimulates phasic contractions in the stomach identical to spontaneous phase III of the MMC.[26] These activity fronts originate in the antrum and propagate into the small intestine and are inducible in adults and children over 32 weeks gestational age.[27] In premature babies less than 32 weeks gestational age, erythromycin increases antral motility but does not induce MMCs.[28] At higher doses, erythromycin produces uncoordinated high-amplitude antral contractions without propagation in healthy individuals.[29]

Low-dose erythromycin is an effective gastric prokinetic and is useful in the treatment of gastroparesis, as well as for placement of transpyloric feeding tubes[13,30] and for elimination of blood from the upper gastrointestinal tract prior to endoscopy for gastrointestinal hemorrhage.[31,32] In a recent systematic review of trials in adult patients, erythromycin was superior to cisapride, metoclopramide, and domperidone for treatment of gastroparesis.[33] In children, oral erythromycin 1 mg/kg is at least as effective as oral metoclopramide 0.15 mg/kg for emptying the stomach prior to emergency surgery.[34] At a higher dose, erythromycin was associated with increased postoperative vomiting,[35] although higher doses may be necessary in treatment of individuals with severely delayed gastric emptying.

Erythromycin has been extensively studied as treatment for feeding intolerance in patients in intensive care and in premature infants.[36–43] Despite the documented lack of effect on MMCs below 32 weeks, studies on premature babies under this gestation age indicate that low-dose erythromycin is effective in reducing the number of days until full enteral feeding is established.[39–41] The authors commented that this may result in a substantial saving on hyperalimentation.[39] Importantly, there were no significant adverse effects. In particular, no increase in necrotizing enterocolitis was seen. Trials of erythromycin in antimicrobial doses have yielded conflicting results but, in general, have also led to improved feed tolerance.[36,37,39,42]

Other Motilides. Current research aims to develop motilin agonists devoid of antibiotic activity. Clinical trials of ABT-229, an erythromycin derivative, demonstrated enhanced gastric emptying in healthy volunteers.[44] However, there was no symptomatic improvement in randomized double-blind placebo-controlled trials in adults with functional dyspepsia [45] or diabetes,[46] and research on ABT-229 as a gastrokinetic agent has been discontinued.[3] Other nonantibiotic erythromycin derivatives under investigation for their prokinetic effects include EM523,[47] EM574,[48] and GM-611.[49]

Octreotide

Somatostatin is a naturally occurring gastrointestinal peptide hormone that has complex actions in the gastrointestinal tract. It can augment or counteract a wide variety of other peptides and can have both prokinetic and antimotility effects. Octreotide is a long-acting synthetic analog of somatostatin that may be administered by intravenous, subcutaneous, or intramuscular injection.

In children with chronic gastrointestinal disorders, octreotide stimulates phase III of the MMC, beginning in the small intestine, and inhibits gastric contractions.[50] The inhibition of antral activity is reversed by feeding[50] or by pretreatment with erythromycin.[51] Octreotide may be of benefit in chronic intestinal pseudo-obstruction, although clinical trials have been limited to adults with connective tissue diseases.

Because octreotide retards gastric emptying, therapy should ideally be given to patients who are receiving post-gastric feeds, such as transpyloric or jejunostomy feeding.

Alternatively, combination therapy with erythromycin may be of benefit because the suppressant effect of octreotide on gastric motility is counteracted by erythromycin.[52] Conversely, octreotide enhances the prokinetic effects of erythromycin in patients with intestinal pseudo-obstruction[52] and on gastric emptying in normal subjects.[53]

Despite stimulating phase III of the MMC, octreotide also has antidiarrheal effects. It inhibits small bowel transit[54] and intestinal and pancreatic secretions. In the colon, octreotide inhibits the postprandial tonic response, whereas phasic pressure activity is increased. Octreotide also has antinociceptive effects that may increase its therapeutic utility in diseases with a component of visceral hyperalgesia.[55]

Because of its inhibitory effects on gastric emptying, octreotide is beneficial in dumping syndrome,[56] reducing the duration of the fed pattern and hastening the return of the MMC after a meal.[57] However, data in children are lacking.

The predominant adverse effects of octreotide include pain at injection sites and gastrointestinal disturbance. Occasionally, hyper- or hypoglycemia, hypertension, headache, dizziness, fatigue, hepatic dysfunction, and cholelithiasis may occur. Theoretically, long-term use of octreotide could inhibit growth by suppressing growth hormone secretion, although this has not occurred in several children treated with octreotide for gastrointestinal diseases.[58–61]

5-HT_4 Agonists

Serotonin (5-HT) is ubiquitous throughout the gastrointestinal tract and is important in modulating motility through its actions at several different receptors (Figure 76.3-1). There is currently intense research interest in developing drugs that manipulate motility by stimulating or antagonizing gastrointestinal serotonin receptors. Several classes of serotonergic agonists and antagonists are discussed in this chapter, including 5-HT_4 agonists and antagonists of 5-HT_3 and 5-$HT_{1B/D}$ receptors. Stimulation of 5-HT_4 receptors initiates peristaltic contraction. These receptors are located on neurons in the myenteric plexus and on smooth muscle cells (see Figure 76.3-1).[62] Stimulation of cholinergic neurons at 5-HT_4 receptors results in acetylcholine release and enhanced contraction.[62]

Cisapride. Cisapride is a partial 5-HT_4 receptor agonist and a 5-HT3 antagonist with some affinity for dopamine D_2, 5-HT_2 receptors, and α-adrenoceptors.[63] Cisapride enhances gastric emptying in normals and in patients with gastroparesis. It increases lower esophageal sphincter tone and improves esophageal acid clearance, reducing esophageal acid exposure. Despite these effects, its clinical utility in unselected children with GERD is not supported by a recent systematic review.[64] This may be because cisapride has no effect on transient lower esophageal sphincter relaxations (TLESRs), the predominant mechanism of gastroesophageal

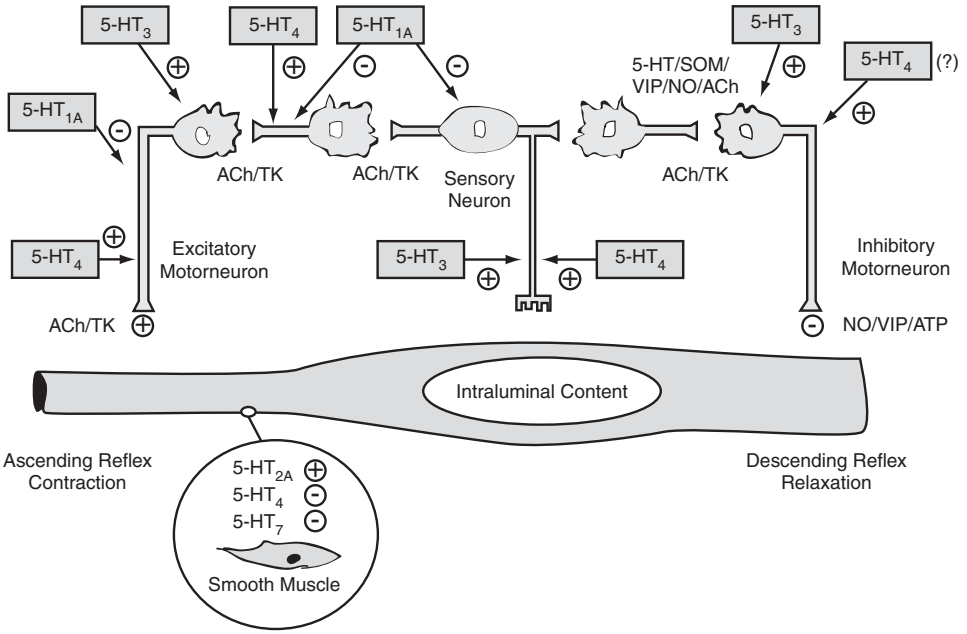

FIGURE 76.3-1 Modulation of intestinal motility by serotonergic receptors. Distention by intraluminal contents stimulates sensory neurons (intrinsic primary afferent neurons), which trigger an ascending excitatory reflex (leading to contraction) and a descending inhibitory reflex (leading to relaxation). Transmitters released by interneurons in the ascending reflex include acetylcholine (ACh) and substance P, whereas descending interneurons belonging to different subpopulations may use serotonin (5-hydroxytryptamine [5-HT]), somatostatin (SOM), vasoactive intestinal polypeptide (VIP), nitric oxide (NO), ACh, and other mediators as transmitters. Excitatory motoneurons release ACh and tachykinins (TK) at the neuromuscular junction, whereas inhibitory motoneurons may release NO, VIP, or adenosine triphosphate (ATP), depending on the gastrointestinal tract level and on the animal species. 5-HT_3 and 5-HT_4 receptors have an excitatory effect on enteric neurones, whereas 5-HT_{1A} receptors inhibit transmitter release. Serotonergic receptors may also directly contract or relax smooth muscle cells via 5-HT_{2A}, 5-HT_4, and 5-HT_7 receptors. + indicates stimulation; – indicates inhibition; (?) indicates limited data. Reproduced with permission from De Ponti F and Tonini M.[62]

reflux (GER).[65,66] Evidence for a beneficial effect of cisapride on reflux and feed intolerance in neonates is similarly contradictory.[67–71] In contrast, cisapride has proven effective in randomized trials in selected subsets of patients with reflux disease. In particular, cisapride improves symptoms, intraesophageal pH indices, and requirements for bronchodilator and steroid medication in patients with chronic respiratory symptoms and GER.[72–74]

Despite its documented efficacy in the treatment of gastroparesis,[33] a recent randomized trial demonstrated that domperidone is more effective for this indication in children.[23] Cisapride stimulates small intestinal motility and has been used in children with chronic intestinal pseudo-obstruction.[75–78] Response to therapy, as measured by the ability to tolerate enteral feeding, was predicted by the presence of phase III of the MMC in antroduodenal motility studies.[78] Cisapride is superior to placebo in the management of constipation.[79] However, owing to the risks of cardiac toxicity, use for this indication should be discouraged.

Cisapride Cardiotoxicity. Cisapride prolongs the Q–T interval in a dose-dependent manner, resulting in potentially fatal arrhythmias, including ventricular tachycardia, ventricular fibrillation, and torsades de pointes. From July 1993 to December 1999, 341 such cases were reported, including 80 deaths. In approximately 85% of these cases, the events occurred when cisapride was used in patients with known risk factors.[80] Patients most at risk include those who are receiving high doses or who have congenital or acquired long Q–T syndrome. Drugs that interfere with the metabolism of cisapride by the cytochrome P-450 (CYP)3A4 system potentiate cisapride cardiotoxicity and are contraindicated in patients on the medication. These include macrolide antibiotics, azole antifungals, and nonsedating antihistamines. Grapefruit juice also increases cisapride bioavailability by inhibiting CYP3A4.[81]

Until recently, it was unclear whether cisapride cardiotoxicity was a result of 5-HT_4 receptor stimulation or the molecular characteristics of cisapride and other members of the benzamide class. Clinical and experimental evidence now supports the latter theory. Clinical trials of tegaserod, a selective 5-HT_4 receptor agonist, showed no alteration of Q–T interval versus placebo in over 2,500 subjects.[82] In contrast, cisapride possesses class III antiarrhythmic properties and prolongs the action potential duration through blockade of distinct voltage-dependent K^+ channels, thus delaying cardiac repolarization and prolonging the Q–T interval.[83] Concurrent use of cisapride is therefore contraindicated in children receiving class III antiarrhythmic agents. To put the potential for cisapride cardiac toxicity in perspective, a critical analysis of safety data by Vandenplas showed that there were two instances of serious ventricular arrhythmia, Q–T prolongation, or sudden death reported for every million patients treated for a month, of which more than 85% could be related to known risk factors.[84]

Importantly, intravenous erythromycin at antimicrobial doses[85,86] and domperidone[87,88] have both been associated with prolongation of Q–Tc and cardiac arrhythmia.

Safety concerns about cisapride cardiotoxicity resulted in its withdrawal from sale in North America, most European countries, and Australasia. In some countries, including the United States, patient access to the drug is now restricted to investigational limited access programs. Patients must meet strict eligibility criteria, have failed other treatment options, and be stringently monitored. In pediatrics, cisapride is available under such programs for refractory GERD, pseudo-obstruction, and neonatal feeding intolerance, although specific details vary between countries.

Other side effects of cisapride include transient diarrhea; flatulence; borborygmi or abdominal cramps; CNS disturbances such as seizures, behavior disturbance, and extrapyramidal effects; and a dose-related increase in urinary frequency. Because of the importance of the liver and kidneys in its metabolism and excretion, the dosage should be reduced in patients with hepatic or renal insufficiency.

Other Benzamide Derivatives. Newer members of the benzamide class, all unavailable in the United States at present, include mosapride, renzapride, levosulpiride, and KDR-5169.[89] KDR-5169 is a 5-HT_4 agonist with dopamine D_2 receptor antagonist properties. In a recent animal study, it was extremely effective in treating gastroparesis, accelerating gastric emptying to supranormal levels.[89] The authors speculated that the increased efficacy of KDR-5169 over cisapride may have been due to more potent antagonism of D_2 receptors because combination therapy with cisapride and domperidone (a D_2 antagonist) has previously been shown to improve gastric emptying versus cisapride alone.[90]

Tegaserod. Tegaserod (HTF 919) is a specific 5-HT_4 antagonist recently licensed for the treatment of constipation-predominant irritable bowel syndrome (IBS-C) in adult women. Like cisapride, it accelerates gastric emptying and promotes gastrointestinal transit.[91,92] Unlike cisapride, tegaserod has no cardiotoxicity,[82] requires only twice-daily dosing, and does not cross the blood-brain barrier to cause CNS side effects. Clinical trials in adults with IBS-C indicate that tegaserod improves constipation, bloating, and overall symptom scores.[4,92] Diarrhea, abdominal cramping, headache, flatulence, and fatigue are the major associated adverse effects, resulting in discontinuation of the medication in 10 to 15% of patients.[93,94] The potential clinical use of tegaserod for GER was studied in adults with mild to moderate GERD. Tegaserod reduced esophageal acid exposure by 50%,[95] possibly by reducing the incidence of postprandial transient lower esophageal relaxations and/or by accelerating gastric emptying.

Prucalopride. Prucalopride (R0-93877) is another 5-HT_4 partial agonist that also accelerates gastrointestinal transit and has proven useful in treatment of constipation in adults. However, further clinical development of this compound has been suspended pending the results of toxicity studies owing to concerns about carcinogenesis in animals and possible cardiac effects.[96]

Bethanecol

Bethanecol is a choline ester that stimulates muscarinic cholinergic receptors. It increases the amplitude of contractions throughout the gastrointestinal tract and raises lower esophageal sphincter tone.[97–99] Contrary to previous reports, it does not accelerate gastric emptying or gastrointestinal transit in adults or children.[7,99,100] Two trials evaluated its utility in pediatric GERD and demonstrated modest benefits, no greater than that achieved with antacids.[101,102] Its usefulness is also limited by side effects, including abdominal cramps, watery diarrhea, bradycardia, hypotension, urination, sweating, and bronchoconstriction.

Neostigmine

Neostigmine is a peripheral anticholinesterase. By inhibiting degradation of acetylcholine, it enhances cholinergic activity at nicotinic receptors in the myenteric plexus and muscarinic receptors on gastrointestinal smooth muscle. Neostigmine accelerates gastrointestinal transit, especially in the colon. It has proven efficacy in reversal of acute colonic pseudo-obstruction (Ogilvie syndrome), with colonic decompression occurring within minutes of drug administration in prospective randomized double-blind placebo-controlled studies.[103,104] Side effects were limited to mild abdominal cramping and bradycardia, which were responsive to atropine. This simple, effective therapy may now become the standard of care for acute colonic pseudo-obstruction in adults[105]; however, further data on pediatric patients are required. It has been suggested that the longer-acting anticholinesterase pyridostigmine may be beneficial for the outpatient management of gastrointestinal motility disturbances.[106]

Baclofen

Although not strictly a prokinetic, baclofen increases lower esophageal sphincter pressure and inhibits TLESRs by simulating the actions of the inhibitory neurotransmitter γ-aminobutyric acid in the brainstem.[107,108] This agent is being explored for its role in reducing gastroesophageal reflux. In the meantime, this mechanism is of potential benefit to neurologically handicapped children receiving baclofen for spasticity, possibly counteracting their known tendency to pathologic GER. Trials of baclofen in pediatric GERD are ongoing.

Other drugs have been shown to inhibit TLESRs and GER, including cholecystokinin A antagonists,[109] atropine,[110] morphine,[111] and nitric oxide synthase inhibitors.[112] The search for specific agents for pharmacologic suppression of TLESRs is currently the subject of intense research activity.

Opioid Agonists

The Mu-opioid agonist fedotozine has both antinociceptive and promotility effects. Although the antinociceptive properties are mediated by stimulation of Mu-opioid receptors, it is likely that the prokinetic effects of fedotozine are due to a different mechanism, direct smooth muscle excitation.[113] In clinical trials of adults with IBS, fedotozine was superior to placebo in reducing abdominal pain and bloating.[114]

Cholecystokinin Antagonists

Although not currently available outside therapeutic trials, this class of agents is expected to reach the public some time in 2004.[4] Cholecystokinin (CCK) is released from the gut in response to fat and protein in food. CCK_A receptors on visceral afferents in the gut mediate sensations of satiety and gastric receptive relaxation via a vagal reflex. Stimulation of CCK_A receptors in the gallbladder and pancreas causes release of digestive juices. Whereas CCK_A receptors mediate stimulation of duodenal activity, CCK_A stimulation relaxes the lower esophageal sphincter, stomach, and colon via a nitric oxide–dependent mechanism.[115–117] CCK_A antagonists such as loxiglumide and dexloxiglumide reduce TLESR, promote gastric emptying, shorten colonic transit time, and increase the number of bowel movements.[4,109,118]

α-Adrenergic Agonist: Phenylephrine

Topical phenylephrine increases anal sphincter tone in normals[119] and in patients with incontinence owing to low resting sphincter pressure.[120] In an initial trial, low concentration (10%) phenylephrine was ineffective in patients with incontinence owing to low resting anal sphincter pressure.[121] However, this concentration was effective in treating fecal incontinence after ileoanal anastomosis.[122] Research on the use of topical phenylephrine for fecal incontinence owing to internal anal sphincter incompetence is ongoing.

ANTISPASMODICS AND ANTIMOTILITY DRUGS

Opioid Agonist: Loperamide

Loperamide is a specific agonist at peripheral Mu-opioid receptors that has both antinociceptive and antimotility actions. Because it does not pass through the blood-brain barrier, CNS effects, including the potential for addiction, do not occur. The overall effect of loperamide is to delay gastrointestinal transit. Although loperamide stimulates gastrointestinal contractions, there is a disruption in the normal propulsive patterns of motility, leading to increased transit time.[3] The safety and efficacy of loperamide in treatment of diarrhea are well established, with the caveat that no hypomotility agent is recommended in the context of acute infective diarrhea in children or dysentery at any age.

Anticholinergics

Anticholinergic medications have traditionally been used as antispasmodics. Blocking the action of acetylcholine at smooth muscle reduces the amplitude of gastrointestinal contractions, and this has been particularly useful in dysmotility syndromes characterized by excessive contractile activity. Older anticholinergic medications include scopolamine, dicyclomine, and hyoscyamine. Their therapeutic utility was limited by their nonspecific actions and unwanted side effects caused by antagonism of muscarinic receptors outside the gastrointestinal tract. These include dry mouth, tachycardia, blurred vision, and urinary difficulties. Toxicity has been a particular problem in children, with reports of serious side effects and anticholinergic poi-

soning.[123–127] Despite their widespread use, there are very few randomized controlled trials of anticholinergic drugs for gastrointestinal motility disorders in children.[128] A recent systematic review of 18 randomized controlled trials of antispasmodic and anticholinergic drugs in IBS concluded that, overall, the trials were of suboptimal methodologic quality. Evidence of effectiveness for these agents in IBS was inconsistent, and adverse effects limited their use.[92]

M_3 Muscarinic Antagonists

Recently, more specific anticholinergic drugs have been developed that target the M_3 muscarinic receptor responsible for gastrointestinal contraction.[129] M_3 receptors are also found in the bladder (and these drugs have been employed in enuresis and urinary incontinence); however, they are not present in the heart, and cardiovascular side effects do not occur.[130] Examples are zamifenacin and darifenacin, which both inhibit contractile activity throughout the small and large bowel.[4] In a multicenter double-blind parallel-group placebo-controlled study of adult patients with IBS, zamifenacin profoundly inhibited colonic motor activity without significant extraintestinal side effects.[130]

Mebeverine

Although mebeverine has some antimuscarinic activity, it is far less potent than atropine. The spasmolytic action of mebeverine is the result of at least three additional mechanisms. Mebeverine has a direct musculotropic action involving calcium ion exchange and stabilization of excitable membranes, a local anesthetic action, and also potentiates sympathetic inhibition of gastrointestinal contraction owing to blockade of noradrenaline uptake into sympathetic nerve endings.[131–133] Mebeverine seems effective in reducing diarrhea and global symptom scores in adults with IBS; however, data from children are scant.[128]

5-HT_3 Antagonists

Alosetron is a 5-HT_3 antagonist that inhibits colonic transit and reduces colorectal hypersensitivity, probably by inhibiting 5-HT_3 receptors on vagal afferents and in the CNS.[4] In the colon, it delays transit and decreases wall tension and sensitivity to distention.[4,92,134] It is effective in female adults with diarrhea-predominant IBS (IBS-D), improving stool consistency and frequency and reducing pain and urgency.[4,92] The most commonly reported side effect was constipation. Owing to reports of rare severe side effects, including ischemic colitis and severe constipation, alosetron was temporarily withdrawn from sale in 2000. In 2002, it was reintroduced for use in women with severe IBS-D who have failed to respond to conventional IBS therapy. The recommended adult dosage is 1 mg twice daily, and the agent should be avoided in patients with constipation.

5-HT_1 Antagonist

Originally developed to treat migraine headaches, the 5-$HT_{1B/D}$ antagonist sumatriptan also has effects on gastrointestinal motility. This agent enhances postprandial gastric fundic relaxation and reduces symptoms of early satiety in patients with functional dyspepsia.[135] Although sumatriptan inhibits gastric motility, it stimulates small intestinal contractions, causing premature phase III of the MMC after subcutaneous injection in fasting individuals.[136] Despite increasing lower esophageal sphincter tone, sumatriptan increases postprandial reflux episodes via an increased incidence of TLESRs and delayed gastric emptying.[137]

Botulinum Toxin

Botulinum toxin (BT) type A is widely used for reduction of muscle spasticity in children with cerebral palsy or strabismus. The toxin binds irreversibly to presynaptic cholinergic nerve terminals, where it prevents the release of acetylcholine, causing paralysis by functional denervation. These effects persist for several months, with reversal dependent on sprouting of new terminal axons.

Gastrointestinal uses of BT revolve around the injection of sphincters[138] and include cricopharyngeal dysphagia,[139] achalasia of the esophagus,[140] spastic esophageal disorders,[141] gastroparesis (pyloric injection),[142] anal achalasia,[143] and fissure in ano.[144] In children with persistent obstructive symptoms after anal pull-through procedures for Hirschsprung disease, BT injection of the internal anal sphincter has been advocated as an effective and atraumatic alternative to myectomy.[145,146] Current pediatric data on the use of BT for gastrointestinal disorders are limited to individual case reports and small series. Larger randomized placebo-controlled trials are needed to fully define efficacy, dosage interval, side-effect profile, and cost effectiveness.

Clinical use of BT in thousands of patients for musculoskeletal disorders and cosmetic applications suggests that BT is extremely well tolerated. Repeated use has been associated with the development of antibodies to BT and reduction of the magnitude and duration of clinical effects. The possibility of distal or systemic paralysis is extremely unlikely given that the estimated lethal dose is 40 U/kg (intramuscularly or intravenously),[147] and doses of 100 U (one vial) or less are sufficient for gastrointestinal interventions. Even if inadvertently injected into the bloodstream, this dose is unlikely to have significant effects.[138] Adverse reactions related to the physiologic effect of BT include occurrence of GER symptoms after lower esophageal sphincter injection. Transient incontinence of flatus and stool has been reported after anal sphincter injection in children with anal hypertonia after surgery for Hirschsprung disease[146] and in adults treated for anal fissure.[148] Adjacent regions may be affected, for example, with the development of dysphagia after head and neck injections for cervical dystonia.

Calcium Channel Blockers

Nonselective: Nifedipine, Verapamil, and Diltiazem. Calcium channel antagonists reduce the amplitude of gastrointestinal contractions.[149,150] There are anecdotal reports of their successful use in children with esophageal motility disorders, such as diffuse esophageal spasm and nutcracker esophagus, and with severe antral spasms.[151–153]

Selective: Pinaverium Bromide. This agent inhibits gastrointestinal contractions by preventing influx of calcium into myocytes. Most of the orally administered dose remains in the gut, resulting in antispasmodic effects at doses that do not cause cardiovascular disturbance.[154] Pinaverium reduces postprandial colonic activity in patients with IBS[155] and has been successful in reducing pain in trials of adult patients with IBS.[156]

Peppermint Oil

In vitro, peppermint oil inhibits smooth muscle contraction in a manner similar to that of the dihydropyridine calcium antagonists by reducing calcium influx.[157] This results in reduced contraction amplitudes at the lower esophageal sphincter,[158] stomach,[159] and small intestine[159] and prevents colonic spasm.[160,161] However, other therapeutic effects are likely. In a study of adults with diffuse esophageal spasm, peppermint oil converted simultaneous to peristaltic contractions and alleviated chest pain, without any reduction of peristaltic amplitude.[162] In a manometric study of peppermint oil and caraway oil, the duration of phase III of the MMC was shortened, in addition to reductions in contraction amplitude and frequency. A nonenteric coated preparation had more rapid effects and more marked suppression of gastric contractions than an enteric coated preparation.[159] In recent systematic reviews, peppermint oil was found to be efficacious in the treatment of IBS in children and adults.[163,164]

Nitrates

Nitric oxide donors such as glyceryl trinitrate and isosorbide dinitrate have been used to reduce the intensity of gastrointestinal contractions in the treatment of fissure in ano, anal achalasia, and spastic disorders of the esophagus.[144,165,166] Side effects such as hypotension and headache are frequently reported and require careful dosage titration.[151] As a result, these agents are probably best used on a short-term or "as required" basis.

Glucagon

Glucagon relaxes smooth muscle throughout the gastrointestinal tract. Pharmacologic uses of glucagon as an antispasmodic include inhibition of small intestinal contractions during endoscopic retrograde cholangiopancreatography and relaxation of the esophagus in cases of impacted foreign bodies. Interestingly, recent prospective randomized double-blind placebo-controlled trials in children and adults failed to show any benefit of glucagon over placebo for this indication.[167,168]

Adrenergic Agonists and Antagonists

α_2-Adrenoceptors mediate sympathetic nervous system inhibition of motility in vivo.[169] α_2-Adrenergic agonists such as clonidine are being investigated for their antidiarrheal and antinociceptive actions.[55] However, the use of clonidine in gastrointestinal motility disturbance has been limited by marked intraindividual variation in response[170] and the potential for systemic hypotension.

The β_2-receptor agonist salbutamol, when administered by an inhaler, is effective in reducing the duration of severe pain in proctalgia fugax.[171–173] However, this agent does not affect resting anal pressure between attacks,[171] and the mechanism of action of salbutamol in this condition is unknown. Because attacks of proctalgia fugax are associated with increased anal tone,[174] salbutamol probably acts to reduce abnormal excessive contractions during attacks.

Other Antispasmodic Drugs on the Horizon

Other antispasmodic, motility-inhibiting drugs currently being developed include neurokinin$_2$ antagonists (such as MEN-10627 and MEN-11420) and β_3-adrenoreceptor agonists (eg, SR-58611A). Both groups are able to decrease painful contractile activity in the gut without significantly affecting other bodily functions.[175]

CONCLUSION

Owing to the recent explosion in knowledge about the ENS and gastrointestinal motility, therapeutic options for patients with motility disturbance are rapidly expanding. New agents are being tested, and many older drugs, previously used for diseases as diverse as asthma, depression, and arterial hypertension, are being assessed for their utility in the gastrointestinal system. Because symptoms of most gastrointestinal motility disorders fluctuate over time and there may be a significant placebo effect on both symptoms and motility indices,[176] any new therapies require thorough evaluation in appropriately sized randomized placebo-controlled trials. As with any chronic disease, the importance of an effective physician-patient relationship in caring for patients with motility disturbance cannot be overstated. Pharmacologic therapy for functional bowel disorders is best administered as part of a biopsychosocial approach.[177]

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4. Pharmacologic Therapy of Exocrine Pancreatic Insufficiency

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The exocrine pancreas is involved in both the digestion and absorption of orally ingested nutrients. Normally, the fluid and exocrine secretions from the pancreas are secreted in great excess following a meal, and, in fact, before clinical manifestations of exocrine pancreatic insufficiency become manifest, over 98% of the gland's function needs to be lost.

Pancreatic fluid has two major components: a fluid consisting primarily of a solution of sodium bicarbonate and an enzyme component consisting of about 20 digestive enzymes and cofactors. The alkaline fluid serves to neutralize gastric acid entering the duodenum and helps to provide an adequate intraluminal pH for the optimal function of the pancreatic digestive enzymes. These enzymes provide the major route for intraluminal digestion of dietary proteins, triglycerides, and carbohydrates and are also involved in the cleavage of certain vitamins, such as A and B_{12}. Therefore, failure of the exocrine pancreas to secrete adequately its enzyme- and electrolyte-rich fluid can lead to major nutritional disturbances that manifest clinically as steatorrhea and azotorrhea, with resultant growth failure.[1] In addition to the obvious lack of intraluminal digestive activity as a result of the enzyme deficiencies, the failure of bicarbonate secretion also has major effects on both intraluminal pH and enzyme activity. An abnormally low pH can be seen in the late postprandial period, which reduces lipid digestion by inactivating pancreatic lipase and also by precipitating bile salts.

Not all diseases involving the exocrine pancrease have equal effects on both the enzyme component and the electrolyte component of the gland's secretion. In general, patients with cystic fibrosis (CF) have major deficits in both enzyme and electrolyte secretion, although there is a wide range of abnormalities; however, patients with Shwachman-Diamond syndrome have intact fluid and electrolyte secretion with marked disturbances in enzyme output.

CF is the most common cause of exocrine pancreatic insufficiency in childhood. Therefore, it is patients with CF who most commonly require oral pancreatic replacement therapy with pancreatic enzymes. Irrespective of the etiology of pancreatic failure, current replacement therapy with oral pancreatic enzymes, although far from ideal in many patients, remains the most important method of correcting the nutritional effects of maldigestion. Despite considerable improvements in the efficacy of pancreatic replacement therapy, it remains difficult to correct malabsorption completely in all patients owing to the many factors adversely affecting the function of exogenously administered enzymes.

Because the major clinical manifestation of pancreatic failure is steatorrhea with large, bulky, malodorous stools, early management protocols of patients with pancreatic insufficiency relied heavily on severe restriction of dietary fat. A low-fat diet did indeed produce socially more acceptable stools, but it also severely restricted calories and essential fatty acids, which contributed significantly to clinical malnutrition and disease morbidity. Use of a low-fat diet in the management of pancreatic failure is no longer considered acceptable; in fact, some centers advocate the use of a high-fat diet, in conjunction with optimal pancreatic enzyme replacement therapy, to maximize total energy absorption.

PANCREATIC ENZYME REPLACEMENT

Extracts of pancreatic enzymes from animal sources have been available for almost a century and have been used clinically for a variety of conditions. However, in spite of their recognized importance, the use of pancreatic enzymes is still not without its difficulties (Table 76.4-1). Commercial enzyme supplements do not have an indefinite shelf life, and, for this reason, patients should be warned not to stockpile large quantities of enzymes. In fact, many commercially available supplements are initially packed with much

TABLE 76.4–1 FACTORS ADVERSELY AFFECTING THE EFFICIENCY OF PANCREATIC ENZYME REPLACEMENT THERAPY

PHARMACOLOGIC PHASE
Enzyme source (porcine, bovine, fungal)
Enzyme stability
Particle size of microspheres
Inadequate enzyme concentration
Inappropriate oral administration
Poor compliance
GASTROINTESTINAL PHASE
Inactivation by gastric acid
Insufficient mixing with chyme
Delay in gastric emptying
Prolonged acidic intraluminal pH
Bile acid precipitation
Abnormal intestinal motility
Proteolytic destruction of lipase

higher protease and lipase values than their listed potencies to allow for this decline. Thomson and colleagues showed a decline of up to 20% in enzyme activity over an 8-month period in several different enzyme preparations, even though all were within the expiration date quoted by the manufacturers.[2] The earliest pancreatic extracts contained low concentrations of active enzymes. Furthermore, only minimal amounts of these were available for intestinal digestion because of gastric inactivation with acid and pepsin, with degradation of lipase and trypsin occurring below pH 4.5 and 3.5, respectively. Even the more active preparations in current use are rapidly degraded in the stomach when unprotected; up to 90% of ingested lipase and 80% of ingested trypsin have been found to be degraded prior to entering the ligament of Treitz.[3]

Broadly speaking, research has focused on three avenues of approach in improving nutrient absorption in patients with pancreatic insufficiency. First, because the older enzyme preparations were highly variable in enzyme content, the more modern approach has been to provide increased concentration of enzyme (up to 20,000 lipase units) in a single capsule or tablet.[4] Second, methods of protecting enzymes from gastric inactivation have been refined. Intensive research has also been aimed at manipulating the acid-alkaline imbalance in both the gastric and intestinal phases of enzyme activity. Third, attempts have been made at improving bile salt function.

Protective barriers were first used to make the enzyme preparations more resistant to acid inactivation. Initially, this was attempted by coating enzyme tablets with an acid-resistant material, but it was soon discovered that these preparations were no better than conventional preparations, and, in some cases, the steatorrhea was worse. This was thought to be due to both inefficient mixing of the tablet with the ingested chyme in the stomach and failure of liberation of the active enzyme in the duodenum secondary to slow release of the active ingredients. In fact, these tablets were not infrequently seen intact in the stools of patients taking them.[5]

To improve delivery of enzymes to the small intestine, a number of commercial pharmaceutical companies developed techniques capable of coating small "microspheres" with an acid-resistant coating.[6–12] The microspheres, in turn, were packaged in a gelatin capsule. The rationale behind this preparation is that the acid-resistant layer around the small spheres prevents acid-peptic degradation within the stomach, but their small size permits passage with chyme into the duodenum. When exposed to duodenal contents with a pH in excess of 5.5, the acid-resistant coating breaks down, releasing active pancreatic enzymes. This exposure may not occur in the proximal duodenum, however, because in CF, the postprandial intraluminal duodenum pH can frequently be below 5.0 for long periods of time. Thus, continued enzyme protection from the highly acidic milieu in the duodenum by the acid-resistant coating may allow for more distal bioavailability. The size of the microspheres also appears to be very important for adequate function. Several groups have shown that digestion in pancreatic insufficiency is more effective with microspheres of less than 1.4 mm compared with larger preparations, supporting the belief that microspheres of this size or smaller will optimally mix with the meal and empty the stomach together with the chyme, improving their digestive efficacy.[4]

Despite these advances in the use of microspheres, there is still some doubt over the reliability of gastric emptying. A study of 12 CF patients has shown considerable variation in gastric emptying and intestinal transit for food and pancreatic microspheres, with enzyme pellets generally emptying from the stomach more rapidly than the food.[13] This mismatch of emptying can be as high as 60 minutes in some patients, which will have obvious ramifications on the amount and timing of the enzyme replacement therapy.

Use of these enteric-coated microspheres has resulted in considerable improvement in fat absorption over that with conventional enzyme therapy.[7] A study has shown that CF patients with refractory malabsorption on conventional enzyme therapy derive significant benefit, with decreased steatorrhea and creatorrhea, using fewer capsules.[7] Other studies have found better compliance and improved absorption with these preparations, except in a minority of patients who appear to have acidic small intestinal contents, thereby preventing dissolution of the acid-resistant coating. Thus, there are few current uses for noncoated enzyme replacement therapy, although even with these modern preparations, some patients still have significant malabsorption.

In addition to acid-peptic denaturation and particle size, rapid proteolytic degradation of lipase, particularly by chymotrypsin, in the proximal small intestine is another important factor that limits the efficacy of pancreatic enzyme replacement therapy. Attempts at protecting lipase from this degradation using protease inhibitors have shown enhancement of lipolysis throughout the entire length of the small intestine.

The number of capsules required depends on the amount of active enzymes in the particular commercial preparation and also on the type and quantity of the meal to be consumed. To abolish malabsorption, the amount and concentration of enzyme present in the duodenum must be 5 to 10% (40–60 IU/mL intraluminal lipase concentration) of the quantities of endogenously secreted enzymes usually present in normal individuals after postprandial stimulation of the pancreas.[3] In an adult, assuming no inactivation of enzymes in the stomach and duodenum, approximately 25,000 to 40,000 U of lipase must be taken with an average meal.[14] In reality, the quantity of enzymes required becomes much higher (perhaps up to 10-fold) if one considers the degree of gastric inactivation and the consumption of a high-energy diet. There is, however, enormous patient to patient variability, and each patient and meal must be considered individually. In general, however, in light of findings regarding fibrosing colonopathy,[15] the daily dose of pancreatic enzymes for most patients should remain below 10,000 U of lipase/kg (Table 76.4–2).

Enzymes derived from porcine sources are the current standard treatment of exocrine insufficiency. Supplementation with enzymes from different species has also been suggested as a method of achieving improved digestion.

TABLE 77.4–2 SUGGESTED DAILY REQUIREMENTS OF PANCREATIC ENZYME REPLACEMENT THERAPY

AGE (YR)	APPROXIMATE DAILY FAT INTAKE (G)*†	DAILY LIPASE UNITS (000S)† (MAXIMUM DAILY DOSE 10,000 U LIPASE/KG/D)
0.0–0.5	25	12.5–25
0.5–1.0	30	15–30
1–3	35	17.5–140
4–6	50	25–200
7–10	60	30–240
11–14	90	45–360
15–18	110	55–440

*Assume 40% of total energy needs.
†In cystic fibrosis, multiply by a factor of 1.5.

Enzymes from bovine sources have been considered but discarded owing to their inferior lipase content and hence increased tablet requirement.[16] Acid-resistant lingual lipase has been proposed as an enzyme worthy of further consideration and investigation. In a preliminary study in animals, lingual lipase was found to be stable in the stomach under both fasting and fed conditions but to be less stable in the duodenum; however, to date no worthwhile clinical trials have been undertaken or reported.[17,18] Lipases derived from fungi have also been examined for their acid-resistant properties, with these experimental studies possibly becoming forerunners of in vivo human work examining pancreatic enzyme supplementation containing "foreign" enzymes. Fungi, such as *Rhizopus arrhizus*, *Candida cylinderaza*, and *Aspergillus niger*, are potential sources of lipase, which differs from lipases of animal origin in that fungi provide greater amounts of acid-stable lipase activity in the stomach; however, they appear to be highly sensitive to denaturation by even low concentrations of bile acids. Despite early promise in vitro, early in vivo studies have failed to extend their clinical usefulness.[19–21]

Lipases derived from bacteria are, however, more promising in their efficacy. Unlike fungal lipases, lipase of bacterial origin appears to be resistant to both acid and alkaline inactivation as well as being stable in the presence of both proteolytic enzymes and bile salts.[22–24] These recent studies have suggested that lipase derived from *Burkholderia plantarii* has far greater lipolytic activity than conventional porcine products, holding out the potential for a dramatic reduction in the quantity of individual tablets required for equivalent lipase activity, but, to date, no human trials have been reported.[23,24]

It is also now possible using recombinant deoxyribonucleic acid (DNA) techniques to produce human acid-stable lipases because the gene for human gastric lipase has now been transfected and expressed using recombinant adenovirus in a variety of animal models.[25,26] Similarly, application of bioengineered, acid-resistant human gastric lipase is also being actively explored in in vitro studies, but, to date, human trials are still pending in these areas.[27,28]

Irrespective of the enzyme preparation used and the amount given, it is imperative that the enzymes be delivered in sufficient amounts to the small intestine to facilitate digestion. It is insufficient simply to take a handful of enzymes at the beginning or end of a meal and hope that this will result in optimal pancreatic replacement. For optimal efficacy, it has been suggested that enzymes be distributed throughout the meal and taken in several small aliquots. This, in theory, allows for adequate dispersal within the stomach throughout the meal and therefore allows for maximum exposure of that particular meal to the ingested enzymes. However, as mentioned above, there is still no certainty that the enzymes will both mix completely with the food and also empty from the stomach uniformly.

MONITORING ENZYME EFFICACY

The simplest method of monitoring the effectiveness of the enzyme replacement therapy is to regularly monitor the patient's growth (in particular weight) and also the nature and consistency of the stools. In general, it is not very difficult to ascertain by history alone if a patient's enzyme dosage is insufficient on the basis of a stool history, but the associated history of abdominal pain is far less helpful. However, it is much more difficult to gauge on the basis of history alone whether a dosage is, in fact, excessive. More precise, yet still very qualitative, to perform is a quantitative assessment of fat absorption pre- and postenzymes with a fecal fat analysis. Each individual requires an objective, quantitative assessment of fecal fat losses as a percentage of fat intake at baseline and after any major adjustments in enzyme replacement therapy. Quantitative fecal fat estimation is the one reliable method, but there are practical limitations. Breath tests for the detection of pancreatic insufficiency have also been developed.[29,30] Cholesteryl octanoate breath testing using a carbon 14 or carbon 13 label has been shown to monitor intraluminal enzymatic activity in both controls and patients with pancreatic insufficiency after treatment with different forms of enzyme replacement. Fecal chymotrypsin and elastase 1 measurements have also been suggested but are still only in research use. Others have suggested that the pancreatic Schilling test is also a means of assessing replacement therapy.[31] This is based on the relationship between the pancreatic output of trypsin and the urinary excretion of cobalamin.

SIDE EFFECTS

Enzyme therapy is not without potential problems in that, being concentrated proteolytic packages, enzymes have the potential for causing quite marked oral excoriation if chewed or held within the mouth too long. This is a particular problem in small infants, in whom gum or mouth injury frequently occurs; with rapid transit through the intestinal tract, anal excoriation has also been observed.

Hyperuricemia and uricosuria are believed to result from the high purine content of the conventional enzyme preparation. Obviously, the greater the dose, the higher the incidence of these biochemical abnormalities. Allergic responses such as bronchospasm, nasal irritation, and repeated coughing may develop, not only in the patients receiving the

enzymes but also in any susceptible caregiver coming into repeated close contact with the enzyme preparations. These allergic reactions were much more prevalent with the nonencapsulated forms of the enzyme preparations.

The antigenicity of the pancreatic extracts should not be underestimated. Anaphylaxis has been observed in patients and caregivers exposed to the enzymes, particularly in a powdered form. Each capsule is a potent source of foreign protein, and small but significant amounts are absorbed into the body. Couper and Quirk and their colleagues studied two groups of CF patients using an enzyme-linked immunosorbent assay[32,33] specifically to detect immunoglobulin G (IgG) antibody directed against porcine trypsin. No antibodies were detected in patients prior to commencement of enzyme replacement, but 96% had developed porcine trypsin-binding IgG within a few years. This antibody production may possibly accentuate immune complex disease progression, which is well known in CF.

After its initial description and significant concerns, fibrosing colonopathy continues to generate debate as to its true place in the cystic fibrosis arena. First reported in 1994, fibrosing colonopathy is characterized by marked submucosal fibrosis with thickening of the muscularis propria and chronic inflammation chiefly affecting the cecum and ascending colon.[34] Typically, fibrosing colonopathy occurs in younger children, although it has been described arising de novo in a young adult patient with CF. Symptoms suggestive of colonic obstruction (abdominal pain and vomiting) occur within a few months after starting high-dose pancreatic enzyme replacement therapy. A systematic review of 114 accredited CF care centers in the United States revealed 31 proven cases of fibrosing colonopathy, with a strong relationship found between the occurrence of the fibrosing colonopathy and the total daily dose of the enzyme replacement.[34] This study showed that for patients taking more than 50,000 U of lipase/kg/d, the incidence of fibrosing colonopathy requiring surgery was about 3.8 per 1,000 patients per year of use, suggesting that most patients should remain below 10,000 U of lipase/kg/d.

The exact etiology of fibrosing colonopathy remains obscure. The appearance of this condition in cystic fibrosis patients initially appeared to coincide with the introduction of the "high-dose" enzyme preparations, with some patients consuming in excess of 20,000 IU lipase/kg/d. Interestingly, since the more conservative use of pancreatic enzymes, in particular, the high-dose preparations, there has been a reduction in the reported frequency of the problem.

Initially, it was suggested that the acid-resistant coating of the microspheres and tablets containing a methacrylic acid copolymer coating, Eudragit L (Röhm GmbH & Co., Darmstadt, Germany), was implicated.[35] Eudragit L has been shown to be toxic to rat intestine, causing mucosal ulceration and submucosal edema and fibrosis identical to the lesions seen in patients described in the literature. More recently, however, fibrosing colonopathy has been reported in patients not using this coating[34] and, in fact, not using pancreatic enzymes at all,[36,37] which highlights the continuing uncertainty of its etiology.

ADJUNCTIVE THERAPY TO ACID-BASE EQUILIBRIUM

The alternative method of improving the efficiency of the ingested pancreatic enzymes has been to modify the acid-base balance within the gastrointestinal tract. The H_2 receptor antagonists, such as cimetidine or ranitidine, have been used to diminish the secretion of gastric acid, thereby successfully decreasing the gastric inactivation of the ingested enzymes with resultant improvement in nutrient absorption.[7,14,38–42] Because enteric-coated microspheres are pH dependent and rely on a luminal pH of greater than 5.5 for dissolution of the acid-resistant coating, it is possible that jejunal hyperacidity may further hinder their activity. It has been shown that postprandial jejunal "hyperacidity" does occur in patients with CF, with 40% of a test meal entering the jejunum at a pH below 5.[38] At this pH, bile acids precipitate out of the aqueous solution, leading to a reduction in the aqueous phase bile acid concentration. In addition, lipase activity, which is extremely pH sensitive, is considerably reduced.

Cimetidine has been shown to increase jejunal pH, thus increasing aqueous phase bile acid concentration.[40] In a study of adult CF patients receiving noncoated enzymes, 60% of the test meal entered the jejunum at a pH less than 5, compared with only 17% in healthy subjects. There was a significant decrease in lipase activity and a decrease in aqueous phase lipid concentration, but the decrease in bile acid precipitation did not reach statistical significance. With the introduction of cimetidine, however, there was significantly less bile acid precipitation, and this resulted in improved lipid solubilization, suggesting that the efficacy of pancreatic enzyme therapy is limited both by exogenous enzyme inactivation in the stomach and by the pH-dependent environment within the proximal small intestine and that these effects were both improved by the addition of cimetidine. Data suggest that patients who had significant steatorrhea while taking enteric-coated microspheres also had improved nutrient absorption with the addition of cimetidine.[41] This improvement could result from both the prevention of gastric inactivitation and the reduction in small bowel hyperacidity levels, thus affecting the solubilization of bile salts.

Because the major effect of the addition of cimetidine appears to be improvement of the small intestinal alkalinity, it is not unreasonable to presume that the addition of antacids of bicarbonate therapy may have some merit. Graham found that the concurrent administration of enzymes with either sodium bicarbonate or aluminium hydroxide yields a greater reduction in steatorrhea than do enzymes alone.[38] Durie and others reported 21 patients with CF in whom sodium bicarbonate (15 g/m^2/24 h) was an effective adjunct to enzyme therapy. These workers found that sodium bicarbonate or cimetidine (20 mg/kg/d) had equivalent beneficial effects as adjuvant therapy, but when both drugs were given simultaneously, there was no further improvement in nutrient absorption.[41] The choice of antacid does appear to be critical.[38]

The proton pump inhibitors, with more potency and duration of action compared to H_2 receptor antagonists,

have also been used as an adjunctive therapy.[43] They have been shown to increase the efficacy of enteric-coated enzyme capsules dramatically and achieve near-normalization of fat absorption.[44,45] In addition to their effect on gastric acid secretion, they also have a profound effect on gastric volume, which may help to prevent dilution of the enzymes. The marked reduction in gastric pH may also see an increased postprandial duodenal pH, assisting in the effectiveness of the enzyme therapy. Further studies have shown that adjuvant therapy with the protein pump inhibitor lansoprazole in young CF patients with persistent fat malabsorption decreased fat losses and improved total body fat. In addition, lung hyperinflation was also decreased in the study patients, which may partly explain the improvement in inspiratory muscle performance.[46]

Yet another approach to adjuvant therapy is with the use of prostaglandin agents.[47] Prostaglandins of the E and I series inhibit basal and stimulate gastric acid secretion both in vivo and in vitro. In the dog, either prostaglandin E_2 or PGI_2 inhibits gastric acid secretion stimulated by food, histamine, pentagastrin, or reserpine. The mechanism by which natural prostaglandins and their analogs inhibit gastric acid secretion is still unknown, but one possibility is that there is direct inhibition of parietal cells by prostaglandins acting from the gastric lumen.[48]

Another mechanism through which prostaglandins might affect gastric secretion is suppression of gastrin release. It has been shown that methylated prostaglandin E analogs given orally in dogs and humans cause a marked suppression of gastrin response to a meal.[49] An important addition to the effect of gastric acid secretion is the effect of prostaglandins, particularly the methylated analogs, in stimulating mucus and bicarbonate secretion. This may account for the reduction in luminal acidity observed with the administration of these prostaglandins.

Prostaglandin therapy may have some inherent advantages over certain H_2 receptor antagonists as adjuvant therapy in CF patients. Cimetidine may interfere with the metabolism of certain drugs by inhibiting cytochrome P-450 oxygenase in the liver.[38] In CF patients, these potential drug interactions may assume some clinical importance by inhibiting metabolism of certain bronchodilators, notably theophylline. Because it has no human interactions with cytochrome P-450, misoprostol may be superior as long-term adjuvant therapy in CF.

BILE ACID DYSFUNCTION

In addition to manipulating the acid-alkaline balance in the upper small intestine, other researchers have explored the possibility of improving nutrient absorption with the addition of exogenous taurine.[50–53] As a result of large fecal losses of bile acids, patients with CF develop an increased ratio of glycine to taurine in conjugated bile acids. It has been proposed that correction of this elevated ratio by oral taurine supplements may improve absorption and, ultimately, nutrition by potentiating bile salt micelle formation. Taurine, which is more soluble in an acidic environment, has been administered to patients with CF in doses of 30 mg/kg/d, and there has been significant improvement in fat absorption in patients on enzyme supplementation.[51] Supplementation with taurine significantly reduced the glycine-to-taurine ratio and bile acid losses in the stools.[51] A further disadvantage of preponderant glycine bile salts conjugates is that they are partly and passively absorbed in the proximal portion of the small intestine. Because taurine conjugates are predominantly absorbed in the ileum and are more resistant to bacterial degradation, they are more available to form mixed micelles with fat that may have escaped intestinal absorption more proximally.

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5. Acid-Peptic Disease

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The clinical spectrum of acid-peptic disease in children includes reflux esophagitis,[1] gastric and duodenal peptic ulcer disease,[2] gastritis and gastropathy,[3] duodenitis, and rare entities such as Zollinger-Ellison syndrome. Several chapters of this book specifically address the pathogenesis, diagnosis, and treatment of some of these diseases (see Chapter 29.1, *Helicobacter pylori* and Peptic Ulcer Disease," and Chapter 29.2, "Other Causes"). The purpose of this chapter is to focus on the pharmacologic aspects of the principal drugs used for acid-peptic diseases, including the mechanism of action, pharmacokinetic and pharmacodynamic data, efficacy, and side effects based mainly on information available in studies on children.

Two major developments have been observed in the management of acid-related disorders during the last 15 years represented by proton pump inhibitors (PPIs) and *Helicobacter pylori*. PPIs irreversibly bind to the H^+-K^+ adenosine triphosphatase (ATPase) enzyme complex and extensively inhibit acid production, revolutionizing the management of hyperacidity diseases. *H. pylori* has been shown to be the leading cause of primary peptic ulcer and chronic-active (type B) antral gastritis.[4] The eradication of this organism dramatically reduces the recurrence of gastric and duodenal ulcers both in adults and in children. These two factors have profoundly changed the natural history and epidemiology of acid-peptic disease in adults, mainly gastroesophageal reflux and peptic ulcer diseases, as well as diagnostic tests and therapeutic strategies, demonstrated by the dramatic decrease of surgical indications in this group of diseases. However, several childhood peculiarities and as yet still unresolved questions limit extensive use of antisecretory drugs in children. Peptic ulcer disease is rare in children, and the role of *H. pylori* in nonulcer dyspepsia in this age group remains a matter of debate. Therefore, recommendations for treatment remain limited to a selected number of patients, and use of PPIs for long-term management of gastroesophageal reflux disease (GERD) is still not recommended in children.

PROTON PUMP INHIBITORS

PPIs (omeprazole, lansoprazole, pantoprazole, rabeprazole, and esomeprazole) inhibit gastric acid secretion by selectively acting on gastric parietal cell H^+-K^+ ATPase, which is the enzyme involved in the last step of acid secretion by parietal cells (Figure 76.5-1).[5]

In contrast to histamine$_2$ (H_2) receptor antagonists, inhibition of gastric acid secretion by PPIs is independent of the pathway of stimulation. PPIs are highly selective and effective in their action and have a few short- and long-term adverse effects. These pharmacologic features have made the development of PPIs the most significant advancement in the management of acid-peptic–related disorders in the last two decades. Although numerous adult studies have been published, there are still few large studies with significant patients enrolled and no randomized controlled comparative studies in childhood.[6] It should be emphasized that almost no clinical or pharmacologic data are available in infants under 1 year of age. Although several different PPIs are available on the market (Table 76.5-1), only two of them, namely omeprazole and lansoprazole, have been studied in childhood. The following section focuses mainly on those drugs that are the only two approved for use in children (with restriction on age, indication, or type of administration according to different countries).

Mode of Action

PPIs form a group of compounds called substituted benzimidazoles, which concentrate within the intracellular canaliculi of parietal cells, irreversibly bind to the H^+-K^+ ATPase enzyme complex, and extensively inhibit acid production. PPIs differ from each other by the molecular structures bound to the pyridine and benzimidazole components of the molecule (Figure 76.5-2).[7] This explains differences in pharmacologic properties, but all of the PPIs

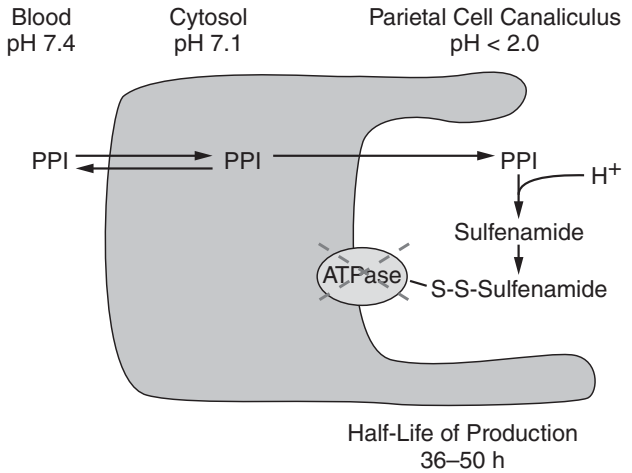


FIGURE 76.5-1 Mechanism of action of antisecretory drugs. ATPase = adenosine triphosphatase; PPI = proton pump inhibitor.

TABLE 76.5-1 DIFFERENT ANTISECRETORY DRUGS AVAILABLE

PROTON PUMP INHIBITORS
Omeprazole
Lansoprazole
Pantoprazole
Rabeprazole
Esomeprazole
H_2 BLOCKERS
Cimetidine
Ranitidine
Famotidine
Nizatidine

have the same mechanism of action. Because they are weakly basic compounds (the pKa value of the pyridine nitrogen being close to 4.0), they are maximally protonated in environments of high acidity (which is exclusively found in the intracellular canaliculi of actively secreting parietal cells and within the stomach cavity). PPIs can be considered prodrugs because in highly acidic environments, protonation of the molecule results in a series of reactions that ultimately produces the active form of the PPI (see Figure 76.5-2).[7] The active cyclic sulfenamide then binds permanently to exposed cysteine thiol groups on the luminal surface of the H^+-K^+ ATPase enzyme. Once covalently bound, the H^+-K^+ ATPase enzyme becomes nonfunctional, and activity returns only by parietal cell synthesis of new H^+-K^+ ATPase enzyme systems. The turnover of the H^+-K^+ ATPase is constant, with a half-life of about 48 hours in adults. The maturation of turnover is unknown in infants and children. The best access of the drug to the H^+-K^+ ATPase situated on the luminal side of the secretory membrane of the gastric parietal cells is provided by a meal, which is the strongest physiologic event inducing the exteriorization of the H^+-K^+ ATPase.[8]

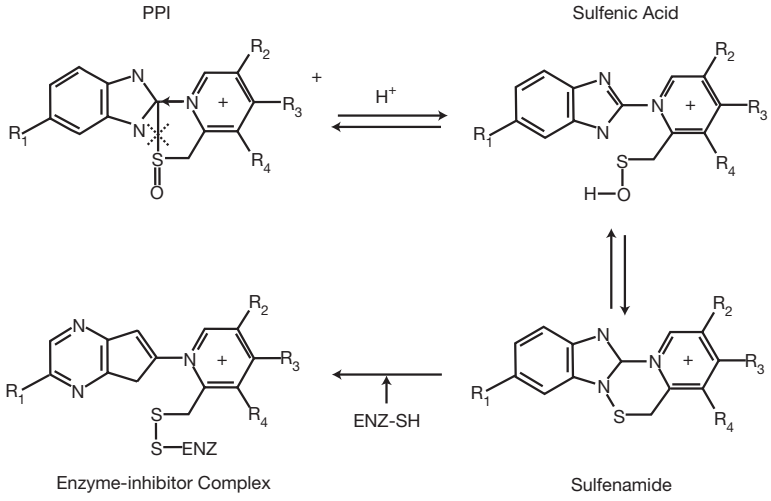
Given orally, PPIs can be prematurely converted to the active form in the acidic environment of the stomach. They are prepared as capsules containing protective enteric-coated granules or as enteric-coated tablets. In these forms, absorption begins only in the higher pH environment of the duodenum. They are almost completely absorbed in the small intestine.[7]

PHARMACOKINETIC PROPERTIES

Most of the data on the pharmacokinetics of PPIs have been obtained in adult volunteers and adult patients with peptic ulcer disease. However, recent studies have provided extensive observations on the pharmacokinetics for omeprazole and lansoprazole in children.[9–16] Data are lacking on rabeprazole and esomeprazole in children; some data have recently been published on pantoprazole.[17]

PPIs are metabolized by the hepatocyte cytochrome P-450 isoforms CYP2C19 and CYP3A4 to inactive metabolites (sulfide, sulfone, and hydroxymetabolites) excreted in urine. A comprehensive comparative review on the pharmacokinetics of omeprazole, lansoprazole, pantoprazole, and rabeprazole has been published on adults.[7] Schematically, the drugs are quickly absorbed (T_{max} = 1–3 hours), with bioavailability varying between them (omeprazole 35–65%, lansoprazole 80–91%, and pantoprazole 57–100%). They are rapidly metabolized (half-time [$T_{\frac{1}{2}}$] = 0.6–2 hours). The antisecretory effect of PPIs is independent from plasma concentration but is correlated with the area under the plasma concentration time curve (AUC).[8] This has also been shown in children.[9]

In addition, in children and adolescents, the pharmacokinetic parameters have been reported in the same range as those in adults for omeprazole[10,11] and lansoprazole,[9,12–14] with variations according to age for omeprazole. Andersson and colleagues have reported a significant difference in $T_{\frac{1}{2}}$ between children aged 1 to 6 years and chil-



	R_1	R_2	R_3	R_4
Omeprazole/Esomeprazole	CH_3O	CH_3	CH_3O	CH_3
Lansoprazole	H	H	CH_3F_2O	CH_3
Pantoprazole	CF_2HO	H	CH_3O	CH_3O
Rabeprazole	H	H	$CH_3O(CH_2)_3O$	CH_3

FIGURE 76.5-2 Chemical formula of proton pump inhibitors (PPIs) and the mechanisms of action of enzyme (H^+-K^+ adenosine triphosphatase [ATPase])-inhibitor complex formation. Reproduced with permission from Gibbons TE and Gold BD.[6]

dren aged 7 to 12 years, with an increasing metabolism of omeprazole in the younger age group.[10] In neonates less than 10 days old, Anderson and colleagues reported a trend toward a prolonged $T_{\frac{1}{2}}$ for intravenous omeprazole.[15] This variability of metabolism related to age has not been reported with lansoprazole, for which data are available in children between the ages of 1 and 17 years.[9,12–14] All of these studies show a pharmacokinetic profile for lansoprazole similar to that of adults. Interindividual variability of pharmacokinetic parameters is wide in adults, as in children, and may explain to some extent the variations observed in the dosage requirement of the PPIs.

For both omeprazole and lansoprazole, there are almost no pharmacokinetic data available for neonates and infants under 1 year of age.

In addition to these maturation differences, pharmacokinetic parameters in children may be affected, as in adults, by genetic variability of the enzyme systems. Indeed, CYP2C19 displays a known genetic polymorphism,[18] and differences in pantoprazole, omeprazole, and lansoprazole disposition have been demonstrated with the AUC that are fivefold higher in poor metabolizers than in extensive metabolizers.[19] Clinically, poor metabolizers (a poor metabolizer phenotype occurs in 1% of blacks, 2–6% of whites, 15% of Chinese, and 23% of Japanese) have been shown to experience superior acid suppression (with omeprazole and lansoprazole) compared with extensive metabolizers, without an increase in the incidence of adverse effects.[7] Thus far, there is no toxicity issue that warrants dosage adjustment of pantoprazole in poor metabolizers.[20]

Other maturation factors may also affect the metabolism of PPIs, such as the rate of renewal of the proton pumps in the parietal cell. In patients with hepatic impairment, the AUC values increased to the same extent as observed in poor metabolizers, with no need to adjust the dosage, although caution should be exercised when giving PPIs to patients with severe hepatic insufficiency. No dosage adjustment of PPIs is required in patients with renal insufficiency[21] or on hemodialysis.[22]

Pharmacodynamic Properties

Pharmacodynamic Efficacy of Omeprazole. Omeprazole is available for oral and intravenous administration. In children, most data were obtained after oral administration, and a mean daily dose of 1 mg/kg body weight was required to obtain a sustained efficacy over 24 hours.[16,23–26] However, in a multicenter study of children aged 1 to 16 years using esophageal pH monitoring below a pH of < 4 for less than 6% of a 24-hour period, the healing dosage varied from 0.7 to 3.5 mg/kg/d. Overall, in this study, more than 75% of the patients required 1.4 mg/kg as the healing dosage.[10]

When the oral route cannot be used, it is necessary to inhibit acid secretion via intravenous administration of omeprazole, which is not approved for use in the United States and Canada. In children, the dose of 40 mg/1.73 m^2 (1.17 mg/kg) was required to achieve a gastric pH over 4 during more than 90% of a 24-hour period following omeprazole intravenous administration.[11]

Pharmacodynamic Efficacy of Lansoprazole. In adults, a daily dosage of 30 mg of lansoprazole, that is, about 17 mg/m^2, has been found to be effective and safe in inhibiting gastric acid secretion and healing acid-related lesions. This dosage was effective in numerous randomized double-blind trials and has been recommended for the treatment of duodenal ulcer and reflux esophagitis in adults.[27] In children, as for omeprazole, the efficacy of lansoprazole on gastric acid secretion studied by gastric pH monitoring has been shown to vary widely among the patients studied: about 40% of children responded to the dose of 0.73 mg/kg (equivalent to the adult dose, ie, 17 mg/m^2), 26% responded to 1.44 mg/kg, and 35% failed to respond to this doubled dose.[9] This variability can be ascribed in part to differences in pharmacokinetics: the AUC of lansoprazole showed a significant positive correlation with gastric acid inhibition, and the AUC was significantly greater and the oral clearance was not significantly lower in the patients who did than in those who did not respond to the lower dose (0.73 mg/kg, ie, 17 mg/m^2). Thus, failure to respond to the lower dose may be ascribed to reduced bioavailability and/or to faster metabolism of the drug. Patients with these characteristics may require a higher dose to achieve the desired antisecretory effect.[9]

Pharmacodynamic Efficacy of Pantoprazole. In a recent study, oral pantoprazole (20 mg daily) provided gastric acid control in 15 pediatric patients with reflux esophagitis.[17]

Clinical Efficacy

The major use of PPIs in pediatrics has been the management of peptic esophagitis and peptic ulcer disease and for eradication of *H. pylori* infection. No controlled or open studies of the use of PPIs in the management of extraesophageal manifestations of gastroesophageal reflux such as otorhinolarygologic or respiratory symptoms have been performed.

Peptic Esophagitis

Most of the studies performed in children with gastroesophageal reflux assessed healing of esophagitis to define the efficacy of omeprazole[23–26,28–31] and lansoprazole.[9,13,32,33] In a review of omeprazole use in the treatment of acid-related disorders in children for the period 1986 to 2000, marketed and extemporaneous formulations of omeprazole were used at dosages of 5 to 80 mg/d (0.2–3.5 mg/kg/d) for periods ranging from 14 days to 36 months. The initial dose most consistently reported to heal esophagitis and provide relief of symptoms of gastroesophageal reflux appears to be 1 mg/kg/d.[34] A similar response was observed with omeprazole[26] among the responders to lansoprazole; 80% of children had healing of their esophagitis after 4 weeks of treatment.[9] Overall, for both omeprazole and lansoprazole, studies performed in children showed that in patients with adequate acid suppression (ie, receiving an appropriate dosage), the healing rate of peptic esophagitis is more than 75% after 4 to 8 weeks of treatment; the clinical symptoms improve in the same time period.[16,17,23–26,28,29,31,32] How-

ever, it should be emphasized that if one considers the initial dose (ie, 0.7–1 mg/kg) in these pediatric studies, the response rate is, as expected, lower (around 50%). In a recent small study, oral pantoprazole (20 mg daily) given to 15 pediatric patients with reflux esophagitis provided healing of the esophagitis in 52% of patients.[17]

Gastric and Duodenal Ulcers. Although rare in pediatrics, PPIs have been used in the treatment of gastric and duodenal peptic ulcers. Low doses (0.3–0.7 mg/kg) were required to achieve healing.[16,25]

H. *pylori* Eradication. Although there are still no clear recommendations on the treatment of *H. pylori* in childhood,[4,35–37] triple therapy using a PPI and two antibacterial agents is considered the treatment of choice.[35,38] However, several nonrandomized studies have also shown a high eradication rate with two antibiotics combining metronidazole or tinidazole and amoxicillin for 2 to 4 weeks.[39] As in adults, treatment failures are mainly explained by poor compliance and resistance of *H. pylori* to antibiotics. The only double-blind trial published in children showed an eradication rate of 74.2% with 1 week of triple therapy including omeprazole, clarithromycin, and amoxicillin compared with an eradication rate of 9.4% in children receiving placebo, clarithromycin, and amoxicillin.[40] This study demonstrated the low eradication rate with two antibiotics used for 1 week, including clarithromycin, and confirmed the efficacy of PPIs associated with antibiotics in *H. pylori* infection in children.

Safety

Short-Term Safety. PPIs are well tolerated by most patients. The principal side effects are mild to moderate headaches, abdominal pain, vomiting, and diarrhea (Table 76.5-2).[9] In the double-blind study previously mentioned comparing omeprazole, clarithromycin, and amoxicillin with placebo, clarithromycin, and amoxicillin in *H. pylori*–infected children, adverse events were reported in 24% of patients in both groups but remained mild.[40] No adverse effect was reported even in children who received a high dose of intravenous omeprazole.[11] A small reversible elevation of transaminases has been reported.

Prolonged periods of hypochlorhydria may lead to gastric bacterial overgrowth, as was noted in adults[41] and neonates.[42] Although the clinical effect of this overgrowth remains unclear, this suggests that in critically ill patients and neonates, if required, the intravenous administration of PPIs should be as short as possible because of the prolonged inhibition of the gastric acid secretion.

TABLE 76.5-2 FREQUENCY OF SIDE EFFECTS ASSOCIATED WITH OMEPRAZOLE AND LANSOPRAZOLE IN CHILDREN

EVENT	OMEPRAZOLE (%)	LANSOPRAZOLE (%)
Headache	0–2	0–3
Constipation	0	0–5
Diarrhea	0–1	0–9
Vomiting	0–0.3	0–4
Dizziness	0	0–2
Insomnia	0	0–2
Total	0–2	0–15

Adapted from Faure C et al,[9] Hassall E et al,[26] Franco MT et al,[32] Tolia V,[33] and Hendriks HJ et al.[121]

Long-Term Safety. Although PPIs have been available for more than 15 years and are widely used in adults, with an excellent long-term safety profile, few data are available on infants and children regarding long-term use (eg, more than 6 months) of these potent acid-suppressing agents. Because of experimental data provided in newborn rats, there are concerns regarding the consequences and effects on the gastric mucosa of the increased gastrin levels (two- to fivefold rise in half of the treated patients). The trophic effects of gastrin lead to stimulation of the enterochromaffin-like cell population and hyperplastic changes in parietal cell mass. Carcinoid tumors have been observed in animals treated lifelong with high-dose omeprazole or an H_2 receptor antagonist. Although enterochromaffin-like hyperplasia has been observed in adults, carcinoid tumors have been reported only once in adults.[43] The trophic action of gastrin on digestive epithelium has not been implicated in the development of gastric or colonic adenocarcinoma in animal and adult safety studies.[44,45] However, fundic polyps and nodules have been reported in children who received omeprazole for more than 6 months.[46] The effect of long-term omeprazole therapy (4–7 years) on the ratio of G (gastrin secretion) to D cells (somatostatin secretion) was studied in 6 children.[47] The mean G-cell number and the ratio of G to D cells showed a significant increase for omeprazole compared with baseline levels. In adults, studies have shown an increased incidence of gastric atrophy associated with long-term use of PPIs, especially in the presence of *H. pylori* infection.[44] However, similar studies are still lacking in children.

Long-term acid suppression may promote the production of *N*-nitrosamine compounds in the stomach secondary to bacterial overgrowth. These compounds are considered carcinogenic.

Suppression of acid secretion may theoretically lead to malabsorption of vitamin B_{12} and maldigestion of proteins. Except for patients with Zollinger-Ellison syndrome, long-term treatment with PPIs is not considered a causal factor of vitamin B_{12} deficiency.[48] Moreover, Evenepoel and colleagues have shown the influence of PPIs on protein digestion.[49] Clinical consequences in children are currently unknown.[50]

In vitro data have shown that osteoclast activity may be inhibited by omeprazole without an influence on bone turnover in children during short-term treatment.[51]

Overall, one should consider that long-term use of PPIs in children is safe but requires careful long-term follow-up.[45]

Drug Interactions. Although most PPIs interact with the cytochrome P-450 system, no clinically important interactions have been observed between PPIs and other drugs.[8,52–55] However, omeprazole may increase the plasma concentration of diazepam, phenytoin, carbamazepine, and warfarin.[7]

Dosage and Administration

PPIs are available per os in capsules containing protective enteric-coated granules or as an enteric-coated tablet. An intravenous formulation is available for omeprazole and pantoprazole. The granules and tablets should not be crushed, chewed, or dissolved because gastric acid secretion may alter the drug's action.

The capsules can be opened, and for children who are unable to swallow capsules or tablets, the microgranules may be administered per os or via a feeding tube, in suspension in an acidic medium such as fruit juice, yogurt, or applesauce. A "homemade" liquid formulation, produced by dissolving the drug in 8.4% bicarbonate solution, has been used in some reports.[56] However, a recent study performed in adults has shown that the pharmacokinetic parameters (absorption) of these simplified suspensions were altered when compared with the regular administration of a capsule. This was particularly true with the simplified omeprazole.[57] Pediatric pharmacokinetic, pharmacodynamic, and efficacy studies with these alternative oral preparations are lacking, preventing them from being recommended. Owing to the activation of the proton pumps in the pre- and postprandial periods, PPIs should be administered just before or during meals.

The intravenous formulation should not be administered per os because gastric acid secretion alters the drug.

Omeprazole. The usual recommended starting dosage of omeprazole is 1 mg/kg once daily (eg, 10 mg for children 10 to 20 kg and 20 mg for children weighing more than 20 kg).

Although not registered in all countries, intravenous omeprazole should be given once daily 40 mg/1.73 m^2 (eg, 1 mg/kg).[11] Because the benefit of a loading dose of intravenous omeprazole was demonstrated in adults,[58] it has been suggested to use a loading dose of 40 mg/0.73 m^2 repeated after 12 hours to achieve a rapid antisecretory effect in similar critical situations in pediatric patients.[11]

Lansoprazole. The usual recommended starting dosage is 1 mg/kg once daily (eg, 15 mg for children ≤ 30 kg in weight and 30 mg for those weighing > 30 kg[59]). This drug is not yet labeled in all countries for use in children.

For both omeprazole and lansoprazole, it should be emphasized that the optimal dose may vary among patients and that, in case of a lack of efficacy, one must be aware that almost 25% of patients may require a double dosage. In the absence of a clinical response to the starting recommended dose, it is suggested to check very carefully with caregivers regarding the mode of administration of the PPIs. If this is correct, doubling the dosage should be suggested.

Pantoprazole. Data are currently lacking on recommended dosages for intravenous pantoprazole in children.

H_2 RECEPTOR ANTAGONISTS

The first H_2 blocker licensed was cimetidine, in 1976, followed by ranitidine and later famotidine and nizatidine (see Table 76.5-1). Experience with other H_2 receptor antagonists, such as roxatidine and ebrotidine, is very limited or nonexistent in children. Although PPIs have now supplanted these drugs for the treatment of acid-peptic disease because of their higher efficacy and excellent tolerance, the large experience of more than 20 years of use of H_2 receptor antagonists on millions of patients has provided considerable insight into the efficacy, pharmacology, and long-term tolerance in adults and children.[60–67] A large number of studies in adults have established that PPIs are more effective in decreasing acid secretion and have better clinical efficacy than H_2 receptor antagonists; however, there are no published articles on children that specifically address this question. Ranitidine, famotidine, and nizatidine are preferred over cimetidine because cimetidine interferes with the cytochrome P-450 enzyme and demonstrates more central nervous system, gastrointestinal, and endocrine side effects than the other H_2 blockers. In parallel with the increase of self-medication of ranitidine in adults, there is also a movement toward the treatment of symptoms in children regardless of the presence or absence of esophagitis. However, the pharmacokinetic and pharmacodynamic effects of over-the-counter H_2 receptor antagonists in the pediatric population remain largely unknown.[63]

Pharmacodynamic Properties

The H_2 receptor antagonists are competitive inhibitors of histamine-stimulated acid secretion; however, they have limited effects on acid secretion that is induced by meals or other stimuli. In most of the pediatric studies, a good correlation has been demonstrated between the median plasma ranitidine concentration and the pharmacodynamic parameter elevation of intragastric pH. The gastric pH typically rose above 4 when the plasma ranitidine concentration approached 100 ng/mL.[60,63,64] Pediatric subjects with suspected abnormal acid reflux aged 4 to 11 years received a single dose of 75 mg of ranitidine. The intragastric pH began to rise approximately 30 minutes after dosing with ranitidine to a peak of pH 4. Ranitidine, 75 mg, significantly increased the intragastric pH throughout the 6-hour evaluation period.[63] The return toward baseline after 5 hours suggests the potential utility of dosing more frequently than every 12 hours. In contrast to PPIs, tolerance and rebound effects may occur with the use of H_2 receptor antagonists. Tolerance to the antisecretory effects of H_2 receptor antagonists has been demonstrated in adults and appears to develop quickly in 3 days. The mechanisms are not fully understood but may be related to the upregulation of enterochromaffin-like cell activity.[68] However, no data are available in pediatrics. The occurrence of a rebound hypersecretion effect should be taken into account when discontinuing the drugs, which therefore should be progressively withdrawn.[69,70]

Pharmacokinetic Properties

Studies of pharmacokinetics of H_2 receptor antagonists have been conducted in infants and children.[60,64,65,67] Overall, the parameters are similar to those of adults, with a reasonable absorption after oral dosing. Absorption is not affected by food. Peak blood levels are achieved within 1 to 3 hours after an oral dose. These drugs are well distributed

throughout the body and cross the blood-brain barrier. After oral administration, cimetidine, ranitidine, and famotidine undergo "first-pass" hepatic metabolic alteration that reduces their bioavailability by 50%. Protein binding is low (15%). H_2 receptor antagonists are eliminated by a combination of renal excretion and hepatic metabolic degradation. Sixty to 80% of orally administered cimetidine and ranitidine is cleared by the liver. In contrast, after intravenous administration, they are eliminated principally through renal excretion. Dose reductions are thus recommended for patients with renal insufficiency.

After a single dose of 75 mg of ranitidine in children aged 4 to 11 years, the median C_{max} value of 477 ng/mL occurred 2.5 hours after dosing, and the median half-life was 2.0 hours.[63] In infants, the half-life is higher (3.5 hours).

Clinical Efficacy

Although no controlled trials are available in children, oral ranitidine therapy has been useful in pediatric practice for the treatment of GERD and peptic ulcer diseases.[60–62] Cucchiara and colleagues showed that high-dose (20 mg/kg/d) ranitidine was as effective as omeprazole in peptic esophagitis.[61]

Tolerability

The side effects of H_2 receptor antagonist are reported in Table 76.5-3. The majority of pediatric clinical trials with ranitidine have reported few side effects or abnormal laboratory values. As for PPIs, raising the gastric pH may result in the overgrowth of pathogenic bacteria in the digestive tract.[41,42] It has been shown in neonates that the length of hospital stay, increased gastric pH, period of antibiotic therapy, and ranitidine use were independently associated with an increased colonization rate.[42]

Interactions between H_2 antagonists and other drugs have been extensively reviewed.[71] It is widely held that cimetidine is a more important antagonist of other drugs than the other H_2 antagonists (Table 76.5-4); however, the clinical relevance of many of the interactions with cimetidine is marginal. In neonates, cimetidine should be used cautiously in patients concurrently receiving theophylline, phenytoin, or caffeine because it may prolong the half-life of those drugs.[72]

Dosage and Administration

The recommended oral dosage of H_2 blockers is summarized in Table 76.5-5. Ranitidine (5–10 mg/kg), given orally daily, divided into two or three doses, produces a symptomatic and endoscopic improvement in erosive esophagitis in children.[73] Pharmacodynamic data on ranitidine suggest that a dosing period of 6 to 9 hours may provide a more effective control of intragastric acidity.[63,73] In patients with renal impairment, the dosage should be adjusted: if the creatinine clearance is 10 to 50 mL/min, the dosage should be decreased to 75% of normal dosage; if the creatinine clearance is < 10 mL/min, the dosage should be decreased to 50% of normal dosage.

The recommended dosages of intravenous cimetidine are 5 to 10 mg/kg/d every 8 to 12 hours in neonates and 10 to 20 mg/kg/d every 6 to 12 hours in infants.[72] Intravenous cimetidine may be infused preferably over 15 to 30 minutes because rapid injection has been associated with cardiac arrhythmias and hypotension. It also may be given by continuous infusion. In premature infants and neonates, ranitidine is usually given intravenously or, rarely, intramuscularly at a dosage of 1 to 2 mg/kg/d divided every 6 hours. The maximal intravenous dosage is 6 mg/kg/d divided every 6 hours. Intravenous continuous infusion is

TABLE 76.5-3 SIDE EFFECTS OF H_2 RECEPTOR ANTAGONIST

SIDE EFFECT	CIMETIDINE	RANITIDINE	FAMOTIDINE	NIZATIDINE
HEMATOLOGY				
Thrombocytopenia	+		+	
Neutropenia	+	+		
Agranulocytosis	+	+		
CARDIAC				
Bradycardia	+	+		
Hypotension	+			
NEUROLOGIC				
Dizziness	+	+	+	+
Headache	+	+	+	+
Confusion	+	+	±	
Sleep disturbance	+			+
GYNECOMASTIA	+			
DIGESTIVE				
Vomiting	+	+	+	+
Diarrhea	+	+	+	+
Constipation		+	+	
Pancreatitis	+	+		
Hepatitis	+	+	+	+
CUTANEOUS				
Rash	+	+	+	+
RENAL				
Interstitial nephritis	+			

TABLE 76.5-4 CLINICALLY SIGNIFICANT DRUG INTERACTIONS WITH ACID-SUPPRESSING AGENTS

DRUGS INTERACTING	CIMETIDINE	OMEPRAZOLE	LANSOPRAZOLE
Cyclosporine	+		
Midazolam	+		
Phenytoin	+	+	+
Theophylline	+		
Warfarin	+	+	

Adapted from Flockhart DA et al[71] and Gold BD and Freston JW.[122]

preferred over intermittent dosing at a dosage of 1.44 to 4 mg/kg/d (maximum 6 mg/kg/d).[72] It can be administered in a parenteral nutrition solution. Famotidine can be given intravenously either at a dosage of 0.3 mg/kg every 8 hours (maximum 2.4 mg/kg/d) or by continuous infusion of 1 to 2 mg/kg/d over 24 hours. It can be administered in a parenteral nutrition solution.[72]

ANTACIDS

Antacids include carbonate and bicarbonate salts (eg, $NaHCO_3$ and Ca^- or $MgCO_3$), alkali complexes of aluminum and/or magnesium (eg, aluminum and magnesium hydroxides), aluminum and magnesium phosphates, magnesium trisilicate, and alginate-based raft-forming formulations. They are used for the symptomatic treatment of heartburn and esophagitis. Experience with antacids is limited in infants. Their efficacy in buffering the gastric acidity is strongly influenced by the time of administration and requires multiple administrations. Dimethicone is used in some regions for regurgitation, although there are no reliable studies demonstrating its efficacy in the treatment of gastroesophageal reflux in infants. Although often classified as an antacid, it acts more as a feed thickener because it contains more than 50% of bean gum and has hardly any acid-neutralizing properties.

Pharmacodynamic Properties

All antacids have a nonsystemic mechanism of action. They chemically neutralize gastric acid. The key therapeutic advantage of antacids is their rapid onset of action. Antacids act within minutes to an elevated intragastric pH above 3.5 and provide symptomatic relief. Hence, their action is limited by the capacity to maintain an elevated pH in the presence of continued physiologic acid secretion and by normal gastric emptying. Alginate-based raft-forming preparations have a quite different mode of action. In the presence of gastric acid, alginates precipitate, forming a gel. Alginate-based raft-forming formulations usually contain sodium or potassium bicarbonate; in the presence of gastric acid, the bicarbonate is converted to carbon dioxide, which becomes entrapped within the gel precipitate, converting it into a foam that floats on the surface of the gastric surface, providing a relative pH-neutral barrier.[74]

Clinical Efficacy

Double-blind studies in adults have shown that alginate-based raft-forming preparations are superior to placebo in relieving the symptoms of heartburn.[74] However, studies in infants and children remain limited (six studies including a total of 303 patients, only one being double blind) and had various study designs (open-label prospective study, comparison of two different dosages of alginate, comparison of placebo, famotidine, or cisapride).[75–80] Their efficacy as monotherapy or in combination with prokinetics for gastroesophageal reflux is not convincing.[81,82]

Tolerability

Because absorption from aluminum-containing antacids may cause serum aluminum concentrations to approach levels reported to cause osteopenia and neurotoxicity, chronic antacid therapy is not recommended.[83] Gaviscon contains a considerable amount of sodium carbonate, so its administration may increase the sodium content of the feeds to an undesirable level, especially in preterm infants (1 g of Gaviscon contains 46 mg of sodium, and the suspension contains twice this amount). Algicon, which has a better taste than Gaviscon, has a lower sodium load but a higher aluminum content. Occasional formation of large bezoar-like masses of agglutinated intragastric material has been reported in association with Gaviscon use.[81,82] Side effects include diarrhea with magnesium-rich preparations and excessive absorption of aluminum in infants.[81,82] The presence of aluminum and magnesium in the majority of antacids means that such products have the potential to chelate drugs in the upper gastrointestinal tract.[71] The

TABLE 76.5-5 RECOMMENDED ORAL DOSAGE OF H_2 RECEPTOR ANTAGONISTS

H^2 RECEPTOR ANTAGONIST	RECOMMENDED DOSAGE IN NEONATES	RECOMMENDED DOSAGE IN CHILDREN	RECOMMENDED DOSAGE IN ADULTS
Cimetidine	5–10 mg/kg/d bid or tid; 10–20 mg/kg/d bid or tid in infants	20–40 mg/kg/d bid or tid	600–1,600 mg/d once or bid or tid
Ranitidine	2–4 mg/kg/d divided every 6 h	5–10 mg/kg/d tid	150–600 mg/d once or bid
Nizatidine	—	10 mg/kg/d bid	150–300 mg/d once or bid
Famotidine	1 to 2 mg/kg/d tid	1 mg/kg/d bid	20–80 mg/d once or bid

Adapted from Bell SG[72] and Rudolph CD et al.[73]

drugs most frequently affected in this way include the quinolone antibacterial agents, didanosine, azithromycin, tetracycline, and the H_2 antagonists.[71] The effects of antacids on the pharmacokinetics of other drugs vary widely according to the type of antacid and time dosage of the administration of the other drug, ranging from no effect to an 85% decrease in bioavailability.[71] For example, a single dose of an aluminum-magnesium antacid was reported to reduce the area under the serum concentration time curve of cimetidine (23%), ranitidine (26%), famotidine (19%), and nizatidine (12%).[84] Separating the administration of antacid from that of another drug by 2 hours usually eliminated any interaction.

Dosage and Administration

Because alginate-based raft-forming preparations need to float on the gastric contents for effectiveness, the time at which this medication is taken is of great importance. Optimal benefit is achieved when alginate-based raft-forming preparations are taken following a meal. Under fasting conditions or when taken just prior to or with a meal, alginate-based raft-forming preparations were reported to empty from the stomach with a $T_{\frac{1}{2}}$ of 20 to 30 minutes.[74] When dosed 30 minutes following a meal, alginate-based formulations empty from the stomach with a $T_{\frac{1}{2}}$ of 180 minutes.

PROSTAGLANDIN ANALOGUES

Misoprostol is a prostaglandin E_1 (PGE_1) analogue that is primarily used to prevent nonsteroidal anti-inflammatory drug (NSAID)-induced gastropathy in patients at high risk. Although initial animal studies showed considerable promise in the treatment of acid-peptic disease, studies in human have not confirmed any clear advantage of the PGE_1 analogues over the H_2 blockers, and their clinical efficacy is now attributed primarily to their antisecretory activity. Since the recent advent of cyclooxygenase 2–selective inhibitors, such drugs are now used less frequently in adults.

Pharmacodynamic Properties

PGE_1 analogues bind to E-type receptors on basolateral parietal cell membranes, distinct from those at which the anti–H_2 receptor antagonists act, thereby inhibiting cyclic adenosine monophosphate–mediated acid production. In addition to blocking acid secretion and gastrin production, these agents may directly enhance mucosal protection through various mechanisms (increase of gastric mucus secretion, bicarbonate secretion, mucosal blood flow, and epithelial regeneration).

Pharmacokinetic Properties

At present, pharmacokinetic data for misoprostol derive from studies in animals and healthy adult volunteers only.[85] Following oral administration, it is rapidly absorbed and de-esterified to its acid form, with peak concentration being reached in 30 to 60 minutes. This free acid metabolite remains as potent as the parent drug in inhibiting acid secretion. It is 85% protein bound. Binding is not affected by age or other drugs. Further metabolism probably takes place in the liver and the kidneys. Biphasic elimination occurs, with a terminal half-life of about 1.5 hours. By 8 hours, 90% of a single oral dose is excreted, mostly in the urine. No parent drug is recovered.[86]

Clinical Efficacy

In adults, studies comparing omeprazole with misoprostol or ranitidine for NSAID ulcer prevention in true NSAID ulcers have shown that omeprazole is equal to full-dose misoprostol for ulcer healing and to the lowest useful dose of misoprostol for ulcer prevention.[87] Misoprostol (at dosages ranging from 400 to 800 µg/d in adults) is an effective form of therapy for preventing NSAID-induced gastroduodenal lesions. However, high-dose misoprostol seems adequate only for the prevention of ulcer complications, mainly in high-risk NSAID users.[88] An analysis of pooled data from comparative studies on omeprazole versus ranitidine, misoprostol, and sucralfate shows a therapeutic advantage in favor of the PPI, ranging from 10 to 40%.[88] In long-term prevention studies, omeprazole (20 mg/d) and pantoprazole (40 mg/d) have also been shown to reduce the risk of gastric and duodenal ulcers and NSAID-related dyspepsia. Current data from recent comparative studies of omeprazole (20 mg/d) versus ranitidine (150 mg/d) and misoprostol (200 µg/d) showed that after 6 months of follow-up, the PPI was significantly superior to control drugs in reducing the risk of both gastric and duodenal ulcers. Thus, available data are undoubtedly in favor of the PPIs as well-tolerated and effective drugs in the prophylaxis and treatment of NSAID-related mucosal lesions in the gastrointestinal tract.[88] Experience in children is much more limited and was mainly focused on children with rheumatologic disease.[89–91] Misoprostol appears to be effective in the treatment of gastrointestinal symptoms in children receiving NSAIDs and to result in a significant increase in the hemoglobin concentration.[90,91] Misoprostol was also shown to improve fat absorption in cystic fibrosis patients with pancreatic insufficiency with residual malabsorption on standard enzyme therapy.[92]

Tolerability

In children, as in adults, misoprostol is usually well tolerated. The side effects are limited and mild. The most frequent adverse event is self-limited diarrhea,[89] and a significant elevation in the eosinophil count has been occasionally reported.[92] Prenatal exposure to misoprostol has been associated with Möbius disease and limb defects. Vascular disruption has been proposed as the mechanism for these teratogenic effects.[93] Apart from a possible interaction with propanolol, no major drug interactions have been reported.[94]

Dosage and Administration

The recommended dosage in adults is 100 µg four times a day for prevention and 200 µg orally four times a day for treatment of NSAID digestive lesions. In children, a mean dosage of 300 $\mu g/m^2/d$ has been proposed.[89]

COATING AGENT

The coating agent sucralfate is a basic aluminum salt of sucrose octasulfate. At an acid pH, it polymerizes to form

a white paste-like substance that adheres selectively to ulcer or erosions via an electrostatic attraction between the negatively charged sucralfate polyanions and the positively charged protein moieties exposed by the inflamed mucosa. At these specific sites, sucralfate acts as a protective barrier by slowing back-diffusion of acid, pepsin, and bile salts. It also directly inhibits the binding of pepsin to ulcer protein and, like cholestyramine, adsorbs free bile salts. Gastric pH does not appear to affect sucralfate binding to the ulcer bed. Other important effects of sucralfate include increased bicarbonate and mucus production, enhanced epithelial cell renewal, and restoration of a normal transmucosal potential difference. Sucralfate also protects the gastric mucosa against damage induced by ethanol, bile acids, and NSAIDs and prevents stress ulceration in critically ill patients. Despite its aluminum hydroxide components, sucralfate does not increase gastric pH or act as an antacid at the usual therapeutic doses. The drug has no apparent effects on gastric acid secretion, gastrin release, or upper gastrointestinal motility. Thus, hypochlorhydria and concomitant bacterial overgrowth do not occur.

Pharmacodynamic Properties

Little, if any, sucralfate is absorbed after oral administration owing to its high polarity and poor solubility. It is absorbed as an aluminum base and sucrose octasulfate, with the latter excreted unchanged in urine because it cannot be metabolized.

Pharmacokinetic Properties

A study of the interaction of gastrointestinal agents in the presence of sucralfate has shown the adsorption of musculotropic and cholinolytic spasmolytic drugs on sucralfate.[95] Similarly, the absorption of certain drugs, such as warfarin, digoxin, and phenytoin, may be decreased when sucralfate is given concurrently. Although aluminum absorption is not significantly increased in patients with normal renal function, it has been shown that the use of sucralfate for stress ulcer prophylaxis in patients requiring hemofiltration results in toxic elevations in plasma aluminum levels.[96] Alternative agents should be considered for prophylaxis in these patients.

Clinical Efficacy

Several studies in adults have shown that sucralfate (1 g orally before meals and at bedtime) is significantly better than placebo and equivalent to cimetidine and ranitidine in the healing of duodenal and gastric ulcers. Maintenance therapy with sucralfate decreases the recurrence rate of both gastric and duodenal ulcers. Sucralfate may also protect the patient from NSAID-induced gastroduodenal lesions. An analysis of pooled data from comparative studies on omeprazole versus ranitidine, misoprostol, and sucralfate shows a therapeutic advantage in favor of the PPI, ranging from 10 to 40% for preventing NSAID-induced gastroduodenal lesions.[88] Sucralfate is also superior to placebo and is as effective as cimetidine in the treatment of reflux esophagitis.[97] Sucralfate can prevent stress ulceration in critically ill patients. Recent data have also shown clinical efficacy of sucralfate topically to treat or prevent mucosal lesions from various origins (ie, stomatitis, mucositis, or pouchitis).[98,99]

Tolerability

Sucralfate is relatively free of side effects, the only major one being constipation, which occurs in about 2 to 3% of patients. Nausea and headaches occur much less frequently. Sucralfate causes bezoars, especially when given to patients in intensive care units (especially in premature and neonates),[100] and diarrhea.[101] Patients with renal failure treated with sucralfate are exposed to aluminum toxicity.[102] Aluminum accumulation has been observed in critically ill children with acute renal failure.[103]

The presence of aluminum and magnesium in sucralfate means that this drug has the potential to chelate drugs in the upper gastrointestinal tract, as for other antacids (see above).[71] Hypophosphatemia may also result from sucralfate's action as a phosphate binder.

The influence of sucralfate on the incidence of ventilator-associated pneumonia and the incidence of upper airway colonization in critically ill children remains poorly studied in childhood.[104]

Dosage and Administration

Sucralfate is available as tablets or suspension, both of which have better acceptance and compliance in children. To minimize the risk of decreased absorption of other drugs given concurrently, it is recommended to take sucralfate at least 2 hours apart from the other drugs.

BISMUTH

Bismuth compounds have been used for centuries to treat various diseases, from digestive diseases (gastric and duodenal ulcer, dyspepsia, infectious diarrhea) to infectious (scabies, malaria, syphilis), skin (eczema, pemphigus), and general disorders (lupus erythematosus, hypertension, edema).[105] Use of bismuth progressively declined in the last 30 to 40 years because its mode of action remained unknown and therapeutic benefits were unproved. Moreover, reports of encephalopathy associated with ingestion of various bismuth salts in large quantities over long periods in France and Australia in the early 1970s contributed to the dramatic reduction of its use and led to the interdiction of use both in adults and children in several countries by the appropriate national drug authorities. In the last decade, a renaissance of the use of some bismuth compounds has taken place. Bismuth subsalicylate and colloidal bismuth subcitrate have been demonstrated to effectively treat traveler's diarrhea[106–108] and *H. pylori* infection in association with antibiotics and/or antisecretory drugs.[39,109,110]

Pharmacodynamic Properties

Bismuth acts to coat areas of mucosal ulceration and inflammation, thereby preventing further epithelial injury by luminal acid and pepsin. Bismuth preparations have also demonstrated inhibitory effects on the growth of *H. pylori* in vitro and in vivo.[105]

Pharmacokinetic Properties

Taken orally, bismuth salts are essentially insoluble in water. Bismuth salts are nearly completely hydrolyzed by gastric hydrochloric acid to form precipitate, namely Bi_2O_3 (oxide), $Bi(OH_3)$ (hydroxide), and BiOCl (hydroxychloride). Small quantities of the ingested bismuth compounds remain nondissociated and enter the small intestine, where reactions with other anions take place to form bismuth subcarbonate and bismuth phosphate salts, which, like their parent compounds, are highly insoluble.[105] Delivered to the colon, the still nondissociated part of the ingested bismuth salts and the generated bismuth compounds react with hydrogen sulfide, which is also insoluble in water. Its black color is responsible for the darkening of the stool that occurs during bismuth salt medication. Because the ingested bismuth salts and the bismuth compounds formed in the gastrointestinal tract are highly insoluble in water, less than 1% of the bismuth is absorbed by the small intestine. The exact site and mechanisms by which bismuth is absorbed by the small intestinal mucosa are not known.[111] Some substances, such as the sulfhydryl group–containing compounds sorbitol and lactic acid, are able to enhance bismuth absorption. Ranitidine may enhance bismuth absorption from colloidal bismuth subcitrate by reducing gastric acidity, maintaining colloidal bismuth subcitrate as a soluble colloid.[112] Enhanced bismuth absorption has been reported in patients with colitis or other mucosal abnormalities.[113] Once absorbed, bismuth is distributed to different organs (kidney, lung, spleen, liver, brain, and muscle). Bismuth is eliminated from the body by two routes: two-thirds in the urine and one-third in the feces.

Clinical Efficacy

Several studies have recently highlighted the therapeutic effects of bismuth salts on infectious diarrhea,[106–108,114] microscopic colitis, and pouchitis. Both bismuth subsalicylate and colloidal bismuth subcitrate have been shown to heal duodenal and gastric ulcers, but the relapse rate when these therapeutic agents are used alone is unacceptably high. In association with antibiotics, bismuth salts have been shown to eradicate *H. pylori* infection, and the relapse rate of primary ulcer is reduced when *H. pylori* is eradicated.[39,109,110] However, no controlled studies in children comparing bismuth association with other therapeutic regimens in *H. pylori* infection or peptic ulcer disease are yet available. Ranitidine bismuth citrate contains 128 mg of bismuth, 110 mg of citrate, and 162 mg of ranitidine in each tablet. The recommended dosage in adults is two tablets per day, in association with two antibiotics (amoxicillin and tinidazole or metronidazole). Experience in children remains very limited.[109]

Tolerability

Bismuth encephalopathy has been reported in adults receiving large quantities of bismuth (most of the time as automedication) for months, even years, mainly with bismuth subgallate. All of these patients had high bismuth concentration in serum, but no relationship could be demonstrated between elevated blood concentrations and the severity of encephalopathy.[115] Individual susceptibility factors have been suggested, a hypothesis being that bacterial overgrowth proved or suspected in most of the cases reported could lead to conversion of bismuth salts to neurotoxic substances in the colon.[105] Observing that neurologic and psychiatric symptoms were described only in patients taking more than 1.5 g of bismuth metal per day, Lechat and Kisch arbitrarily stated that a dose less than 1.5 g of bismuth metal is safe in adults.[116] In the same way, comparing the bismuth blood concentration of asymptomatic adult patients on bismuth therapy with that of patients with bismuth encephalopathy, Hillemand and colleagues concluded that a bismuth blood concentration below 50 µg/L should be regarded as a safe level.[117] Such studies are completely lacking in childhood. Frequent side effects are dark stools, blackening of the tongue, and diarrhea.[109]

In children, rare cases of acute renal failure after overdose have been reported.[118,119]

Dosage and Administration

Most of the national drug agencies in Europe and other developed countries prohibit the use of bismuth salts in children. Colloidal bismuth subcitrate and bismuth subsalicylate are, however, the most popular forms of bismuth used in children. A liquid 1.75% preparation of bismuth subsalicylate is usually prescribed at a dosage of 30 mL taken orally four times daily for a period of 6 weeks.[120] Colloidal bismuth subcitrate tablets are given at a dosage of 480 mg of Bi_2O_3/1.73 m^2 of body surface three to four times daily for 4 to 6 weeks.[110]

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6. *Alternative Medical Treatment*

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The term complementary and alternative medicine (CAM) is commonly used to encompass approaches to health care not included in our understanding of "conventional" medicine. There is no sharp demarcation between the two, but perhaps it is easier to define CAM if we define what falls within the domain of conventional medicine. "Western," "mainstream," "orthodox," and "allopathic" medicine are all terms synonymous with conventional medicine. Interestingly, the boundaries of conventional medicine may vary greatly between countries, cultures, classes, and individuals. These boundaries also change with time because many alternative therapies are gradually adopted by conventional practitioners. Probably the best example of such change is the use of probiotics. These have become much more common in conventional medicine and are now considered much less of an "alternative" therapy. This ever-changing interface makes it all the more important for practitioners of conventional medicine to be alert to practices that may, in time, start appearing among their own therapies. Needless to say, there is an almost insatiable demand for therapies that lie just outside the domain of conventional medicine. Patients may place enormous, and frequently inappropriate, faith in these alternative therapies, which, by their very nature, may inspire hope beyond the rational expectations one might have of any conventional therapy.

Perhaps the most comprehensive definition of CAM was first coined by O'Connor, and it has since been adopted both by the National Centre for Complementary and Alternative Medicine (NCCAM) in the United States and the Cochrane database in the United Kingdom[1]:

> Complementary and Alternative Medicine (CAM) is a broad domain of healing resources that encompasses all health systems, modalities, and practices and their accompanying theories and beliefs, other than those intrinsic to the politically dominant health system of a particular society or culture in a given historical period. CAM includes all such practices and ideas self defined by their users as preventing or treating illness or promoting health and well-being. Boundaries within CAM and between the CAM domain and that of the dominant system are not always sharp or fixed.[1]

More recently, the term "integrative" medicine has been used. This style of practice combines the use of mainstream medical therapies with those CAM therapies for which there is high-quality scientific evidence on safety and efficacy. The focus is therefore more on the quality of available evidence for a particular therapy rather than on the type of therapy or the practitioner prescribing it.

Over the last decade, use of CAMs has increased worldwide. In 1990, the US population was making more visits to practitioners of alternative medicine than to their primary care physicians. By 1997, out of pocket expenditure for alternative medicines in the United States had increased by over 45% to about 27 billion dollars, similar to out of pocket expenditure for all US physician services.[2]

Whereas several studies have now addressed the use of CAM in adult patients, few have done so in children. Spigelblatt and colleagues showed that 11% of over 1,000 Canadian children attending routine outpatient clinics were using CAM, with chiropractic, homeopathy, naturopathy, and acupuncture accounting for more than 80% of use.[3] In this study, children who used CAM were older than nonusers, had better educated mothers, and tended to have parents who also used CAM. Almost 90% of parents opted to use conventional medicines first, suggesting that CAM be used in conjunction with it. In a review of Australian children with asthma aged 1 to 16 years, 55% used an additional alternative therapy, whereas 46% of those with childhood-onset malignancies used at least one form of CAM.[4,5]

For the purposes of this chapter, we are not including pre- and probiotic use in our definition of CAM. Both entities have, over the last few years, moved squarely into conventional gastroenterologic practice. However, in some fields, such as constipation, they are still felt to be complementary therapies, and, as such, we briefly discuss them. The maintenance of a normal healthy gut flora has become central to the management of many gastrointestinal pathologies, and the manipulation of gut flora with pre- and probiotics is dealt with elsewhere in the text in much greater detail.

USE OF CAM IN GASTROINTESTINAL DISORDERS

In this chapter, we focus on the currently available evidence for CAM use in the areas of inflammatory bowel disease (IBD), constipation, acute gastroenteritis, and liver disease.

Search Strategy

We conducted a thorough search of the literature using the following on-line databases: *MEDLINE*, *PsycINFO*, *Allied and Complementary Medicine*, *Psychological Abstracts*, *CINAHL*, and the *Cochrane Database of Systematic Reviews*. The MeSH terms "homeopathy," "'naturopathy," "comple-

mentary therapies," "alternative therapies," "acupuncture," "acupuncture therapy," "'spiritual therapies," "'music therapy," "laughter therapy," "aromatherapy," "medicine, herbal," "'medicine, chinese traditional," "drugs, chinese herbal," "medicine, ayurvedic," "massage," and "phytotherapy" were used in a *MEDLINE* search, in conjunction with the term "gastrointestinal diseases." Almost 2,500 references were found, with 203 being clinical trials published in English with an available abstract. All of the latter were reviewed to select 58 articles that were felt to be relevant to this publication. These references were then reviewed in full where possible. Additional references were identified by manual searching of bibliographies from recent review articles, and further citations were received from experts in the field.

Inflammatory Bowel Disease

There is considerable evidence of CAM use in patients with IBD. However, there are very few studies documenting the efficacy in different disease types. There are no studies of efficacy in children with IBD, yet there is some preliminary information on the prevalence of its use in this population.

Heuschkel and colleagues carried out a survey of CAM use in children and young adults with IBD in three centers of pediatric gastroenterology (Boston, Detroit, and London, UK)[6]; 208 questionnaires were completed, and the frequency of CAM use in this population was 41%. The most common therapies were megavitamin therapy (19%), dietary supplements (17%), and herbal medicines (14%). Parental CAM use and the number of adverse effects from conventional medicines were predictors of CAM use (odds ratio [OR] = 1.9, 95% confidence interval [CI] = 1.2–3.1, $p = .02$; OR = 1.3, 95% CI = 1.2–1.5, $p < .001$, respectively). The most important reasons respondents gave for using CAM were side effects from prescribed medicines, prescribed medicines not working as well as they had hoped, and hoping for a cure. Almost 60% of respondents not taking CAM were interested in learning more about it. These results are broadly similar to those found in adults with IBD, in which 51% of patients reported some CAM use in the previous 2 years.[7]

Verhoef and colleagues recently explored how often CAM providers see patients with IBD and how ready they were to recommend therapies.[8] The group interviewed 66 chiropractors, 19 pharmacists, 16 herbalists, and 15 health food store employees in Calgary, Alberta. Most respondents had seen patients with IBD, and over 80% of each group, except pharmacists (only 10%), would treat these patients or recommend a therapy. Almost all chiropractors used spinal manipulation, whereas other practitioners recommended a wide variety of interventions. Most of the respondents viewed their own recommendations as moderate to very effective. It is clear from these types of data that a significant amount of information is being given to adults (and probably children) with IBD outside the physician-patient consultation.[9] Much of this information is also not discussed with the practitioners prescribing conventional therapies, potentially leading to significant drug interactions and to issues around long-term compliance with orthodox therapies.

Very few rigorous human intervention studies have been carried out with CAM, and almost all of these have been done with adult patients. There is also some limited laboratory and animal evidence accumulating, providing initial insights into the possible mechanisms underlying specific therapies.

Recently, Langmead and colleagues reviewed the in vitro antioxidant effects of six herbal remedies used by patients with IBD; these included slippery elm (derived from the bark of the slippery elm tree), fenugreek (an ayurvedic therapy), Mexican yam (a tropical staple), devil's claw (the root of an African flower), tormentil (a European flower), and Wei Tong Ning (a traditional Chinese herbal therapy).[10] All of these herbs are likely to contain numerous antioxidant compounds. Orange juice was used as a phytic control and 5-acetylsalicylic acid (5-ASA) as a positive control. Like 5-ASA, slippery elm, devil's claw, tormentil, and Wei Tong Ning all had dose-dependent antioxidant properties, whereas all of the herbs and 5-ASA also had dose-dependent peroxyl-radical scavenging effects. Detection of reactive oxygen metabolites was significantly reduced if inflamed mucosal biopsies were incubated with 1 in 100 dilutions of all herbs except Mexican yam and orange juice. This in vitro evidence provides intriguing evidence that substances present in certain diets or used as alternative therapies may have direct anti-inflammatory effects on an inflamed mucosa.

There is some work in animals focusing on individual herbs and plant compounds, assessing their efficacy in different mouse models of colitis. Among others, *Cordia myxa* fruit, polygalae root, and tryptanthrin all appear to have anti-inflammatory effects, with down-regulation of interferon-γ occurring in several disease models.[11–13]

There is further evidence from animal work that polyphenols in green tea, already thought to have some preventive role in carcinogenesis, may regulate tumor necrosis factor-α (TNF-α) production by their effect on the core molecule nuclear factor-κB.[14,15] A dose of 0.5 g/kg body weight of green tea polyphenols reduced the serum TNF-α concentrations by 80% and prevented induced lethality in mice. A further study has extended these findings, showing that the spontaneous colitis of interleukin-2 knockout mice is ameliorated by regular supplementation with green tea polyphenols over a 6-week period.[16]

Two studies report the efficacy of the Chinese herbal remedies Kui Jie Qing (KJQ) and Yukui Tang in the treatment of active ulcerative colitis (UC).[17] The authors used KJQ enemas for 20 days in 95 patients with UC, comparing the response with that of 11 controls. They showed 95% "effectiveness" compared with 53% using sulfasalazine, prednisolone tablets, and KJQ enemas. In a second study, 118 patients with UC received KJQ orally with herbal decoction enemas and 15 mg oral prednisolone, neomycin sulfate, and sulfasalazine. This group was compared with 86 patients receiving a similar treatment but without KJQ. Again, an "efficacy" rate of 84% was reported in the treated group compared with 60% in controls.

A randomized, double-blind study treated 153 patients with active UC in three different groups. Group 1 received

Jian Pi Ling and a retention enema of *Radix sophorae flavescentis* and Flos Sophora (RSF-FS) per night. Group 2 received sulfasalazine plus a retention enema of dexamethasone, whereas group 3 was randomized to placebo plus the same retention enema (RSF-FS) given to group 1.[18] The remission rates of groups 1 and 3 were 53.1% and 19.0%, respectively, with only 27.7% responding to treatment with conventional medications. The outcome measures in both of these studies were not clearly stated and were all recorded in an unblinded fashion, hence casting some doubt on the dramatic results.

An oral formulation of the Indian gum resin *Boswellia serrata* (900 mg/d) was compared with oral sulfasalazine (3 g/d) in the treatment of moderately active UC. Both treatments were effective in this study, with 14 of 20 of those receiving *B. serrata* achieving a clinical remission at 6 weeks compared with 4 of 10 given sulfasalazine.[19] Boswellic acids are thought to inhibit leukotriene synthesis by noncompetitive inhibition of 5-lipoxygenase, thereby leading to their anti-inflammatory effects on chronic inflammation in UC.[20]

Wheat grass juice has been reported as effective compared with placebo in a small but carefully conducted study on 24 adults with left-sided UC.[21] Patients treated with 100 mL of wheat grass juice for 1 month had significant improvements in rectal bleeding, abdominal pain, and disease activity scores. In addition, there was a trend toward sigmoidoscopic improvement at follow-up. Again, it is the antioxidant and anti-inflammatory properties of components such as the flavonoids, which are known to be active in the arachidonic acid pathway, that are felt to be responsible for the therapeutic effects.

There may be further mechanisms at play in some food derivatives. A pilot study, still awaiting placebo-controlled confirmation, has shown germinated barley foodstuff to be effective in achieving both clinical and endoscopic improvement in adults with treatment-resistant active UC. Much, if not all, of the effect of this treatment may be due to the inherent prebiotic properties, which increase colonic short-chain fatty acid concentrations significantly.[22]

There is an almost complete absence of well-controlled trials assessing the impact of neuromodulation on patients with IBD. Patients and their physicians have long been aware of the association between emotion, stress, and disease activity in IBD. An early randomized controlled trial on the impact of stress management in 80 patients with IBD did suggest a significant reduction in disease-specific activity. There was also a reduction in stress indices in adults who received six classes on stress management.[23]

There are only anecdotal reports of either mind-body influences or stress contributing to the degree of mucosal inflammation in IBD. Whorwell and colleagues did study the effect of three hypnotically induced emotions (excitement, anger, and happiness) on distal colonic motility in 18 adults with irritable bowel syndrome.[24] Anger and excitement increased the colonic motility index, pulse, and respiratory rate, providing some hard, albeit limited, data on this association. Further studies are required to identify suitable patients and provide more convincing evidence on the efficacy of these therapies.

Constipation

There is already a complete dearth of good-quality data on the conventional therapies used in the management of constipation in children, so it will come as no surprise that there are no controlled prospective trials on any complementary therapies and that all available evidence is anecdotal.

Anthraquinones are known to be the active components in senna and aloe (sap of aloe leaves) and have stimulant laxative properties. Their abuse, however, has been associated with an increased risk of colorectal cancer (OR = 3.4).[25] Ispaghula husk and the plant polysaccharide psyllium can be used as effective bulking agents. Odes and Madar compared capsules containing celandine, aloe vera, and psyllium with placebo in a randomized controlled trial of 35 adults over 28 days. The treated group had an increase in bowel movements, softer stools, and less laxative dependence than the placebo group, yet abdominal pain remained unchanged in both groups.[26]

A Thai plant containing anthraquinone, *Cassia alata*, was tested against *Mist. alba* and placebo in a blinded controlled multicenter trial. Eighty adults with a 72-hour history of constipation were randomized into three groups, and each received a single dose of treatment. Over 80% of adults in the *Cassia alata* and *Mist. alba* groups had passed stool within 24 hours compared with 18% in the placebo group.[27]

The centuries-old liquid ayurvedic medicine *Misrakasneham* (containing a mixture of herbs, castor oil, and ghee) was compared with a conventional senna-based laxative in a controlled trial for treatment of opioid-induced constipation in adults with advanced cancer.[28] Fifty patients were randomly allocated to the treatment group, receiving the treatment in incremental doses if necessary over 14 days. After the study period, 69% and 85% of the senna and the ayurvedic therapy groups, respectively, reported satisfactory bowel movements, a difference that was not statistically significant. However, the *Misrakasneham* was well tolerated, quicker acting, and cheaper than the senna.

Brazelli and Griffiths, in a Cochrane review, looked at the effects of behavioral and/or cognitive interventions for the management of defecation disorders in children.[29] Sixteen randomized trials with a total of 843 children met the inclusion criteria. Sample sizes were generally small. Interventions varied among trials, and few outcomes were shared by trials addressing the same comparisons. The synthesis of data from eight trials showed higher rather than lower rates of persisting problems up to 12 months when biofeedback was added to conventional treatment (OR = 1.34, 95% CI = 0.92–1.94). In two trials, significantly more encopretic children receiving behavioral intervention plus laxative therapy improved at both the 6-month (OR = 0.51, 95% CI = 0.29–0.89) and the 12-month follow-up (OR = 0.52, 95% CI = 0.30–0.93) compared with those receiving behavioral intervention alone. Similarly, in another trial, the addition of behavior modifications to laxative therapy was associated with a marked reduction in children's soiling episodes (OR = 0.14, 95% CI = 0.04–0.51). The reviewers concluded that there is no evidence that biofeedback training adds any benefit to conventional treatment in the management of encopre-

sis and constipation in children. However, there is some evidence that behavioral intervention plus laxative therapy, rather than behavioral intervention or laxative therapy alone, improves continence in children with primary and secondary encopresis.

Probiotics have been used for treatment of constipation, but there are no reports in the literature of their use in children. There is one randomized controlled trial reporting their use in the elderly. Ouwehand and colleagues enrolled 28 elderly subjects in an open-label parallel study.[30] In addition to a placebo group, two groups received different strains of probiotic over a 4-week period. The subjects receiving the *Lactobacillus rhamnosus/Propionibacterium freudenreichii*–supplemented juice exhibited a 24% increase in defecation frequency, although there was no overall reduction in laxative use. Trials are now in progress assessing the impact of nonabsorbable prebiotic sugars such as inulin and fructo-oligosaccharides on children with idiopathic constipation.

Abdominal massage has been used for centuries in the treatment of constipation. It has recently regained popularity, and Ernst completed a systematic review of controlled clinical trials of this therapy.[31] He was able to select only four studies from the available literature, yet even these were heterogeneous in trial design, patient sample, and type of massage used. He concluded that massage therapy may be a promising treatment for chronic constipation, yet, inevitably, more rigorous trials should evaluate its true value.

Dolk and colleagues assessed the effect of yoga on the puborectalis muscle in nine patients with severe defecation difficulties secondary to puborectalis dysfunction (puborectalis paradox).[32] Having had electromyography (EMG) of the striated anal sphincter muscles, patients were offered training in yogic techniques of relaxation and muscle control to change the activity of the pelvic floor muscles during attempted defecation. Five patients completed the training program of 20 2-hour sessions and were re-examined clinically and with EMG. One patient regained a normal EMG pattern, but none of the patients improved clinically.

Kesselring and colleagues reported on the role of foot reflexology (FR) on well-being, voiding, bowel movements, pain, and/or sleep in women who underwent an abdominal operation.[33] One hundred and thirty subjects were randomized into three groups. For 5 days, they were exposed to 15 minutes of FR, foot/leg massages (FMs), or interview alone, respectively. The results showed that women in the FR group were more able to void without problems after the indwelling catheter had been removed than were women in the comparison groups. There was also a tendency in the FR group for the indwelling catheter to be removed earlier than in the other groups. In comparison, the FR subjects slept worse than the others. FM showed significant results in subjective measurements of well-being, pain, and sleep.

Liver Disease

Medicinal properties of the milk thistle plant (*Silybum marianum*) have been extensively researched. The active ingredient is silymarin, a mixture of at least four closely related flavonolignans, which appears to be antihepatotoxic owing to the antioxidant- and membrane-stabilizing properties of its components.[34]

The most dramatic reports of its use are in patients poisoned with the deathcap mushroom *Amanita phalloides*. In animal studies, dogs were given lethal doses of *A. phalloides*, 85 mg/kg, and were then randomized to receive either silymarin or placebo 5 to 24 hours after ingestion. None died in the treated group compared with 4 of 12 in the untreated group. This was supported in the treated group by improvement in hepatic enzymes and less necrosis on liver biopsy.[35] There are no randomized trials in humans, but there are various case series and retrospective studies supporting the usefulness of milk thistle in treatment of amanita poisoning.[36]

Liu and colleagues reported the efficacy and safety of Chinese herbal remedies in the treatment of asymptomatic carriers of hepatitis B.[37] Despite some methodologic problems, the "Jianpi Wenshen recipe" appeared to be significantly better than interferon for clearance of serum hepatitis B surface antigen (HBsAg) (relative risk [RR] = 2.40, 95% CI = 1.01–5.72) and hepatitis B e antigen (HBeAg) (RR = 2.03, 95% CI = 0.98–4.20), as well as in the seroconversion of HBeAg to anti-HB_e (RR = 2.54, 95% CI = 1.13–5.70).

In a further Cochrane review of Chinese medicinal herbs in the treatment of chronic hepatitis B infection, of nine randomized trials included in the review, only one was considered to have adequate methodologic quality.[38] Ten different medicinal herbs were tested in these nine trials. Fuzheng Jiedu Tang was significantly better in clearing serum HBsAg, HBeAg, and hepatitis B virus deoxyribonucleic acid (DNA) compared with placebo. In a further randomized trial of 94 patients, kurorinone was found to be as effective as interferon-α in clearing HBeAg and hepatitis B virus DNA over a 12-month follow-up period.[39]

In the treatment of hepatitis C, 4 of 10 trials were considered to be of adequate quality for assessment.[40] When Bing Gan Tang was used in conjunction with interferon-α, it cleared serum hepatitis C virus ribonucleic acid (RNA) (RR = 2.54, 95% CI = 1.43–4.49) with normalization of serum alanine aminotransferase (ALT) (RR = 2.54, 95% CI = 1.43–4.49) better than interferon-α alone. The herbal mixture "Yi Zhu decoction," when compared with glycyrrhizin plus ribavirin, was significantly better at clearing serum hepatitis C virus RNA and normalizing ALT levels. In addition, Yi Er Gan Tang had a significant advantage over silymarin plus glucurolactone in normalizing serum ALT.

Although many of these data are intriguing, the lack of prospective randomized studies seriously limits any widespread clinical application. Caution is also indicated in view of the completely unknown adverse-effect profile of these medications.

Ayurvedic medications have also been used in the treatment of hepatitis. Despite initial hopes that the herb *Phyllanthus amarus* might reduce the carriage of surface antigen in carriers of hepatitis B, other studies have failed to show a similar improvement.[41,42] One study has shown *Phyllanthus urinaria* to be more effective than *P. amarus*.[43]

Acute Gastroenteritis

There is now extensive evidence in the literature about the use of probiotics in the treatment of acute gastroenteritis, especially in children. This is covered in greater detail elsewhere in the text.

Homeopathy is widely used in the treatment of gastroenteritis in children, and Jacobs and colleagues reported two double-blind placebo-controlled randomized trials to assess its efficacy in the treatment of childhood diarrhea.[44] Eighty-one children from Leon, Nicaragua, age 6 months to 5 years of age, received individualized homeopathic medicine daily over a period of 5 days. Standard treatment with oral rehydration treatment was also given. There was a statistically significant decrease in both the duration of diarrhea (less than three unformed stools daily for 2 consecutive days) and in the number of stools per day between the two groups after 72 hours of treatment. The same study was then repeated in 126 children from Kathmandu, Nepal.[45] This study revealed similar results, and a Kaplan-Meier survival analysis of the duration of diarrhea showed an 18.4% greater probability that a child would be free of diarrhea by day 5 with homeopathic treatment ($p = .036$).

Izadnia and colleagues reported the effective use of brewer's yeast for treatment of *Clostridium difficile* gastroenteritis in rats, with both brewer's yeast and *Saccharomyces boulardii* attenuating *C. difficile*–induced colonic secretion in the rat.[46] Hyperimmune cow's colostrum has also been used with some success in randomized trials of *Rotavirus* diarrhea in children.[47] However, bovine immunoglobulins are more easily digested by human trypsin and chymotrypsin and should thus have little or no effect on small intestinal function.[48]

Loeb and colleagues have shown, again in a randomized trial, that a tannin-rich carob pod, together with oral rehydration solution, led to a significantly quicker resolution of viral or bacterial diarrhea compared with oral rehydration solution alone.[49]

A combination of berberine and tetracycline was shown to significantly decrease the volume and frequency of diarrheal stools, the duration of diarrhea, and the requirement for intravenous and oral rehydration fluid in adults with cholera. However, neither berberine nor tetracycline was found to be better than placebo in treating noncholera diarrhea.[50]

Although not strictly used in acute infective gastroenteritis, psyllium was successful in reducing symptoms in 20 of 23 children with chronic nonspecific diarrhea.[51] Wenzl and colleagues showed that stool looseness is determined by the ratio of water to insoluble solids in the stools; therefore, diarrhea can also be the result of low output of insoluble solids, and psyllium may act by increasing these insoluble solids.[52]

ADVERSE EFFECTS

It is a popular belief that CAM therapies are natural products and therefore safe. Although this may apply to many CAM therapies, the recent explosion in CAM use has been paralleled by an increasing number of serious adverse events reported in the world literature. In the absence of any large prospective studies that systematically collect and report safety data, the vast majority of adverse events are reported as case reports, therefore clearly providing a gross underestimate of the actual incidence of adverse events related to CAM use.

Bensoussan and colleagues conducted a survey of reported adverse events in the use of traditional Chinese medicine in Australia.[53] Practitioners did report some adverse events with use of acupuncture (fainting, nausea and vomiting, and increased pain) and consumption of Chinese herbal medicines. However, these side effects were very uncommon when compared with those of practices in conventional medicine. An average of one adverse event was reported for 8 to 9 months of full-time practice (ie, 1 adverse event in 633 consultations). This type of study is clearly flawed in many ways, with significant bias again likely to significantly underestimate the incidence of adverse events. There is thus little reassurance both for practitioners of conventional medicine and for the consumers of CAM therapies that these therapies are as free from hazard as they are often purported to be.

Although most herbal remedies used in children at home appear to be safe, allergic reactions and cases of anaphylaxis have been reported.[54] The most frequently reported adverse effects include diarrhea, nausea, and vomiting, and although many herbs preferentially affect the digestive tract, most of their adverse effects are self-limiting.[55]

Adverse effects on liver function may be more serious, with hepatotoxicity secondary to ingestion of Chinese herbs being well described (Table 76.6-1). In Africa and Central America, toxicity can be endemic in areas where particular plants are consumed on a daily basis. Liver transplant has been necessary in cases of fulminant hepatic failure secondary to herbal remedies.[56]

The potential toxicity of pyrrolizidine alkaloids in herbal teas was recognized over 40 years ago. These can result in centrilobular necrosis, portal hypertension, veno-occlusive disease, and an increased risk of hepatocellular carcinoma.[57] In cases of suspected poisoning, it is important that some of the ingested product is retrieved and analyzed. It may be useful to save blood and urine for later analysis. Once the offending agent is identified and further intake is prevented, treatment of these cases is mostly supportive. Suspected poisoning should always be considered in patients presenting with unexplained hepatitis, although the offending constituent is identified in a minority of cases only.

In view of the widespread and self-directed use of herbal preparations, consumers and clinicians alike need to be increasingly aware of these potentially hepatotoxic products.

McGuire and colleagues reported a case of fatal hypermagnesemia secondary to the cardiac complications of high doses of magnesium oxide.[58] This had been given to a child with mental retardation, spastic quadriplegia, and seizures as part of a regimen of megavitamin and megamineral therapy and without the knowledge of the patient's physician.

Homeopathic medicines are generally considered to be nontoxic owing to the degree of dilution these medicines

TABLE 76.6-1 LIST OF HERBAL TREATMENTS WITH ADVERSE EFFECTS ON THE LIVER

BOTANICAL NAME	COMMON NAME	USE	SIDE EFFECTS	REFERENCE
Teucrium chamaedrys	Germander	Antipyretic, obesity	Hepatitis	69
Lycopodium serratum	Jin Bu Huan	Analgesic	Hepatitis	70
Cassia augustifolia	Senna	Laxative	Hepatitis	71
Atractylis gummifera	White chameleon	Antipyretic purgative	Hepatitis	72
Larrea trientata	Chapparal	Anti-inflammatory	Cholestatic hepatitis	73
Senecio crotolaria	Herbal tea	Tonic	Veno-occlusive disease	74

undergo. Massage therapies are also generally considered to be safe, although a hepatic hematoma has been reported after deep abdominal massage.[59]

In 1986, Stryker and colleagues reported six cases of acute hepatitis B after receiving acupuncture treatment in a chiropractic clinic over a period of 6 months.[60] In these cases, acupuncture needles were reused after overnight sterilization using 1:750 solution of benzalkonium chloride. Practitioners now use disposable needles to eliminate this risk.

Dunbabin and colleagues reported a case of lead poisoning in a 37-year-old man after the intake of some Indian ayurvedic medications. The tablets contained a very high concentration of lead, and the patient required chelation therapy.[61]

The majority of complementary therapies are taken as adjuncts to conventional therapies. As a result of this, and the fact that very few patients inform their physicians of concomitant use, there is now an increasing list of known interactions between certain remedies and commonly prescribed medications in pediatric gastroenterology (Table 76.6-2).

Plants undergo several processes before manufacturing of the final product. During this period, they are at risk of adulteration, deterioration, and contamination. Adulteration is more likely to occur with certain herbs, which are expensive and in short supply. Many countries of origin do not have a well-enforced code of good manufacturing practice, and remedies may be adulterated, with steroid and nonsteroidal anti-inflammatory drugs being the most commonly detected.

Plants may be misidentified at the time of picking, their true names misspelled or even mistranscribed during export. Proper identification of herbs is, of course, particularly important, particularly for the accurate reporting of adverse effects.[62]

The final concentration and quality of the active ingredient are determined by many different factors, not least of which are the local environmental conditions (availability of water, sunshine, and rainfall in some areas). Stringent collection and packaging measures with batch to batch analysis of the active ingredient in the final product are important.

Ginenoside is the biologically active glycosylated steroid in ginseng, which is commonly used and prescribed. It was examined in 50 commercial brands sold in 11 countries. In 44 of these products, the concentration of ginenoside ranged from 1.9 to 9% w/w; six products contained no ginenoside, with one of these six containing large amounts of ephedrine.[63] A series of five adults developed heavy metal poisoning in the United Kingdom from Indian ethnic remedies; the products used had lead concentrations varying from 6 to 60% w/w.[64]

In addition to the contamination of products, ingredients may be substituted without adequate research into their safety or efficacy. *Stephania tetrandra* was an active ingredient in a Belgian slimming treatment. This was, however, replaced by *Aristolochia fangchi* without a change in the brand name, and its use resulted in nine cases of rapidly progressive interstitial nephritis in young

TABLE 76.6-2 INTERACTIONS BETWEEN CONVENTIONAL DRUGS USED IN PEDIATRIC GASTROENTEROLOGY AND HERBAL REMEDIES

HERB	CONVENTIONAL DRUG	CONSEQUENCE/EFFECT ON CONVENTIONAL THERAPY
Licorice	Spironolactone, prednisolone	Antagonistic effect; hypokalemia; increased salt and water retention
Echinacea	Anabolic steroids, methotrexate, immunosuppressants	Hepatotoxicity; theoretically may interfere because echinacea is an immune stimulant
St. John's wort	Cyclosporine	Reduced blood levels
Feverfew, garlic, ginkgo, ginger, and ginseng	Warfarin	Increased effect
St. John's wort, saw palmetto (containing tannic acid)	Iron	Inhibits absorption
Ephedra	Corticosteroids	Increased excretion
Xaio chai hu tang (sho-saiko-to)	Corticosteroids	Reduced blood levels

Adapted from Zou L et al,[75] Miller,[76] and Fugh-Berman A.[77]
Known herbal treatments that are cytochrome P-450 inhibitors: (1) ginkgo biloba (ginkgolic acids I and II); (2) kava (desmethoxyyangonin, dihydromethysticin, and methysticin); (3) garlic (allicin); (4) evening primrose oil (*cis*-linoleic acid); (5) St. John's wort (hyperforin and quercetin).

women. Laboratory analysis revealed it to contain a nephrotoxic component, aristolochic acid. To date, 80 cases have been identified. More than half of these patients have developed renal failure.[65]

Although the production of many CAM therapies is now more closely scrutinized, their final distribution can lead to further misinformation and subsequent confusion for the consumer. With the explosion of information sources on the Internet, it has become impossible to control or regulate many of the claims being made about remedies that are now sourced from around the world. Ernst and Schmidt investigated Internet advice offered by "medical herbalists" to a pregnant woman for the herbal treatment of morning sickness.[66] Search engines were used to find relevant Web sites, and all potential electronic mail addresses were contacted. Herbalists gave a wide range of differing advice, with less than one in three cautioning potential consumers about adverse effects.

Many complementary therapies, particularly those involving the administration of products encompassed by the term "dietary supplement," can continue to avoid the costly validation and safety studies that are required of conventional pharmaceutical products by relying on legislation passed in the United States in 1994 (Dietary Supplement Health and Education Act, 1994). Although this loophole exists, it is unlikely that manufacturers of dietary supplements will embrace the high cost of the formal drug trials and the research and development that are required of their colleagues in the pharmaceutical industry.

THE FUTURE OF CAM

LEGISLATION

United States. CAM use has been increasing rapidly in the United States. The majority of expenditure for these therapies is made by individual third-party payers. This fact and the lack of evidence-based practice prompted Congress to create the NCCAM at the National Institutes of Health. NCCAM's mission is "to explore complementary and alternative healing practices in the context of rigorous science; to educate and train CAM researchers and to disseminate authoritative information to the public and professionals."[67] Although the budget for NCCAM has increased to 70 million dollars in the year 2000, it is still insufficient for exhaustive studies in all complementary therapies. Currently, scientific research is broadly aimed at publicly relevant therapies. These include, among others, characterizing the active components of cranberry that may help prevent urinary tract infections, finding out more about meditation through the use of functional magnetic resonance imaging, investigating the biologic and chemical activity of red clover for use in women's cardiovascular health, and studying the effects of ginseng in an animal model of diabetes.

United Kingdom. In contrast to the United States and most European countries, there are almost no regulations governing the work of CAM practitioners in the United Kingdom. In 1997 and 2000, the Centre for Complementary Health Studies (UK) surveyed over 50,000 practitioners from 140 professional health bodies. There was a great variation in practicing standards. Homeopathic, osteopathic, and chiropractic practice have their own self-regulated professional bodies, and there has now been a move in the House of Lords that training courses for different CAM practitioners should become standardized and implemented by their respective professional bodies. There has also been a move to establish a body similar to the NCCAM to sponsor well-funded good-quality research into complementary therapies.

RESEARCH INTO CAM

Research in CAM generally suffers from different difficulties than does research into conventional medicines. Whereas in conventional medicine, direct comparison of the efficacy of two drugs is frequently possible, this is often not feasible with CAM therapies. Therapies in CAM may be based on whole systems; for example, ayurvedic medical therapy may include a combination of oral medicines, massage, and yoga exercises. This makes comparative studies very difficult because only certain aspects of a therapeutic approach are generally tested at any one time against a certain conventional medicine. Randomization leads to the paradox that much of CAM therapy relies on patient choice and individually tailored treatment programs, thereby making larger randomized studies extremely difficult to complete. Furthermore, diagnoses in CAM frequently rely on reported symptoms rather than on the pathogenesis of an underlying disease. Thus, two patients with the same disease process but differing symptoms might receive entirely different complementary therapies. Nonetheless, studies must continue to be carefully designed and carried out to overcome the above problems, thus ensuring that the best possible evidence informs our clinical decisions.

CONCLUSIONS

A Cochrane analysis of the literature for articles published on CAM from 1966 through 1996 shows that the total number of articles listed in *MEDLINE* rose to a peak of 400,000 additions by 1996, with only 1,500 per annum being indexed under alternative medicine.[68] Before 1986, only 2% of the latter were clinical trials in alternative medicine, with an increase to 10% by 1996. With the worldwide growth in interest, it is imperative that research on complementary medicines should be conducted using stringent criteria, as used in research on conventional therapies. Only in this way will it be possible to fully exploit the wealth of products and therapies at our disposal. The trend toward a more evidence-based approach in this discipline is vital if the move toward a more integrated type of health care provision is to occur.

It is now clear that clinicians can no longer ignore the potential benefits and occasional risks of CAM, making it necessary for us to familiarize ourselves with all of the therapies that our patients may be taking.

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7. Adherence to Medical Regimens

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Nonadherence to (noncompliance with) medical recommendations is a leading cause of morbidity and mortality in a wide array of disease processes and in all age groups.[1–8] These diseases include disorders of the gastrointestinal tract.[9–13] Nonadherence is specifically thought to be prevalent during the adolescent years.[1,10,11,14] Hence, it is frequently encountered by pediatricians. Adherence is especially important in patients who have a chronic medical illness, who need to adhere to medications and dietary regimens over a long period of time, and whose health outcome is closely related to following the recommendations.

Extant data strongly suggest that nonadherence is a significant cause of morbidity and mortality in several patient groups, such as post-transplant patients.[2,3,15–18] Yet the assessment and treatment of nonadherence are rarely approached in a systematic way in clinical practice.

This chapter reviews the definition and characteristics of nonadherence, its prevalence, ways to assess it, developmental considerations in adherence behavior, risk factors that predispose a patient to become nonadherent, and methods that are thought to improve adherence. Most of the discussion is not disease specific. However, specific examples, or a concise summary of issues that are unique to children who suffer from specific illnesses of the gastrointestinal tract, are included when relevant.

Nonadherence is a leading reason for poor outcome in a variety of disease processes. It can be improved in many cases. It is important to study it and to incorporate adherence assessments and interventions into routine practice. Very little has been done to date to address this important phenomenon in a concerted fashion. This chapter is intended to increase awareness of nonadherence as a mediator of outcome and to provide a framework for a rational approach to its assessment and treatment.

DEFINITION

Nonadherence to medical recommendations happens when a patient does not follow the recommendations that are given by physicians, nurses, or other medical professionals. Nonadherence could be related to a wide array of recommendations. These could be, for example, dietary recommendations, scheduling recommendations (clinic visits, imaging tests), and recommendations to take medications. Adherence to one type of recommendation (ie, coming to clinical visits as scheduled) does not necessarily imply adherence to another type of recommendation (ie, taking the medications as prescribed[19]). The treatment of gastrointestinal illnesses, such as celiac disease, frequently includes a dietary modification, which is a lifestyle change. Adherence to such lifestyle changes may be quite different from adherence to medications, which does not require a major change in lifestyle and habits. Even within one general type of recommendation, adherence may vary (ie, a patient may adhere to an immunosuppressant regimen but not to a prescribed vitamin pill, even though both are prescribed by the same clinician). Hence, adherence should be defined narrowly, in relation to a specific recommendation. I decided to focus the discussion in this chapter primarily on nonadherence to medications. This is done for clarity and also because more data are available on this particular type of adherence.

A strict definition of nonadherence is of very limited use in clinical practice. This is because it is quite likely that every once in a while, a patient who suffers from a chronic medical condition will not take the medications exactly as prescribed, and most clinicians will not call this behavior "nonadherence." The following example illustrates this principle: a patient who had a liver transplant has been prescribed tacrolimus every 12 hours. This patient occasionally (ie, every weekend) takes the medications on a slightly different schedule (ie, 8 am and 10 pm instead of 7 am and 7 pm). Most clinicians will not consider this slight variation to be nonadherence, and most would probably not think that this patient should be treated for it. Yet, by a strict definition, during the weekend this patient is nonadherent to the recommendation that the medication should be taken every 12 hours.

Hence, there should be a defined threshold beneath which a nonadherent behavior is acceptable. This threshold has not been prospectively determined in any gastrointestinal disease process. It is possible that certain illnesses require different levels of adherence. For example, a patient who is suffering from autoimmune hepatitis and has been transplanted will be expected to adhere to an immunosuppressant regimen more closely than a patient who had extrahepatic biliary atresia and was transplanted as well. This is because the disease process could recur in autoimmune hepatitis but not in extrahepatic biliary atresia, and strict adherence is mandatory to avert that possibility in the first, but not the second, patient.[18]

One way in which an adherence threshold level can be determined (ie, "it is okay for this patient to forget to take the medication once a week but not twice a week") is by determining at what point medical adverse events are likely. The danger of waiting for the actual occurrence of the adverse event to make this determination is obvious. Rather than waiting until some medical emergency has happened owing to nonadherence (ie, a flare of Crohn disease), a better method would be to predetermine a level of acceptable adherence for each patient or group of patients, assess it frequently, and try to act *before* the medical event has happened.

The way in which nonadherence is defined could have far-reaching consequences. For example, studies done by pharmaceutical companies sometimes set a threshold of 70% adherence for inclusion in a study that examines the effects of a certain medication against a placebo. Although it is indeed imperative to include only patients who actually take the medications in such studies, this approach is not supported by specific evidence as far as gastrointestinal illnesses in children are concerned. There are no data that demonstrate that 70%, or any particular percentage, should be the adherence threshold in any of these illnesses. Furthermore, this arbitrary cutoff point skews the study population by excluding nonadherent patients from the final analysis. Hence, if a medication seems less potent or not potent when used in standard practice rather than in the context of a research protocol, nonadherence could be a contributing factor.

To summarize, nonadherence should be defined in relation to a specific recommendation. It should include a threshold (ie, What degree of nonadherence is permissible?). In determining the threshold, the clinician should take the illness involved into account. Because no empiric data are available regarding the desirable threshold, clinicians must use their judgment and experience to decide what level of adherence is acceptable for each patient.

EXTENT OF THE PROBLEM

Nonadherence to medical recommendations has been reported to be a leading cause of morbidity and mortality in several chronic disease processes, such as ulcerative colitis,[12,13] celiac disease,[9–11] and survivors of liver transplant.[16] Nonadherence may be associated with grave consequences, and it may be quite frequent in children and adolescents (see below). A recent study reported 34 episodes of nonadherence among 28 pediatric liver transplant recipients, leading to acute cellular rejection and recurrent hepatitis in 16 patients, death in 2 patients, and loss of 5 transplants.[16] Our retrospective study of adolescent liver transplant recipients showed that a protracted course of nonadherence led to retransplant and death in several patients.[18] Interestingly, in that study, the rate of documented nonadherence exceeded 50% of the sampled adolescents. Among ulcerative colitis patients, nonadherence to medications was associated with a fivefold increase in the risk of recurrent disease by the end of 12 months of follow-up.[13] These and other data strongly suggest that nonadherence is a common and dangerous phenomenon in patients who are suffering from chronic illnesses of the gastrointestinal tract.

Yet nonadherence is rarely systematically addressed in clinical practice. The following sections review some of the difficulties that are encountered on the way to a rational approach to the detection and treatment of nonadherence.

DEVELOPMENTAL CONSIDERATIONS

A child's cognitive, emotional, and social development may alter adherence behavior. It is quite possible that the same child will be nonadherent at one point and adherent at another, in tandem with specific maturational and developmental stages. The following are a few specific examples of how different developmental stages[1,14] can affect nonadherence, its assessment, and its treatment.

One point is the shift of control, or responsibility, from parent to child. At a certain age or developmental stage, the child becomes responsible for taking his/her medications. Before that stage, it is the responsibility of the parent to administer them. It is obvious that in the treatment of an 8-month-old infant, the parents are the focus of assessment and treatment if nonadherence becomes a problem. In such a case, it is the parent's adherence to administering the medications that should be addressed. On the other hand, a parent may have very little input in affecting the adherence of an 18-year-old adolescent who is in college and is not living with the parent. Hence, somewhere between infancy and young adulthood, the main burden of adherence shifts from parent to child. Assessment and treatment of nonadherence should also shift their focus at that stage. Yet there are virtually no published empiric data about the average age at which this shift occurs. In a survey of 81 patients and their caretakers that was done at the Mount Sinai Medical Center's liver post-transplant clinic, the median age at which parents and children reported that the responsibility for taking the medications is in "transition" was 12 years old, but there was significant variability (range of reported age of transition = 9 to 16 years old). In this cohort, therefore, early adolescence is the time at which the shift in responsibility occurs. Therefore, it may be useful to review the medication regimen in detail with the child and family as the child approaches this age, perhaps even in the context of a dedicated "transitional" visit.

Another important aspect is the development of a child's body image as an essential part of emerging self-esteem during adolescence. Physically disabled children, who may suffer, for example, from disfiguring postoperative scars, reduced weight, or delayed hormonal maturation (as in cases of malnutrition or gut absorptive defects), may not be able to form a positive body image of themselves.[20–22] These patients may become nonadherent to recommendations that are perceived as disfiguring, such as prescriptions of steroid medications and surgery.[22]

Lastly, because of the significant psychosocial changes that children go through on the way to becoming young adults, nonadherence in a child should be viewed as a potentially transient problem. It depends on the child's particular circumstances, cognitive abilities, and emotional status at each point in time. Hence, the labeling of a patient, rather than the patient's behavior, as "nonadherent" is rarely justified. Therefore, clinicians should be encouraged to keep trying to improve adherence by offering counseling, advice, or sometimes specific psychiatric help. At some point, a child may become receptive to a treatment modality that has not been successful before because of different circumstances or cognitive abilities. The following case report illustrates this general principle:

M is a 21-year-old patient who had her first liver transplant when she was 15 years old owing to fulminant hepatic failure caused by a psychoactive medication used to treat attention-deficit/hyperactivity disorder. Following the transplant, she was recovering well until about 1-year post-transplant, when a rejection episode was diagnosed by biopsy, following an elevation in hepatic enzymes. The patient's blood level of tacrolimus at admission was zero, and M disclosed that she was not taking her medication as prescribed. A psychiatric consultation was obtained, and a depressive disorder was diagnosed. The adoptive parents, at the time, were overwhelmed and stated that they could not be responsible for monitoring the child's adherence. Family sessions were conducted, an antidepressant was prescribed, and the patient was discharged. Following discharge, adherence was monitored closely by direct questioning at each clinic visit and medication blood levels. A psychosocial intervention included family sessions and educational efforts aimed at the parents and the child. For 2 years, the medical course was unremarkable. The psychiatric symptoms improved, and adherence was restored. However, when M approached 18 years of age, she moved out of her parents' home to live with her boyfriend. She started to miss clinic visits and seemed more depressed, and her medication blood levels became erratic. She was eventually admitted again for a second rejection episode related to nonadherence. Two more episodes followed in the span of less than a year. An antidepressant was prescribed but not taken. Because of progressive liver damage, retransplant became the only way to keep this patient alive. An extensive psychosocial evaluation was conducted again, and the patient was diagnosed with a depressive disorder (adjustment disorder with depressed mood). The boyfriend's parents, who were supportive but not well informed about the patient's condition, were engaged in sessions and encouraged to take an active supportive role. They ensured adherence to medications, including adherence to the antidepressant. The patient became adherent again for several months prior to a retransplant. One year following the second transplant, the patient was taking her medications and was doing well both medically and psychologically. A re-evaluation found that her mood disorder was in remission.

ASSESSMENT

There is no gold standard for the measurement of adherence, and each proposed method has its shortcomings. Table 76.7-1 summarizes frequently used methods, their strengths, and their weaknesses. Below are specific comments about several methods that are in use.

SELF-REPORT

When compared with objective measures of adherence, data suggest that self-reports are not a sensitive and reliable way in which to assess adherence.[23–26] Self-reports that are obtained in the context of an interview may be an exception.[27,28] Self-reports may still have a place in assessment because a patient's report that he/she *does not* take the medications is generally considered reliable.[26] The part that is not thought to be reliable is the patient's report that he/she *is* taking the medication. Hence, self-report measures may be considered a relatively easy screening method that will identify some but not all cases of nonadherence.

Pill Counts

Pill counts, obtained through a manual count at every clinic visit, are an objective method. A patient may engage in a variety of behaviors that would invalidate this method as a measure of adherence. For example, a patient may remove pills but not take them or take the correct number of pills at an incorrect time.[19] This method will therefore identify patients who are nonadherent, provided that they are not actively concealing it. Routine use of this method is time-consuming. It has not been empirically validated in children who suffer from gastrointestinal illnesses.

Electronic Event Monitoring Devices

Electronic event monitoring devices (eg, MEMS Caps, a product of AARDEX/APREX, Switzerland) are pill boxes with electronic caps that register each opening of the device (for dispensation of a pill). A dedicated software translates the pill box readings into an output chart that gives information about the number and timing of openings. Figure 76.7-1 represents the MEMS readings that were obtained for the patient who was described above shortly before retransplant. It is easy to note that adherence was restored in that patient in December 2001 following the intervention.

Although this method has been described as the state-of-the-art adherence monitoring method, it is not free of bias. Patients may open the pill box but not remove a pill, or they may discard the pill after removing it. Electronic monitoring provides a way to ascertain precisely when a bottle was opened. However, data are lacking about the importance of this precise information. It is possible that taking an immunosuppressant 10 hours as opposed to 12 hours apart does not really constitute a significant adherence problem. The precise threshold at which timing becomes important is not well defined. Finally, although electronic monitoring carries the promise of an objective method that is much harder to "fool" than pill counts, its use was not validated against other acceptable methods of adherence monitoring or clinical outcome in children who suffer from gastrointestinal illnesses.

Prescription Refill Rates

Prescription refill data have been used for the detection of nonadherence[29] and, in some instances, have been reported to be more reliable than patients' self-reports.[30] To use this method, the clinician needs to be able to communicate with the pharmacy, or pharmacies, that the patient is using. Patients are likely to request a refill a few days before they run out of their medications, so the refill rate is only a crude estimate of the time the patient ran out of his/her supply. Further, refilling a prescription is not synonymous with having taken the previously prescribed dose. Hence, prescription refill rates are an objective but crude, and sometimes inaccurate, method of detection.

TABLE 76.7-1 METHODS THAT ARE FREQUENTLY USED TO ASSESS ADHERENCE

ADHERENCE ASSESSMENT METHOD	STRENGTHS	WEAKNESSES	COMMENT
Subjective methods	Easy to obtain	Less reliable than objective methods	
Patient/parent self-report	(1) Easy to obtain; (2) specific (if the patient reports not taking the medication, this report is usually reliable)	Not sensitive (a report that the patient is taking the medication may not be reliable)	Interview results are probably more reliable than questionnaire results
Clinician report	Easy to obtain	(1) Not reliable in predicting nonadherence as judged by objective measures; (2) clinicians' disagreement is a problem (different adherence ratings by different clinicians)	Correlates with health outcome measures (clinicians are more likely to detect nonadherence after an adverse health event has happened as a result of the nonadherence)
Objective methods	More reliable than most subjective methods	Require time and resources	
Medication levels (in blood, urine, or saliva)	Indicates actual intake	(1) Costly; (2) some medications do not have a readily available assay; (3) requires obtaining a biologic specimen; (4) level may at times be affected by metabolic processes, not just by intake	Measurement of the degree of fluctuation between individual levels may be a more accurate representation of adherence over time
Measurement of metabolites of a medication	Reflects intake during a relatively long period of time (not just a single dose)	(1) Costly; (2) assay may not be available; (3) metabolism may be affected by factors other than intake	For example, azathioprine metabolites
Pill counts	Easy to perform and the least expensive method	Patients may discard pills without taking them	
Electronic monitoring devices	Provide specific data about the time in which a medication was taken	Patients may discard pills without taking them (but it is harder to "fool" the electronic device than it is to "fool" a pill counter)	May be too sensitive in some instances (ie, it does not really matter, sometimes, when exactly a patient has taken the medication)
Assessment of the biologic effect of the medication	Addresses the desired outcome of a medication and hence also assesses the effectiveness of the regimen	(1) A lack of biologic effect could be due to other factors (such as a biologic resistance to the medications); (2) assays may not be available	An example: an assessment of thromboxane production values in patients who are prescribed aspirin
Prescription refills	Inexpensive	May not reflect actual intake	

Medication Blood Levels

Medication blood levels may be used for determination of nonadherence. The use of a blood level drawn only once may be misleading. This is because some fluctuation is permissible. An incidence of forgetting to take the medication once may be erroneously counted as nonadherence when using this method. My colleagues and I therefore previously argued that an evaluation of the fluctuation of medication blood levels over time is a better predictor of nonadherence, except in the rare case of a patient who is never taking the medication (this patient will have a consistent level of zero without any fluctuation).[31] We compared standard deviations of consecutive blood levels in pediatric liver transplant recipients.[31] A higher standard deviation and, therefore, more fluctuation between individual measures was deemed to be indicative of nonadherence and was shown to be consistent with a panel assessment of adherence in the same subjects. It is important to note that this method assumes that medication blood levels are closely related to intake. This was shown to be the case for tacrolimus but is not true for cyclosporine.[31] Hence, fluctuations in cyclosporine blood levels cannot be used as a reliable adherence detection method. This method will not identify patients who take their medication only prior to clinic visits ("white coat adherence"[32]).

Metabolites

Metabolites are levels of a medication degradation or metabolic products. Metabolite levels of azathioprine have been used to assess the degree of adherence to this medication.[33] The benefit of using this method is that metabolites accumulate over time, and hence their level reflects the level of medication intake over a period of time, not just recent intake (as is the case with medication blood levels). This method may be less sensitive than others in that only a significant deviation from the prescribed regimen will be detected. It is also usually quite expensive. Further, drug metabolism may be affected by factors other than intake (eg, level of activity of an enzyme that is responsible for the metabolite that is being measured) and may therefore differ between patients.

Clinicians' Assessment of Adherence

Clinicians' assessment of adherence, although used in a few adherence studies, was, in fact, not shown to be a particularly reliable method for the detection of nonadherence.[19,34] It may be more reliable in the most severe cases, and it does

take into account the health status of these patients. My colleagues and I previously reported a perfect agreement in a post hoc blind assessment of the most severe cases of nonadherence in pediatric liver transplant patients among a clinician panel composed of two hepatologists and a nurse.[31] Agreement was reached only for the most severe cases, however. The use of clinicians' assessments of adherence has not been validated in children who suffer from gastrointestinal illness. Taken together, extant data suggest that clinicians' assessments are not a particularly reliable method of detection of nonadherence, with the possible exception of the detection of the most severe cases.

Finally, nonadherence has been described as dynamic in nature.[19] A patient may present as nonadherent at one point in time and adherent at another. Therefore, clinicians' assessments, or indeed any method used to measure adherence, must be examined repeatedly over time rather than at only one time point.

RISK FACTORS

Many risk factors have been reported to be associated with nonadherence to medications.[1,2,14,18] Only some of these are reviewed below. The factors are grouped into those that are associated with the illness, the treatment, the patient, the social milieu, and the clinic/clinician. These groups overlap, and factors may belong to more than one group.

Factors Related to the Illness

Specific medical illnesses may predispose a patient to become nonadherent by virtue of their course or associated features. For example, some medical illnesses are associated with cognitive decline (eg, subacute hepatic encephalopathy as a complication of chronic liver disease). Nonadherence to medications was found to be increased in instances in which the cognitive abilities of a patient are diminished.[35–37] Therefore, diseases that are associated with cognitive decline may be associated with nonadherence owing to this decline. Another example relates to the course of the illness. The management of adherence in patients who suffer from a chronic (as opposed to acute) medical condition is thought to be different in focus and duration.[38] Thus, the characteristics of the illness process, such as chronicity, may influence the risk of nonadherence.

Factors Related to the Treatment

The characteristics of the treatment regimen may determine the likelihood that a patient will adhere to it. Treatments that are time-consuming, require a high level of organization (ie, multidrug regimens), and require a high level of motivation all carry an increased risk of nonadherence.[37,39,40] Treatments that have a severe spectrum of side effects were sometimes,[41–43] but not always,[18,44,45] reported to carry a higher risk of nonadherence; for specific regimens or illnesses, side effects may not independently constitute a significant risk factor for nonadherence. The nature of the recommendation is also important; lifestyle changes (ie, dietary) are thought to be hard to achieve, perhaps because lifestyle changes require a more complex behavior.[46]

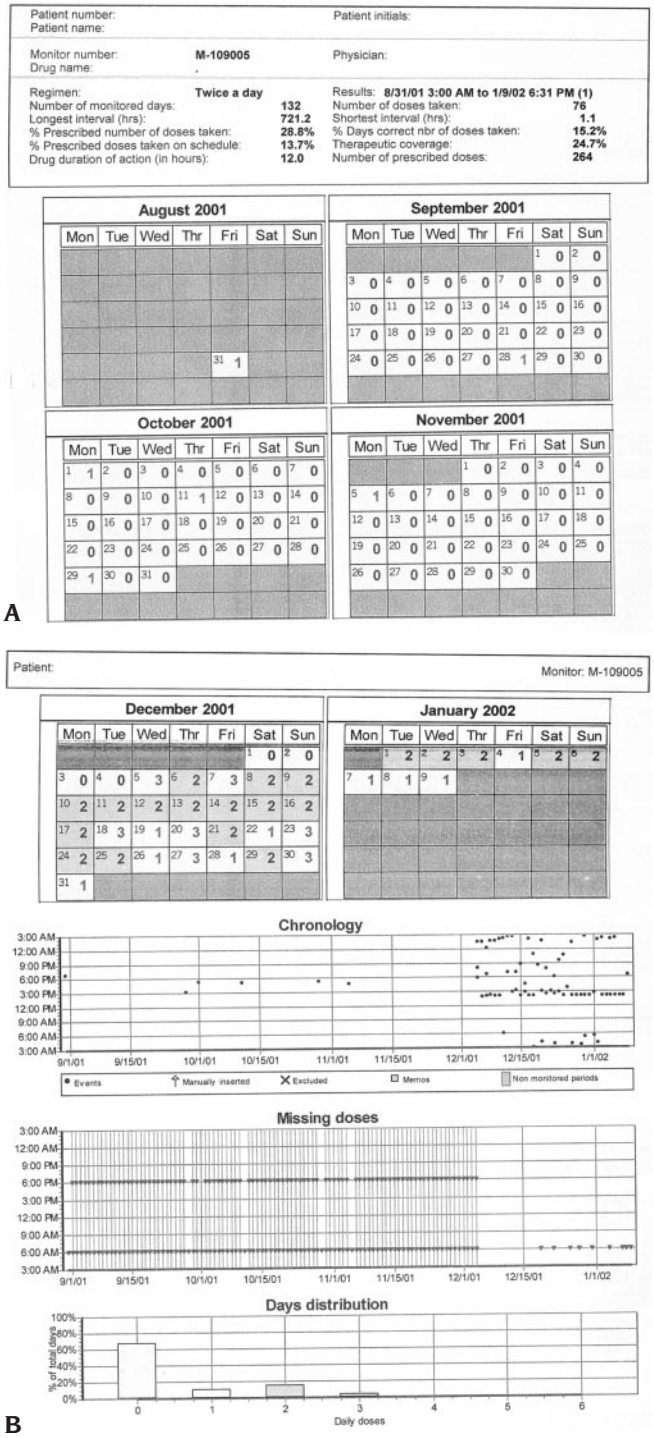

FIGURE 76.7-1 Output data from an electronic monitoring device (MEMS-IV, child resistant, a product of AARDEX/AAPREX). The device was used to monitor adherence to tacrolimus in the patient whose history is presented in the chapter. *A* includes basic patient information (deleted in this case), information about the medication that was used, basic statistics about the number and timing of doses, and data for September through November 2001. Each square represents the number of times the medication bottle was opened on a given day (should be twice a day for this regimen). *B* includes the calendar reports for December and January, the time-scatter (chronology) of the doses that were taken, and a representation of the missing doses. This patient was nonadherent until the beginning of December 2001, at which point she started taking the medications. An intensive psychosocial intervention occurred during the last week of November.

Factors Related to the Patient

Psychopathology, for example, the occurrence of depressive disorders[47–49] and post-traumatic stress disorder,[31,50] was consistently found to be related to nonadherence. A history of child abuse,[18] drug use,[18] reported feeling of "lack of control,"[51–53] and the use of avoidant coping[50,53] have all been implicated in nonadherence.

Social Factors

In young children, it is plausible that any of the above factors that apply to a child's parent will constitute a risk factor for the child's nonadherence (ie, parental psychopathology, depression, etc). A lower socioeconomic status is sometimes implicated as a risk factor.[54–57] A lack of social support (ie, only one parent lives at home or no caretaker lives at home) is also a predictor of nonadherence.[18,50,58]

Factors Related to the Clinic or the Clinician

A lack of empathy on the part of the clinician and a lack of trust between the patient and the clinician have been implicated in patient's nonadherence.[59–60] Lack of appropriate information about a medication is also a plausible reason for nonadherence, but lack of information alone may not be a sufficient cause for nonadherence in many cases.[61] Finally, the characteristics of the clinic itself (ie, time spent with patients, kind of illness that is addressed, awareness of adherence as a therapeutic goal, convenience of clinic hours, gender of clinicians) can be important to the development of adherence behavior in patients.[62]

TREATMENT

Treatment of nonadherence is best conceptualized as a stratified effort. It should begin with preventive efforts that are aimed at every patient and are expected to improve adherence in the clinic or practice as a whole. The preventive effort should also create a mechanism for early identification of nonadherence that needs to be further addressed. Specialized treatment strategies for identified or suspected cases that have not improved by using the general preventive model should then be offered.

Preventive Measures

The hallmarks of this part of an effort to improve adherence are (1) creation of a systematic method to assess patient's adherence as part of the general clinical care (ie, routinely asking about it, routine medication blood level determinations); (2) provision of general and specific education about medication-taking that is repeated frequently and targeted to the developmental stage of the child at the time the education is given; and (3) prospective assessment of risk factors that are known or suspected to be related to nonadherence and addressing these risk factors as they become known and before nonadherence develops.

Specialized Treatments

Nonadherence can be managed in several ways once diagnosed or even when suspected but not confirmed. Available treatment strategies have been grouped below to treatments that focus on patient education and awareness, treatments that focus on the adherence behavior itself, and treatments that seek to improve risk factors that are considered to be the main reason for nonadherence in a particular patient. These specialized treatment strategies are time-consuming and sometimes require a highly skilled individual to deliver it. However, the provision of such care may have a profound impact on adherence and outcome in selected patients.

Patient and Parent Education

It is hoped that education about the illness is provided to all patients, adherent or not, during the routine medical management of their illness. This section addresses a more intensive educational approach intended for confirmed or suspected nonadherent patients. The components of this approach are the assessment of the patient's (and parent's) actual understanding of the prescribed regimen, its administration, and the reasons for it; the correction of any misinformed notions that are discovered; and an open discussion about the ways in which the medication is being taken, how it can be better integrated into a patients' lifestyle, and what concessions or resources are needed to make medication-taking possible. Thus, education in this model is an interactive process in which the clinician tries to identify the cognitive and procedural needs of the patient and address them. Such an approach may also increase the patient's confidence in the clinician and enhance the collaboration between the two. Educational approaches have been shown to have limited but significant effects on adherence.[63,64] Because they are relatively straightforward and not labor intensive, they should be attempted in most cases as a first line of treatment. However, it should be emphasized that education alone is not sufficient in many instances.[61]

Behavioral Modification Strategies

In this model, the adherence behavior itself is addressed. Behavior modification strategies can be used to implement a reward system for adherence (ie, a patient who is suffering from Crohn disease is rewarded with a sticker every day in which he is able to take his medications without being reminded, by an extra hour of play before bedtime on the weekend if all of the week's stickers have been collected). Parents are sometimes reluctant to implement behavioral techniques because they think that the child is suffering so much already and do not want to start being cruel or engage in strict disciplinary action against him/her. A medically ill child, however, frequently must follow very strict nutritional, medication-taking, and scheduling routines, for which a high level of discipline is required. The point to emphasize is that reinforcements are the best way to achieve a behavioral goal. Negative reinforcements or "strict discipline" is not always necessary.

Behavioral methods could also include a change in the frequency in which the patient is seen in the clinic to improve the physician's control on adherence (ie, more frequent assessments of medication metabolites and liver enzyme profiles in a pediatric hepatology clinic). Behav-

ioral methods may sometimes necessitate the involvement of another caretaker or a therapist if the primary caretaker is unable to implement these methods consistently. For example, a school nurse may be enlisted to monitor and implement adherence to medications that are taken during school hours. Behavior modification strategies have been shown to dramatically improve an array of behaviors and have been reported as helpful in improving adherence.[65,66] However, these methods have not been rigorously evaluated in the improvement of adherence in children who are suffering from diseases of the gastrointestinal tract.

Although behavioral methods may well be successful in improving adherence, they require a specifically trained individual who is typically required to serve as a "coach" to patients and parents. This individual may not be readily available in many clinics. Also, the implementation of a successful behavioral plan may be harder to achieve in a context in which a child is chronically ill and a parent is reluctant to be "cruel," as mentioned above. Finally, because strict adherence to the behavioral intervention is required for success, it may at times be hard to implement these methods in patients who are nonadherent to recommendations to begin with.

Strategies Aimed at the Improvement or Elimination of Risk Factors

There are many known or suspected risk factors for nonadherence, as mentioned above. A thorough discussion of methods that may be used to address each of these risks is well beyond the scope of the present review. The main point is that significant risk factors do exist and that these may be highly modifiable.

For example, the treatment of a specific psychiatric disorder such as a major depressive disorder or posttraumatic stress disorder[31,67] may improve adherence. Improvement of social stressors and provision of safety, such as the treatment of ongoing child abuse,[18] may be reasonably expected to improve adherence as well. The identification and management of risk factors related to the child and his/her environment should therefore be attempted. In a child, the assessment of risk should extend at least to the primary caretakers as well.

CONCLUSIONS

Nonadherence to medical recommendations is a significant, and mostly modifiable, risk factor for increased morbidity and poor outcome in many patient groups. The assessment of nonadherence and its management in children are complicated and not well standardized. The child's developmental stage affects adherence behavior, and the target for intervention frequently includes not only the child but also the caretakers. There are no "gold standard" methods for assessment of nonadherence. Yet several objective and subjective methods do exist. The management of nonadherence includes preventive, nonspecific efforts that start with proper patient education and rapid identification of suspected cases and specialized treatment strategies that focus on education, behavior management, and addressing specific risk factors. Knowledge of the methods that are described in this review is expected to give practitioners the initial tools and confidence to deal with nonadherence as an important aspect of patient care.

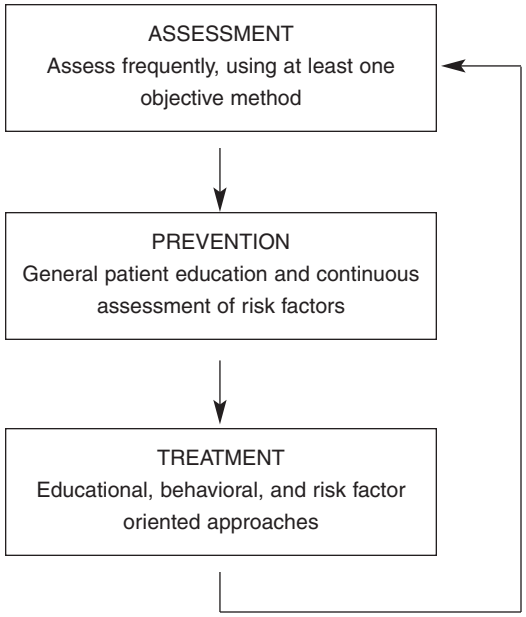

FIGURE 76.7-2 Suggested clinical approach to the management of nonadherence.

The present textbook includes, in most chapters, a discussion of effective and sometimes innovative treatment strategies for children who suffer from gastrointestinal illnesses. These treatments include medications, dietary restrictions, surgical procedures, and other modalities. Although areas of medicine have advanced tremendously in the development of such strategies, these treatments will remain effective in practice only if they are adhered to. Extant data establish that complete adherence is, in fact, very hard to attain in clinical settings. Therefore, adherence should not be assumed. Rather, the attainment of adherence to recommendations must be included as a treatment goal. Figure 76.7-2 summarizes in a concise algorithm the clinical approach that was described in this chapter. Implementation of this approach would ensure a clinical focus on adherence. It could also facilitate research efforts that will provide crucial information about the validity and applicability of assessment and treatment methods that were discussed in this chapter.

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CHAPTER 77

MANAGEMENT OF SURGICAL PATIENTS

1. *Complications after Gastrointestinal Surgery*: A *Medical* Perspective

Samuel Nurko, MD, MPH

Advances in pediatric surgery and in postoperative care have allowed the survival of children who were born with complex congenital anomalies. As more children survive and grow older, new long-term medical problems are arising, and new therapies are often needed. The pediatric gastroenterologist has to deal with some of these specific problems, particularly as they relate to the surgical correction of esophageal, hepatobilliary, intestinal, or anorectal malformations.

The purpose of this chapter is to describe some of the long-term medical complications seen in children after surgery in the alimentary tract. The following discussion deals specifically with representative problems after surgical therapy for gastroesophageal reflux (GER), imperforate anus, and Hirschsprung disease. The long-term problems after the correction of other surgical conditions such as tracheoesophageal fistulae and hepatobiliary malformations, as well as the treatment of short gut, are dealt with in other chapters.

SURGERY FOR GER

Antireflux surgery is a successful way to treat intractable GER. Fundoplication is the third most commonly performed general surgical procedure in some institutions,[1,2] and the Nissen operation is the most common type performed (Figure 77.1-1).[3,4] In both children and adults, postoperative results after a Nissen operation are satisfactory in 74 to 94% of patients.[1–3,5–7] Operative mortality is low, usually less than 1%.[2] A substantial late death rate (16–24%) has been reported in some series, but this is usually secondary to the underlying diseases.[1–3,6,7]

Even though many centers report excellent results, it is known that antireflux surgery can have a significant amount of side effects, varying from minor to severe (Tables 77.1-1 and 77.1-2).[2,4,8–10] A recent multicenter retrospective review of 7,467 patients (56% neurologically normal and 44% neurologically abnormal) operated on over 20 years reported that in the neurologically normal children, there was a good to excellent result in 95%, with major complications in 4.2%. These compared with a

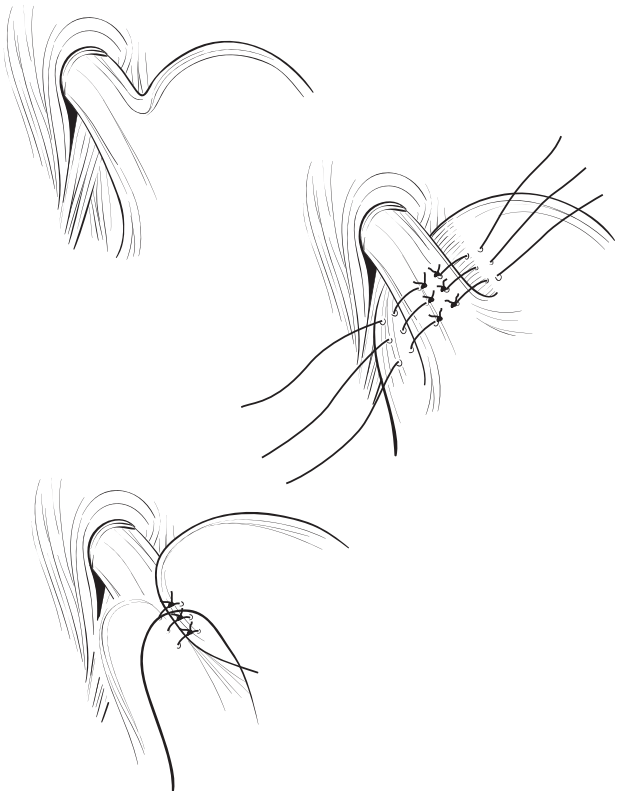


FIGURE 77.1-1 Nissen fundoplication. The fundus of the stomach is seen to be wrapped like a collar around the area of the lower esophageal sphincter. Adapted from Smout AJ, Akkermans LM. Normal and disturbed motility of the gastrointestinal tract. Petersfield, Hampshire (UK): Biomedical Publishing; 1992.

TABLE 77.1-1 LONG-TERM COMPLICATIONS AFTER FUNDOPLICATION

Small intestinal obstruction
Recurrence of symptoms and reappearance of gastroesophageal reflux
Dysphagia
Gas bloat syndrome
Herniation of the wrap
Fistula formation
Dumping syndrome

reported 84.6% good result in those with neurologic problems and a complication rate of 12.8%. The most common complications reported were recurrent reflux from wrap disruption in 7.1%, respiratory symptoms in 4.4%, gas bloat in 3.6%, and intestinal obstruction in 2.6%. Postoperative deaths occurred in 0.07% of normal versus 0.8% of neurologically abnormal children, whereas the incidence of reoperation was 3.6% in normal and 11.8% in neurologically abnormal patients.[2]

Children with underlying neurologic abnormalities have a higher incidence of complications.[1,2,4,8,11–14] Some authors have suggested that they have more than twice the complication rate, three times the morbidity, and four times the reoperation rate.[4,14] Some authors have attempted to do a direct comparison of the outcome between children with and without neurologic problems. Dedinsky and colleagues reported a large series of 429 fundoplications, of which 297 were done in children who were neurologically impaired.[15] This last group accounted for all 4 postoperative deaths, 24 of 28 wrap herniations, and most of the reoperations. Similar findings were reported by Pearl and colleagues when they compared the outcome in 81 normal children with the outcome in 153 patients who were neurologically impaired.[14] They showed that there was a morbidity of 12% versus 24%, a rate of reoperation for a failed fundoplication in 5% versus 19%, and an aspiration-induced mortality of 1% versus 9%. Because of all of the above findings, it has even been suggested that neurologic status is the major predictive factor of failure of antireflux surgery in children.[14]

Another high-risk population in which the outcome after fundoplication is not as good as that in neurologically normal children is those patients with esophageal atresia repair.[16] It has been reported that only 40% have excellent results and that many require reoperation.[3,4,17,18] The use of partial wraps has therefore been advocated in these patients.[17]

The surgical approach to children who require fundoplication has been changing in recent years with the advent of minimally invasive surgery.[19] The experience with laparoscopic procedures in children has been accumulating in recent years, and the results are similar to those found in adults.[19–26] Laparoscopic surgery has been successful even in small babies (3 kg), children less than 1 year,[24] children with neurologic problems,[22] and children with respiratory disease.[27] There are no prospective studies in children that have compared the open with the laparoscopic technique. There are limited studies in which the laparoscopic technique has been compared with retrospective "open" procedures.[20] Collins and colleagues, in a cohort of 120 children, compared the operative time, complication rate, and hospital stay between children who had a laparoscopic procedure and a retrospective control group who had undergone an open operation.[20] They found a slightly lower complication rate and a significant decrease in hospitalization after laparoscopy. In a large series of 220 patients aged 5 days to 18 years, an intraoperative complication rate of 2.6% and a postoperative complication rate of 7.3%, which included 7 patients with a breakdown of the fundoplication, 5 patients with gagging and retching, 2 patients with gastroparesis, and 4 patients with severe dysphagia, were reported.[19] It is becoming apparent that laparoscopic fundoplication is the procedure of choice in many centers and is slowly becoming the norm.

The most common problems after fundoplication are shown in Table 77.1-1 and are similar independently if the operation is open or performed laparoscopically.[20,21,28] The physiopathology of these symptoms may be multifactorial, and Table 77.1-2 suggests the different mechanisms by which some of the most common symptoms may be produced.

SMALL BOWEL OBSTRUCTION

This is one of the potentially serious complications after fundoplication.[2,3,15,29–31] Ashcraft reported in a recompilation of series with Nissen fundoplication that from a total of 1,319 patients, 43 had small bowel obstruction (3.2%),[29] and of 7,467 patients, Fonkalsrud and colleagues described it in 2.6%.[2] It has been suggested that the incidence of this complication is higher if other procedures are performed at the same time as the fundoplication. In these instances, the incidence has been reported to be as high as 10% compared with an incidence of 1.8% when only a fundoplication is performed.[29,32] This complication needs to be recognized promptly because it can be associated with significant morbidity and mortality. It has been reported that from one-third to one-fifth of patients who develop intestinal

TABLE 77.1-2 COMMON CLINICAL SYMPTOMS AFTER FUNDOPLICATION AND THE POSSIBILITIES THAT NEED TO BE CONSIDERED

VOMITING	DYSPHAGIA	GAS BLOAT	IRRITABILITY
Gastroesophageal reflux	Tight fundoplication	Tight fundoplication	Gastroesophageal reflux
Tight fundoplication	Peptic stricture	Delayed gastric emptying	Wrap herniation
Wrap herniation	Primary motility problem	Impaired gastric accommodation	Dumping syndrome
Small bowel obstruction		Visceral hyperalgesia	Small bowel obstruction
		Small bowel obstruction	Visceral hyperalgesia

obstruction may die if it is not promptly recognized. It can occur in the immediate postoperative period or many years thereafter. This complication has to be suspected when there is abdominal distention and pain, persistent vomiting (if the fundoplication is loose enough), and evidence of obstruction. Sometimes it is difficult to diagnose, particularly in severely impaired patients or when the patient cannot vomit, so there has to be a high index of suspicion. A delay in diagnosis and treatment will inevitably lead to bowel necrosis and death, and the treatment is surgical.

REAPPEARANCE OF GER

The return of symptoms compatible with GER usually indicates that the operation has failed. The incidence of GER can occur in the immediate postoperative period (surgical failure) or much later. The information about surgical failure is limited. In one series of 385 children evaluated by pH probe within 12 weeks postoperatively, GER was documented in 2.9%.[33] Most late cases in which symptoms recur happen in the first 1 to 2 years after the operation,[2,6,31] and it has been reported that in up to one-third of patients, the symptoms of GER become apparent after an episode of forceful emesis.[31] The incidence varies among series. It was described in 7.1% of 7,467 patients[2] and in 29 (12%) of 242 patients.[13]

Caniano and colleagues were able to identify the cause for the appearance of recurrent GER in 86% of children in their series: "slipped" fundoplication in 15, no fundoplication visualized in 2, and paraesophageal hernia in 1.[31] It is not known why wrap disruption occurs, but mental retardation, pulmonary dysfunction, and the presence of a seizure disorder are all risk factors.[13] It is also possible that the surgical technique may also have an influence, with the possibility that an inadequate mobilization of the gastroesophageal junction, fundus, and cardia has occurred, particularly in children with increased intra-abdominal pressure owing to movement disorders, aerophagia, or constipation.[13]

If the patient returns with symptoms that are compatible with reflux, an upper gastrointestinal (UGI) series needs to be obtained (Figure 77.1-2). The presence or absence of the wrap should be determined, and the functional integrity can be grossly examined. If there is evidence of wrap disruption (see Figure 77.1-2B) and of free-flowing reflux, it can be assumed that the fundoplication is not working, and antireflux therapy should be initiated. If it is necessary to judge the state of the esophageal mucosa, an endoscopy can then be performed. If, on the other hand, the UGI seems to show an intact wrap (see Figure 77.1-2A) and no evidence of GER, a pH probe study or an esophageal impedance will actually show the amount of reflux the patient is experiencing and give an assessment of acid clearance. An endoscopic procedure will then show if there has been esophageal damage. There is no need to perform esophageal manometry in these patients. Manometric studies should be performed, however, if a new surgical procedure is being contemplated or if there is the possibility that the patient has a primary esophageal motility disorder.

Once the diagnosis of recurrent GER is made, aggressive medical therapy needs to be instituted,[4,10] and a trial of gastrojejunal feeds may be necessary in those patients at risk of pulmonary complications, particularly in children with severe neurologic problems (see Figure 77.1-2).

If the reflux proves to be refractory, the patient seems to be aspirating, or new complications arise (eg, Barrett esophagus), a reoperation should be considered. In cases in which the main indication of a failed fundoplication and the need for reoperation is the presence of respiratory problems, a full assessment of oral motor function needs to be done because some of the symptoms may be due to inability to handle oral secretions, particularly in neurologically impaired children. The use of jejunal feedings can be used as a therapeutic trial before a reoperation is performed. Also, in those high-risk patients with recurrent GER and aspiration secondary to poor oral-motor function, the long-term use of jejunal feedings may be the best option because the performance of a fundoplication may alter esophageal clearance of saliva and exacerbate the problem. This is particularly important in patients with documented esophageal dysmotility.

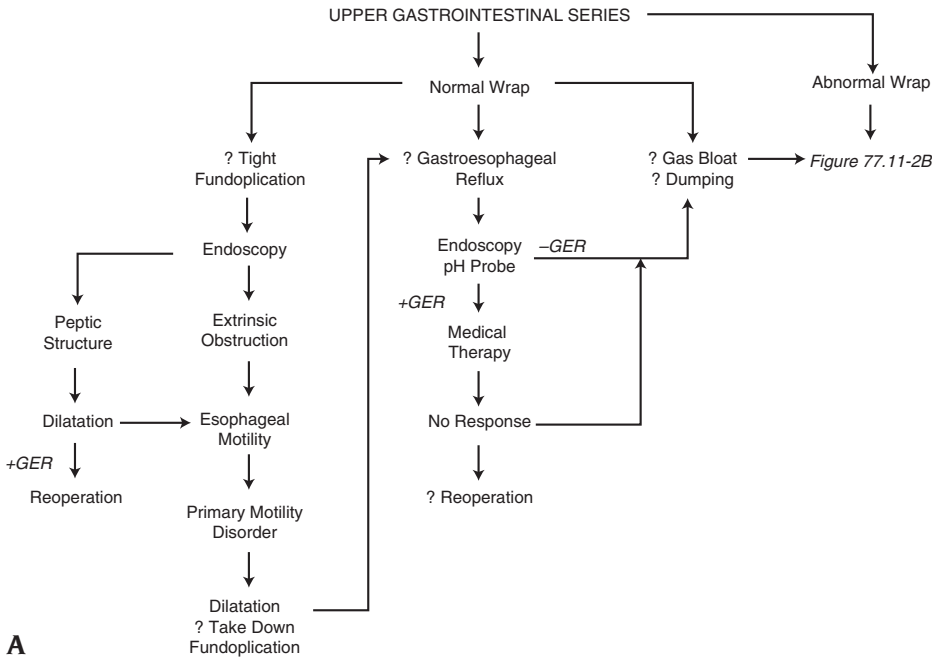
Wheatley and colleagues described their experience in the treatment of 29 patients with recurrent GER after fundoplication.[13] They found that medical management was successful in controlling the symptoms in 11 of 29 (38%) patients. In another study, Caniano and colleagues reported that 21 of 364 (6%) patients who had a fundoplication required a reoperation because of GER recurrence.[31]

DYSPHAGIA

Dysphagia has been the most common problem after the Nissen operation and the symptom most commonly associated with long-term unsatisfactory results.[5,6,34] Some authors have suggested that more than 50% of patients have some degree of solid food dysphagia even after a follow-up of 20 years, but most report an incidence that varies from 0 to 40%.[6,35] The exact incidence of postoperative dysphagia is difficult to establish, but it has been suggested that its prevalence is higher in those children who have undergone a Nissen fundoplication when compared with those who have undergone fundoplications that are considered "more floppy," such as Thal, Toupet, or Rosetti.[36] In the largest pediatric series of 7,467 patients, dysphagia attributed to esophageal obstruction was reported in 2.4%.[2]

The dysphagia may be related to either a wrap that is too tight around an esophagus with good peristaltic function or secondary to a functional obstruction created by the inability of a damaged esophagus to produce enough force to propel the food into the stomach (see Table 77.1-2 and Figure 77.1-2A).

The role that preoperative esophageal peristalsis plays in the development of dysphagia has been controversial.[34,37] It has been suggested that even though there are no prospective data, care should be exercised when a fundoplication is performed in patients with abnormal esophageal peristalsis.[34,37] Low and colleagues reported in a series of patients who underwent secondary operations



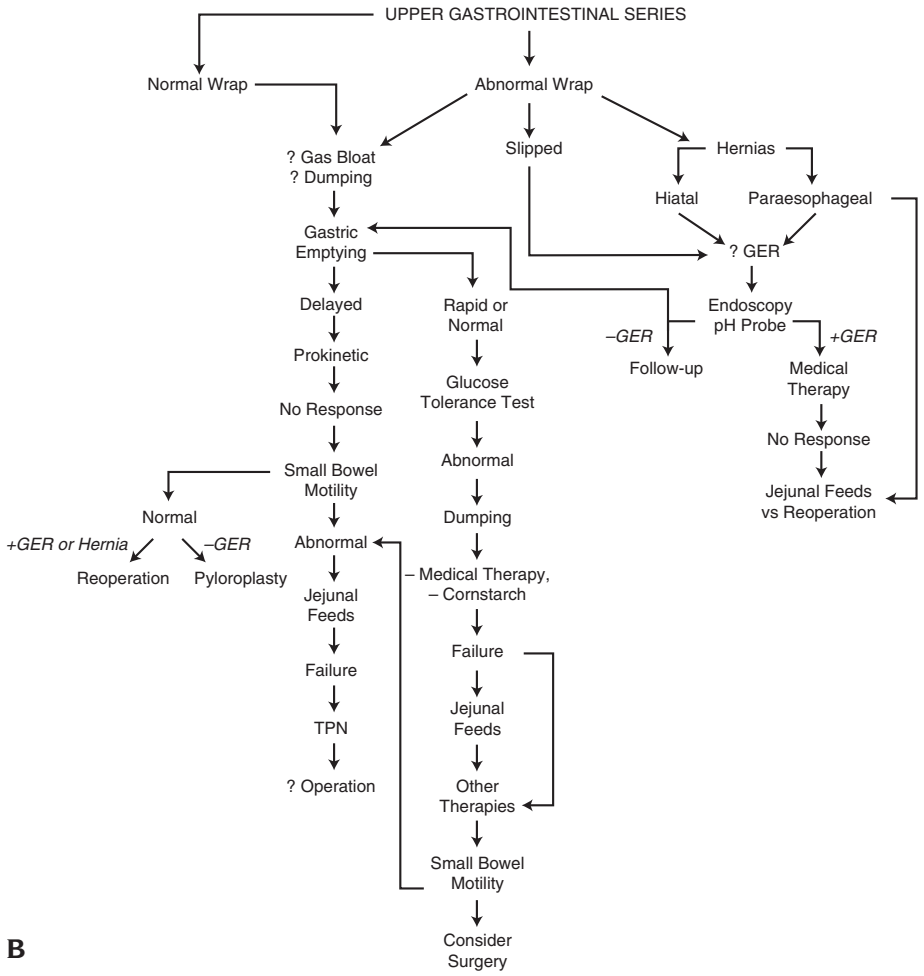


FIGURE 77.1-2 Algorithm for the evaluation and treatment of patients with problems after fundoplication. *A*, An approach in which the upper gastrointestinal series (UGI) has shown the presence of a normal wrap. *B*, This figure focuses on patients in whom the UGI has shown an abnormal wrap. GER = gastroesophageal reflux; TPN = total parenteral nutrition.

for failed Nissen procedures that six patients presenting with severe postoperative dysphagia had evidence of primary esophageal motility disorders (four with collagen vascular diseases and two with achalasia) that were not diagnosed before surgery, [38] indicating the importance of assessing esophageal motility before the operation.[37] In children, this problem is commonly found after patients with scleroderma or tracheoesophageal fistulae have undergone fundoplications.[17] Also, because of the possibility of creating a functional obstruction in a dysmotil esophagus, an esophageal motility test should be performed before the operation in those children in whom esophageal dysfunction is suspected.[37]

The relationship between preoperative esophageal motor abnormalities and postoperative dysphagia has been questioned.[34,39,40] A study after a laparoscopic Nissen operation in 81 adults who had baseline esophageal motility showed no difference in the prevalence of dysphagia up to a year after the operation (12.5% vs 15%) when comparing the 48 patients with normal motility with the 33 patients with abnormal esophageal function.[34] The authors suggested that there was poor correlation between the preoperative manometry and outcome and that abnormal esophageal peristalsis is not a contraindication to performing the operation.[34] Another recent prospective randomized clinical trial of 200 patients randomized either to Nissen (360°) or Toupet fundoplication (270°) studied esophageal motility before and after the surgery. They found that preoperative esophageal dismotility reflected more severe disease but did not affect postoperative clinical outcome. In 85%, the motility remained unchanged because it was not corrected by the fundoplication (independent of the surgical procedure performed). In 20 patients, the motility improved, whereas in 9 patients, it worsened. The authors concluded that preoperative esophageal dysmotility requires no tailoring of the surgical management.[39]

The dysphagia in many cases is transitory[5] but at times can be very severe, leading to significant dietary restrictions. The presence of dysphagia in the immediate postoperative period may be secondary to edema or transient esophageal hypoperistalsis (particularly if the operation is performed through the thoracic approach).[3] The management has to be conservative, allowing time for the edema to subside. However, if the dysphagia persists or is severe, it may be necessary to consider that the operation is too tight, and further evaluation will be necessary.

The best way to investigate patients with dysphagia is to perform a barium study to fully assess the anatomy (see Figure 77.1-2A). This study will help delineate the wrap and assess if there is obstruction. It can also detect the presence of peptic strictures. A better functional assessment of emptying can be performed with esophageal scintigraphy and can be a useful way to follow patients if dilatations are performed. An esophageal motility should be performed in cases with severe symptoms. This allows the definition of lower esophageal pressure and identifies the presence or lack of peristalsis. Endoscopy may be useful to assess if there is fibrosis or other complications from GER (eg, peptic stricture, esophagitis, Barrett adenocarcinoma). If there is evidence of poor emptying and primary motility disorders, attempts to lower the functional obstruction should be undertaken. If the motility is normal, dilatation should be attempted, and if dilatation is necessary soon after the operation, great caution needs to be exerted because it has been shown that if done forcefully, it can lead to disruption of the repair.[35] The results after endoscopic dilatation in adults have been satisfactory.[35] In a series of 35 patients, dysphagia resolved in 52%, and there were no disruptions of the fundoplictions or GER. The results varied according to the radiologic appearance of the fundoplication. In those patients with a slipped operation, endoscopy relieved the dysphagia in only 27%, as opposed to 67% of those with intact fundoplication.[35] If symptoms persist after dilatation or the patient has other symptoms associated with a tight wrap (such as gas bloat), a revision of the surgery may be necessary. In general, this is not very common. Spitz and colleagues reported that only 1.2% of patients required a reoperation because of dysphagia.[12]

GAS BLOAT SYNDROME

Gas bloat syndrome is characterized by distention, inability to vomit, abdominal pain, and, in children, severe retching, gagging, and irritability.[11] Its duration is variable, but it can last for many hours and be severe enough for patients to seek medical attention. Many long-term studies of patients who have undergone Nissen fundoplication have reported that gas bloat syndrome can occur from 2.8%[3] to 50%[6] of cases. The exact incidence of this problem is difficult to assess, particularly because some surgeons routinely add a decompression gastrostomy at the time of the operation.[29] In one study of 106 patients, 2 required the placement of a late gastrostomy because of this problem,[41] and Fonkalsrud and colleagues reported that even though they routinely use a gastrostomy in children less than 3 years, gas bloat still developed in 12 patients following removal of the tube.[42] The problem with gastric distention cannot be minimized because it has been reported that death can occur secondary to gastric necrosis.[43] In their report of 7,467 patients, Fonkalsrud and colleagues reported gas bloat syndrome in 3.6% (2–10%) and suggested that the incidence was higher in those centers in which the gastric emptying procedure was rarely used.[2]

The physiopathology is not well characterized.[6] Patients who develop the syndrome tend to have GER associated with activation of the emetic reflex (pallor, sweating, retching, and forceful vomiting).[11] Most likely it is related to the presence of an increased amount of gastric air,[44] compounded by an inability to vomit or belch, impaired gastric accommodation,[5,45] gastric hypersensitivity,[5] and slow gastric emptying.[3,5,6]

It has been shown that many children with gas bloat syndrome have delayed gastric emptying (see Figure 77.1-2B).[3] In a retrospective review of fundoplications performed in 92 patients with delayed gastric emptying, the authors compared the outcome between those who also had a gastric emptying procedure and those who did not. They showed a higher incidence of recurrent reflux in

those without a gastric emptying procedure (35 vs 18%).[46] Because of the possibility of this association, the performance of a pyloroplasty in conjunction with a fundoplication has been suggested when delayed gastric emptying is found preoperatively.[3,13,47]

It has also been shown that fundoplication may induce gastric myoelectrical disturbances that may correlate with the development of retching.[48] In one study, the authors showed that children who retch preoperatively were three times more likely to retch after the operation and that 25% of neurologically impaired children may start to retch after the operation. Sixty percent of neurologically impaired children had gastric dysrhythmias before the operation compared with 20% of neurologically normal children. These findings suggest a loss of central inhibitory mechanisms that may result in the inappropriate activation of the emetic reflex, which may be heightened by antireflux surgery.[48]

The role that small bowel motility plays in the development of this symptom is also not clear. In one study, 25 of 28 symptomatic children after fundoplication had abnormalities in antroduodenal motility. The most common abnormality found was an absence of the migrating motor complex in 12 children, whereas 6 children had postprandial hypomotility; other nonspecific abnormalities included clustered, retrograde, and tonic contractions.[49] It is unclear if the abnormalities were present before the operation or are a result of it, although the authors suggested that because the abnormalities found were similar to those seen in chronic intestinal pseudo-obstruction, it is likely that they predated the operation, suggesting that those children had a more generalized gastrointestinal dysfunction and not only GER. In that case, the performance of a fundoplication, with the elimination of the ability to vomit, could be expected to worsen symptoms such as retching and abdominal distention. This observation needs to be taken into account when the performance of a fundoplication is being considered in children with generalized symptoms of gastrointestinal dysfunction, and an antroduodenal motility may be a useful test to perform in those cases before the operation to exclude the possibility of pseudo-obstruction.

Recently, it has been suggested that gastric hypersensitivy may also play a role. Studies using the Barostat technique have documented that adult patients after fundoplication may have a lower threshold for postprandial discomfort and abnormal gastric postprandial relaxation.[5,50] This represents an emerging field of study, and treatments to decrease the hyperalgesia may be needed.

The medical therapy for gas bloat syndrome has included the use of motility and simethicone-containing agents. Cisapride has been used with success, particularly in children with delayed gastric emptying. Attempts to decrease air swallowing should be undertaken, and the status of vagal function can be determined by using sham feeding or Congo red testing. The tests have been found to be useful in adults, but no information is available in children.[6]

The inability to vomit and belch is rarely incapacitating enough to require another operation. At times, it may be necessary to perform a temporary gastrostomy.[3] It has been reported that the symptoms disappear if the fundoplication is undone, although if the patient has underlying dysmotility or there was vagal nerve damage, they may persist after reoperation.[29] If there is evidence of delayed gastric emptying, it is possible to consider the performance of a pyloroplasty[3,42] without taking down the fundoplication (see Figure 77.1-2B).

Treatments to decrease the gastric hyperalgesia and improve gastric accommodation may be beneficial.[5] Because 5-hydroxytryptamine $(HT)_1$ receptors may be involved in gastric accommodation,[51] the use of 5-HT_1 agonists may be attempted. Cyproheptadine may be tried. In adults, 5-HT_{1D} agonists such as sumatriptan or 5-HT_{1DA} agonists such as buspirone have been attempted with some success in patients with dyspepsia as a way to increase postprandial fundic relaxation.[5,51,52] The visceral hyperalgesia may also be treated with medications such as anticholinergic agents.[5] Because tricyclic antidepressants such as amitriptyline have been shown to be useful in the treatment of the visceral hyperalgesia that can be seen in functional bowel disorders in adults,[5,53] they may be useful in children with severe hyperalgesia postfundoplication.[5] Future studies with new medications that change the threshold of sensation, such as 5-HT_3 antagonists or 5-HT_4 agonists, will be conducted in the coming years and will determine if these medications will be useful in the treatment of these children.[5]

HERNIAS AND OTHER PROBLEMS WITH THE FUNDOPLICATION

Herniation of the fundoplication is another common complication, and it represents the most common indication for late reoperation. Spitz and colleagues described their findings during reoperation of those patients in whom the initial operation failed and found that the most frequent finding in 25 patients (14%) was a prolapse of the wrap into the posterior mediastinum through an enlarged hiatus with or without a paraesophageal hernia.[12] In only two patients, the wrap was too tight, and in only two patients, the wrap was partially disrupted, leading to recurrent reflux. There are two principal types of herniation distinguished by the localization of the gastroesophageal junction.[54] In the first type, there is herniation of the entire wrap and gastroesophageal junction into the chest,[54] usually presenting as recurrent GER, and in the second or paraesophageal type, there is a posterolateral herniation of a portion of the wrap, with the gastroesophageal junction remaining within the abdomen (see Figure 77.1-2B). This latter type seems not to be associated with reflux but has the risk of incarceration/strangulation or bleeding.[30]

It can be argued that the herniation of the entire wrap usually indicates a failure of the surgery. Some studies, however, have shown that not all patients with this problem have recurrent symptoms of reflux.[6] If this type of hernia is found, the patient needs to be worked up for GER, and the presence of other symptoms should be ascertained (see Figure 77.1-2B). In the absence of any significant symptoms or of pathologic reflux, its presence is probably

not important, but if significant problems are associated with its presence, a new operation may be necessary.

The significance of a paraesophageal hernia is more important. It is more common,[14] it is the primary reason for reoperation in some series,[3,14,30] and its appearance seems to be related to a failure to perform an adequate crural repair.[13,30,54] Pearl and colleagues reported after doing a recompilation of 2,142 cases postfundoplication that a wrap herniation occurred in 117 cases (18.3%) and accounted for 63% of reoperations.[14] Fonskalrud and colleagues reported that 11 (2.6%) of their patients developed this complication and that all required surgical correction.[42] It has also been reported that in neurologically impaired children, herniation occurred in 38%[14] and that the incidence of wrap breakdown/paraesophageal hernia and small bowel obstruction after Nissen fundoplication in this group of children is disturbingly high.[1,14]

The paraesophageal hernias usually increase over time and can produce symptoms, which may include GER, dysphagia, chest pain, bleeding from the hernia sac, or even ischemia of the gastric segment involving the hernia.[1,14] In smaller children or children with severe neurologic impairment, the only manifestation may be severe irritability. The demonstration of these hernias is done with the performance of a UGI (see Figure 77.1-2B). This test allows the visualization of any obstruction to esophageal emptying and will help delineate gastric anatomy; the presence, status, and location of the fundoplication; and any other gastric problem. Endoscopy may help evaluate the state of the herniated gastric mucosa and if there is ischemic damage.

The treatment of paraesophageal hernias has to be tailored to the individual patient (see Figure 77.1-2B). If there is evidence of recurrence of GER, aggressive medical therapy needs to be started, but if the associated symptoms are severe or there is evidence of inflammation or bleeding in the herniated segment, a reoperation needs to be performed. If the hernia is small and the patient is asymptomatic, conservative management with close follow-up should be undertaken.

FISTULA FORMATION

The performance of a Nissen fundoplication has been associated with the occurrence of fistula formation. The fistulae usually occur between the fundic wrap and other abdominal and mediastinal structures. Gastrodiaphragmatic, gastroaortic, gastrobronchial, gastrocardiac, and gastrocutaneous fistulae have been described.[14,55] The clinical presentation varies depending on the localization of the fistula, but it may be life-threatening when it involves the great vessels. When these are diagnosed, they need to be surgically treated.

DUMPING SYNDROME

Dumping syndrome refers to the symptom complex that results from the rapid transit of food into the small bowel[56–60] and is one of the most common causes of morbidity after gastric surgery.[61] It has been estimated that between 25 and 50% of all patients who have undergone some type of gastric surgery have some symptoms of dumping, although only 1 to 5% have serious disabling symptoms.[58,61] In children, dumping has been described almost exclusively as a postoperative complication of Nissen fundoplication, but the exact incidence is not known.[2,56,57,59–66] In the article by Fonskalrud and colleagues, they described a postoperative transient dumping syndrome in 0.9% of 7,467 fundoplications (0–5%).[2] In a study of 50 patients, Samuk and colleagues reported dumping in 30% (15 patients).[59]

The syndrome is characterized by both gastrointestinal and vasomotor complaints (see Table 77.1-2). Gastrointestinal symptoms include postprandial fullness, crampy abdominal pain, nausea, vomiting, and explosive diarrhea. In younger children, aversion to food, failure to thrive, and retching may be part of the clinical picture.[56,57,60,62] Vasomotor symptoms include diaphoresis, weakness, dizziness, flushing, palpitation, and a desire to lie down. In infants and children, the typical symptoms appear during or after feeding and include irritability, pallor, perspiration, tachycardia, lethargy, diarrhea, and vomiting.

Dumping has been classified into early and late forms based on the timing of onset of symptoms after a meal.[56,57,61] The early symptoms occur soon after eating (10–30 minutes) and can be a mixture of both gastrointestinal and vasomotor complaints. These include abdominal distention and discomfort, nausea, borborygmus, tachycardia, pallor, diaphoresis, somnolence, and, occasionally, syncope.[56–58] The late symptoms, in contrast, are mainly vasomotor and occur from 2 to 3 hours after eating. These include diaphoresis, weakness, dizziness, flushing, palpitations, and, usually, hypoglycemia.[56–58,60,61]

The pathophysiology of dumping syndrome is multifactorial. It seems to be related to alterations in gastric emptying, and its incidence and severity appear to be proportional to the rate of emptying.[56–58,60,61] The symptoms of early dumping are usually produced by the rapid emptying of hyperosmolar chyme into the small bowel. The osmotic effects of these foodstuffs drag large quantities of fluid from the intravascular space into the bowel, resulting in rapid small bowel distention and an increase in both the amplitude and the frequency of bowel contractions.[56,57,60,61] This bowel distention may be responsible for the gastrointestinal symptoms, such as diarrhea, bloating, and crampy abdominal pain. This sequestration of fluid into the bowel depletes circulating blood volume[60,61] and may be responsible for the vasomotor symptoms. The postprandial release of gut hormones is also enhanced,[56–58,60] and the release of enteroglucagon, glucose-dependent insulinotropic peptide, pancreatide polypeptide, vasoactive intestinal polypeptide, gastrin-releasing peptide, serotonin, bradykinin, motilin, and neurotensin is higher in patients with dumping syndrome than in asymptomatic patients after gastric surgery.[61]

Late dumping symptoms seem to be related to the development of hypoglycemia.[56–58,60,62] It has been suggested that the rapid gastric emptying results in the delivery of unusually high concentrations of carbohydrates to the small bowel, leading to hyperglycemia and to an exuberant post-

prandial insulin release.[58,60] This insulin release results in late hypoglycemia, which leads to vasomotor symptoms.[58,61] The hypoglycemia may persist after the disappearance of the circulating insulin, suggesting that the counterregulatory response to low blood sugar may also be inadequate.[56–58,60]

The reactive hypoglycemia that is observed is probably related to a continuing cellular glucose uptake after insulin has been cleared from the circulation.[58,60] Once the hypoglycemia has developed, spontaneous corrections do not generally occur, particularly in children.[58] Rivkees and Crawford showed that the glucagon levels did not increase in response to hypoglycemia during challenge tests, and their data suggest that the counterregulation was disturbed primarily because of the blunted response of glucagon.[58] This lack of glucagon response may be related to the release of other incretin hormones, such as glucagon-like peptide, which has been shown to be elevated in patients with dumping syndrome. The reactive hypoglycemia may also be related to the ingestion of other dietary components, such as protein.[60]

As can be appreciated, the symptoms of dumping syndrome are nonspecific. The diagnosis has to be suspected in patients who have had a Nissen fundoplication and present with any of the gastrointestinal or vasomotor complaints mentioned above (see Table 77.1-2). The possibility of late hypoglycemia has to be considered, and direct questions about the presence of diaphoresis, irritability, or lethargy need to be asked. In those patients who have a G tube, its position needs to be determined because if the tube has migrated into the duodenum, the patient may present with a dumping-like picture that is related to the administration of the feeds directly into the duodenum.[60] Because of the nonspecific nature of the symptoms, and particularly because dumping can present like other complications mentioned in this chapter, the workup has to include a UGI to evaluate the anatomy and the status of the fundoplication, as well as to establish if a pyloroplasty was performed (see Figure 77.1-2B). The measurement of gastric emptying is very useful.[56] A gastric emptying scan will show a rapid gastric emptying time, although it is worth mentioning that it may be normal if the test meal is of insufficient volume to reproduce the patient's symptoms.[56,59,67]

The measurement of serum glucose in the first hour after meals will usually reveal the presence of hyperglycemia and serves as a good screening measurement. The presence of late hypoglycemia is also an indicator that the patient may be suffering from "late dumping." The diagnosis can be made accurately by using a glucose tolerance test (see Figure 77.1-2B).[56–60,62] Ideally, it can be combined with simultaneous measurements of insulin, so the presence of the hypoglycemia can then be correlated to the insulin levels.

Once the diagnosis has been made, treatment needs to be instituted. Dietary manipulation is the mainstay of therapy and the most effective way to control the symptoms and avoid the late hypoglycemia.[56] When the symptoms are not very severe, it is recommended that the patients eat small frequent "dry" meals and avoid simple sugars. It has also been suggested to add fiber and to increase complex carbohydrates (such as raw vegetables), dietary proteins (such as fish and chicken), and fat (to gain more calories and to decrease gastric emptying).[56,57,59–62] In children, particularly those with neurologic problems and an inability to eat complex meals and those who are fed mainly liquid diets through a gastrostomy tube, the dietary therapy is much more complicated.[60,62] Attempts to reduce the volume of the feeding, either by continuous infusion or by more frequent feedings, should be done first and can be successful in some patients.[58,60] At times, it is necessary to change the feeding regimen and give the infusions over 24 hours because the dumping may reappear if the feeding volume is increased to give only nightly feedings. Taking into account the proposed physiopathology for the occurrence of the delayed hypoglycemia, attempts to reduce the hyperinsulinism have been undertaken.[60] This has been successfully done with the use of formulas with added uncooked cornstarch, which permits the delivery of small amounts of glucose at a steady rate over a long period of time.[60,62,65] Gitzelmann and Hirsig compared the effects of the administration of a formula with cooked or uncooked starch in two infants with dumping and showed that only the uncooked starch controlled the late hypoglycemia and dumping syndrome.[65] Usually, the formula used has to contain the lowest amount of refined carbohydrate, and the uncooked corn starch is added to provide the equivalent to hepatic glucose production,[68] the same way it is added in patients with glycogen storage disease. The use of uncooked cornstarch usually allows the patient to be bolus fed, avoiding the initial hyperglycemia and the delayed hypoglycemia.[60,62,65,69] Other dietary additives, such as pectin, guar gum, and glucomannan, have also been tried.[60,61]

In an effort to reduce late hypoglycemia, acarbose, an α-glucosidase inhibitor, has been used.[57,70] Acarbose delays the conversion of oligosaccharides to monosaccharides and attenuates postprandial increases in blood glucose.[57,70] Ng and colleagues reported on the successful use of acarbose for the treatment of hypoglycemia.[57] They reported six children in whom acarbose was started at 12.5 mg and increased upward until hypoglycemia was controlled. The final dose ranged from 12.5 to 50 mg per feeding. The only side effect was flatulence, but other side effects can include diarrhea and abdominal distention.[57] It is recommended that if acarbose will be used, liver function tests need to be monitored because there have been reports of elevated enzymes during its administration.[57]

Because the physiopathology of dumping is multifactorial, it would be simplistic to think that if one deals only with the glucose homeostasis problems, most of the symptoms, particularly of early dumping, will be controlled. These are probably more related to the duodenal distention and to the gut hormone production mentioned above. Recently, octreotide acetate (Sandoz, East Hanover, NJ), a long-acting somatostatin analogue, has been used with some success in adults with severe dumping syndrome.[61,71] It probably acts by slowing gastric emptying, inhibiting insulin release, and decreasing enteric peptide secretion.[61,71] Several anecdotal reports and four controlled randomized trials have documented the short-term

efficacy of octreotide treatment in patients with severe dumping syndrome.[61,71] In general, octreotide improves the symptoms in more than 90% of patients with severe symptoms. In all studies, the acute administration significantly reduced the symptoms and the scores, but it is unclear if the chronic administration will prove to be as beneficial as the acute therapy. No pediatric experience is available in dumping syndrome, but octreotide has been safely used for the treatment of other pediatric conditions, particularly gastrointestinal bleeding.[72]

If the symptoms are severe and intractable, a surgical option may be considered. Many different options have been designed, including procedures to decrease gastric emptying, such as a reconstruction of the pyloroplasty, or if the patient has had a gastroenteroanastomosis, a reduction of the size of the stoma.

REOPERATION FOR FAILED PRIMARY ANTIREFLUX REPAIRS

The selection of patients who need further surgical therapy remains a challenging problem.[2,6,46] Reoperative antireflux surgery is complicated and difficult and should be preceded by a complete investigation to ensure that symptom interpretation is correct and that no other coexistent abnormality in gastric emptying or antroduodenal or esophageal motility exists (see Figure 77.1-2). Preoperative investigation should include UGI, endoscopy, gastric emptying analysis, 24-hour pH probe, and esophageal motility. If a more diffuse motility problem is suspected, an antroduodenal motility test should also be performed, and a glucose tolerance test should be undertaken if dumping is a possibility. An assessment of oral pharyngeal coordination and the ability to swallow needs to be done, and a trial of jejunal feeds should be initiated when it is not clear if the symptoms are related only to GER or also to an inability to handle oral secretions. Frequently, it is beneficial to use jejunal feedings while the patient is being evaluated, and in some children, the use of gastrojejunal tubes may be a good long-term option.[46] Different authors have reported that a second operation is required from 1.6 to 12% in children without other underlying problems,[13,31,46,73] although in neurologically impaired children, the incidence of reoperation has been as high as 16%.[31,73] In a series of 7,467 children, Fonkalsrud and colleagues reported a reoperation rate in 3.6% (2–10%) of neurologically normal children and 11.8% (6–24%) in those with neurologic impairment.[2]

The results of a second operation will also vary depending on the underlying problem. Studies have shown that the increase in preoperative complications, including complete vagotomy, is significantly increased during the second procedure.[38] In children, the incidence of postoperative complications after reoperation also seems to be higher. Caniano and colleagues reported that a second operation was associated with a 14% incidence of intraoperative complications and a 43% incidence of postoperative morbidity.[31] The main complications were prolonged ileus, pneumonia, small bowel obstruction (19%), wound infection, and pneumothorax, and intraoperative blood loss was substantially higher. Most authors report that for children who have undergone a second operation, the results have also been satisfactory from 70 to 80%.[2,13]

EVALUATION OF PATIENTS WITH PROBLEMS AFTER A FUNDOPLICATION

Table 77.1-2 presents the different possibilities that the clinician needs to entertain when confronted with a patient who has problems after fundoplication. Figure 77.1-2 shows an algorithm for the evaluation and treatment of children who present with complications after fundoplication.

Briefly, after a careful analysis of the symptoms, the workup can start with the performance of a UGI. As shown in Figure 77.1-2A, if there is evidence that the wrap is too tight and that there may be a functional obstruction to esophageal emptying, an endoscopy should be performed to see if there is a peptic stricture or an extrinsic compression. If there is no evidence of stricture, an esophageal motility should be performed to assess the possibility of a primary motility disorder. If after the UGI the wrap is intact and there does not seem to be a functional obstruction, the workup needs to proceed, depending on the main symptoms. If the symptoms are mainly those compatible with recurrence of GER, an endoscopy and pH probe or esophageal impedance should be performed (see Figure 77.1-2A). If, on the other hand, the main symptoms are related to gas bloat or possible dumping, gastric emptying should be performed first (see Figure 77.1-2B).

As shown in the algorithm, if there is evidence of GER, medical therapy should be undertaken, and if there is no response, a new operation should be considered. On the other hand, when evaluating for gas bloat (see Figure 77.1-2B), if the gastric emptying is delayed, a prokinetic agent should be tried. If there is no response, a pyloroplasty can be considered, but a small bowel motility study should be performed first to exclude the presence of pseudo-obstruction. If there is evidence of rapid emptying, the most likely diagnosis is dumping syndrome, and a glucose tolerance test should be done (see Figure 77.1-2B).

As can be appreciated in Figure 77.1-2B, if after the UGI there is evidence that the wrap has either slipped or there is evidence of a hernia, further evaluation for GER or the presence of complications should be undertaken, and if there is evidence of recurrence, bleeding, or mucosal compromise, a reoperation should be performed. In all cases, the administration of jejunal feedings needs to be carefully considered as an alternative to a reoperation, particularly in those children with severe esophageal dysmotility, high surgical risk, or chronic aspiration of their own oral secretions.

HIRSCHSPRUNG DISEASE

Hirschsprung disease is a congenital illness in which varying degrees of aganglionosis occur in distal segments of the intestinal tract.[74] Because these abnormal segments are unable to relax during peristalsis, they are spastic and pro-

duce mechanical obstruction.[75–77] The treatment of Hirschsprung disease is surgical.[78–81] The basic principle for definitive surgical therapy is resection of the aganglionic segment followed by a pull-through of ganglionic bowel down to the anus.[75,82] Surgery for Hirschsprung disease generally results in a satisfactory outcome,[79,82,83] and it has been suggested that the outcome after each commonly performed procedure is comparable.[79,82,83] There are, however, some patients who continue to have long-term difficulties (Table 77.1-3). Some studies have suggested that the prevalence of problems is much higher than previously anticipated.[84–87] The most common symptoms are diarrhea, constipation, and, sometimes, intermittent colitis (see Table 77.1-3).[84,87–89]

The most commonly used operations include the Swenson (rectosigmoidectomy), Duhamel (retrorectal transanal pull-through), and Soave (endorectal pull-through) (Figure 77.1-3). In the Swenson pull-through (rectosigmoidectomy), the rectum is removed and the normal ganglionic bowel is anastomosed to a 1 to 2 cm rectal cuff.[76,90,91] It requires a combined abdominoperineal approach, is probably the most difficult, and requires extensive pelvic dissection, so injury to the sacral innervation of the bladder and ejaculatory mechanisms is possible (see Figure 77.1-3). In the Duhamel (retrorectal transanal pull-through) procedure, the aganglionic rectum is left in place, and normal ganglionic bowel is pulled down behind the rectum and through an incision in the posterior rectal wall at the level of the internal sphincter.[92–94] The original Duhamel procedure was an anastomosis of the ganglionated proximal bowel to the closed native rectum at the anal verge. Dilatation of the defunctioned rectum by fecal retention in the blind loop led to the Martin modification, which added a proximal suture anastomosis of anterior native rectum to the pulled-through colon, following which the septum was crushed by a spur clamp.[82] A rectum of expanded size with an anterior aganglionic wall and a posterior ganglionic wall is therefore created.[82] This operation eliminates the need for much of the pelvic dissection needed in the Swenson procedure (see Figure 77.1-3).[94] The endorectal pull-through, as originally described by Soave and modified by Bole, is the third alternative.[82] In the modified Soave procedure, there is no need to do any pelvic dissection. In this procedure, the mucosal lining of the rectum is removed, the ganglionic colon is pulled through the rectal muscular tube, and a primary anastomosis is done within 1 cm of the anal verge (see Figure 77.1-3).[82,95,96] The modified Soave procedure is easy to perform, and with it there is no need to do any pelvic dissection.

The outcome after these procedures is similar. It is difficult to compare the results obtained with the different operations because they have usually been done by different surgeons, in different institutions, and at different times, and it is possible that the incidence of complications after the different procedures is closely related to the skill of the individual surgeon.[89] There are some reports in which the experience with individual operations performed by the same surgeon have been reported, and these are useful to obtain an idea of the type of long-term complications that

TABLE 77.1-3 COMMON SYMPTOMS AFTER REPAIR OF HIRSCHSPRUNG DISEASE

OBSTRUCTION
Anatomic
Anal stenosis
Functional
Residual aganglionosis
New aganglionosis
Neuronal intestinal dysplasia
Dysmotility
FECAL INCONTINENCE
Overflow incontinence from constipation
Abnormal sphincteric function after surgery
Diarrhea
ENTERCOLITIS
Bacterial
Clostridium difficile
Other

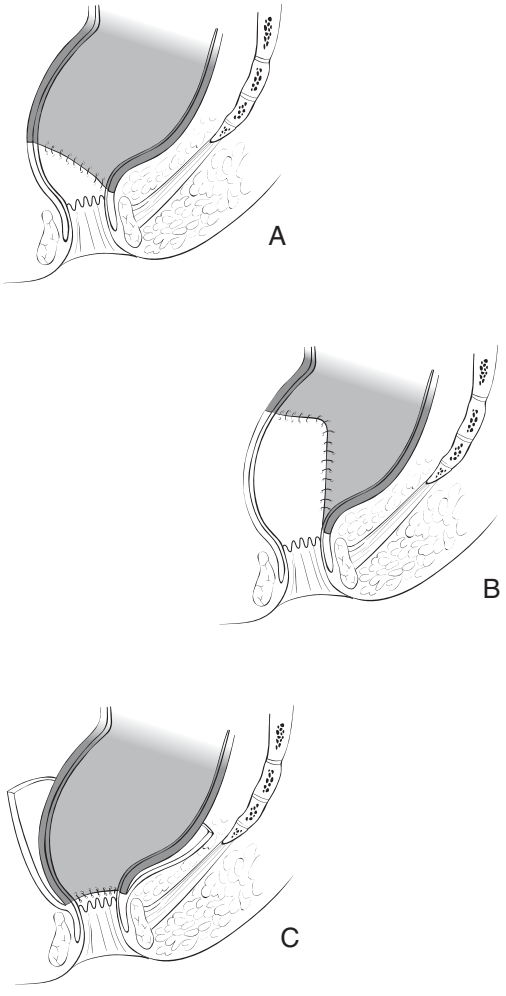


FIGURE 77.1-3 Graphic representation in lateral view of the three major operative procedures for Hirschsprung disease. *A*, Swenson; *B*, Duhamel/Martin; *C*, Soave/Boley. The unshaded native rectum is aganglionic, and the shaded pulled-through bowel contains ganglion cell. Adapted from Philippart AI.[82]

can be seen with them. In a large multicenter report of 880 Swenson procedures that spanned four decades, it was reported that there was a 1% mortality, with a 6% incidence of an anastomotic leak and a late incidence of strictures of 8%.[97] Soiling was significant in 13%, and in 39%, enterocolitis was observed, leading to a 7% incidence of secondary sphincterotomies. They reported that 20 years after the operation, 90% had satisfactory bowel function.

There are several studies that describe the long-term results after Duhamel.[98–99] Ehrenpreis and colleagues reported their long-term experience after the Duhamel procedure.[98,99] The overall mortality rate in 352 operations was 2.8%, of which the late mortality was 1.1%. The most common postoperative complication was fecaloma formation, particularly in the early periods after the operation. In their first follow-up report, they found a fecaloma in 9 of 30 patients and fecal incontinence in 12 of 30 patients.[98] On long-term follow-up, however, 15 years later, fecaloma formation was not a significant problem (1/10), and fecal incontinence had decreased to 2 of 30 patients.[74] These findings suggest that over time, patients who undergo the Duhamel procedure tend to improve. In a recent long-term study, 91 children post–Duhamel procedure were compared with 22 healthy children. Outcome scores were significantly worse in the Hirschsprung disease group, and only 42% of patients overall and 79% of those above 14 years had a satisfactory outcome.[100] Some have suggested that the Duhamel procedure is associated with less risk of overall complications but may have a greater risk for postoperative enterocolitis.[92]

Tariq and colleagues described their long-term experience after the Soave procedure.[96] They described the follow-up of 53 survivors, and 18% had diarrhea with intermittent incontinence and 9.4% required a second pull-through, in this case a Duhamel procedure. Other series have described that up to 22% of the patients suffered from constipation at 3 years, with 18% with diarrhea and incontinence, although 82% had a satisfactory result.[101] After endorectal pull-through, it has also been reported that anastomotic stenosis occurs with an incidence that varies from 9 to 24%[96,101,102] and that even after dilatation, a mild residual stricture persists in 3%.[101]

As can be appreciated, it is difficult to directly compare the results of various operations. There are few truly comparative studies. Probably the largest compilation of patients, 1,196 children, and comparison between treatments were reported by Kleinhaus and colleagues.[89] They obtained information in an extensive survey of the members of the surgical section of the American Academy of Pediatrics. They reported results in 390 patients after the Swenson procedure, 339 patients after the Duhamel procedure, and 187 patients after the modified Soave procedure. Complications after the procedures included a disrupted anastomosis in 11.2% after the Swenson, 2.4% after the Duhamel, and 5.8% after the Soave procedure. This carried a mortality of 11% after the Swenson procedure, and 27% of patients with disrupted anastomosis after the Swenson procedure required a major surgical procedure. Anal stenosis was mild (requiring only dilatation) in 5.2% after the Swenson, 2.9% after the Duhamel, and 5.2% after the Soave procedure and severe (requiring reoperation) in 4.3%, 2.6%, and 4.2%, respectively. After the Swenson procedure, there was a 15.6% incidence of postoperative enterocolitis and 3.2% of incontinence compared with 5.9% and 1.1% after the Duhamel procedure and 2.1% and 1.1% after the Soave procedure.

Most studies report short-term follow-up, and there are few long-term studies. Long-term survival is excellent, although sudden death from enterocolitis may occur years after successful surgical reconstruction.[103,104] Most series describe an overall good outcome in more than 90% of the patients, but many recent studies have shown a higher than anticipated incidence of problems, particularly persistent obstruction, fecal incontinence, or enterocolitis (see Table 77.1-3),[75,79,84,105] and they seem to indicate that any operation is associated with long-term morbidity.[100,106,107] In a series that followed patients from 1 to 30 years, Mishalany and Wooley reported the follow-up of 62 patients, of whom 14 had a Duhamel, 15 a Swenson, and 33 a Soave pull-through.[88] Approximately 23 to 50% felt that they had normal bowel movements, and the rest had various degrees of problems in defecation. Subjectively, half of the patients in the Duhamel and Soave groups and one-third in the Swenson group considered their stooling pattern normal. Of the whole group, 18 had one bowel movement per day, 15 had one every other or more days, and 29 had an increased frequency ranging from 2 to 7 per day. In 20 patients, there was evidence of postoperative enterocolitis, regardless of the type of operation (3 after the Duhamel, 7 after the Swenson, and 11 after the Soave procedure). Another study of the long-term quality of life in 178 patients showed that 76% had between one and five stools a day and 58% had normal stool consistency.[79] Full fecal continence was present in 75%, whereas 19% had minor degrees of leakage, and the rest had severe problems. The degree of stool control improved with age. Whereas 52% were fully continent below 4 years of age, 87.5% between age 9 and 12 years had full control. Neurologic impairment and length of the agangliotic segment beyond the rectosigmoid appeared to influence functional outcome. Enterocolitis was present in 16% at presentation and in 6% postoperatively. The long-term functional results were comparable for the Soave and Duhamel procedures, but children after the Swenson procedure had less favorable results. Growth was similar to the normal population, although younger patients had a tendency to be smaller. Delayed developmental milestones were present in 8%. Satisfactory school performance was achieved in 74%. Ninety-four percent of the patients appeared to be well adjusted, and five patients had severe behavioral problems. After the Soave procedure, there was a lower incidence of constipation, sexual dysfunction, and micturition problems. Duhamel patients tended to have more constipation, whereas the Swenson procedure was associated with more abdominal distention, micturition problems, and cuff strictures.

In another long-term study of 19 adolescents, it was found that 32% had significant impairment of continence

but no more psychopathology or psychosocial dysfunction when compared with healthy controls. Fecal incontinence was associated with poorer psychosocial functioning and parental criticism, and psychosocial functioning was significantly correlated with the degree of fecal incontinence.[106] Another long-term follow-up of 45 patients after the Swenson procedure showed that 51.1% had bowel dysfunction, with 37.8% suffering from fecal soiling. Because of poor fecal continence, 55.7% had to restrict their foods, 13.3% had school absence, and 15.6% had problems with peer relationships.[84]

COMMON PROBLEMS FOUND AFTER SURGICAL TREATMENT FOR HIRSCHSPRUNG DISEASE

Obstructive Symptoms

From the postoperative symptoms that can be found in children who have undergone surgical treatment for Hirschsprung disease, the presence of recurrent obstruction is one of the most common and difficult to manage (see Table 77.1-3).[75] Obstructive symptoms may be related either to an anatomic problem that is producing an obstruction[108] or to functional alterations (Figure 77.1-4).[79,109]

The most common anatomic problem that can be encountered is the presence of anal stenosis.[79,89] This complication seems to be more common after Soave pull-through and can usually be managed only with a dilatation program, although a secondary surgical procedure may be necessary.[89] Kleinhaus and colleagues reported that anal stenosis occurred in 5.2% after the Swenson procedure, 2.9% after the Duhamel procedure, and 19% after the Soave procedure.[89] The incidence of reoperation because of the presence of stenosis also varied between techniques, being 4.3%, 2.6%, and 5.2% after the Swenson, Duhamel, and Soave procedures, respectively. Postoperative strictures can also occur at other sites, particularly in the segment that has been pulled through. These are most likely related to ischemic events; they respond rarely to dilatation and usually require surgical correction.

Most patients with obstructive symptoms do not have stenosis. A variety of functional problems that can be found are related to the residual abnormal function of the intestine after surgical correction (see Figure 77.1-4).[79,106] Moore and colleagues found that of 107 patients followed for at least 4 years, 14.9% had recurrent episodes of gaseous distention and symptoms suggestive of persisting obstruction in the absence of an anatomically defined problem.[83]

The first consideration must be that the patient continues to have the presence of residual aganglionosis because of an inadequate initial repair. The exact incidence of this complication is difficult to establish because it depends on the surgeon and the surgical technique. Soave found that in 5 of 271 patients, the aganglionic segment was not completely removed proximally at the time of the initial operation.[102] This possibility needs to be excluded early in the evaluation of these children, and a barium enema will show if there is evidence of a transition zone. Often it is necessary to perform rectal biopsies to establish the presence of ganglion cells. It is important to remember that in some corrective surgeries, such as the Duhamel, a piece of aganglionic segment is always left as part of the surgical technique, so I recommend obtaining biopsies in all four quadrants. It can therefore be appreciated that the decision about what to do if there are no ganglion cells in the biopsies depends on the type of initial operation that the patient

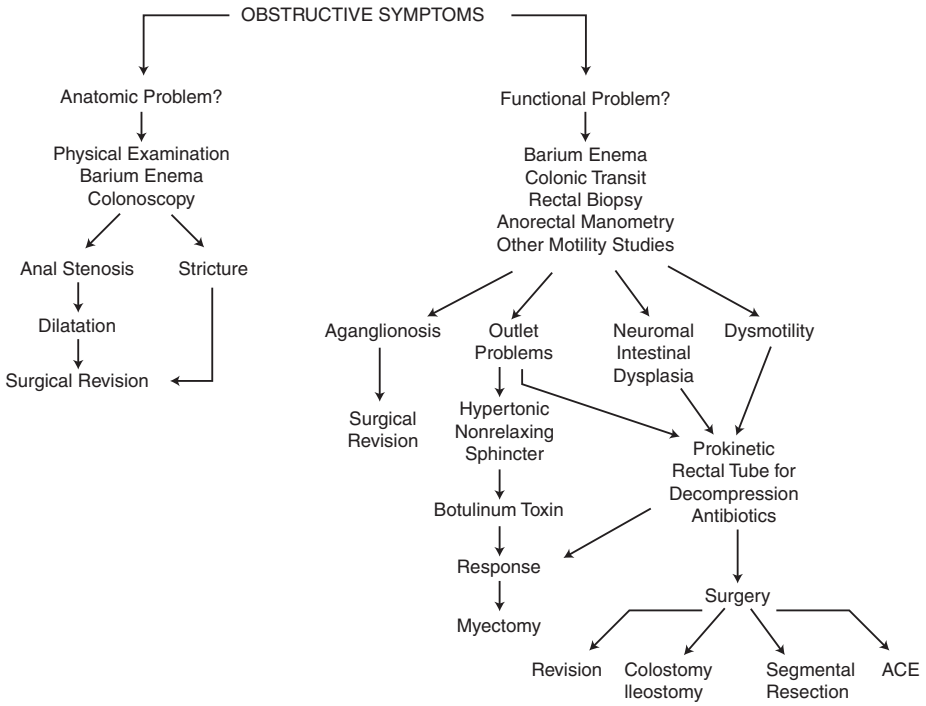


FIGURE 77.1-4 Algorithm for the evaluation and treatment of patients with obstructive symptoms after surgical repair for Hirschsprung disease. ACE = antegrade colonic enemas.

underwent. If there is evidence that a full aganglionic segment was left behind, the treatment needs to be surgical.

The finding of acquired or secondary aganglionosis following pull-through procedures is a rare occurrence, but it has been well described.[83,110] The patients described have developed obstructive symptoms that on evaluation were found to have aganglionosis in a pulled-through bowel that had previously been found to have ganglion cells. This complication has been reported following any of the different corrective procedures,[110] and it should be considered in any patient operated on for Hirschsprung disease in whom recurrent or obstructive symptoms persist. Multiple theories have been proposed for this development, but most authors believe that it is secondary to an increased susceptibility of neural tissues (including the plexuses of ganglion cells) to a hypoxic insult.[83,110] An alternative explanation is that there is postoperative cell death caused by the pull-through of abnormally innervated bowel (eg, the transitional zone or neuronal intestinal dysplasia [NID]).[109] If aganglionosis is suspected, a barium enema may show a transition zone, and rectal suction biopsies at different levels will be necessary to confirm it.

Some reports have suggested that NID type B may be present in more than 20% of cases with Hirschsprung disease.[83,109,111–114] Anorectal manometry is not useful in the diagnosis of NID,[115,116] so a full-thickness rectal biopsy is necessary.[111] The value of a suction rectal biopsy for the diagnosis of NID is controversial, and recent studies have shown that they may not be useful in its recognition.[111,117] Furthermore, there is still controversy regarding the significance of NID or if it truly represents a distinct clinicopathologic entity.[111,114,117] Of 47 cases of Hirschsprung disease, Hanimann and colleagues reported that 11 patients (23%) had associated NID and that after a mean follow-up of 5 years, there were no differences in the symptoms when comparing patients with NID with those without it.[112] Recent studies have reported that histologic criteria were not helpful in predicting clinical outcome and suggested that the finding of NID should not influence clinical management.[111,117]

Independent of the controversy, it is clear that some children may have abnormalities in the histologic examination of the residual colon and that these histologic abnormalities may be associated with symptoms.[113,117,118] Therefore, in children with obstructive symptoms after operation, it is advisable to perform a full-thickness rectal biopsy and to stain it with acetylcholinesterase or other special stainings. In these children, conservative management is indicated.[111,115,116] However, if symptoms are severe and dysmotility is clearly associated with the abnormal segment, surgical resection may be necessary.[118,119]

Persistent internal anal sphincter (IAS) dysfunction is another reason for the obstructive symptoms. This dysfunction is sometimes referred to as "internal sphincter achalasia"[120] and is related to specific abnormalities in IAS innervation. Some studies suggest that there is an intrinsic problem in the IAS in which there is an inability to respond to nitric oxide.[77] The above finding, namely that nitric oxide fails to relax the IAS of patients with Hirschsprung disease, suggests that a primary defect within or around the myocytes of the sphincter and a separate abnormality of the IAS is compatible with some of the long-term obstructive symptoms that are frequently seen.[77,79,86,120,121] It is possible that the IAS pressure produces a functional outflow obstruction that, with time, leads to colonic dilatation and a less efficient peristalsis to expel stool.[83,120,122] The persistent chronic obstruction from the IAS may also lead to recurrent enterocolitis or bacterial overgrowth with stasis.[83,120,122] It is sometimes difficult to establish who are the patients in whom the symptoms are related to IAS problems. In one study in which children with obstructive symptoms were compared with those without symptoms, IAS pressure and other manometric findings were the same.[83] In some patients with obstructive symptoms, however, the high-pressure zone was longer, the sphincter pressure was higher, and there was evidence of prolonged transit times.[83] Because of these sphincteric abnormalities, some authors have suggested that an internal sphincter myotomy or myectomy needs to be performed in those patients with postoperative obstructive symptoms that have not responded to medical management.[100,22,123] Myectomy has been shown to significantly decrease IAS pressure.[123] Even though the initial experience reported a poor response after myectomy,[96] recent reports have shown it to be useful in the treatment of these children.[122,123] Sphincter-dividing procedures are not always effective; they affect the sphincter permanently and can rarely be associated with fecal incontinence. There is now growing experience that the injection of intrasphincteric botulinum toxin (Botox) can be used to temporarily decrease the pressure of the sphincter.[86,120] The mechanism of action of *Clostridium botulinum* toxin has been studied extensively.[86] Botulinum toxin has been used extensively in children with skeletal muscle problems and recently in the treatment of achalasia[124] and patients with chronic anal fissures.[125] Because it weakens the muscle in a transient fashion, it could be used to produce a "medical" myectomy, therefore allowing the evaluation of sphincter pressure reduction without permanent sphincter destruction.[86,120] In a prospective study of 18 children who were injected with botulinum toxin, the authors reported that 4 had no improvement in bowel function, 2 had improvement for less than 1 month, 7 had improvement for 1 to 6 months, and 5 had improvement more than 6 months. Nine of those with symptomatic improvement longer than 1 month had sphincter pressures measured, with a documented decrease in eight. Five with no significant clinical improvement had pressure measurements, with a decrease in three. There were no adverse effects associated with botulinum toxin injection. Four children had new encopresis postinjection, which was mild and resolved in each case. Repeated injections were often necessary.[86,120] The botulinum toxin injection seems to be safe, and no major side effects have been reported when it is used in skeletal muscle or the lower esophageal sphincter. Therefore, it is possible that botulinum toxin is useful as a way of predicting which children may benefit from myectomy or as a therapeutic modality in selected patients, in particular those with recurrent enterocolitis or intractable obstructive symptoms.

The obstructive symptoms may also be related to abnormal upper gastrointestinal motility.[87,126,127] It has been shown that years after the surgical resection, children with Hirschsprung disease can have evidence of esophageal, gastric, or small bowel dysfunction, suggesting that children with Hirschsprung disease may have underlying motility disorders in segments not involved by aganglionosis.[87,126–128]

The establishment of the cause of obstruction can usually be accomplished with the use of simple tests (see Figure 77.1-4). As can be appreciated, the treatment will depend on the etiology (see Figure 77.1-4). In general, and while the workup is in progress, close attention to rectal decompression and treatment of symptoms suggestive of enterocolitis are necessary. A barium enema will help define the anatomy and the appearance of the pull-through and determine if there is a stricture, a new transition zone, or a megacolon. Radiopaque transit studies will detect delays. A rectal biopsy with acetylcholinesterase staining will allow the detection of residual or new aganglionosis or neuronal dysplasia, and anorectal and colonic manometry will allow further assessment of motor function.[128,129]

Using the techniques mentioned above to evaluate patients with obstructive symptoms, Moore and colleagues found that of 107 patients followed at least 4 years, 14.9% had recurrent episodes of gaseous distention and symptoms suggestive of persisting obstruction, in the absence of an anatomically defined problem, and they all had evidence of radiologic megacolon and delayed colonic transit.[83] The reason for this delay was related not only to findings in anorectal function but also probably to abnormal or residual disease in the intestinal wall. Anorectal manometry detected four patients in whom the high-pressure zone was too long, and they all responded to myectomy. They performed histologic evaluation of rectal biopsies and found that in 56% of the patients with obstructive symptoms, there were changes compatible with NID,[109,118] and in another 25% of patients, they detected postoperative aganglionosis. The authors concluded that an aggressive and systematic approach for the evaluation of patients with obstructive symptoms allows the determination of the etiology in most patients and therefore guides their treatment. Recently, in an effort to define the physiology underlying the persistent symptoms in children with Hirschsprung disease, Di Lorenzo and colleagues suggested that colonic motility helps explain the pathophysiology and direct the treatment.[128] They studied 46 symptomatic patients and identified 4 motility patterns: (1) high-amplitude propagated contractions (HAPCs) migrating through the neorectum to the anal sphincter, associated with fecal soiling in 18 patients; (2) normal colonic motility associated with retentive posturing in 9 patients; (3) absence of HAPCs or persistent simultaneous contractions over two or more recording sites in 15 patients; and (4) normal colonic motility and a hypertensive anal sphincter (80 mm Hg) in 4 patients. As can be appreciated, the treatment of obstructive symptoms depends on the etiology (see Figure 77.1-4). If there is an anatomic problem (eg, a stricture), it needs to be corrected. If there is no evidence of anatomic problems and the colonic transit study and anorectal manometry indicate that the obstruction is distal, aganglionosis needs to be considered.[83,102] Biopsies of the distal anastomotic site need to be performed. If aganglionosis is present, the aganglionic segment needs to be removed, or if it is short, a myectomy may be considered.[83] If the patient has neuronal dysplasia, I recommend the use of a prokinetic agent, and I and others have had good results with cisapride.[83] If the anorectal manometry indicates a nonrelaxing sphincter, suggesting that the obstruction is at the level of the IAS, steps to decrease sphincter pressure need to be undertaken.[83] A trial of botulinum toxin injection should be undertaken.[86,120] Repeated injections may be necessary. If the injection of botulinum toxin fails, a myectomy needs to be considered.[130] If there is evidence of aganglionosis, the segment needs to be resected.[130,131] At times, it may be necessary to perform a new pull-through procedure, particularly if there is residual aganglionosis, stricture, or a fistula or if the pull-through anastomosis is too high.[130] In one series, 23 of 68 patients after a Soave procedure and 15 of 39 after a Duhamel procedure required a reoperation. For unclear reasons in this series, the incidence of reoperation is very high, but, overall, 90% were cured after the reoperation.[130] In another report of 22 reoperations followed for a mean 6.5 years, 75% showed good results.[132]

Finally, it may also be necessary to resect dysfunctional bowel.[128] It has been postulated that colonic motility allows the detection of the abnormal segments guiding the surgical intervention.[128] In their series, 15 patients with abnormal colonic motility underwent colonic resection of the abnormal segment with good results. Long-term follow-up and larger studies are needed before the exact role that colonic resections will play in the management of these patients is defined. Finally, for patients with severe problems, it may be necessary at times to create a colostomy or ileostomy.

In recent years, antegrade colonic enemas (ACEs) have been developed as an effective way of treating constipation and fecal incontinence in different conditions, including Hirschsprung disease.[133–135] The ACE procedure produces a continent conduit from the skin to the cecum that can be catheterized for self-administration of enemas.[135–139] In short-term studies, the ACE has been shown to be very effective in children with Hirschsprung disease in whom a permanent colostomy was being considered for the treatment of refractory constipation or incontinence.[135,136] Data for long-term follow-up are not yet available, and close follow-up is needed because in some patients, the enemas may not give the desired results because they are working against a hypertonic, nonrelaxing sphincter.

Fecal Incontinence

Another common problem that may be encountered is fecal incontinence in the absence of fecal impaction (see Table 77.1-3).[84,105,140] Even though long-term studies usually report fecal incontinence in 2.5 to 13% of patients,[75,104,140] a study designed specifically to establish the extent of incontinence found it in 80% of patients.[105] In 53%, it was significant, and in 27%, it was less severe. Contrary to other

reports, the incontinence did not diminish with increasing age.[89] The mean age of the patients was 10.1 ± 3.6 years, and there were no differences in the age at the definitive surgery, sex, extent of aganglionosis, type of surgery, or early or late postoperative complications when comparing those with or without incontinence. The survey of the surgical section of the American Academy of Pediatrics reported that fecal incontinence occurred in 3.2% of patients after the Swenson procedure compared with 1.1% after Duhamel and 2.1% after Soave.[89] One long-term study has indicated that of 282 patients after the Swenson procedure, 90% had normal bowel habits, 3.2% had permanent soiling, and 2 patients had a permanent colostomy because of the incontinence,[90] whereas another study has found that in 185 cases after the Duhamel procedure, 8% had severe incontinence and 27% had used enemas in the past.[141] It has been reported that after the Soave procedure, 18% had diarrhea and incontinence, although 82% had a satisfactory result.[101] Mishalany and Wooley reported the follow-up of 62 patients after different procedures (14 Duhamel, 15 Swenson, and 33 Soave).[88] Approximately 23 to 50% felt that they had normal bowel movements, and the rest had various degrees of problems in defecation. Subjectively, half of the patients in the Duhamel and Soave groups and one-third in the Swenson group considered their stooling pattern normal. Approximately 50% were not totally continent. Half of the Duhamel, one-third of the Swenson, and slightly more than half of the Soave group considered themselves completely continent, and the incontinence ranged from moderate soiling several times a day in 28 patients to total incontinence in 3 patients.

The physiopathology of the incontinence not associated with constipation is not well understood. By doing anorectal manometry, Mishalany and Wooley found that 10% of patients were not able to increase external sphincter contraction and that 50% after the Duhamel or the Swenson procedure and 30% after the Soave procedure experienced an inability to have rectal sensation.[88] Both of those abnormalities have been shown in other populations to be associated with fecal incontinence.[142,143] In a study of 54 adult patients (mean age 29 years), there was a positive correlation between functional outcome and anal resting pressure. The low resting pressure reflects IAS dysfunction, which may be caused by operative trauma.[140] Other abnormalities that have been associated with incontinence in Hirschsprung disease patients are related to the ability of the IAS to relax after balloon distention. The presence of the rectoanal inhibitory reflex has been variable; it is usually absent and in most studies does not seem to be correlated with outcome.[83,88] Recently, Di Lorenzo and colleagues suggested that fecal incontinence can result from the presence of HAPCs that migrate all the way through the neorectum to the anal sphincter.[128] In normal patients, the HAPCs stop in the sigmoid, never reaching the rectum. In their series, 18 children had fecal soiling that occurred when an HAPC was present and migrated all the way down through the neorectum.

Independent of the physiopathology, the treatment of the fecal incontinence is complex. The main objective is to produce social continence. Biofeedback has been used successfully in these children and has focused on trying to improve sensation abnormalities and increase muscle strength. Other treatments that have been used have included the use of enemas, the bowel management tube,[144] or, recently, ACEs.[145]

When evaluating patients with fecal incontinence, the first thing that needs to be established is if this is related to overflow incontinence and encopresis or abnormalities in anorectal or colonic function. The use of physical examination, colonic transit studies, abdominal radiography, and particularly manometry will allow this differentiation. If constipation seems to be the cause, laxatives need to be initiated. On the other hand, if there is fecal incontinence not related to constipation, enemas to maintain the rectosigmoid empty, together with diet manipulation, and biofeedback therapy need to be instituted. In those patients in whom the HAPCs migrate all the way down through the neorectum, an anticholinergic agent or loperamide may be useful.[128] Particularly in patients with total colonic aganglionosis, the use of loperamide may be needed[146] at the same time as the use of rectal tubes allows complete evacuation.

Enterocolitis

Enterocolitis continues to be the major cause of both morbidity and mortality in Hirschsprung disease.[103,104,147–149] It has been shown that this can occur before or after surgical treatment[103,104,148,149] in 2 to 33% of patients, with a mortality that ranges from 0 to 30%.[75,147–150] In a retrospective review of 105 cases, enterocolitis occurred in 32%.[148,149] It has been suggested that postoperative development of this complication is the most reliable indicator of the successful relief of the low intestinal obstruction present in this patients.[89,148,149,151] In the survey of the surgical section of the American Academy of Pediatrics, it was reported to be present in 15% after Swenson repair, in 5.9% after Duhamel repair, and in 1 to 2% after a modified Soave procedure.[89] Klein and Phillipart reported that enterocolitis occurred a mean of 0.51 episodes per patient after the Swenson procedure, 1 episode per patient after the Duhamel procedure, and 0.21 episodes after the Soave procedure.[94] Enterocolitis tended to be more frequent in premature infants and patients with long-segment Hirschsprung disease. The usual clinical presentation consisted of fever, abdominal pain, and diarrhea. In a recent study of 168 patients with Hirschsprung disease, enterocolitis presented with abdominal distention in 83%, explosive diarrhea in 69%, vomiting in 51%, fever in 34%, lethargy in 27%, rectal bleeding in 5%, and colonic perforation in 2.5%.[150] Chronic diarrhea was present in 54% and delayed growth in 44%. The occurrence of explosive diarrhea in any patient with Hirschsprung disease should suggest the diagnosis, even in the absence of systemic symptoms.[103,104,147,149,150] The presence of postoperative enterocolitis needs to be recognized promptly because the child can present initially with mild symptoms that are followed by a rapid fulminating course that may lead to death.[103,104,147,149,150] The pathogenesis is not fully understood.[149,152] In a retrospective review of cases with postoper-

ative enterocolitis, the risk was significantly increased by mechanical factors related to anastomotic complications and intestinal obstruction.[148] In general, it appears that fecal stasis facilitates bacterial overgrowth and subsequent mucosal invasion. There may be an association between enterocolitis and the presence of *Clostridium difficile*,[149,151] although recent reports indicate that in up to two-thirds of patients, stool cultures are negative.[150] The enterocolitis secondary to *C. difficile* may be more fulminant,[103] with rapid progression, shock and prostration, and, eventually, death.[103,149,151] Other organisms that have been associated with enterocolitis include rotavirus, retrovirus, *Pseudomonas*, or *Escherichia coli*.

The diagnosis of enterocolitis needs to be suspected early. Appropriate stool tests for identification of *C. difficile* and stool cultures for other pathogens need to be obtained. The diagnosis may be helped by using abdominal radiographs, in which the presence of an intestinal cutoff sign and at least two air-fluid levels strongly suggests the diagnosis.[147,150] The barium enema usually shows colonic dilatation and modularity and speculation of the rectal mucosa, with significant narrowing.[147,150] Endoscopic examination will show colitis and will detect the presence of pseudomembranes. Conservative management in the hospital is usually necessary. The treatment of choice includes fluid and electrolyte support, antibiotics, and the use of transrectal decompression either by tube or by sphincter dilatation.[75,147,149,150,153] Antibiotics against *C. difficile* and bowel flora should be empirically started as soon as the appropriate cultures are obtained.[147,153] The rectal decompression may need to be accompanied with the use of saline irrigation to evacuate the retained stool and gas.[75,103,149,150,153] It has also been described that some patients have frequent relapses, and this is usually related to chronic obstruction from a nonrelaxing sphincter.[120,149] It has been suggested that in them, the chronic or intermittent use of metronidazole may be beneficial,[149] and the administration of botulinum toxin has been successful in the prevention of the recurrent episodes. Some authors have advocated the use of a myectomy.[149] If the problem persists, it may be necessary to consider a surgical approach, which may include a diverting ostomy.

Total Colonic Hirschsprung Disease

Patients with transition zones in the small bowel account for 5 to 10% in most large series.[154,155] The complications found in patients with total colonic Hirschsprung disease are more severe when compared with those with shorter segments.[154,155] Preoperative complications continue to plague these patients,[154,155] and their mortality continues to be high.[89,154,155] In the survey of the American Academy of Pediatrics, there was a mortality of 47% (42 of 90).[89] Improvements in parenteral and enteral nutrition, as well as in surgical techniques, have recently decreased it.[154,155]

The surgical therapy initially includes and ileostomy. Later an endorectal pull-through, along with total colectomy, and the long side-to-side anastomosis with aganglionic bowel are the most commonly used procedures.[154,155] In a recent review of the treatment of 48 patients, 6% had died early, and 85% underwent a pull-through (38 with the Duhamel procedure, with 13 having the Martin modification and 3 a Soave procedure).[155] Long-term follow-up was available in 27 patients, of whom 19 (70%) required additional surgical procedures. In six patients, a permanent stoma was necessary. Two patients who underwent the Martin modification required resection of the side-to-side anastomosis because of intractable diarrhea, and the authors concluded that there was no advantage to its performance. At 5-year follow-up, 82% had fecal incontinence, but by 10 and 15 years, the incontinence had decreased to 57% and 33%, respectively. At 15 years, over half of the patients were below the 2nd percentile for weight and one-quarter were below the second percentile for height.[155] Fecal incontinence is common, and failure to thrive and malnutrition have been described, as well as disturbances in electrolyte balance, lipid metabolism, and vitamin B_{12} absorption.[154] One of the main problems in these children is residual obstruction that leads to intestinal dilatation, diarrhea, and postoperative enterocolitis. In these patients, intermittent antibiotics and a rectal tube with irrigation need to be used chronically for decompression. If it is clear that the patient has a functional obstruction at the level of the sphincter, a procedure to relieve the obstruction may be indicated. Until recently, a myectomy was the procedure of choice, but the use of botulinum toxin is an alternative that will provide only a temporal effect.[120] These interventions need to be performed in patients who have been fully evaluated because fecal incontinence may result after the procedure. With aggressive follow-up and nutritional support, as well as treatment of obstructive episodes, most patients who survive will eventually attain normal growth and the ability to feed enterally.

IMPERFORATE ANUS

The treatment of children born with anorectal malformations continues to be a challenging problem.[156–158] The term "anorectal malformation" encompasses multiple congenital defects with varying degrees of involvement, and many authors have stressed the complexity of the anatomic, physiologic, psychological, and social aspects that come into play in the management of these children.[158–161] The main objective of treatment is to achieve fecal continence.[157] Proposed treatments for these malformations have included simple perineal operations for benign defects ("low"), abdominal pull-through for more complicated defects ("high"), the sacral approach devised to preserve the puborectalis muscle (Figure 77.1-5), and combined approaches such as the abdominoperineal, sacroabdominoperineal, or sacroperineal approaches.[161,162] Even though today's surgical management permits the survival of virtually all patients,[158,161] it has been reported that only 25 to 75% of the operated patients have an acceptable stool continence following surgery,[156,161,163] particularly those patients with the high-type anomalies.[157] The highest incidence of incontinence seems to occur in male patients who have a rectobladder-neck fistula.[156,158,164] These patients usually have very poor muscle structures, a flat (round) bottom, and a narrow pelvis, with little space

for satisfactory levator reconstruction behind the rectum.[156–158] Recent developments in the surgical technique, particularly the development of the posterior sagittal anorectoplasty,[156,157,161,165–167] have improved some of the results, but fecal incontinence and stricture formation continue to be a problem after the newer operations.[156,158,168]

After long-term follow-up, postoperative results in children with imperforate anus vary according to the type of the original malformation and probably according to the age of the patient. It has usually been mentioned that fecal continence and bowel control improve with time and usually reach their maximum around puberty.[157–159] It has been the usual experience that patients with a good anatomy and adequate treatment become continent much earlier,[157,159] whereas those with the worst results usually do not improve spontaneously. In general, even after the new operations, it has been suggested that 25% of children will suffer from fecal incontinence, and 25 to 30% will have other forms of defecation disorders, such as constipation, soiling, and incontinence associated with episodes of diarrhea.[156,157] In the longest series, Pena and Hong described their experience with 1,192 children whose imperforate anus was repaired using the posterior sagittal approach.[158] They found that 75% had voluntary control of the bowel movements, with half of that group still soiling their underwear occasionally. Therefore, only 37.5% of all cases were considered totally continent.[158] Constipation was the most common sequela, and urinary incontinence was common after the repair of cloacas. Twenty-five percent suffered from fecal incontinence but improved once they were subjected to an aggressive bowel program, including an appendicostomy.[158] Molander and Frenckner described the long-term follow-up (18–35 years) of 29 patients operated on for high imperforate anus.[166] They found that 9 had a permanent colostomy as a consequence of severe fecal incontinence, 6 were the only totally continent patients, 6 had occasional accidents that made them wear sanitary napkins, 2 had constant incontinence, and 4 had a moderate degree of incontinence. In another report with an 8- to 20-year follow-up of 104 children with imperforate anus, Holschneider found that only 6 of 69 patients with a high anomaly had a normal or near-normal continence and that 20 of 104 children had uncorrectable urinary incontinence, suggesting also that a high percentage of these patients continues to have severe problems after long-term follow-up and does not improve spontaneously.[167]

Most information comes from those children with high malformations, and it has been suggested that those with low malformations have a better prognosis. Recent studies, however, suggest that they continue to have long-term problems, particularly constipation.[158,169–171] Ludman and Spitz reported that 28% had compromised continence, with up to 25% continuing to experience difficulty with constipation and 7% requiring enemas on a regular basis.[170] A recent long-term report of 44 girls with low malformations reported that 89% were successfully toilet trained but that 47% experience at least occasional soiling or episodes of fecal incontinence.[171] In a study that compared bowel function in 40 patients with low malformations with that of healthy controls, Rintala and colleagues found that 52% had a normal function indistinguishable from the controls.[169] Constipation requiring dietary or medical treatment was present in 42% compared with 7% of the controls, whereas soiling was present in 10%.

Long-term problems with fecal incontinence can have major effects on the development of the children.[107,161,163,172] In a recent long-term survey, Glinn-Pease and colleagues reported that almost half (47%) of parents report problems with bowel function (constipation, diarrhea, or soiling).[161] Most children had a normal growth and were of average intelligence, and scores for math and reading, as well as of adaptive behavior, were age appropriate. They found that 18% had learning disabilities and 18% had some degree of social maladjustment. Of interest is the fact that children with fecal incontinence represented 60% of the patients with behavior problems, suggesting that the frequent association of fecal incontinence with behavioral dysfunction may indicate that these children may benefit from psychological testing. In an interesting study in which 61 patients were observed for 2.5 to 24 years, Ditesehim and Templeton showed that in chil-

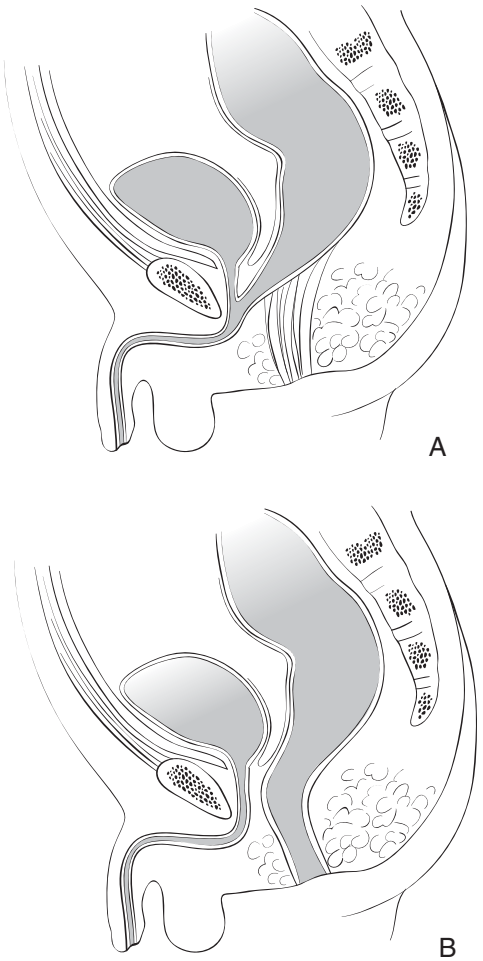


FIGURE 77.1-5 Schematic representation of a rectourethral fistula preoperatively and postoperatively. *A*, Rectourethral bulbar fistula. *B*, Repaired defect. Adapted from Pena A. Atlas: surgical management of anorectal malformations. Heidelberg (Germany): Springler Verlag; 1990.

dren older than 10 years, the quality of life was directly related to their fecal continence, whereas in younger children, their quality of life was better than their continence, indicating that in the younger children, the families tend to be more patient and use special stratagems to minimize incontinence problems: liners in the underpants, enemas, meticulous perineal hygiene, and avoidance of certain foods.[159] It seemed that after age 10, children with incontinence could not be shielded by parents and were not well tolerated by teachers and peers. In fact, in this older group, only 5 of 30 children had greater social adaptability scores than fecal continence scores, and children with a poor fecal continence score faced such severe social problems that they often requested aggressive medical or surgical interventions, including the performance of a colostomy. This study emphasizes the importance of continuing follow-up of these children and for aggressive evaluation of children with fecal incontinence. It is then clear that fecal incontinence is a socially disabling problem in children and remains a challenging problem.[107,156,157,159,163] A recent report of 33 adolescents status post repair of imperforate anus, with a median age of 15 years, found that 73% had flatus incontinence, whereas 70% had persistent dysfunction with staining. Fifty-eight percent met the criteria for a psychiatric diagnosis, and impairment of psychosocial function was found in 73%. The degree of psychosocial impairment correlated significantly with fecal incontinence and flatus incontinence.[163] The authors pointed out that in addition to soiling, staining and fear of flatus are associated with psychiatric and psychosocial dysfunction.[163]

The genesis of these problems is not clear.[107] In a study that compared outcome between children with Hirschsprung disease and children with low anorectal malformations, it was found that even though the degree of fecal dysfunction was similar in both groups, there were differences when comparing both groups. The duration of anal-invasive treatment procedures, particularly anal dilatation, was the most significant negative predictor of the adolescent's mental health, whereas chronic family difficulties and parental warmth, together with the current bowel function variables, best explained the variance in psychosocial outcome.[107] The authors concluded that anal dilatation and continence dysfunction have a negative impact on mental health and psychosocial functioning.[107] They suggest that the use of anal dilatations needs to be reevaluated, particularly in older children.

A recent study of 160 children 6 to 18 years with fecal incontinence after surgery found that the way in which children dealt with the problem could be grouped into three phases.[172] In phase 1, around 6 to 7 years of age, boys were largely unaware of the unsociable nature of their condition, whereas girls were sensitive and withdrawn. In phase 2, between 8 and 11 years of age, boys used overt denial, whereas girls used secretiveness. In phase 3, from 12 years to adolescence, both sexes were marked by continued covert denial and eventual acceptance of their disability.[172] The authors pointed out that the coping mechanisms reflected a complex interrelationship between the characteristics of the child, the family, the social environment, and the unsociable and embarrassing nature of fecal incontinence. Parents' perceptions of how others would react to a child with incontinence influence the children's coping behaviors. Frequently, the emphasis on the need to be secretive (to avoid ridicule) exacerbated the child's awareness of having a shameful disability.[172] Coping with children with fecal incontinence also poses a number of problems to teachers and schools. These children generally look healthy and do not have a visible handicap, and the condition is usually considered taboo. Despite efforts by all concerned, keeping the child's condition secret is difficult, and for a number of children, the problems at school become so acute that some children are removed from it.[172]

MANAGEMENT OF COMMON PROBLEMS

Fecal Incontinence

The management of fecal incontinence is difficult.[135,156,157,160,161,173] Multiple attempts to control it have been done, starting with medical manipulations,[156,157,161] followed by surgical options such as colostomy, grafts, or reoperations.[156,157,164,174]

The physiopathology of fecal incontinence is multifactorial and is not well understood. It has been suggested that good bowel control is the result of the integrity of anatomic structures and the physiologic mechanisms involving three main factors: sensation, bowel motility, and voluntary muscles.[156,157] The presence of fecal incontinence could be related, on the one hand, to abnormalities in the muscle or its innervation[157,168,175] or intrarectal sensation but on the other hand to overflow incontinence from constipation and a lack of bowel motility.[156,176] Pena and colleagues suggested that patients with incontinence can be classified into the following groups: (1) those with a poor surgical repair who may require a reoperation, (2) those with poor functional prognosis who mostly have a tendency either to diarrhea or to constipation, and (3) those with overflow secondary to a dilated sigmoid colon. This distinction is important because the approach and treatment of the patient vary according to the nature of the problem (Figure 77.1-6).[157]

One of the main problems in the evaluation of fecal soiling is that even though there are many tests that try to obtain an objective assessment of anorectal function (eg, defecography, barium enema, manometry, anal endosonography, electromyography [EMG], nuclear medicine, computed tomography [CT], and magnetic resonance imaging [MRI]), each one studies only a specific aspect of a very complex function.[157,177] To evaluate the state of pelvic and anal muscles, as well as the position of the rectum after the operation, imaging techniques such as CT scan[168] and MRI have been used.[177,178] It has been suggested when comparing both techniques that MRI may be superior in the delineation of the pelvic muscles, and another advantage may be that it allows the detection of other unsuspected malformations, particularly tethered cord, or urinary tract problems and also because it does not involve ionizing radiation.[156,178] In general, it has been suggested that the

main advantage of the imaging techniques rests in their ability to help evaluate patients who have been previously operated on and are having fecal incontinence because it allows the differentiation between poorly developed muscles and improper placement of the neorectum.[159,178]

Anal endosonography has also been shown to be useful in these patients.[177,179] In a study comparing anal endosonography with EMG and anorectal manometry in 15 patients, endosonography detected the external anal sphincter in all patients. The distribution image was inadequate in high anomalies. For the external anal sphincter, the endosonographic findings corresponded well to the EMG. The IAS was identified in five patients with high anomalies and in one patient with intermediate anomalies, although only one of the six patients had relaxation by manometry. The authors suggested that the IAS findings did not correspond to the manometry.[177]

However, even though these techniques are very useful in delineating the anatomy, there does not seem to be a good correlation between the anatomic findings and the degree of fecal continence.[158,180] Anorectal manometry is a useful technique to evaluate the state of intrarectal pressure and sensation, as well as of the voluntary muscles.[143,181–185] I and others have found that patients with repaired imperforate anus have significant abnormalities in anorectal function.[143,182–185] The threshold of sensation and the maximum tolerable pressure in the high type are significantly higher than those in the low type were, and the rectal compliance in the high type is significantly lower than that in the low type. It has been found that patients with imperforate anus had a shorter and weaker intra-anal pressure and that patients with high anomalies had abnormalities in voluntary control and sensation.[183,186] Iwai showed an increase in the EMG activity of the external sphincter after voluntary contraction in the patients with imperforate anus, independent of the type of the malformation.[182] Molander and Frenckner suggested that in normal children, fecal continence correlated with the presence of the inflation reflex,[187] and they found that those postoperative patients in whom the inflation reflex was demonstrated had good Kelly scores, independent of the type of anorectal malformation. They also found that in contrast to normal subjects, the electrical activity of the external anal sphincter in children with high anomalies remained stationary in spite of further rectal filling. More sophisticated EMG studies showed that the number of spike bursts in the high and intermediate anomalies was significantly higher than those in the low type. A recent study of 45 patients measured pudendal nerve motor latency, spinoanal responses, and evoked potentials of the cauda equina.[85] The authors showed that patients with imperforate anus had latencies that were significantly prolonged when compared with controls.[85] Compared with normal values, the spinal central conduction time in patients was increased by 278%,

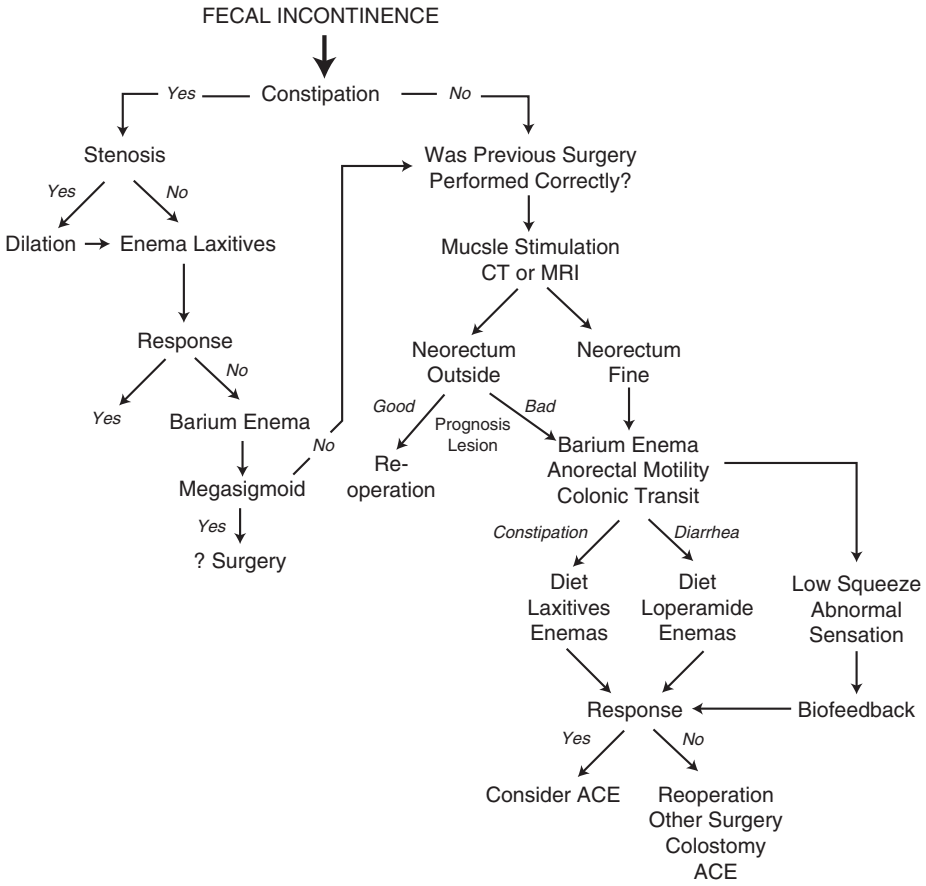


FIGURE 77.1-6 Algorithm for the evaluation and treatment of patients with fecal incontinence after surgery to correct imperforate anus. ACE = antegrade colonic enemas; CT = computed tomography; MRI = magnetic resonance imaging.

the latency of pudendoanal reflex by 232%, the latency of spinoanal response by 180%, and the latency of cauda equina evoked potential by 146%. There was also a negative correlation between the latencies and the clinical scores. The authors concluded that these patients have widespread and serious lumbar and sacral nerve lesions that probably have an impact on fecal continence.[85]

There are few long-term functional studies. Iwai and colleagues studied 27 patients and compared manometric results after surgery with those obtained 3 years later.[188] Manometrically, they found that 7 of 11 patients with a high anomaly had a high-pressure zone in the anal canal and only 1 of 11 patients had an anorectal reflex on the first examination and 3 on the second examination. All patients with intermediate and low anomalies had an anorectal reflex present. Overall, the authors concluded that incontinent patients had a lower anorectal pressure than controls or continent patients. Hedlund and colleagues described the long-term manometric investigation in 30 patients 5 to 10 years after posterior sagittal anorectoplasty.[184] They found that the sensation was within the normal range and that 9 of 30 patients had a rectoanal inhibitory reflex. They also found that soiling was common in patients with low anal resting tone and low squeeze pressures.

It can therefore be concluded from all of the above studies that fecal incontinence is usually related to low voluntary and intra-anal pressure and to abnormal sensation.[166] The role that colonic motility plays in these patients is also becoming increasingly more clear. It has been suggested that abnormal colonic transit is present in patients with either high or low anomalies.[189] The authors found that after using the saturation technique, patients had a significantly longer transit time compared with controls.[189] There was no difference when comparing total transit between those with low or high anomalies, but patients with low malformations have delays in the rectosigmoid segments, whereas those with high anomalies have a more generalized delay.[189] The authors found that the functional outcome was strongly related to the degree of these motility disturbances. Recently, by using colonic motility, it has been suggested that another mechanism that exacerbates incontinence is the presence of HAPCs that migrate in 80% of the subjects to the neorectum.[175] This suggests that it is possible that fecal soiling occurs when the HAPCs propel stool to a low-pressure anus that is unable to accommodate to maintain continence.[175]

Most of the above discussion has been related to the fact that the fecal incontinence of these children is usually the result of abnormalities in anorectal function. There are cases, however, in which there is fecal soiling as a result of overflow incontinence from constipation.

Constipation

Constipation can be a significant functional long-term problem after the posterior sagittal approach or in any operation in which the rectum has been preserved.[156–58,169,174,176,190] In a series of 1,192 patients, Pena and Hong found a 48% incidence of constipation.[158] This varied according to the type of malformation: vestibular fistula, 61%; bulbar urethral fistula, 59%; rectal atresia/stenosis, 57%; imperforate anus without fistula, 55%; perineal fistula, 50%; long cloaca, 48%; prostatic fistula, 45%; short cloaca, 39%; and bladder-neck fistula, 15%.[158]

The physiopathology of the constipation is probably multifactorial.[157] Abnormal myenteric plexus innervation and smooth muscle have been described in rectal dissections.[190] There are cases in which the distal rectosigmoid may become atonic and baggy.[156,157,169,174,190,191] The degrees of rectal dilatation and dysfunction vary, and it has been shown by Rintala and colleagues that the grade of rectal dilatation prior to closure of the colostomy had a positive correlation with the severity of the constipation and that the occurrence of constipation was clearly related to the presence of a functioning IAS.[176] The main problem with the atonic rectum is that it is nonfunctional and has no peristaltic activity,[157,191] so in those patients with atonic rectum in which there is IAS activity, there may be a functional obstruction to rectal emptying causing significant constipation.

The presence of Hirschsprung disease needs to be excluded because aganglionosis has been observed in some patients with imperforate anus.[192]

Treatment of the Fecal Incontinence. The treatment of fecal incontinence has to be tailored to the specific problem presented by the patient. When examining a patient, one has to make the distinction between the different groups (see Figure 77.1-6). This distinction can usually be made on clinical grounds and using simple tests. If the subject has good bowel control potential, good sphincters, a good sacrum, and a malpositioned rectum, a reoperation needs to be considered. One must suspect that overflow incontinence from constipation is present when the child presents with a history of malformation with a "good" prognosis. A colonic transit study may be performed and a barium enema obtained to detect the presence of a megarectum and megasigmoids. On the other hand, if the impression is that the patient is suffering from fecal incontinence not related to constipation, it may be necessary to perform MRI or CT to further delineate the anatomy, to confirm that the neorectum is well placed, and to exclude a tethered cord.[193] If the previous operation is satisfactory, an anorectal manometry will then be necessary to delineate the degree of anorectal abnormalities and the possibility of performing biofeedback therapy.

If one suspects that the fecal soiling is secondary to overflow incontinence from constipation, then medical treatment with diet and bulk laxatives, followed by stimulant laxatives and enemas, needs to be instituted.[157,175] A multidisciplinary approach that also includes behavioral interventions has also been shown to be effective.[173] In those patients with megasigmoid and intractable symptoms even after aggressive medical therapy, resection of the baggy atonic rectosigmoid or sigmoid may be the only effective treatment.[156,157,174,191] Rintala and colleagues reported that 13 of 26 patients responded favorably to dietary manipulations and the use of bulk laxatives, and the researchers were later able to wean all medications.[176] In 11 patients, these measures failed, so enemas and stimulant laxatives were necessary; of those patients, 6 could not wean the medica-

tion without experiencing a relapse. Finally, two patients did not respond to medical therapy and underwent surgical resection, which relieved the constipation, although they continued to have inadequate fecal continence and to use enemas to stay clean. On the other hand, Cheu and Grosfeld reported three children with intractable constipation in whom the resection of the baggy rectum resulted in disappearance of the constipation and therefore in a normal fecal continence.[191] Pena and colleagues have questioned the wisdom of dissecting both the sigmoid and the rectum, and they reported the successful treatment of three patients in whom they only resected the sigmoid, preserving the rectum.[157,174] These authors suggested that all patients with fecal incontinence should have a barium enema, particularly those born with malformations and with the potential for good continence, and that if a megasigmoid is found and medical therapy fails, a sigmoid resection with rectal preservation should be performed without subjecting the patient to another pull-through.

On the other hand, if the soiling is the result of a lack of good muscles, an abnormal sacrum, a defect with poor prognosis, or an abnormal sensation, an aggressive bowel program needs to be instituted.[157,175] An attempt to make the stools more solid should be undertaken because liquid stools usually leak out without the patient's perception. The problem tends to be worse in those patients who were subjected to an operation in which the rectosigmoid was resected, such as after abdominoperineal procedures and endorectal dissections. These patients usually suffer from "diarrhea" and increased colonic motility and pass stool constantly. One approach to these patients involves the use of antimotility agents such as loperamide,[157,194,195] which decreases colonic transit and changes anorectal function, together with the use of bulking agents in the diet. In a study in which loperamide was administered to eight patients with fecal incontinence after rectoplasty for high imperforate anus, four had a significant decrease in the amount of soiling.[195]

Also, besides decreasing stool output, an attempt to keep the distal part of the colon empty needs to be undertaken, and the use of enemas and suppositories is useful.[157,158] At times, it is necessary to use large enemas or colonic irrigation.[157,158] Pena and colleagues have developed a program in which the parents are taught to clean the colon every day with the use of an enema or some form of colonic irrigation.[157,158] The problem is usually much more difficult to control in those children with absent sacrum and worse neurogenic abnormalities because they are unable to hold the enemas or the suppositories. For these patients, the use of a continence enema has proven very beneficial.[144] The success of the retrograde application of enemas or suppositories to achieve continence has changed the quality of life of these patients. However, the problem with this approach lies in the fact that a helper is needed, so independence is not achieved.

Pena and colleagues found that of 172 patients with fecal incontinence who were not candidates for a reoperation or a sigmoid resection, 44 had predominantly constipation and incontinence, whereas 128 had a tendency to diarrhea.[157] The first group was treated with enemas, with a 93% success rate, whereas those in the second group were treated with constipated diets and drugs to slow down the motility. The authors reported a success rate of 88%.

Another option for the treatment of fecal incontinence is biofeedback therapy.[196] Even though the mechanisms of fecal continence are still not well defined, studies in patients with fecal incontinence owing to a variety of different etiologies have suggested that it tends to occur in patients in whom the maximum squeeze pressure is low or in whom the sensation threshold is high. Attempts to correct both abnormalities have usually led to an improvement in the fecal incontinence.[142,156,181,197] Biofeedback for fecal incontinence has been successfully used in the treatment of patients with peripheral nerve impairment, such as diabetes mellitus and multiple sclerosis, myelomeningocele,[142] or after anorectal surgery.[181] In children, it has been successfully used for the treatment of myelomeningocele,[142] in patients with ileoanal anastomosis,[197] and, recently, in children with imperforate anus.[181,196] Most studies suggest that approximately 50 to 90% of patients with incontinence respond to biofeedback,[198,199] and in a review of all published studies in which biofeedback was performed in adults to treat fecal incontinence, the technique was successful in 79.8% (in 257/322 patients).[198]

The information on the use of biofeedback for the treatment of imperforate anus is limited. Arnbjornsson and colleagues performed biofeedback training in patients with incontinence.[165] They found a correlation between clinical improvement and EMG increase after voluntary contraction and EMG tonic activity, with 7 of 11 patients showing a decrease in fecal incontinence, 1 worsening, and 4 no change. No correlation was made with pressure changes. Worona and colleagues have also studied the effect of a biofeedback program on the manometric and clinical outcome of 54 patients with imperforate anus and fecal incontinence.[181] After multimodal treatment, including biofeedback, there was a significant improvement in 82% of the patients, and 34.5% became completely continent. There was a significant decrease in the number of accidents/day, laxatives/week used, number of enemas/week, and sensation threshold and a significant increase in squeeze pressure, total duration of squeeze, and duration of maximum squeeze. Ninety-two percent of the patients maintained their improvement for more than 6 months. Newer biofeedback techniques have used portable EMG machines with similar results.[196,200]

When all attempts to improve the fecal incontinence have failed, the option of a repeat surgical procedure needs to be considered.[201] This is particularly important in those children who were operated on years ago, for whom the performance of a posterior sagittal anorectoplasty may be beneficial, or in those in whom the initial repair might have been technically inadequate.[157] Candidates for a reoperation are those with good sphincters, a good sacrum, a good prognosis–type defect, and a mislocated rectum.[157] Brain and Kiely recently described 12 patients who underwent the procedure because of severe fecal incontinence after the original anal reconstruction.[202] They found that only two patients achieved good results, two others

improved, and the rest remained incontinent. On the other hand, Pena and colleagues found that patients with a normal sacrum and fecal incontinence operated on elsewhere who underwent a posterior sagittal anorectoplasty achieved marked improvement in 45%, some improvement in 37%, and no improvement in 18%.[157,203] In contrast, a marked improvement in only 20%, some improvement in 30%, and no improvement in 50% were achieved in patients with an abnormal sacrum.[157] A report of 20 patients who were reoperated on showed that of 16 incontinent patients, 12 achieved continence and 4 some improvement; of those with fecal impaction, 2 achieved daily bowel movements, and 2 remain with mild constipation.[201] Resection of a dilated sigmoid (megasigmoid) has been suggested for those patients in whom there is overflow incontinence, good sphincters, a good sacrum, a good prognosis defect, and a preserved rectum.[157]

Knowing the success that the application of retrograde methods has on producing evacuation and continence, the idea of performing procedures that will allow the anterograde delivery of the enemas was brought forward, and the ACE procedure was proposed. As mentioned above, the ACE procedure has been developed to produce a continent conduit from the skin to the cecum that can be catheterized for self-administration of enemas.[131,139,145,157,204,205] The procedure can be performed with open surgery,[145,157] laparoscopically,[206] or percutaneously either by radiologic methods[131,135,136] or endoscopy.[137] The main disadvantage of the surgical approach is that it requires a laparoscopy or laparotomy, which, in many of the children who require this procedure, involves major surgery because they have usually had other surgical procedures. The advantage is that it can be done at the same time as the creation of a urinary conduit and that there is no indwelling device. The percutaneous approach can be done either radiologically[136] or endoscopically,[137] and its advantage is that it does not involve surgery, so the recovery time is very fast. The main disadvantage is that a device is left in place, which is not always acceptable to the patients.

Independently of the technique used, the patient is then able to administer an enema in an antegrade fashion. This allows the patient to be predictable and independent. The ACE procedure has been used in children with fecal incontinence secondary to different etiologies[135,136] but most commonly with spina bifida,[139,205,207] other neurogenic problems,[135] and also imperforate anus.[136,145,156–158,204,207] Even though some have suggested that the procedure should be performed only in those patients in whom the fecal incontinence and constipation have been controlled with bowel management,[156–158] recent experience has shown that it is also successful in those who do not respond to enema therapy[136] or even in those children with a poor prognosis.[204]

Overall success rates have been described as ranging from 60 to 90%. In a review of the literature, of 149 reported patients, 88% were completely continent and 12% were partially continent.[135] The most common solutions used are polyethylene glycol, tap water, saline, and phosphate enemas.[135,139,157,158,204] The use of tap water has been associated with hyponatremia,[133] so it is preferable to administer saline. The type, volume, and frequency must be individualized for each patient,[135] although they usually vary from 400 to 2,500 mL.[133,135] The patient sits on the toilet, administers the irrigation, and usually has complete evacuation in 30 to 60 minutes.[135] The most common complications have been stomal stenosis,[134] leakage at the surgical site,[145] or difficulty with catheterization.[208]

Recently, it has been suggested that the placement of an irrigation button in the sigmoid may be as effective as the ACE performed in the cecum.[138,209] The experience is very limited. The main theoretic disadvantage of pursuing that approach is that, most likely, laxatives will still be needed to allow the stool to move from the cecum to the sigmoid colon. More information is needed before this procedure can be recommended.

Several other surgical procedures have been recommended for patients with postoperative incontinence. Some are designed to increase the anorectal angle or reinforce the existing musculature.[210] Some authors have advocated the use of gracilis muscle transposition for the treatment of children with intractable fecal incontinence and evidence of a lack of muscle function.[210] The results have been mixed. In one series, however, Sonnino and colleagues reported their long-term experience in seven patients with gracilis transposition in whom there was severe fecal incontinence, a lack of adequate sphincteric function, and a properly positioned neorectum before the new procedure.[210] They all became continent after the procedure with a mean follow-up of 0.5 to 12.5 years. It is important to note, however, that this type of operation should not be performed if one determines that the neorectum is malpositioned, in which case, a posterior sagittal approach as a redo operation is still recommended.

For those patients who have failed all therapies and continue to have severe fecal incontinence, a permanent colostomy may be necessary.[157,158] Even though the performance of a colostomy may represent a chronic disability and loss of hope of a perfect cure; it may reduce the practical and emotional strain of soiling and invasive treatment procedures, both for the patients and the parents.[107] In fact, Lask and colleagues reported in a controlled study of psychosocial adjustment to stoma surgery for childhood inflammatory bowel disease that there was no evidence that the surgery was psychologically harmful to psychosocial adjustment, self-esteem, or quality of life.[211] They concluded that as long as children are well prepared and followed up carefully and sensitively, stoma surgery should not be deferred for fear of adverse psychological consequences.

It is difficult to estimate the exact incidence of children who have required colostomy. In a long-term follow-up reported by Molander and Frenckner, 9 of 29 patients had a permanent colostomy; the patients in this study, however, were operated on before the new techniques for the treatment of these children were available.[166] In a series of 348 patients, Pena and colleagues reported that they had to perform this procedure in 7 children.[157] Of those, 6 had diarrhea and incontinence as the main symptoms.

Summary on the Approach to the Patient with Fecal Incontinence. Figure 77.1-6 presents an algorithm for the treatment and evaluation of children with postoperative problems. When a patient is referred for the evaluation of fecal incontinence, the following questions need to be addressed:

1. Is the patient having overflow incontinence from constipation? If so, is there a massive dilatation of the rectosigmoid?
2. What is the status of the sacrum? Does the patient have more than two or three missing vertebrae?
3. What is the state of anorectal functioning? Is there a tethered spinal cord?
4. Was the original repair done properly? Is there evidence of stenosis or malposition of the neorectum?

In general, this information can be obtained from the history and physical examination and with the performance of some basic tests. The physical examination should provide information about the state of the anoplasty (particularly as it relates to stenosis), the presence of the midline groove and the anal dimple, and the status of the perineal musculature. A thorough neurologic examination will provide information about deficits in the sacral innervation. The abdominal examination will show the amount of fecal material that is present and should detect the presence of big stool masses.

A plain abdominal radiograph will provide information related to the amount of fecal material present. An anorectal manometry will be useful to evaluate intrarectal sensation, as well as the functioning of the IAS and the strength of the squeeze pressure.

If the patient has severe incontinence that is resistant to medical therapy and surgical options are being considered, the performance of a barium enema may be useful. This test will detect the presence of rectosigmoid dilatation in those patients with severe overflow incontinence from a nonfunctioning segment. In those patients in whom the sigmoid was resected in the initial pull-through, the barium enema will usually show a nondistended colon with normal haustration down to the perineum.

If it is necessary to establish the position of the neorectum in relation to the pelvic musculature, an MRI will be useful. An MRI will also show the presence of spinal cord tethering. At times, it may be necessary to perform electrical stimulation of the perineal region to try to identify where the muscle contractions are occurring in relation to the pull-through.

All of the information obtained after evaluation will allow a better understanding of the physiopathology of the fecal incontinence of the patient. If there is overflow incontinence, attempts to increase stool evacuation need to be undertaken. This may involve dilatation of the anus (if it is stenotic), the use of laxatives and particularly enemas, or sigmoid resection if the constipation is intractable and there is massive dilatation by barium enema. On the other hand, if the patient has fecal incontinence without constipation and a properly performed operation, a biofeedback and behavioral program should be undertaken. This should be accompanied by the use of bulking agents, colonic irrigations, and, usually, loperamide. If one is successful in maintaining a clean rectosigmoid, the incontinence will usually be controlled, independently of the muscle abnormalities. The performance of an ACE procedure needs to be considered, both in those who have achieved continence with enemas and in those in whom the bowel program has not been successful. In those patients with lesions that have been associated with a good prognosis, if the fecal incontinence persists or if there is evidence that the neorectum may not be positioned properly, a redo operation should be considered.[157] Finally, if the neorectum is in a good position but the patient has sacral abnormalities, poor muscles, and a flat perineum and continues to have incontinence independently of the therapy, the possibility of a different type of surgical procedure or the creation of a permanent colostomy will need to be considered.

SUMMARY AND CONCLUSIONS

As more children with complex congenital anomalies survive, new and long-term medical problems are arising. An attempt has been made in this chapter to analyze the long-term complications after surgery for some common and representative pediatric surgical procedures that are directly related to the gastrointestinal tract. The main focus has been to describe usual postoperative problems after surgery for gastroesophageal reflux, Hirschsprung disease, and imperforate anus. Practical aspects regarding their clinical presentation have been reviewed, and suggestions for the evaluation and therapy of these children have been proposed (see Figures 77.1-2, 77.1-4, and 77.1-6). Some general principles can be mentioned. Throughout the years, it has been learned that even though the surgical procedures are usually necessary, there has to be a balance between an attempt to follow physiologic principles and avoiding the creation of more problems. This may not always be possible, particularly when the patient's deficits are extensive, as in cases of fundoplication in children with severe neurologic impairment and esophageal dysmotility, patients with high imperforate anus without sacrum, or patients with total colonic aganglionosis. In most cases, however, recent advances have allowed for a better postoperative outcome, particularly in the area of anorectal malformations.

The pediatric gastroenterologist therefore needs to be familiar with the type of surgical procedures that can be performed, their indications, and their most common postoperative problems. An attempt should be made to participate with our surgical colleagues in deciding the best approach to therapy, particularly after the initial surgical procedures have failed.

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2. The Pediatric Ostomy

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A stoma can be a beneficial and life-changing surgical intervention for many chronic and acute gastrointestinal disease processes. It is a safe and effective way of diverting the fecal stream and allowing the bowel to heal while continuing enteral feeding. Indications vary depending on the age of the child, but a poorly created, poorly sited stoma can be a nightmare for both children and their family.

The first recorded formation of a colostomy on an infant was by the French surgeon Duret, in 1798,[1] when he successfully carried out the procedure on a 3-day-old infant with an imperforate anus. Although the basic principles and surgical technique have changed little over time, probably the single most significant development in pediatric stoma care has been the shift in emphasis from the earliest attempts of surgical intervention to cure or control the disease to the acknowledgment of the importance of the patient's quality of life after surgery. The identified need for education and support of the child and family before and after surgery has led to the creation of multidisciplinary teams of pediatric surgeons, pediatric stoma care nurses, dietitians, and gastroenterologists, all of whom play an important part in the rehabilitation of the child and family back into the community.

The aim of this chapter is to give an overview of the indications for stoma formation, describe the common types of stomas, and provide surgical tips for creating a good stoma. Practical advice will be included on coping with stomal complications and an understanding of the effect that a stoma has on both the child and the family, in particular the adolescent, who faces disturbance of body image and difficulty with emerging sexuality.

NUMBER OF PATIENTS

It is estimated that as many as 80,000 people in the United Kingdom have a stoma,[2] with only a small percentage of these being pediatric patients. Anecdotal evidence suggests that each regional center within the United Kingdom will see 40 to 100 new cases each year across the pediatric age range. The majority of stomas (76%) are formed in the first 6 weeks of life, 5% from 3 months to 1 year of age, 8% from 2 to 7 years of age, and the remaining 11% from the age of 8 to 16 years.[3]

TYPES OF STOMAS

The term *stoma* derives from the Greek meaning "mouth" or "opening" and is described as "an artificial opening established surgically between an organ and the exterior."[4]

Jejunostomy

The highest-output stoma is a jejunostomy, in which the stoma is sited in the jejunum. It is usually problematic owing to high fluid and electrolyte loss; therefore, enteral feeding cannot be established.

Ileostomy

An ileostomy is a stoma formed in the ileum and can be either divided (ie, two ends spatially separated) or formed as a loop. It is our view that the bowel is not completely defunctioned by a loop because spillover of feces can occur, but it does give a blowhole effect.

Colostomy

A colostomy is the formation of a stoma into the colon, and it is commonly sited in either the transverse or sigmoid colon and again can either be divided or formed as a loop. The lower down the bowel the stoma is placed, the less difficulty is faced with sodium and fluid imbalance because of the retained length of functioning bowel.

Antegrade Colonic Enema

The antegrade colonic enema was first developed by Malone in which one end of the appendix is reimplanted in a nonrefluxing manner into the cecum and the other end is brought out onto the abdominal wall as a continence stoma.[5] Surgical variations have been developed on this technique, but the basic function is to form an access port into the cecum. An alternative is the cecostomy button.[6] The procedure may be considered in children with anorectal anomalies, Hirschsprung disease, or spina bifida or in those children with chronic idiopathic constipation as an alternative to a permanent stoma or rectal washouts.

INDICATIONS FOR STOMA FORMATION

The Neonate

An acute stoma may be necessary in the following neonatal conditions: necrotizing enterocolitis, anorectal anom-

alies, Hirschsprung disease, complicated meconium ileus or failure to decompress the bowel after administration of Gastrografin, some intestinal atresias, and milk curd obstruction. Acute, short-term stomas may be situated within the wound to avoid a further site and therefore additional abdominal scars. Often the size of the abdomen in preterm babies makes siting near the umbilicus unavoidable, although still workable, and therefore avoids a second wound. Whatever the type of stoma, there needs to be an appreciation of the fragility of the blood supply to the mesentery of the neonatal bowel, with meticulous dissection and as limited as possible devascularization of the ends of the bowel. Historically this was why loop stomas were preferred.[7]

A neonate with gut dysmotility associated with, for example, gastroschisis or malrotation may need an end ileostomy to allow enteral nutrition to be established while the small bowel recovers function, without having to work against an ileocecal valve and unused colon. This may be required for several years and therefore should be properly sited by the stoma care nurse if possible.

The Older Child

An acute stoma in the older child may be necessary in the following conditions: trauma sustained either rectally or abdominally; some intra-abdominal catastrophes such as intussusception or volvulus associated with malrotation, in which viability of the bowel is doubtful; ulcerative colitis with toxic megacolon or intractable bleeding; perianal sepsis in the immunocompromised oncology child or those with severe neutropenic enterocolitis associated with therapy; acute abdominal abscess; or perforation in Crohn disease.

A semiurgent stoma may be considered in the following conditions: chronic constipation, pseudo-obstruction, children with Hirschsprung disease in whom the bowel has not decompressed, and children born with anorectal anomalies who continue to have problems with constant soiling. Severe refractory ulcerative colitis will require a subtotal colectomy and formation of an end ileostomy, as will familial adenomatous polyposis[8] because of the risk of malignancy.

PREOPERATIVE CARE

Stoma Siting

It is important that, whenever practicable, patients have access to a stoma care nurse specialist for information, advice, and siting preoperatively. This is not always achievable because many stomas in children are formed as an emergency; therefore, there is little time for discussion and siting in relation to the position of the stoma, but the following principles of stoma siting should be adhered to whenever possible (Table 77.2-1).

Careful siting should involve the cooperation and consent of the patient (depending on the child's age). It is important to remember that the shape of the abdomen alters when the patient is lying, sitting, or standing and in thin or obese children. Each stoma site therefore needs to be tailored to the individual's needs, and, when possible, patients should be given the chance to familiarize themselves with the stoma bag by applying it to the site, so as to predict any potential problems with positioning. As Black noted, "There is nothing that can compensate for a badly sited stoma, achieving the correct site will have an enormous influence on the individual's post-operative rehabilitation, ability to manage his/her own care and ultimately their quality of life."[9]

Bowel Preparation

For the emergency stoma, there is no time to adequately prepare the bowel. For those children undergoing elective surgery, 24 to 48 hours of clear fluids and oral laxatives are often adequate; however, rectal washouts may be required to empty the bowel, but these should not be attempted on those at risk of perforation (eg, the immunocompromised child or the child with diffuse inflammatory bowel disease).

Psychological Preparation

The majority of congenital abnormalities requiring stoma formation are not apparent until after birth, therefore allowing parents no time to prepare for the consequences of giving birth to a baby with a disability.[10] It is often difficult for parents to understand the medical terminology being presented to them. Every family will have its own means of understanding the information provided, and, wherever possible, time should be allowed for questioning, preparation, and acceptance of the need for surgery. Sometimes this is realistically impossible when faced with a sick neonate or an adolescent trauma victim.

Even in these emergency situations, parents are entitled to feel that they have adequate information about the procedure to make an informed choice about their child's care. Parenthood can be traumatic and stressful at the best of times, and the support received from staff in the initial stages can make all the difference[11] as to their acceptance of the stoma after surgery.

When stoma surgery is planned, the method of preparation used should be related to the time frame available and the age of the child. A number of books and dolls are available through various organizations (see contact addresses) that can allow even a young child to come to some level of understanding of what is about to happen. Experienced play therapists and pediatric nurses can use play acting and painting to help the child become involved in the decision-making process.[12]

TABLE 77.2-1 AREAS TO AVOID IN STOMA SITING

Waistline
Hip bones
Previous scars
Groin area
Fat folds and bulges
Umbilicus
Under pendulous breasts
Areas of skin irritation (psoriasis, eczema, skin allergies)
Positioning of artificial limbs
Areas in which there would be difficulties if weight gain or loss occurred

Postoperatively, the older child who has suffered the consequences of inflammatory bowel disease, with pain, repeated hospitalization, polypharmacy, and exclusion from their peers, may see surgery as a relief and an opportunity to start living again. The desire for a cure often clouds many teenagers' judgment as to their true feelings about their altered body image, which tends to emerge months after surgery, when the true picture of their new life becomes apparent.

Access to the Internet can provide teenagers with information that they may feel too embarrassed to ask for (Table 77.2-2), and many self-help organizations have sites for the young adult ostomist. Approximately 20% of patients with a stoma experience clinically significant psychological symptoms postoperatively; commonly, these are anxiety and adjustment disorders.[13]

CULTURAL ISSUES

When caring for the child and family, it is important to take into account their religious, cultural, and individual needs. There may be a number of questions, such as "Is there any animal content in the makeup of the appliance being used?" According to Muslim culture, the left hand is used for cleansing and the right hand is used for meeting and greeting. This therefore requires special allowance when teaching stoma care if the left hand is the nondominant hand. The prolonged fasting during Ramadan can result in increased levels of dehydration and constipation. Children with a stoma can often be exempt from their religious duties at this time or at least be allowed some flexibility. Discussion with their religious leader can help in assistance and guidance.

COMPLICATIONS OF STOMA SURGERY: PRACTICAL ADVICE

Many of the complications listed below can be avoided by careful consideration of the site of the stoma and care being taken by the surgeon when surgically creating the stoma spout itself and its positioning in relation to the abdominal wound. Different complications can be seen at different time scales following surgery.

Avascular Necrosis

Stomas initially are often dusky and bruised in appearance, but many recover without any problems. Technical attention must be paid to preserving a good blood supply to the active stoma by not strangulating it either with a misplaced suture, closing the abdomen too tightly, or by placing the mesentery under tension. The mucus fistula can be placed in slightly less healthy bowel, understanding that it may fibrose down, but it will preserve bowel length that would otherwise have been sacrificed in closure.

Stomas in neonates are usually covered only with a small piece of Jelonet (Smith and Nephew Healthcare Ltd, Hull, UK) and gauze to allow clear observation until the stoma becomes active. The first appliance in all age groups should always be clear-fronted and drainable with no flatus filter; this allows clear visual access and the ability to record the volume of stool passed and monitor any wind produced.

Care should be taken to protect the stoma in neonates when undergoing phototherapy because "sunburn" of the mucosa (black appearance) can result, and incubators can often dry out the stoma. Covering the stoma with Jelonet and gauze can prevent this.

Dehiscence

Complete dehiscence is when the stoma wound breaks open and a further portion of the bowel can extrude from the wound. This is unusual and requires surgical revision, and it is more likely to occur if the two stoma ends are crowded into too small a wound so that skin bridges between them fail to heal. Wound dehiscence is more likely if there is heavy soiling with fecal matter and a malnourished child or premature baby. A superficial dehiscence is more common and usually responds to topical packing, without revision being required.

Bleeding

Bleeding can be peristomal, from erosions or ulcers (usually secondary to bag placement and local trauma). Parents should be warned to expect some bleeding when the stoma is wiped clean because of surface trauma or if the infant is crying when the appliance is being changed because tension on the sutures occurs owing to the increase in intra-abdominal pressure.

Bleeding may be a symptom of the intrinsic pathology of the sick child, but it is important to beware of parastomal varices that can accompany total parenteral nutrition–induced liver failure and portal hypertension. These can bleed exsanguinating amounts of blood rapidly. Local pressure and resuscitation with comprehensive blood products can be lifesaving.

Stenosis

Ischemia to the bowel end is one of the most common causes of stenosis. Closing the abdominal wall too tightly

TABLE 77.2-2 WEB ORGANIZATIONS

ORGANIZATION	WEB SITE
United Ostomy Association	www.uoa.org
International Ostomy Association	www.ostomyinternational.org
Wound, Ostomy and Continence Nurses Association	www.wocn.org
The Mark Allen Group Organisation (specialist stoma care nursing)	www.internurse.com/stomacare.cfm
Living with a colostomy	www.ostomy.fsnet.co.uk
The Continence Foundation	www.continence-foundation.org.uk
The Simon Foundation for Continence	www.simonfoundation.org-html

around the stoma can cause an ischemic effect and can also tighten the abdominal exit site, causing stenosis.

A narrowing of the outlet of the stoma through the abdominal wall can cause an obstruction, often with output fluctuating from little to excessive. Translocation of bacteria can occur at the site, and the patient can become toxic and unwell, often with subsequent line sepsis. If this is suspected, the stoma should be digitally probed to check its patency and may require dilation (not in the neonate), although surgical revision is usually required.

A loopogram radiologic study can show dilatation, but it is a good precaution to cover the procedure with intravenous antibiotics to avoid translocation of bacteria and septicemia.

Necrosis following an infection around the stoma can result in narrowing at the entrance, causing acute abdominal pain and no activity or difficulty in passing stool. Colostomy patients should be advised to keep the stool soft by consuming additional fluids (especially in warm weather) and avoid a high-fiber or bulky diet.

Prolapse

Prolapse of the bowel can be quite dramatic (Figure 77.2-1). This is more common with loop enterostomies, especially in the distal limb, but can occur with end stomas, especially those in a very dilated bowel as it returns to normal size. Fixing the bowel to the muscle fascia in four quadrants goes some way to preventing prolapse.

Small prolapses can be ignored if easily reduced, but an irreducible stoma needs attention to avoid bowel compromise: application of fine sugar granules followed by manual reduction or warm saline gauze and slow pressure usually achieves a result. The sugar causes a fluid shift across the bowel wall via an osmotic gradient, causing the stoma to shrink.[14]

Failure to reduce the stoma requires urgent surgical intervention and, occasionally, revision. A chronic prolapse can be encircled with a nonabsorbable suture, as in a Thiersch suture for rectal prolapse. Refashioning of the stoma is sometimes the only option, and, if required, a new site is preferable. Adolescents who have difficulty with accepting their body image because of the prolapsed stoma may request revision.

When the stoma has prolapsed, it may be difficult to apply the original size appliance; therefore, increasing the size of the aperture can sometimes prevent the stoma from suffering trauma to its edges.

Retraction

Retraction of the stoma can be due to inadequate length of bowel pulled under tension, subsequent adhesions, or the disease process recurring, such as Crohn strictures. The stoma can become indrawn and flush with the skin, causing excoriation and problems with leakage. Although retraction can sometimes resolve spontaneously, revision may be required to make management of the stoma easier, and adequate spouting initially can make this less of a problem, even for colostomies.

Another cause can be from the child gaining a lot of weight after surgery, causing the stoma to become level with the abdominal wall; therefore, the feces has difficulty draining away from the appliance, causing pooling of stool (pancaking) under the appliance. In older children, a convex appliance can assist in pushing the stoma out and thus creating a moat effect to promote better drainage (pastes may help make the appliance stickier).

Incisional or Parastomal Hernia

This is not a big problem in young people because of the natural strength of the abdominal wall, but it may become a problem in those who have undergone repeated abdominal surgery or in those who are overweight.

The weakness in the closure of the abdominal wall adjacent to the stoma can result in an incisional hernia. This is more common in a very dilated bowel that returns to size, but it is also more frequently seen in children with Down syndrome who have required stoma formation owing to poor collagen and therefore poor healing. If troublesome, revision may be required; otherwise, it can be ignored, although bag application can be difficult.

Intestinal Fistula

A leak of intestinal contents from a fistula adjacent to the stoma can result from an anchoring suture placed too deeply or from the disease process recurring in Crohn disease. Intra-abdominal fistulae need to be revised urgently, but a small external fistula can be of little consequence.

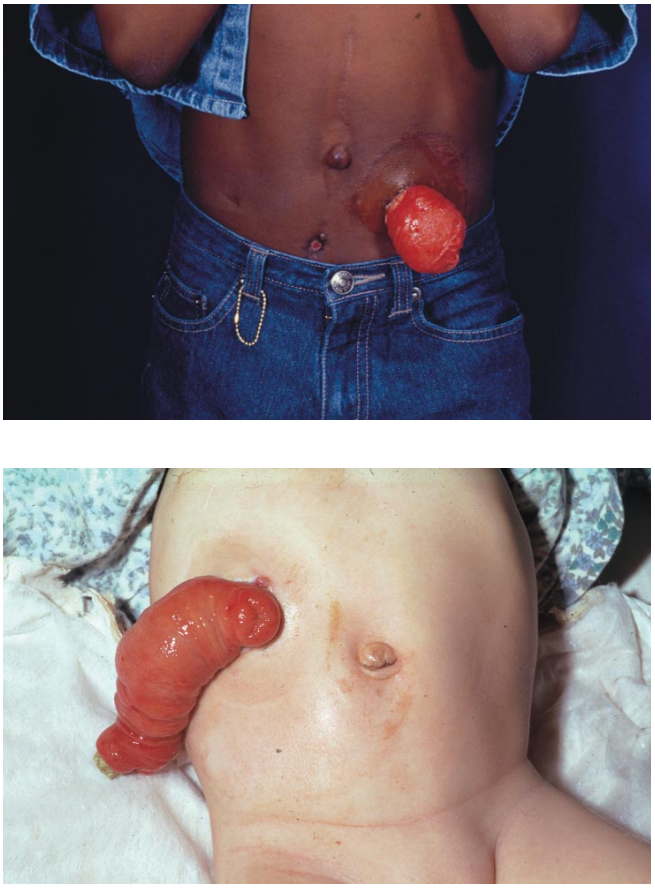

FIGURE 77.2-1 End sigmoid colostomy prolapse in two children.

Mucocutaneous Separation

This results when the stoma edge comes away from the surrounding skin, leaving a shallow cavity around the stoma. It is a particular problem in neonates owing to insufficient bowel mobilization at the time of surgery, causing undue tension on the stoma sutures, which anchor it to the skin. Sore skin around the stoma can result from leakage of stool under the flange; the developing trough can be filled with hydrocolloid gel or paste to promote granulation tissue, and the appliance aperture can be cut to incorporate this.

Dehydration and Electrolyte Imbalance

Every effort should be made to site the stoma as far as practicable down the bowel, and in the immediate postoperative period, close attention to fluid balance must be maintained to avoid electrolyte imbalance. Once on a full enteral diet, the urine should be monitored to ensure that adequate sodium levels are maintained. Supportive parenteral nutrition or fluids may be required for some time in children with acute high intestinal stomas. Enteral nutrition in children with jejunostomies should be given as a low continuous volume to keep the enterohepatic circulation going and decrease cholestasis; it also maintains nutrition to the enterocytes but avoids excessive fluid problems by limiting the input.

Oral sodium supplements are needed almost always in children with ileostomies and even with some colostomies. The infant's or child's growth can be severely impaired by low total-body sodium.

Diversional Colitis

This can occur in the defunctioning bowel owing to stagnation of the contents and a lack of topical nutrition to the enterocytes, with the child experiencing painful discharge and bleeding. Short-chain fatty acids can be instilled into the defunctioning limb with a good result; this is usually given daily for 1 week, with the amount dependent on the length of bowel involved, and then repeated as necessary until under control.[15]

The passage of mucus from the rectum is not uncommon, with older children reporting the urge to defecate. For the occasional rectal discharge, a plain saline washout through the defunctioning stoma or rectum may alleviate the problem, although too frequent washouts can have the undesirable effect of increasing mucus production. Bleeding from the rectum may be an indication of recurrence or continuation of active inflammatory bowel disease.

Skin Problems

An entire atlas has been developed about abdominal stomas and their skin disorders.[16] Soreness can result from an allergic reaction or sensitivity to the adhesive part of the appliance (contact dermatitis), with the outline of the appliance clearly visible following removal. Changing the appliance used can usually resolve the problem; otherwise, barrier films or hydrococolloid dressing used under the appliance can reduce contact and therefore the likelihood of a reaction. Topical steroids should be used with extreme caution because they can result in the peristomal skin becoming very fragile.

Fungal infections often respond to topical nystatin or cotrimoxazole, or a single dose of fluconazole may be required orally if severe.

Effluent dermatitis, inflammation, or excoriation caused by leakage of stool directly onto the peristomal skin is more common in ileostomy patients owing to the liquid consistency damaging the skin on contact. Gut enzymes, particularly protease and amylase found in ileostomy output, can cause fecal irritation by damaging the horny layer of the skin.[17] The cause can be simply that the shape of the stoma has changed, thus allowing fecal fluid to leak under the flange. If this occurs, barrier films can be used to protect the skin.

Poor technique in the fitting of the appliance or the changing shape of the abdomen as children grow may result in the development of troughs and ridges, which prevent the appliance from lying flat against the skin. Pastes or seals can be used to fill uneven areas, allowing the appliance a flat, even surface to which to adhere.

Wet skin under an appliance can be dried using calamine lotion, also giving a soothing effect when the area is itchy. Preexisting skin conditions such as eczema can cause reactions and itchiness from some appliances, and often trial and error is the only way to achieve a solution.

The adhesive quality of the appliance can be increased by warming the appliance either in clasped hands, leaving it on a radiator for a short period of time, or by the use of a hairdryer before sticking it to the skin.

Overfull bags can increase the likelihood of leakage because the weight of the stool pulls the flange away from the skin. Patient education should include the importance of emptying the skin before it becomes no more than two-thirds full.

Granulation tissue may be "normal" and can be controlled with topical oxytetracycline and hydrocortisone (Terra-Cortril), silver nitrate application (with caution), or the regular use of dexamethasone, famycetin, and gramicidin (Sofradex) ointment.

CHOOSING THE RIGHT STOMA APPLIANCE

In the past decade, the choice of stoma appliances for the infant or child has increased dramatically. The following issues should be considered when offering advice to the new ostomist (Table 77.2-3):

- Stoma appliances are made either as a one-piece system; incorporating both the flange and bag (either drainable or closed), or a two-piece system with a separate flange and bag that connect together.

TABLE 77.2-3 FACTORS INFLUENCING CHOICE OF STOMA APPLIANCE

Age and size of the infant/child
Type of stoma: colostomy/ileostomy
Site/position of stoma
Physical/social activities
Dexterity of the child

- One-piece drainable appliances are particularly suitable for infants and younger children who do not have a high level of output. As children grow and their diet becomes more varied, the volume of effluent increases, and with increasing levels of activity, the support and flexibility of a two-piece system may be more suitable, allowing the bag to be changed without the need of the flange to be removed.
- Bags can be drainable for high-output stomas, or if the child has difficulty in emptying the bag, a nondrainable system may be more appropriate. The size or capacity of bags can be varied depending on the activities undertaken (a larger bag may be useful overnight to decrease the need for emptying).
- To combat the effects of flatulence, "flatus filters" are available in many drainable and nondrainable appliances. These do become ineffective when wet; therefore, the outlet should be covered when bathing or swimming.
- Convexity appliances have integral convexity and help create a seal for use with retracted stomas. This system is not recommended in young children because of the risk of pressure ulcer formation, and if used long term in young children, there is also some concern about interruption of muscle growth.
- A wide range of appliances and accessories is available that will cover the individual requirements of most patients. Before finalizing any appliance orders, the opportunity to try different products should be offered because the consumer should have ultimate choice on the system used.

DISCHARGE PLANNING

As the child recovers from surgery, plans should be made for the child to return home. Teaching should involve basic stoma care, knowledge of the practical skills required to change an appliance, possible complications, and whom to reach for advice (Table 77.2-4).

Regular contact should be maintained until the family has the necessary confidence to be totally independent at home. It should be clear to the family how and where additional supplies can be obtained from community services.

Ideally, home visits after discharge offer the family an opportunity to discuss issues that they may have felt unable to question while in the hospital environment. It also provides an opportunity to review bathroom facilities, which may need simple adaptation, and general storage advice for the new equipment.

TABLE 77.2-4 DISCHARGE PLANNING

Discharge teaching should involve
Normal stoma function
Preparation, application, and removal of appliance
Skin care
Where to obtain supplies
Disposal of used appliances
Exemption from prescription charges
Recognizing complications and whom to contact for advice
Dealing with potential problems, eg, leakage, sore skin, odor, and flatus

EDUCATIONAL SUPPORT

Under the guidelines of The United Kingdom Education Act (1981), all children are entitled to an education, and it is the duty of their local educational authority to facilitate the necessary support. The reality is that parents are often "on call" to troubleshoot any problems that occur while the child is at school. The pediatric stoma nurse can offer the child and teachers support in terms of reintegration into the school environment and can educate teaching assistants and carers.

Most head teachers are only too willing to provide access to private toilets away from the main children's facilities, allowing the child to empty or change the appliance in private. The adolescent may have serious problems returning to school with a stoma, and avoidance behavior is common. Postoperative psychological support is often necessary to overcome this awkward period.

NUTRITIONAL CONSIDERATIONS

Concerns about eating and the associated reaction of the stoma are common among new ostomists. Initially, they are constantly aware when the stoma acts and, as a result, are cautious about eating certain foods. Flatus is produced more readily when eating pulses, baked beans, fizzy drinks, and, in some, chocolate. Eating slowly and chewing food well can reduce the amount of air swallowed.

Effluent will contain more fluid when eating spicy foods, fruit, and green vegetables. Eggs, boiled rice, sweetcorn, bananas, and mushrooms will thicken the stool and, in some children, lead to constipation. Foods that are known to cause problems should not be avoided, but by emptying the appliance more frequently or wearing an appliance to accommodate increased output, mealtimes should be more relaxed. It takes about 16 to 22 hours from food being swallowed to it being seen in a colostomy bag—less time with an ileostomy.

Children should be encouraged to experiment to see how their stoma reacts to different food items so that they can remain in control. A healthful balanced diet should always be encouraged. Useful tips in having a stoma can be gleaned from other patients and their families (Table 77.2-5).

TRANSITION OF CARE

The preparation to move from pediatric care to adult-based services can cause great anxiety to many families. For many parents, the security blanket of a pediatric service has enabled them to manage their children's chronic health needs and feel comfortable when attending clinic appointments. The handover of care should be planned and coordinated if possible in a combined adolescent clinic, giving the family the opportunity to meet the new team while maintaining links with the old.

TABLE 77.2-5 HELPFUL HINTS FROM OUR PATIENTS

When gaining confidence in fitting a new bag use a mirror so that you can see what you are doing.
Clean the stoma with gauze swabs because cotton wool balls can leave strands, preventing the bag from sticking.
Storage of appliances should be in a cool, dry area (steamy bathrooms affect the adhesive).
Always have some bags ready cut for those emergency changes; they will always happen.
It is up to you who you tell about your stoma!
To improve the stickiness of the appliance, warm it in clasped hands before applying.
To enable you to see what you are doing, use a clothes-peg to hold clothing out of the way.
If the smell is too offensive, use scented candles in the bathroom when changing the bag.
After the recovery phase of surgery, your stoma's activity will become more predictable. Often it is most active after breakfast.
Beer can give you wind!

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3. Psychological Aspects

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The psychological effects on pediatric patients who are preparing for or have experienced gastrointestinal (GI) surgery are rich in scope and poor in documentation.[1] Although virtually all clinicians agree that GI surgery poses potentially significant psychological and emotional risks for children and adolescents, the medical and psychological literature addressing these issues continually emphasizes the need for more rigorous studies to ascertain which interventions are most useful for this patient population. Although pre- and postoperative preparations most often include a developmentally driven attempt to demystify the hospital and the operation itself, the existing studies lack methodologic consistency and concrete guidelines.[1,2] Indeed, consulting the existing literature alone leaves one uncertain of how best to help children and adolescents through the ordeals of major and minor GI surgeries.

However, the lack of methodologic consistency contrasts sharply with the wealth of clinical experience that many mental health clinicians have accrued in working with medically ill young people. With regard to GI surgery, a coherent picture of the psychic effects of surgical intervention on younger patients is complicated by the premorbid psychiatric and psychological state, which is itself often a function of the underlying chronic GI illness. Patients with inflammatory bowel disease (IBD), for example, will potentially suffer different psychological effects than patients who are awaiting liver transplant or who suffer from GI tumors. The variety of children in general and the breadth of medical illness in particular make generalizations about this patient population especially challenging.

Nevertheless, clinical experience and a growing appreciation for a biopsychosocial approach *do* yield important guidelines for the care of these young patients. With these concerns in mind, this chapter addresses both general developmentally based observations regarding pediatric patients undergoing GI surgery and specific psychological aspects of common surgical issues seen in patients with IBD and those who receive corticosteroid therapies.

DEVELOPMENTAL APPROACH

The most efficient and accurate approach to medically ill children and adolescents involves careful attention to developmental principles.[3] The effects of major surgical intervention will be different for toddlers than for teenagers, and clinicians must take these differences into account when assessing children in these settings. Although an exhaustive review of child and adolescent development is beyond the scope of this chapter, a brief summary of important differences among children of different ages is important.

Infants and toddlers lack the cognitive skills to understand the gravity of their condition. They rely on their parents and caregivers to relieve immediate suffering and discomfort, and they experience much of the surgical world in terms of the discomfort engendered and the limitations imposed.

Perhaps most importantly, the youngest patients rely on their parents and primary caregivers to keep them safe and to soothe them when they are troubled. Infant–parent attachment can be particularly compromised by severe medical illness, and the hospital staff should work to foster a milieu that allows for as much contact as possible between infant and parent. Parents should remain as close as possible and should interact as much as possible. In this light, fundamental issues such as parent–child physical contact, feeding, and, if possible, breastfeeding should be preserved as much as possible. However, potential exceptions include using parents to restrain older and struggling infants and toddlers during painful procedures. This practice is potentially extremely traumatic for the child and the parent and may prolong and make more dangerous the procedure in question.

As toddlers age, they develop the egocentrism that often defines younger children. They view all events as relating directly to themselves and interpret both bad and good experiences as a function of their own actions. Thus, the toddler often associates unrelated incidents as being causally related, a cognitive style that developmentalists refer to as associative thinking. In the setting of illness severe enough to warrant surgical intervention, toddlers (as well as older patients in more subtle ways) are particularly vulnerable to concluding that their own transgressions have led to their medical predicament. To protect against the damage that these conceptualizations can create, it is often helpful to clarify as much as possible the etiology of a given medical condition and to uncover and correct misconceptions about the cause of illness.

Separation from caregivers and anxieties around bedtime are some of the more common psychological stressors affecting toddlers as they prepare for or recover from surgical procedures. In addition, because they have only recently come to understand that they have control over their body, toddlers often experience insults such as blood draws and even physical examinations as assaults on their entire physical being. To the extent that they can be gently

and consistently prepared before procedures and examinations, they will be protected from a state of constant hyperarousal as they prepare to defend themselves from the onslaught of physicians and technicians.

As children age, their cognitive capacities rapidly develop. They enjoy a growing sense of mastery in understanding the world around them, categorizing and labeling many of the central features of their lives. They will collect baseball cards, know their telephone number, and memorize the names of countless dinosaurs. Medically ill children will often take great pride and comfort in knowing the names of their diseases, of their medications, and of complicated surgical procedures. If a child in this setting expresses interest in what is happening medically, it is developmentally useful to answer as concretely as possible. Simple cause and effect are hallmarks of preadolescent cognitive development, and children in this age group understand, for example, that germs cause disease and that taking out something bad (like a splinter, in more benign settings) can lead to feeling better. However, this cognitive style can lead to psychological distress when there is no clear cause for a given illness. Always tuned into media and popular culture, children may wonder, for example, how they developed cancer without smoking or why they need to go back to the operating room if they diligently followed their postoperative instructions. Parents and clinicians must balance a child's desire to understand the world with helping the child to endure the ambiguities that we all find difficult to tolerate with regard to medical illness.

As children enter adolescence, they develop an increasing capacity to think abstractly and form multiple points of view. An appreciation for rules that more or less dominates preadolescent children develops into a tendency to question rules and restrictions. In this setting, adolescents begin to form a coherent sense of who they are and what matters to them. Although this sense of self seems constantly in flux to many adults, the intense exploration of different values and ideals is a normal adolescent obsession and should not necessarily be taken as a sign of serious difficulty. In addition, adolescents begin to separate themselves from their families of origin. For example, when they are in need, adolescents may increasingly turn to friends or mentors instead of parents. All of these forces can ultimately manifest themselves as apparent rebellion and "acting out" from the rules and values of their own families.

For medically ill children, it is vital that these normal adolescent tendencies not be expressed in terms of worsening medical compliance, both in and out of the hospital. The teenager with a new liver, for example, *must* take her medicines and must *never* experiment with alcohol. Helping adolescent patients to accept their limitations, to understand the necessities of their medical regimen, and to find alternative methods of expressing their individuality is absolutely essential to the successful management of the medically ill teenager.

Having stressed the discrete stages of normal development, it is paramount that one remember that developmental approaches are not static and often overlap. It is not unusual for teenagers to feel that their past transgressions have caused their newly diagnosed malignancy. Indeed, it is their realization that such thoughts are juvenile and immature that compounds their shame as they try to make sense of their predicament. In a similar light, GI concerns such as bowel mastery and control of one's flatulence are mastered early in normal child development. To the extent that GI surgery can compromise bowel control, children and adolescents may find themselves with soiled pants and regressed anxieties. In this sense, earlier developmental concerns are revisited on the recovering patient.

Finally, children often compartmentalize their concerns. There is nothing that is necessarily worrisome about the youngster who worries more about a soccer game than his/her upcoming surgery. Only in rare and specific circumstances is it helpful to force a young patient who appears unbothered to discuss his/her medical condition.

SPECIFIC TREATMENT ISSUES

Given the number and variety of pediatric GI procedures, it is somewhat difficult to generalize with regard to the psychological effects of invasive GI surgery. However, a number of considerations specific to certain GI illnesses have a direct and discernable effect on the psychological well-being of pediatric patients.

For example, children who undergo surgery in the setting of IBD often take varying doses of steroids and immunosuppressing agents. Similar medication regimens exist for children who undergo liver transplant and a scattering of other autoimmune diseases that affect the GI system. Although these medications are extremely important to the medical well-being of the patient, they also carry the potential for substantial psychological risk. Furthermore, virtually every serious GI condition has, in and of itself, potential psychiatric sequelae. As IBD constitutes perhaps the most common indication for pediatric GI surgery, it is helpful for clinicians working with this population to familiarize themselves with both the psychological risks of this condition as they pertain to surgery and the risks associated with medication regimens.

UNIQUE EMOTIONAL STRESSORS IN PEDIATRIC IBD

Children and families coping with IBD face unique emotional stressors and social challenges. When a child with recurrent debilitating colitis has an increase in symptoms, including refractory diarrhea and possibly stooling accidents, it may be intermittently necessary for parents to help with their child's basic toileting hygiene for the first time since toilet-training. As parents feel increasingly helpless as the disease progresses, there is a risk that they may become overcontrolling and overinvolved in an attempt to manage the unmanageable. The pediatric patient with colitis also faces some unusual psychological challenges. For a child who is appropriately increasing her sense of independence as she gets older, a family focus on bowel habits and bowel control may feel overwhelming and humiliating. The familial stance that is most psychologically supportive balances

the child's emotional needs, autonomy, and privacy. Often psychological support provided by a social worker, psychologist, or child psychiatrist can help the family refine an approach that best supports the patient's personal growth.

The social ramifications of colitis are unique. Compared with asthma, which is a common childhood disease that is well known and does not involve any specific toileting needs, colitis is not a disease familiar to most children. It is not an easy disease to explain to one's friends.

When the disease is active, the child's activities may be greatly curtailed owing to abdominal pain or frequent stooling. Embarrassed by their disorder, children may go to great lengths to hide their symptoms from their peers. Children with varied stages of disease, pre- and postoperatively, often worry about soiling their underwear if they do not make it to the bathroom on time. If bathroom access at school is limited, the child may start refusing to go to school to avoid the impending stooling disaster. Importantly, these symptoms can potentially worsen as a function of anxiety regarding an upcoming surgery or as a result of the surgery itself. It is extremely important, therefore, that clinicians be aware of these issues and facilitate the child's development in the context of his/her medical condition.

SCHOOL AND CAMP INTERVENTIONS THAT FACILITATE EMOTIONAL GROWTH AND MINIMIZE ANXIETY

A physician caring for a child with colitis can provide some sound advice about the basic social interventions that can increase a child's confidence at school. Children with IBD should be allowed open access to school bathrooms. They should also be allowed to use the bathroom in the nurse's office if necessary. (Whereas other bathrooms at school may have frequent toilet paper shortages, this one tends to be well equipped.) These children should not be required to ask the teacher or to obtain the one bathroom pass allotted for the classroom to leave the room; they may need every moment available to make it to the bathroom without soiling. Some schools provide a special hall/bathroom pass for these children that they can carry with them at all times.

In some cases, increased academic support may also be indicated. For children unable to carry heavy books back and forth from home to school, two sets of books should be provided. If the diarrhea or abdominal pain increases around a project due date, teachers should be flexible and provide due date extensions as needed. In addition, if a child needs to rush to a bathroom during a testing situation, he should not be penalized and should be allowed extra time to finish the examination.

Whatever unique setup is organized, the planning should be done privately with the children's teacher. If these safeguards are not in place, these children may worry about their bowel habits during the school day rather than focus on school activities. Knowing that they have open access to available restrooms and that the school will be appropriately flexible if their symptoms affect their academic availability can be a great relief that supports normal cognitive and social development.

For camping or portable toilet situations, children with IBD should be armed with flushable wipes and sterile gel hand cleanser to use as needed. For outside activities that may have limited toilet availability, such as rope courses in summer camps, children with IBD should be first in line to complete the activity so that they can make it to the bathroom after the event if necessary. For rowing or other boating events without available bathrooms, the counselor in charge needs to be informed of the patient's medical limitations before the activity rather than during the event because of an acute toilet need. In general, attention should be paid to anticipating and then preventing problems rather than ignoring the possible hazardous situations that may crop up.

POTENTIAL PSYCHIATRIC AND COGNITIVE EFFECTS OF CORTICOSTEROIDS

Many of the GI conditions that lead to surgical interventions are treated with corticosteroids. These medications often carry with them profound psychological impact secondary to side effects such as changes in facial characteristics and weight. Furthermore, these worries can be easily compounded if the child also suffers from psychiatric symptoms caused by the steroids themselves. Finally, patients can exhibit severe psychiatric symptoms as a result of steroid treatment without having a past history of psychiatric vulnerability or psychiatric family history.

Corticosteroids can cause a wide range of psychiatric symptoms that may occur at any stage of treatment, including treatment withdrawal.[4,5] Usually, the worst symptoms are dose related and notable within a few weeks of therapy. During steroid withdrawal, depression and fatigue have also been documented and are not always coincident with evidence of hypothalamic pituitary axis suppression.

Mood lability, irritability, depression, anxiety, and mania have been documented in children and adults during steroid treatment, even at modest doses. Depressed children may become more withdrawn and irritable and have trouble sleeping, eating, concentrating, and feeling motivated. Manic children may develop sleep difficulties and experience increased activity and impulsivity and worsening distractibility. Their mood can be euphoric or extremely irritable.

Patients with a history of post-traumatic stress disorder may have increased depressive symptoms or intrusive trauma memories during steroid treatment. Further, adolescent patients with a previously undisclosed trauma have been noted to become symptomatic during steroid therapy.

Finally, some children may experience cognitive difficulties as a result of steroid treatment. Difficulties with short-term memory and concentration have been reported in the absence of mood symptoms, and often these effects dissipate when the steroid dose is decreased.[4]

NUTRITIONAL SUPPORT

Patients who require GI surgical intervention will sometimes require some form of nutritional support for pro-

longed periods. Although one can imagine the potential psychological effects of these nutritional interventions, again, there is relatively little literature exploring these issues.

As noted above, children with illnesses such as severe IBD already run significant psychological risk. The developmental and emotional toll of these conditions is derived from a combination of the disease symptoms themselves, underlying psychiatric predispositions, and side effects to potentially psychotropic agents such as corticosteroids. Although it is clear that nutritional support can also affect emotional well-being, the etiology of psychic distress as a result of total parenteral nutrition or tube feedings is more subtle and poorly studied. Existing literature suggests relatively good emotional adjustment, although these studies are hampered by the lack of an adequate comparison group and by unclear measurements of psychological effects.[6]

Adequate exploration of these issues is also hampered by the lack of a clear etiologic connection between nutritional support and emotional well-being. Although corticosteroids, for example, directly affect mood and anxiety, evidence for a specific psychotropic effect related to successful nutritional support is somewhat lacking. However, for children who develop vitamin and mineral deficiencies during parenteral or enteral support, there is the possibility that psychological effects will develop as a direct consequence of these nutritional shortcomings.[7,8] In addition, for children already uncomfortable with the developmental and psychological burden of their GI illness, the motivation, patience, and perseverance necessary for successful total parenteral nutrition or enteral support can be particularly challenging.

PSYCHOLOGICAL EFFECTS OF OSTOMIES

Children whose medical conditions necessitate ostomy placement also may suffer emotionally and developmentally as a result of this intervention. Again, a developmental approach is necessary when understanding the potential psychological implications. For younger children, the growing sense of autonomy and pride with regard to appreciation of and control over one's own body is potentially compromised by the placement of ostomy itself. For older children, the growing emphasis on peer conformity and appearance is further threatened by this intervention. As with much of the psychiatric literature concerning severe GI illness, there is a relative shortage of systematic investigations into the effects of ostomy placement in pediatric populations. Nevertheless, much of the literature does support significant need for emotional support and psychoeducation to ensure favorable outcomes.[9–11]

MISCELLANEOUS TREATMENT ISSUES

Other important issues relevant to this patient population include the drug–drug interactions in children who are taking multiple medications. Although an exhaustive list of these interactions is beyond the scope of this discussion, some specific treatment issues deserve mentioning.

First, given the spread of alternative therapies, especially with regard to psychiatric symptoms, it is always important to ask parents and patients whether they take any agents that are categorized as herbal or homeopathic. Although both parents and patients alike may view these agents as benign, many of these therapies have the potential to complicate treatment. For example, St. John's wort, an extremely popular herbal medication purchased at health food stores and taken for depression, also interferes with cyclosporine metabolism.[12] Clinicians have worried about graft rejection in patients who began St. John's wort and subsequently increased the metabolism of their prescribed immunosuppressing agents.

In general, taking a careful psychosocial and medical history helps to make patients and their families more comfortable discussing psychological issues and allows the clinician to screen for psychological distress. Having said this, it is extremely important that seemingly psychological symptoms be examined from multiple perspectives. For example, the youngster who seems lethargic, even in the setting of active depression, should also be examined for a worsening medical condition. All of the antidepressants and psychotherapy in the world will not fully motivate an anemic or otherwise medically compromised child. To this end, a team approach is absolutely essential for the overall health of these young patients.

PRE- AND POSTOPERATIVE MANAGEMENT

As noted above, there is limited methodologic consistency with regard to how best to prepare children and adolescents for GI surgery. Investigations have focused on measurements of pre- and postoperative psychological distress, postoperative pain, overall psychological adjustment, and the extent to which parents effectively cope.[1,2,13]

Studies suggest that 40 to 60% of children experience significant psychological and behavioral distress prior to surgery.[1] However, many studies do not describe in detail the scope or characteristics of these behavioral difficulties. Nevertheless, although these percentages refer to the almost 3 million children per year who undergo any type of surgery, it is reasonable to conclude that all surgeries, including GI procedures, are potentially extremely distressing to children and adolescents.[1]

In spite of these findings, there is little consistency among the approaches used to help quell these concerns. Some institutions use preoperative anxiolytic pharmacologic management, whereas others pursue a psychoeducational approach, preparing youngsters as best as possible for what they can expect both pre- and post-operatively. Many institutions use some combination of behavioral and pharmacologic management, and regimens are often a function of individual preferences among clinicians and, hopefully, a careful assessment of the needs of patients and their families. One large meta-analysis notes that some institutions have favored an overall decrease in preoperative psychological management.[1] These hospitals and clinicians acknowledge that although some form of preoperative management is potentially very helpful, the lack of consistent data supporting any one method of intervention makes much of these endeavors somewhat suspect.[1]

Other investigations have centered on postsurgery analgesic requirements and use, as well as the relative characteristics of parents as they cope with their child's illness and procedures. For example, some small studies note that variables affecting pain medication requirements include the relative invasiveness of surgery, the preoperative psychological state of the patient, and the extent to which parents felt anxious or concerned about the upcoming procedure.[13]

As noted above, the heterogeneity of procedures that can be characterized as GI surgery makes generalizations difficult, and many smaller investigations suggest findings that one can cautiously extrapolate from the specific conditions that they discuss to the more general concerns of the surgical pediatric patient. For instance, a study examining 101 pediatric liver transplant patients found that earlier transplants correlated with increased aggressive behavior and sexual dysfunction.[14] Furthermore, this study found that the surgery itself continued to be an important psychological part of the child's sense of self many years after the procedure. A similar study examined results from psychological projective testing in post–liver transplant children, noting increased negative self-focus and depressive episodes in this patient population.[15] One study has found that children were relatively well psychologically and developmentally when the treating team used a multidisciplinary approach.[16] Although many of these findings seem specific to the extraordinary circumstances surrounding any transplant, it is reasonable to expect earlier major procedures in some instances to interrupt development sufficiently to confer significant and lasting psychological effects. More studies are badly needed to confirm these hypotheses.

Similarly, it is also possible to extrapolate information from other severe illnesses in children to the overall understanding of how pediatric patients might respond to GI surgery. An important finding over the last few years has been a growing recognition that pediatric patients who undergo invasive and side effect–laden treatments such as chemotherapy and massive surgeries are at risk for the development of post-traumatic syndromes, cognitive decline, and depression. In addition, because underlying psychiatric predispositions often express themselves in the setting of external stressors, it is likely that many children will experience their first presentation of major psychiatric conditions such as mood and anxiety disorders in the setting of the stress of a surgical procedure.[17,18] Indeed, one study examining risk factors for the development of post-traumatic stress disorder in children with cancer noted that the most robust predictor was the extent to which the parents felt traumatized. Thus, it behooves any treatment team to soothe the fears and anxieties of parents as well as patients.[19,20]

At this point, it should be noted that there are many relevant studies listed in psychological databases such as *PsychINFO* and relatively few studies in more medically oriented sites such as *MEDLINE* and *Index Medicus*. Indeed, many of the most intriguing investigations are listed as doctoral dissertations, unpublished in other formats as of yet. Clearly, the case can be made that the medical establishment has neglected a more systematic interest in the psychological effects of GI surgery on children.

CONCLUSION

Why the dearth of knowledge? Some clinicians suggest that recent improvements in the overall treatment of drastically ill children have spawned a new interest in understanding the psychosocial effects of severe medical illness in pediatric populations. There may have been a tendency in the past to simply ignore or pay little heed to psychological distress, given other, more pressing medical concerns.[21] After any medical condition serious enough to warrant surgery, many parents and clinicians are, understandably, delighted that the child is simply alive and functioning. This can lead to a relative lack of emphasis on psychological well-being, and many consultation-liaison psychiatrists, psychologists, and pediatricians are only now systematically examining the effects of major medical interventions, such as GI surgeries, on the psychological state of their younger patients. Future studies will undoubtedly yield more specific and important findings, filling an important gap in the understanding of a child's experience in the surgical setting.

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INTRODUCTION

PEDIATRIC GASTROENTEROLOGY: A SUBSPECIALTY IN PEDIATRICS "COMES OF AGE"

W. Allan Walker, MD
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Early in the twentieth century, pediatrics emerged as a distinct medical specialty, largely focused on infectious diseases and the nutritional support of infants and young children. Pediatric subspecialties have evolved even more recently, over the past 50 years. For the young pediatrician just entering a fellowship in pediatric gastroenterology, it probably seems as if the subspecialty has existed forever. Few realize that even 35 to 40 years ago, those who wished to become pediatric gastroenterologists had to leave pediatrics and enter adult fellowship programs to obtain formal training. In spite of its "youth," the discipline of pediatric gastroenterology, hepatology, and nutrition has established itself scientifically and clinically as a major contributor to the care and well-being of infants, children, and adolescents.

In this introductory chapter of the fourth edition of this textbook, we attempt to summarize the historical evolution of our field, highlighting some of those disorders that have their onset during infancy or childhood or are unique to that age period and that have spurred the development of the discipline. We also review the defining events in the establishment of the subspecialty and speculate on the directions it may follow into the new millennium. All of this serves as a preface to a significantly expanded fourth edition of this textbook. All chapters have been revised and updated to reflect the advances in science and clinical practice that have occurred since the publication of the third edition. The textbook has also been significantly expanded to reflect the extraordinary advances in our understanding of the development and pathophysiology of the gastrointestinal tract at the cellular and molecular levels.

DEVELOPMENT OF PEDIATRIC GASTROENTEROLOGY

The concept of organ-specific subspecialties in medicine began at the end of the nineteenth century. For example, the American Gastroenterological Association was established in 1897 in Philadelphia.[1] It took almost another century before such subspecialties of pediatrics were established. The European and North American societies of pediatric gastroenterology were incorporated in 1967 and 1973, respectively, and, more recently, the Asian Pan Pacific and Latin American societies formally organized. A major impediment to the development of this field was a notion among pediatricians that gastrointestinal conditions could be fully addressed within the purview of general pediatrics. Although this undoubtedly is true for common acute gastrointestinal conditions (eg, neonatal regurgitation and rotavirus gastroenteritis), it quickly became clear that the diagnosis and treatment of chronic, complex, and often debilitating conditions affecting the liver, pancreas, gastrointestinal tract, and nutritional status of pediatric patients required specific knowledge and experience, acquired through specialized training. Heretofore, this need had been filled by gastroenterologists in internal medicine. In general, this approach was acceptable as long as it involved an older child or adolescent. However, these individuals had little or no expertise in managing young patients with gastrointestinal problems unique to pediatrics, for example, inborn errors in bilirubin metabolism, congenital intestinal absorptive defects and other metabolic diseases (eg, α_1-antitrypsin disease [α_1-AT]), and congenital malformations of the liver and gastrointestinal tract. To better serve these patients, pediatricians who left pediatrics to train in adult gastroenterology adapted this training to the care of infants and children. In the early 1970s, small selective divisions of pediatric gastroenterology and nutrition were established in North America and Europe by pediatricians trained in adult gastroenterology programs. These divisions became nascent training centers for future pediatric gastroenterologists. In three short decades, the field has evolved to become an established pediatric subspecialty worldwide.

Evolution of Diagnostic and Therapeutic Approaches to Gastrointestinal Disease States in Children

Table 1, adapted from a recent comprehensive review of the development of pediatric gastroenterology, hepatol-

TABLE 1 MEDICAL EVENTS LEADING TO THE EMERGENCE OF PEDIATRIC GASTROENTEROLOGY

Small intestinal biopsy
Parenteral nutrition
Safe pediatric ileocolonoscopy
Percutaneous liver biopsy
Pathophysiology of hereditary disorders of absorption
Rise of gut immunology and food allergy reseach
Oral rehydration
Advances in pediatric surgery
Portoenterostomy (Kasai) procedure for biliary atresia
Organ transplant
Liver
Small bowel
Pathophysiology of metabolic liver disease (eg, α_1-antitrypsin genetic defect)
Genetic basis for disease
Cystic fibrosis
α_1-Antitrypsin disease
Crohn disease

Adapted from Walker-Smith J and Walker WA.[2]

ogy, and nutrition,[2] lists diagnostic techniques and therapeutic approaches that have helped shape the unique expertise of the pediatric gastroenterologist. We highlight a few representative conditions from this list below. A complete discussion of each disorder is found in subsequent chapters in the book.

Celiac Disease

Celiac disease is a unique genetically predisposed condition that, although lifelong, often presents in infancy and childhood. In postwar Holland, Dicke demonstrated that this chronic, malabsorptive condition associated with severe "failure to thrive" was caused by intolerance to gluten, a major constituent of several grains, particularly wheat.[3] This observation, that a common condition in infants and children was not an infectious gastroenteritis but instead a food intolerance, launched pediatric gastroenterology as a subspecialty of pediatrics in Europe. In 1957, Drs. Jack Sakula, a pediatrician, and Margot Shiner, a gastroenterologist, published a diagnostic technique that could be used to diagnose celiac disease.[4] By successfully modifying the intestinal biopsy capsule designed for adults with a smaller portal, they could obviate intestinal perforation in infants and children and obtain a safe biopsy of intestinal mucosa for chemical or morphologic analysis. This diagnostic procedure, coupled with advances in microanalysis of mucosal enzymes[5] and breath testing, has been the basis for the diagnosis and management of celiac disease as well as acquired and congenital disorders of carbohydrate digestion and absorption for the last three decades.

More recently, celiac disease has been linked to specific human leukocyte antigen (HLA) loci (DQ2 and DQ8), suggesting a genetically based underlying immunologic defect.[6] Currently, it is considered to be an autoimmune disease, with transglutaminase as the putative autoantigen.[7] With modern infant feeding guidelines, including exclusive breastfeeding for 3 or more months, the classic presentation of celiac disease in early infancy (chronic diarrhea or malabsorption associated with severe failure to thrive) is much less common today. As serologic tests with high sensitivity and specificity for celiac disease have come into routine use (IgA antiendomysial and tissue transglutaminase antibodies), subclinical celiac disease and atypical presentations of the disorder have been recognized, and the true prevalence of this disorder has significantly increased.[8] These advances in noninvasive diagnostic tests, along with a better understanding of the pathogenesis of celiac disease, have allowed the pediatric gastroenterologist to manage the disease and its complications more efficiently (see Chapter 44.1, "Celiac Disease").

α1-Antitrypsin Deficiency Disease

In 1969, Sharp and colleagues reported that the inherited disorder α_1-AT deficiency was associated with neonatal hepatitis syndrome, leading to cirrhosis.[9] Subsequently, when the percutaneous liver biopsy technique was adapted for use in infants and children,[3] this condition could be distinguished from other neonatal hepatic conditions, such as biliary atresia. In follow-up studies by Sharp and others, a mutant form of α_1-AT (PiZZ) was identified as a sequestered protein in the endoplasmic reticulum of hepatocytes, and this histopathologic feature became diagnostic of the disease.[10,11] Initially, α_1-AT–associated hepatitis was thought to be due to a failure of its release into the hepatic interstitium to neutralize proteases in the portal circulation that could cause hepatocellular damage.[12] The hepatic lesion has been ascribed to hepatocyte toxicity caused by the retained mutant form of α_1-AT in hepatocytes.[13] However, using sophisticated "cutting edge" cell biologic techniques, Perlmutter and colleagues and others have suggested that the process is much more complex and involves not only accumulation of misfolded mutant forms of α_1-AT in hepatocyte endoplasmic reticulum but also disruption of other subcellular functions leading to liver damage.[14,15] They also suggest that genetic variants of α_1-AT can define phenotypic hepatic disease expression.[16] This common metabolic liver disease can now be distinguished from other liver conditions (eg, tyrosinemia, biliary atresia) and managed in an appropriate prospective manner. Of interest, α_1-AT–deficient liver disease is a common cause of liver transplant in pediatric patients.[17]

Cystic Fibrosis

All now recognize that cystic fibrosis is a genetically determined condition that often presents with pancreatic insufficiency, malabsorption, progressive, obstructive lung disease, and, in some, progressive biliary cirrhosis. Initially, with an incomplete understanding of the pathogenesis of this condition, patients died in childhood from severe lung failure.[18] However, the recent discovery that the cellular membrane transporter of chloride is defective in patients with cystic fibrosis, as well as cloning of the cystic fibrosis transmembrane conductance regulator,[19] promises more effective approaches to management of the pulmonary and gastrointestinal complication of this disorder. The association of genotypes with phenotypes in patients with cystic fibrosis has the potential to facilitate

the diagnosis and management of patients with cystic fibrosis.[20] The obvious extension of having cloned the gene is the potential for gene therapy to prevent the severe complications of the disease. Until this is established, however, patients with progressive disease leading to lung and liver failure may undergo combined lung and liver transplant as the ultimate "rescue" therapy. In addition, new therapeutic approaches to enhancing membrane chloride transport and to the prevention of *Pseudomonas* and other infections of the lung have met with some success.[21] This condition is also an example of a unique pancreatic disorder that presents in infancy and childhood. A complete discussion of the molecular biology of this disorder, its diagnosis, and approaches to treatment is found in Chapter 65.1, "Cystic Fibrosis."

Intrahepatic Cholestasis

Major advances have occurred in our understanding of the molecular mechanisms responsible for the formation and transport of bile in the liver and biliary tract. These are fully described in Chapter 55.6, "Biliary Transport." The recent discovery that the hydrolysis of adenosine triphosphate (ATP) is necessary for cannalicular transport of bile salts led to the finding that bile acid transporters are members of the ATP-binding cassette transporter superfamily of proteins. Many of these proteins, such as the bile salt export pump, have now been identified. Disorders such as Byler disease can now be renamed according to the defects in transport proteins that are responsible for them. This elucidation of the molecular and physiologic process of bile formation sets the stage for extraordinary advances in the diagnosis and treatment of these inherited disorders, as well as a whole host of disorders characterized by cholestasis found in both children and adults.

Technology

Technologic advances have clearly enhanced the ability to diagnose and treat many disorders of the liver, pancreas, and gastrointestinal tract that occur in infancy and childhood. These include the development of improved flexible instruments to perform safe upper tract and ileocolonoscopies on infants and small children.[22] Noninvasive ultrasonography, magnetic resonance imaging (MRI), and other radiologic imaging techniques have evolved to an extraordinary degree over the past quarter decade and have supported the earlier and safer diagnosis of life-threatening disorders of the liver, pancreas, and gastrointestinal tract even in fetal life. The use of parenteral nutrition to support the nutritional needs of infants and children with intractable diarrhea or short-bowel syndrome has been lifesaving.[23] The survival of patients supported by parenteral nutrition has also permitted in-depth molecular and physiologic investigations of a number of disorders, such as microvillus inclusion disease, epithelial cell dysplasia, and chronic intestinal pseudo-obstruction syndrome. Improvements in pediatric surgery and the establishment of liver and small intestinal transplant programs[24] have also helped save the lives of children with major inherited anomalies of the gut and liver and have established the pediatric gastroenterologist/hepatologist as an important member of the tertiary care team at major medical centers.

MAJOR DEFINING EVENTS IN THE SUBSPECIALTY

Centers of Excellence

In the early 1960s, small pediatrics-based centers of excellence in the care of children with gastrointestinal disorders formed and focused on common gastrointestinal problems unique to the referral region. For example, several centers in Europe and the United Kingdom specialized in the care of patients with celiac disease, whereas others in France and the United Kingdom began to attract infants with chronic, life-threatening liver diseases. Ultimately, these centers began to provide care for a broader-based spectrum of gastrointestinal disorders. With increasing interest in pediatric gastroenterology among academic pediatric centers, small "splinter groups" (eg, the "Pediatric Gut Club") began to have organized symposia at national pediatric meetings or as satellite symposia at meetings of adult gastroenterologists. As interest in this pediatric subspecialty grew, the need for a formally organized society emerged.

Pediatric Gastroenterology Societies

The natural evolution of the increasing interest in pediatric gastroenterology was the establishment of formal societies in the late 1960s or early 1970s. The European Society for Pediatric Gastroenterology was established in 1967 and held its first independent meeting in Paris in 1968 with representatives from the Netherlands, Sweden, France, Italy, and the United Kingdom.[25] This society grew steadily over the next three decades into the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN), which exists today (currently with almost 600 members). The formal organization of a society has been a catalyst for increased numbers of European and United Kingdom pediatricians choosing to exclusively practice or conduct research in gastrointestinal diseases. Among other benefits, it has also led to the establishment of guidelines for the diagnosis and management of several common pediatric gastrointestinal diseases (eg, celiac disease, gastrointestinal allergy, neonatal hyperbilirubinemia).

The North American Society of Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN) was established in 1973 as an extension of the informal Pediatric Gut Club. It sponsored an evening symposium at the annual meeting of the Society for Pediatric Research and American Pediatric Society. As additional physicians with an interest in pediatric gastroenterology or with formal gastrointestinal training joined the Society, the venue for its annual symposium moved to the American Gastroenterological Association's (AGA) national meeting. Finally, in 1984, the Society established its own independent annual meeting, which has occurred yearly since then, either separately or in conjunction with the ESPGHAN. The establishment of a formal society for pediatric gastroenterology in North America has led to subspecialty boards

and multiple formally constituted and recognized training programs (3 years duration) approved by the Accreditation Council on Graduate Medical Education (ACGME). NASPGHAN now has over 1,000 members. In the last two decades, additional international societies have been established in other parts of the world. In 1974, the Latin Society for Pediatric Gastroenterology and Nutrition (LASPGAN) was founded to formalize interaction among pediatric gastroenterologists in countries in South and Central America and to establish exchanges with NASPGHAN.

In like manner, in 1993, the Asian Pan Pacific Society of Pediatric Gastroenterology (APPSPGN) was established as an extension of a group of pediatricians interested in gastroenterology in Asia who had been informally meeting since the 1960s. This society began holding large meetings every other year in venues throughout Asia. In 1994, the Commonwealth Association of Pediatric Gastroenterology and Nutrition (CAPGAN) was founded to include both developing and industrialized countries that are or were part of the British Commonwealth. With the recognition that these new societies represented almost 2,500 physicians worldwide, the decision was made to hold a world congress every 4 years. This began with a highly successful first world congress held in Boston, Massachusetts, in 2000, which had over 3,000 attendees. The next world congress will be in Paris, France, in 2004 and is expected to have even more attendees. These meetings resulted in position papers defining and outlining management of several gastrointestinal conditions that are accepted as standard practice guidelines worldwide.[26] In addition, a Federation of Societies of Pediatric Gastroenterology, Hepatology and Nutrition was formed to coordinate the relationships and joint activities of the four major regional societies. A major benefit of these newly organized relationships has been the fostering of collaborative studies and exchange training programs, as well as greater awareness of major gastrointestinal health problems in developing countries. Recently, the National Institutes of Health (NIH) has made substantial efforts to foster collaborative studies of pediatric gastrointestinal and liver disorders as well. This includes multicenter programs to study acute liver failure, biliary atresia, and nonalcoholic fatty liver disease. These NIH-sponsored programs represent a major milestone in the development of pediatric gastroenterology.

Journal of Pediatric Gastroenterology and Nutrition

In 1982, the *Journal of Pediatric Gastroenterology and Nutrition* was first published as a private journal. In 1991, the journal became the official journal of ESPGAN and NASPGHAN, and, in 1995, the other two societies, LASPGN and APPSPGN, became affiliates with representation on the editorial board. This journal has expanded and now receives manuscripts from clinical and basic research physician scientists worldwide. Through this journal, societies can publish position papers, supplements from major meetings, abstracts from national meetings, and medical opinions from leaders in the field. It has also helped to establish the field of pediatric gastroenterology in the eyes of the medical community worldwide.

Role of Textbooks

Several major textbooks have been published in the field of pediatric gastroenterology. The first textbook in pediatric gastroenterology was published by Roy and colleagues in 1971.[27] In Europe, Anderson and Burke published a similar general text in 1975,[28] and Walker-Smith published a specialized text on diseases of the small intestine in childhood,[29] whereas Gryboski wrote a textbook describing gastrointestinal problems in infancy.[30] The first edition of our textbook was published in 1991,[31] and this effort continues today with the publication of this fourth edition. The proliferation of textbooks of pediatric gastroenterology, hepatology, and nutrition, as well as others that focus on pediatric gastroenterology, hepatology, or nutrition, underscores the assimilation of knowledge on disease states that define the subspecialty of pediatric gastroenterology. The amount of information and expanding literature in this field is accumulating at such a pace that we have decided to update our textbook with each world congress in an effort to provide a current and comprehensive reference for physicians caring for children with gastrointestinal problems.

THE DEVELOPING CHILD: A UNIQUE CHALLENGE TO THE PEDIATRIC GASTROENTEROLOGIST

In the introductory chapter to the third edition of this textbook, Dick Hamilton emphasized that the pediatric gastroenterologist must have an in-depth knowledge of the morphologic and functional development of the gastrointestinal tract to understand and manage gastrointestinal symptoms and disorders in pediatric patients. [32] For example, many infants may have regurgitation during early feeding in infancy.[33] This condition is usually not pathologic and improves with time as the gastrointestinal tract matures. At a later stage in life, however, the same persistent symptoms might portend serious disease such as obstruction or inflammation.[34] Without a complete knowledge of developing gut function in infancy, inappropriate diagnostic interventions could be instituted and unnecessary invasive therapy undertaken.

In contrast, a missed diagnosis of a serious gastrointestinal condition that affects gastrointestinal function could lead to severe "failure to thrive" and, if a chronic condition ensues, to growth retardation. Microvillus inclusion disease, Hirschsprung disease, and other congenital disorders of gut and liver function are examples of disorders that have been identified as a result of an expanding understanding of gut morphogenesis. Furthermore, a chronic condition such as inflammatory bowel disease that interferes with the child's emotional and physical development during the vulnerable period of adolescence might have a lifelong impact on adult stature and psychosocial stability if not treated properly. These problems need a specialized knowledge of the normal expected pattern of physical and psychological development in the pediatric patient and an

appreciation for the impact of chronic or serious illness on the child's quality of life. The pediatric gastroenterologist therefore needs to understand his or her patient population, know the unique age-related conditions that can affect the gastrointestinal tract, and be able to distinguish symptoms and signs that are related to gut development from that of serious disease. Said in another way, certain gastrointestinal signs occurring during different periods of life (infancy, childhood, and adolescence) often evoke a very different differential diagnosis and approach.

Two examples may underscore the uniqueness of caring for pediatric patients with gastrointestinal problems. A major diagnostic problem occurring during childhood that requires careful evaluation by the pediatric gastroenterologist is the child with recurrent abdominal pain (RAP). In most instances, RAP is not due to a serious "organic" gastrointestinal condition but instead may relate to stresses in the child's life, such as school adjustment problems, learning difficulties, a dysfunctional family situation, or a low pain threshold, all manifesting themselves as recurrent gastrointestinal symptoms (eg, pain, diarrhea, constipation).[35] The pediatric gastroenterologist (like his or her counterparts in internal medicine) must be able to separate complaints that originate from a serious physical gastrointestinal disorder (eg, early inflammatory bowel disease, *Helicobacter pylori* gastritis) from functional, often age-related, complaints. He or she must carefully decide on appropriate diagnostic tests without being too invasive or "feeding" into the child's or parent's fear of serious illness. If the symptoms of abdominal pain are diagnosed as functional RAP, the pediatric gastroenterologist must reassure the patient and parents without offending them and prepare an acceptable approach to management. The thoughtful consideration of RAP in this textbook provides a major insight into the diagnosis and management of this condition (see Chapter 14, "Abdominal Pain").

Another condition unique to pediatric gastroenterology is "breast milk colitis."[36] This condition presents with bloody diarrhea in infancy in young infants who are exclusively breastfed. Parents anxious about caring for a vulnerable newborn may overreact, fearing the worst for their baby. As pediatric gastroenterologists, we know that the immaturely developed mucosal immune system in neonates, activated by foreign proteins (cow's milk proteins) present in breast milk from the maternal diet and breast milk cytokines and other active immune factors, can temporarily cause sigmoid-rectal inflammation and blood in the stools. The condition is transient and requires either limiting the maternal diet or temporarily taking the infant off breast milk. Over time, the condition clears, and no residual intestinal problems persist. Our expanding understanding of the maturation and physiology of the gut immune system and the presence and function of biologically active factors in breast milk are areas of unique interest and importance to the pediatric gastroenterologist. With this knowledge and experience, infants with this and other immune-related inflammatory conditions can be spared unnecessary investigation and treatments. What we have just briefly described are but a few examples that underscore the need for subspecialists with formal credentials in pediatric gastroenterology and nutrition.

FUTURE OF THE SUBSPECIALTY: PEDIATRIC GASTROENTEROLOGY IN THE TWENTY-FIRST CENTURY

Having provided evidence for our specialty having "come of age" as an established component of pediatric medicine, let us speculate about the future of our field during the twenty-first century. Advances in medicine and science will make the future practice of pediatric gastroenterology an exciting and fulfilling professional experience. In the last few years, the Human Genome Project has been completed. With this database, we can systematically begin to identify genes that are responsible for organ development, as well as those that cause or influence the development of complex gastrointestinal conditions (eg, *CARD15/NOD2* mutations and Crohn disease). Because so many of the disorders seen by pediatric gastroenterologists have an inherited basis, they serve as biologic examples that lead to significant discoveries regarding the mechanisms of disorders that spread well beyond the sphere of pediatric gastroenterology.

As a result of the advances in molecular genetics, we will also be in a better position to make earlier diagnoses of chronic debilitating conditions (eg, cystic fibrosis, inflammatory bowel disease, celiac disease) before they cause irreversible damage to the pediatric patient. By providing genotype-phenotype associations with genetically determined diseases, we can modify the patient's environment to minimize the severity of disease expression. Patients can be tested for genetic responses to therapeutic interventions (eg, use of 6-mercaptopurine in inflammatory bowel disease patients). Effective gene therapy is clearly within sight. Using molecular and cellular biologic techniques, we can define the precise cellular mechanism of disease and ultimately develop genetic profiles that will allow patients to be identified before disease expression and its consequences occur. The challenge, of course, will be to identify only those who would actually develop disease and not to mislabel the otherwise healthy carrier of "disease-related" genes.

We have now begun to understand how microorganisms and their molecular patterns mediate health and disease in the gastrointestinal tract.[37] Several years ago, a molecular pattern recognition receptor for endotoxin (lipopolysaccharide [LPS]) was identified on intestinal lymphoid cells. This receptor was termed the Toll-like receptor (TLR) because of its conserved structure similar to the Toll receptor in *Drosophila melanogaster*, which determines organism orientation and innate immunity. This receptor was noted to have an intracellular signal transduction schema similar to the interleukin-1β receptor. Interaction with LPS resulted in an up-regulation of nuclear factor κB, an inflammatory cytokine transcription factor, which generated the inflammatory response to endotoxin. This discovery has led to a better understanding of the mechanism of endotoxin-induced shock and new approaches to its prevention. The TLR that principally interacts with LPS

is termed TLR4. In addition to this receptor, which has been cloned, nine other TLRs have been identified and cloned, and other molecular patterns of both gram-positive and gram-negative bacteria, as well as viruses, have been identified as their ligands. This genomic/molecular biologic discovery has opened up the field of innate immunity and has identified the role of the gut epithelium in its effector response. We now have a better sense of how intestinal pathogens and commensal organisms communicate with the gut through the epithelial surface lining or underlying lymphoid elements, with very important future health care implications for controlling infections that originate in the intestine and elsewhere.

As techniques for diagnosis improve and become less invasive (eg, MRI), we can pinpoint gastrointestinal lesions earlier and in smaller infants. New targeted forms of therapy that pose no health risk can be used to mitigate or even prevent disease (eg, probiotics and protective nutrients for preventing inflammatory conditions of the intestine). With a better understanding of the molecular mechanisms of disease and developmental pharmacology, drugs can be designed to treat a condition and, in particular, a specific patient without extensive adverse side effects. These are but a few of the many examples to be found in the following chapters of this textbook of extraordinary advances that will influence the direction of care of pediatric patients with gastrointestinal, liver, pancreatic, and nutritional disorders over the coming years.

SUMMARY AND CONCLUSIONS

In this introductory chapter of the fourth edition of this textbook, we have provided evidence to support the "coming of age" of the subspecialty of pediatric gastroenterology. In a brief summary, we have presented some of the defining "developmental" steps that have led to the formal establishment of the subspecialty of pediatric gastroenterology, hepatology, and nutrition. The importance of understanding the physical and emotional development of the infant and child cannot be overemphasized in the context of investigating and treating gastrointestinal conditions in pediatric patients. Thus, the pediatric gastroenterologist must have a complete understanding of pediatric development and gastrointestinal diseases unique to specific age groups, not only to optimally diagnose and manage symptoms and disorders but also to enhance the quality of life for children and adolescents.

The growth of our subspecialty has been extraordinary to be a part of and to behold. This is a very exciting time for medicine in general, no less so for those who have chosen to focus on the care of infants and children with disorders of the liver, pancreas, and gastrointestinal tract. The growth velocity of the knowledge base in this subspecialty is still increasing, as if it was still in its infancy. It has clearly matured beyond that stage. The current state of the art in the understanding and management of gastrointestinal disorders in pediatric patients is set forth in the current edition of this textbook. We also look forward enthusiastically to future editions and the wonderful, even amazing, advances that they will document.

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Persistent Diarrhea

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Liver Tumors

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Postnatal Infections of the Liver: AIDS and Other Immune Disorders

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Drug Therapy: Modulation of Intestinal Flora: Probiotics

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Pediatric Gastroenterology: A Subspecialty in Pediatrics "Comes of Age"

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Enteropathy: Celiac Disease

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Treatment of End-Stage Liver Disease

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Approach to Neonatal Cholestasis

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Gastrointestinal Bleeding: Lower Gastrointestinal Bleeding

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Congenital Disease of Dysfunction and Absorption: Genetically Determined Disaccharidase Deficiency

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Intestinal Tumors: Other Neoplasms

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Munchausen Syndrome by Proxy: Factitious Disorder by Proxy

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DEDICATION

To Michael (Pic) Walker and Heather McDonald Walker in celebration of their union and as role models for life.

WAW

I wish to acknowledge my cherished French colleagues, professors Jean Rey, Claude Ricour, and Jacques Schmitz, who encouraged me to enter the exciting field of research and clinical practice in pediatric gastroenterology and nutrition, and Professor Allan Walker, who invited me to be one of the two European associate editors of this fourth edition. To my wife Véronique and our children, Pierre-Arthur, Charles, Alix, and Marine, who have supported me in my work for so many years.

OJG

To Allan Walker, who has inspired all of us in this discipline of pediatric gastroenterology and nutrition with his energy, wisdom, and imagination to dedicate our efforts to improving the lives of children with gastrointestinal, liver, pancreatic, and nutritional disorders; who has been and continues to be my mentor and close friend and a source of sage advice for over 25 years; and who remains the standard bearer for those who wish to advance the science and practice of medicine for pediatric patients. With deep gratitude.

REK

To Megan, David, and Rachael for their support.

PMS

To Julia and Vita with love.

IRS

To my wife Abigail and my daughters Caitlin and Elizabeth for their constant love and support, without which this and many of my other professional activities would not have been possible, and to my many mentors and colleagues in pediatric hepatology, including, most notably, Drs. Frederick Suchy, Peter Whitington, and Alex Mowat.

BLS

PREFACE TO THE FIRST EDITION

Over the last two decades, the field of pediatric gastroenterology has developed from an obscure subspecialty to an essential component of every major academic pediatric program throughout the world. Among the many pediatric texts available, none deals extensively with the pathophysiologic basis of gastrointestinal disease in children of all ages. Contributors to this text have been asked to undertake their writing with a plan to fill this void, extending pathophysiologic considerations to their coverage of diagnosis and management as well. In tandem with development of the subspecialty the literature of gastrointestinal and hepatic entities as they pertain to the pediatric patient has grown. Accordingly, we have prepared an approach to the subject that should provide a reference text for pediatricians, gastroenterologists, and pediatric gastroenterologists alike.

This new multivolume textbook is dedicated to establishing a comprehensive approach to pediatric gastroenterology. Each author was carefully selected to provide an authoritative, comprehensive, and complete account of his assigned topic. We have devised an approach to dealing with the families of children with gastrointestinal diseases, and a pathophysiologic section examines cardinal manifestations of gastrointestinal disease as well as the development of the gastrointestinal tract. These sections help to augment an in-depth approach to disease manifestations and management. A careful and unique approach to diagnosis of gastrointestinal diseases in children follows. Finally, the principles of therapy are explored. We hope and expect that this collective approach will be beneficial to all physicians dealing with gastrointestinal problems in children.

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J. Richard Hamilton, M.D., FRCPC
John A. Walker-Smith, M.D., (Syd.), F.R.C.P. (Lon., Edin.), F.R.A.C.P.
John B. Watkins, M.D.
1991

PREFACE TO THE FOURTH EDITION

The fourth edition of this textbook, published in time for the Second World Congress of Pediatric Gastroenterology, Hepatology, and Nutrition, to be held in Paris, France, in July 2004, has been planned to update pediatricians caring for children with gastrointestinal and liver diseases worldwide and to provide the most recent, cutting-edge developments in our field. In a recent article in *Pediatric Research* reviewing the development of our subspecialty, it was stated that evidence for the field "having arrived" as a discipline in pediatric medicine was the publication of a textbook exclusively devoted to pediatric gastroenterology.[1] This edition of the textbook has made major strides in covering the most important aspects of physiology and pathophysiology, clinical presentation of disease, clinical manifestations and management, diagnostic approach, and

principles of therapy. As genetics and molecular biology, as well as areas of interest in certain aspects of disease, have expanded, we have added additional chapters to comprehensively cover these new developments. Conversely, as diseases have become less prevalent, we have decreased their emphasis in the textbook. Hence, we believe that the current edition is up to date with the needs of the field.

With this edition, the editorship of the textbook is being passed to a new generation of experts in the field. We thank Drs. Durie, Hamilton, Walker-Smith, and Watkins for their contributions over the last three editions. Dr. Walker has continued with the fourth edition to pave the way for the new editors: Drs. Kleinman and Shneider from the United States, Dr. Sherman from Canada, and Drs. Goulet and Sanderson from Europe. It is hoped that this textbook will continue in perpetuity under their able leadership.

To keep up with the ever-increasing growth of information in this rapidly advancing field, we have chosen to produce a fourth edition in 2004. The size and content of each section have been modified. This revision is dedicated to the maintenance of a comprehensive approach to the practice of pediatric gastroenterology. Each author, whether newly chosen or retained from the third edition, has been selected because of a particular expertise in a specific field. Each author has provided an authoritative and comprehensive account of his or her topic. For example, in the fourth edition, we have separated pathophysiology from clinical syndromes by establishing Section 2, "Clinical Presentation of Disease," and Section 3, "Clinical Manifestations and Management." We have also added new chapters on study design (outcomes, methodology, and ethics) to the diagnosis section entitled "Diagnosis of Gastrointestinal Diseases" to reflect increasing interest in clinical investigation and the evidence-based approach to disease management. We have also added a section on prebiotics and probiotics to reflect this alternative medical approach to the treatment of pediatric gastrointestinal diseases and have also extended the section devoted to clinical nutrition. We believe that these modifications have resulted in a more "user-friendly" textbook.

To give the readership a perspective on the evolution of this textbook, we have included the preface from the first edition published almost 13 years ago. It has been an exciting adventure for the editors to contribute in a small way to the overall development of the practice of gastroenterology by pediatricians. We hope that you enjoy this latest edition.

The editors wish to again thank Ms. Suzzette McCarron for her organizational talents and her ability to liaise among authors, editors, and the publisher. Without her extensive efforts, this textbook would never have been possible. The editors are also grateful to Mr. Brian Decker, Patricia Bindner, and the able staff of BC Decker Inc for their help and support in further developing this edition and in the publication of this textbook.

W. Allan Walker
Olivier J. Goulet
Ronald E. Kleinman
Ian R. Sanderson
Philip M. Sherman
Benjamin L. Shneider
July 2004

REFERENCE

1. Walker-Smith JA, Walker WA. The development of pediatric gastroenterology: a historical overview. Pediatr Res 2003;53:706–15.

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